PROCEEDINGS OF THE FAO/UNEP/IAEA CONSULTATION MEETING ON THE ACCUMULATION AND TRANSFORMATION OF CHEMICAL CONTAMINANTS BY BIOTIC AND ABIOTIC PROCESSES IN THE MARINE ENVIRONMENT (La Spezia, Italy, 24-28 September 1990)

Edited by G.P. Gabrielides

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Ce volume constitue le cinquante-neuvième numéro de la série des Rapports techniques du Plan d’action pour la Méditerranée.

Cette série comprend certains rapports élaborés au cours de diverses activités menées dans le cadre des composantes du Plan d’action pour la Méditerranée: Programme de surveillance continue et de recherche en matière de pollution (MED POL), Plan Bleu, Programme d’actions prioritaires, Aires spécialement protégées et Centre régional méditerranéen pour l’intervention d’urgence contre la pollution marine accidentelle.
PREFACE

The present volume of the MAP Technical Reports Series contains the proceedings of the Consultation Meeting on the Accumulation and Transformation of Chemical Contaminants by Biotic and Abiotic Processes in the Marine Environment which was jointly convened by FAO, UNEP and IAEA and hosted by ENEA, La Spezia, Italy, from 24-28 September 1990. The meeting was organised in the framework of the research component of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL - Phase II) and specifically of Research Area IV "Fates/Environmental transformation".

The key-note paper as well as the other 22 papers presented at the meeting appear in full (in alphabetical order of the senior author's name) as Annex III to the report while the discussions which took place and the recommendations appear in the main body of the report. The papers were reviewed by other participants of the meeting. The views expressed in the papers are those of the authors and do not necessarily represent the views of either FAO, UNEP or IAEA. The meeting has not only provided a forum for Mediterranean scientists to present their work but has also given an excellent opportunity for an exchange of views on the problems encountered by scientists working internationally in this field.

During the discussion the term transformation was taken to encompass not only changes in molecular structure but also changes in the physical matrix and the medium in which a chemical entity is found. The meeting also recognized the difficulty of treating separately biotic and abiotic transformations since they are often indistinguishable in nature. A large part of the discussions concentrated on methodological problems while participation in international intercomparison exercises was considered mandatory for all those involved in the collection and provision of data on contaminant levels. Interdisciplinary research was strongly encouraged in environmental studies.

Final editing and compilation of this volume was done by Mr. G.P. Gabrielides, FAO Senior Fishery Officer (Marine Pollution) at the Co-ordinating Unit for the Mediterranean Action Plan, while Ms Vanta Papapanagiotou was responsible for the typing.
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INTRODUCTION

The present Consultation Meeting on the Accumulation and Transformation of Chemical Contaminants by Biotic and Abiotic Processes in the Marine Environment was jointly convened by FAO, UNEP and IAEA in the framework of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean (MED POL - Phase II) which constitutes the scientific and technical component of the Mediterranean Action Plan. One of the main objectives of the MED POL programme is to generate information that can be used for the progressive technical implementation of the Land-based Sources Protocol which has already been ratified by almost all of the Mediterranean States. The study of the transformation of the chemical substances in the marine environment is essential for understanding their biogeochemical cycles and their fate is one of the criteria considered when taking management decisions for marine pollution control.

Following a kind invitation by ENEA the meeting took place at the Marine Environment Research Centre of Santa Teresa near Lerici, La Spezia, Italy, from 24-28 September 1990. It was attended by 30 participants from France, Greece, Israel, Italy, Spain, Syria, Tunisia, Turkey, U.K., U.S.A. and Yugoslavia as well as by representatives of the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency. The list of participants appears as Annex I.

1. OPENING OF THE MEETING (Agenda item 1)

The meeting was opened by Mr. G.P. Gabrielides, FAO Senior Fishery Officer (Marine Pollution), on behalf of the Food and Agriculture Organization of the United Nations and the Co-ordinating Unit for the Mediterranean Action Plan, and by Mr. M. Bernhard on behalf of the Director of the host Institution. After welcoming the participants and thanking the local organizers for accepting to host the meeting, Mr. Gabrielides outlined the importance of studying the environmental transformation of chemical substances in marine pollution work and expressed the wish that the meeting would motivate and encourage Mediterranean scientists who now limit their studies to concentration levels to give more emphasis to transformations.

Mr. Bernhard welcomed the participants and expressed his satisfaction that the meeting was hosted by his Institution. He briefly explained ENEA's activities and the involvement of the Centre in the MED POL programme.

2. BACKGROUND AND SCOPE (Agenda item 2)

Mr. Gabrielides outlined the background and scope of the meeting. In doing so, he referred to the previous MED POL activities in this field and specifically to the old research activity K "Biogeochemical cycle of selected pollutants". A number of research projects are now under way in Mediterranean Institutions in the framework of Research Area IV "Fates/Environmental transformation" of the MED POL programme and the present meeting provides a forum for the principal investigators of these projects to present their work. In addition,
the meeting will give an opportunity for an exchange of views on the problems encountered by scientists working internationally in this field. Finally, the meeting will be required to make recommendations for future research needs.

3. ELECTION OF OFFICERS (Agenda item 3)

The meeting unanimously elected Dr. Michael Bernhard, Scientific Adviser at ENEA, as Chairperson, Mr. Michael Scoullos, Professor at the University of Athens, as Vice-Chairperson and Mr. James Lamond, Research Scientist at the Napier Polytechnic, Edinburgh, Scotland (presently at University of Crete, Greece) as Rapporteur. Mr. Gabriel P. Gabriellides, FAO Senior Fishery Officer (Marine Pollution) at the Coordinating Unit for the Mediterranean Action Plan, acted as Technical Secretary of the meeting.

4. ADOPTION OF THE AGENDA (Agenda item 4)

The provisional agenda as proposed by the Secretariat was accepted without modifications. It appears as Annex II.

5. ORGANIZATION OF THE WORK (Agenda item 5)

The meeting decided to work in plenary but break up into two groups for the discussion of agenda item 7. The working hours were agreed to be 09:00-13:00 hrs for the morning session and 14:30-17:30 hrs for the afternoon session. Thursday afternoon was reserved for the writing up of the report.

6. PRESENTATION OF PAPERS (Agenda item 6)

During the meeting 23 papers were presented covering various aspects of the accumulation and transformation of chemical contaminants by biotic and abiotic processes in the marine environment. The papers presented appear in full as Annex III.

The keynote paper outlining the current status, trends and prospects of chemical and biotic transformations of marine contaminants was presented by Dr. F.E. Brinkman, of the U.S. National Institute of Standards and Technology.

The importance of the ability to analytically determine both very low concentrations (10^-15 molar) and rapidly reacting chemical species in future research was stressed in conjunction with the recognition of the need for a predictive capability in such environmental interactions with respect to both abiotic and biotic components.

Subsequent papers highlighted aspects of environmental transformations of mercury/methylmercury, TBT, aromatic and chlorinated hydrocarbons and surface active substances as well as biotic and abiotic interactions of a range of heavy metals.
7. THE ROLE OF TRANSFORMATION AND BIOACCUMULATION OF CHEMICAL CONTAMINANTS IN THE MARINE ENVIRONMENT VIS-A-VIS POLLUTION CONTROL (Agenda item 7)

In discussing the definition of transformation several types of transformations were identified. It was agreed that the term transformation should encompass not only changes in molecular structure but also changes in the physical matrix and the medium in which a chemical entity is found. Such matrices can include not only inorganic fluvial and airborne particulates but also dissolved and particulate organic matter, living organisms such as phytoplankton and zooplankton and biogenic materials. The term "medium" can be understood to include freshwater, oxic and anoxic seawater, the gastrointestinal tracts of biota and even physiological fluids and cell cytoplasm. The recalcitrance of organic groups such as PCBs, pesticides and detergents arising from anthropogenic activities and interacting within these media was identified as a problem occurring largely at localised pollution zones with mainly lower-level 'disturbances' arising on a global level.

The meeting recognised the difficulty of treating separately biotic and abiotic transformations since they are often indistinguishable in nature but it was agreed that an attempt should be made to differentiate between the two in the discussions and in this report. Similarly the need to distinguish between man-made contaminants and elevated occurrences of natural materials was recognised, as was the importance of interfaces in acting as sites for adsorption and desorption between biotic and abiotic phases.

7.1 Biological transformation of contaminants

Both active and passive processes were identified whereby biota can mediate contaminant transformation. Contamination is essentially a matter of degree; many metals are required by living organisms for biological processes e.g. iron for oxygen and electron transport, cobalt in vitamin B_{12}, zinc in DNA and in enzymes, calcium, sodium and potassium in muscle and nerve tissues. When present in excessive amounts such metals can become toxic and need to be classified as contaminants. Degradation of organic contaminants by the biotic phase was defined as the alteration of the chemical structure of a contaminant leading to partial or complete decomposition of the parent compound.

It was recognised that, in the case of certain chemical species such as PAH, the production of mutagenic metabolites increases the need for assessment of the significance of their production, presence and removal.

a) Alteration of environment by biota

Biota can indirectly mediate contaminant transformation by altering their environment e.g. during algal blooms anoxic conditions can build up in the water column due to excessive oxygen consumption. Excretion of waste products, such as carbon dioxide and ammonia, can directly influence contaminant speciation through changes in pH, etc. Phytoplankton are known to excrete complex molecules such as sugars and polysaccharides into their medium. Such exo-metabolites can complex
metals and may render them more or less bioavailable to other organisms. The important part played by organic material associated with the particulate phase, incorporating ranges of particle size distributions, is of prime importance in determining biotic and abiotic interactions.

b) Physical interactions

Biota can also mediate contaminant transformation by providing reactive surfaces where metals and organics may be adsorbed. Such biota can also transport contaminants to depth and sea-bottom sediments. In this respect the inclusion of deep-sea areas in pollution studies is necessary.

One biological activity which is particularly important in contaminant transformation is the accumulation of contaminants which are associated with particulate matter (whether inorganic or organic) by filter-feeders and grazers. These organisms serve, by ingesting small particulates and by producing rapidly-sinking fecal pellets, not only to transform the physical matrix and perhaps the chemical structure of the contaminant, but also to transport rapidly the contaminant to depth. During their passage through the water column, associated metals can be remobilised by physical disaggregation of the pellet, by leaching and dissolution, and by biological activity in the pellet matrix. Such sinking pellets are also very efficient scavengers of contaminants and have been shown to remove metals and organics from the water column, through which they fall, and to deliver them to bottom sediments.

c) Metabolism

Perhaps the major role of biota in contaminant transformation is through metabolism. The meeting agreed that a need exists for more detailed scientific knowledge on the metabolism by organisms of persistent organic contaminants including PCBs, PAH and pesticides. As far as metals are concerned, simple chemical reactions such as methylation, demethylation, oxidation and reduction tend to be very important. Several such examples were discussed including the methylation of mercury and arsenic, the solubilization of non-toxic chromium (III) through oxidation reactions and the reduction of the toxic Cr(VI) to Cr(III).

In eukaryotes, metals can be transformed not only during accumulation and metabolism in the tissues but also during passage through the gastrointestinal tract. Little is known of the processes operating on metal contaminants during digestion and the need to clarify the effects of such transformations, physical and chemical, was emphasized. A considerable research effort has been expended on metal uptake and accumulation in eukaryotes. Much is known, mainly from laboratory studies, of the rates and the routes of accumulation. Less is known of the transformation of chemical species of metal contaminants within tissues and physiological fluids. Uptake and accumulation of persistent organics directly or through the food chain has also been widely studied and the need for a better understanding of in vivo processes (by the identification and quantification of metabolites) is recognised as an important area of research activity.
Sequestration of contaminants by inclusion in lysosomes, by binding to metallothioneins and by deposition in mineral granules is a well known phenomenon in marine organisms. The contaminants are effectively rendered unavailable to other cellular components.

At the microbial level the meeting discussed how some bacteria are resistant to metal contaminants by envelope impermeability which excludes the contaminants from their interior. Other bacteria can accumulate contaminants but, possessing certain enzymes, can transform the contaminants. In this context, the group discussed not only contaminant toxicity but also contaminant mobility induced by both biomethylation and biohydridization yielding volatile hydrophobic metal toxicants.

Mercury is an example of a metal which, when methylated becomes more toxic. Arsenic, on the other hand becomes less toxic when methylated. Bacteria can oxidise As(III) to As(V). Bacterial transformation of tin species was also discussed. Monoalkyl tin species are adsorbed to particulate matter and rapidly sink through the water column. Dimethyl and trimethyl tin do not adsorb and thus can be shown to remain in the water column. Bacteria can also form several selenium species, including elemental selenium. Perhaps the most significant of these is dimethyl selenium oxide which is extremely volatile.

The meeting identified the adoption of a multidisciplinary approach, based on the use of data on the chemical structure, reactivity and partition properties and incorporating biological parameters in exposure and effects evaluation in future hazard and risk assessment studies.

7.2 Abiotic transformation of contaminants

When discussing abiotic transformation of contaminants the meeting recognised that metals are not present in water as simple ions but have extremely complex coordination chemistries. In freshwater, many metals exist as divalent cations ions coordinating four or six neutral H₂O groups. In seawater, a number of H₂O groups are replaced by chloride and hydroxyl ions with resultant changes in charge, molecular weight, size, hydrophobicity, volatility, etc. Thus at the freshwater-seawater interface some extremely complex reactions occur which, though we can often understand, we cannot always predict. Changes in metal bioavailability can accompany the transformation. Important abiotic transformations of organic contaminants (such as those occurring in DDT and dieldrin) require further study of processes such as photochemical reactions and the solubilization of contaminants by surface-active substances.

Our understanding of such reactions is derived from well-controlled laboratory experiments. In the environment, there is no such control and many competing reactions occur.

The chloride ion in seawater was described as an extremely efficient coordinator which can bind different metals, through molecular bridges, closer than they would exist even in alloys.
Association of metal contaminants with natural and man-made particles is dependent on the particle type and source. Some particles can be sources of metals e.g. volcanic ash and fluvial deposits from mineral-rich areas. Many particles contain bubbles or act as seeds for bubble-formation. Since the surface microlayers of bubbles and particles are prime sites of metal transformation, the meeting recognised that our understanding of processes operating at these sites need to be improved.

Another area where abiotic transformation is important is the oxic-anoxic interface in the water column and in the sediment column. Oxidation-reduction reactions occurring at this interface not only can they remove metals from solution but can also remobilise metals from particles. We are only beginning to recognise that anoxic conditions can build-up in the water column on small scales (temporal and spatial) and we need better methodology to detect the advent of such conditions and to predict their effects.

Many chemical equilibria result in the formation of a bioavailable species. If such a species is removed from the system e.g. through accumulation by biota, then the chemical equilibrium results in the formation of more of the bioavailable species with the result that non-bioavailable species eventually enter the food chain through simple chemical equilibrium considerations.

Unfortunately for those who wish to interpret nature, the above was recognised as a simplification since not only one but many of such reactions may occur simultaneously.

7.3 Strategy of environmental management/pollution control

One of the most important prerequisites for pollution control is to understand the system reliably. Simple laboratory approaches can help but cannot replace environmental measurements. It cannot be overstressed that natural systems are complex almost, if not altogether, beyond our current level of understanding.

Several approaches were identified by the meeting:

a) The most likely matrices and pathways should be identified for various contaminants so that the concept of "proper measurements, properly applied" can be implemented.

b) Quality assurance is a necessity. Participation in intercalibration exercises ensures that data is realistic and comparable to that of other investigators based upon reliable interchangeable reference or standard materials. An added advantage of intercalibration programmes is that defects in analytical methods can be identified and corrected for. It is recognized that sampling can contaminate transformation. Sampling techniques and analytical techniques should be optimised to minimise contamination, sampling variability and analytical variability. The development of direct and in-situ non-destructive measurements should be encouraged.
c) On the basis of the development of scientific knowledge on the fate and significance of contaminants in the environment the meeting recommended periodic review of existing legislation, and the update of accepted environmental quality criteria in line with scientific developments.

As marine environment pollution is not limited to national boundaries, an international approach to environmental management is required. The meeting recognised the importance of a preventative rather than curative approach in pollution control.

Since control measures often take several years to have an observable effect, it is essential to establish specimen banks so that realistic samples will be available many years later. Such a mechanism should ensure that temporal changes which are measured are real and are not due to changes in methodology and instrumentation. High levels of "noise" in relating ecological and toxicological data was cited as a particular problem in relation to developing an interdisciplinary approach to environmental pollution strategy. There is a fundamental need for the collation of good data on ecological systems if extrapolation to include contaminant effects are to be realistic.

A final point relating to biotic transformation of contaminants in relation to pollution management was the potential use of biota to clean up contaminated environments. For example, cyanobacteria are being used to remove metals from industrial effluents just as oil-degrading bacteria are used for oil spill clean-up.

It was recognised that future progress would best be achieved through interdisciplinary research efforts. One of the lessons learned during the meeting was that exchange of ideas between scientists with different backgrounds and expertise can be a very fruitful process not only in improving sampling and analytical techniques but also in interpreting data. Artificial imposition of "chemical" or "biological" or "geological" strategies and philosophies on an heterogeneous natural environment is effective up to a point but the meeting felt that this point has now been surpassed. Co-ordinated research efforts, with a sharing of knowledge, facilities and equipment, was felt by the meeting to be the optimal strategy for future developments.

8. FUTURE RESEARCH NEEDS (Agenda item 8)

The most important strategy for future research was felt to be an interdisciplinary approach. However several specific areas were identified where such an approach should be focussed.

(a) Better characterization of particulate matter, both man-made and natural, is required. This should include both qualitative and quantitative aspects.

(b) Development of new direct and non-destructive analytical methodologies and improvement of existing ones are needed to identify different chemical species in the natural environment and in the tissues of marine organisms.
(c) Sampling techniques should be improved and standardised at several levels.

   (i) to minimise, since it is impossible to eliminate, variability,
   (ii) to obtain samples which are suitable for bioassay work,
   (iii) to facilitate spatial and temporal comparisons.

   Efforts should be made to obtain representative samples.

(d) Study of the behaviour of contaminants at cellular and subcellular levels. Elucidation of mechanisms of toxicity is important not only to determine but also to devise appropriate bioassays to measure contaminant effects.

(e) Study of the degradation of organic contaminants especially those whose metabolites are expected to accumulate to a higher extent and be more toxic than the parent compound.

(f) Ecotoxicological studies should focus on areas such as synergistic contaminant effects.

(g) More attention should be paid to the role of organic material associated with the particulate phase and especially in relation to variations in carbon particle size and distribution relationships.

(h) The role of micro-organisms in metal transformation is slowly being given the recognition it deserves. More attention should be focussed on prokaryote interactions with metal contaminants and on factors modulating their transformation.

(i) A better understanding of natural processes operating in uncontaminated environments would greatly aid the interpretation of data from contaminated environments. Even though contaminated environments receive more attention, uncontaminated ones should not be ignored.

(j) Information on processes operating at the air-sea interface is still limited. Considering the quantities of contaminants entering the marine environment from the atmosphere and the extremely high concentrations of contaminants in the upper 200 micrometres of the water column, our understanding of this microlayer should be enhanced.

(k) More attention should be given to designing laboratory experiments by taking into consideration the parameters existing in the natural environment so that a more realistic simulation of the natural conditions is achieved. Additional work employing mesocosm or even microcosm experiments in the field itself is needed.

(l) Our understanding of the meaning of high contaminant levels in various matrices and their relation to measured effects should be improved. The interpretation of data in the context of environmental impact needs to be improved at population, organism, cellular and sub-cellular level.
(m) As a corollary to (l) above, the meeting felt that too much attention was being paid to making measurements and not enough attention was paid to the interpretation of results in terms of environmental risk.

(n) A better understanding of the environmental behaviour of contaminants and especially of the mechanisms involved in their distribution and fate is necessary for the prediction of the environmental target.

9. RECOMMENDATIONS (Agenda item 9)

The meeting made the following recommendations:

a) Interdisciplinary research should be strongly encouraged in environmental studies. Expertise in biochemistry, geology and physical oceanography were quoted as examples to be included in such research programmes.

b) Increased communication and collaboration between scientists should be encouraged especially between advanced and less advanced laboratories in developing countries. The provision of equipment alone is not considered satisfactory and joint programmes should also be initiated and promoted. The organization of international meetings was considered a good way of getting appropriate scientists together.

c) Bioassay techniques should be developed to aid the interpretation of contaminant effects.

d) Sampling techniques need to be improved and to be standardised—particularly where bioassays need to be performed on the samples.

e) A wider use of statistical techniques should be encouraged in the interpretation of the data.

f) Participation in international intercomparison exercises should be mandatory for all scientists involved in the collection and provision of data on contaminant levels especially based on interlaboratory comparisons defined by mutual standard reference materials.

g) A specimen bank should be established which can store, for decades if necessary, realistic environmental samples, for the use of programme participants.

10. ANY OTHER MATTER (Agenda item 10)

The publication of the proceedings was brought up under this agenda item. The Technical Secretary explained that the report of the meeting will be published very soon as a preliminary document. The papers presented will be published in full either in the FAO Fisheries Reports Series or the MAP Technical Reports Series. Participants were encouraged to also publish their papers in international scientific journals.
It was agreed that the papers should be reviewed by other participants of the meeting. People wishing to submit a revised version of their paper before this review, should do so as soon as possible but not later than the end of October 1990.

11. ADOPTION OF THE REPORT (Agenda item 11)

The present report was adopted by the meeting on Friday, 28 September 1990.

12. CLOSURE OF THE MEETING (Agenda item 12)

In his closing remarks, the Technical Secretary expressed satisfaction for the results of the meeting and the constructive spirit in which it was conducted. He also thanked the participants, the Officers of the meeting, the guest-speaker and everybody else who contributed directly or indirectly in the success of the meeting. Special thanks were expressed to BNEA for the excellent hospitality.

An exchange of courtesies followed after which the Chairperson closed the meeting.
ANNEX I

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ANNEX II

AGENDA OF THE MEETING

1. Opening of the meeting
2. Background and scope
3. Election of officers
4. Adoption of the agenda
5. Organization of the work
6. Presentation of papers
7. The role of transformation and bioaccumulation of chemical contaminants in the marine environment vis-a-vis pollution control
8. Future research needs
9. Recommendations
10. Any other matter
11. Adoption of the report
12. Closure of the meeting
ANNEX III

PAPERS PRESENTED AT THE MEETING
1. INTRODUCTION

1.1 Chemical basis and scope

We humans exist globally and must seek continued healthy existence on our unique aquatic planet that features both abiotic and biotic geointerfacial transformations and mobilization of a full portion of the known assembly of elements in the Periodic Table, in both monomolecular and macromolecular size ranges (Caldwell et al., 1985). Such diverse global activity of often huge amounts of both essential and toxic elements represents the now well established field of biogeochemistry (Hutzinger, 1986-5), and also provides the basis and scope of crucial monitoring of crustal phenomena that ultimately assures practical and accurate environmental assays (Pawlowski et al., 1984). Clearly, such a broad involvement of both cellular (biotic) and exocellular (abiotic) elemental changes within and across typical land- or sea-based geological interfaces, such as soils and sediments, groundwaters, aquatic microlayers, membranes, gases, or particles, demands reliable chemical monitoring capable of ultratrace multielemental-specific detection that can provide essential quantitative molecular speciation of key diagnostic transformed transport chemoagents (Bernhard et al., 1986). Such biotic or abiotic transformations of certain key elements definitely require rigorous assessment of both naturally-occurring and man-made alterations of local land-sea sites invoking consideration of both dangerous and detoxified pollutants (Chakrabarty, 1982) as well as possible essential or nutrient products (Bowen, 1979).

1.2 Molecular monitoring development

The past two decades have realistically evolved quality ultratrace, element-specific measurements dealing reliably with the scale and diversity of biotic and abiotic sources (Brinckman and Iverson, 1975; Brinckman et al., 1976), along with mobilizations and bioaccumulations analyzed kinetically (Hawker and Connell, 1985; Bacci et al., 1990). Figure 1 which summarizes the compartmentally defined, broad interrelationships between key matrices via real world kinetic pathways that reflect both natural and man-made sources or acceptors, also recognizes the critical rate-determining needs for diverse site evaluations, always in combination with globally prevalent "steady state" solutions represented by fresh and marine waters. Such
OCEANIC MEASUREMENT COMPARTMENTS:

Transitions through which Materials Cycle

![Diagram showing transitions between compartments involving volatilization, dynamic 'steady state' solution, biotic concentration, precipitation & sequestering, natural and anthropogenic sources.]

Fig. 1 Relevant to the scope and nature of key environmental compartments requiring monitoring for assay of abiotic and biotic contaminants transformations, the kinetically active "steady state solutions" available on-site at crucial land-air-sea locations form the basis for state-of-art ultratrace molecular speciation.
environmental monitoring now must always deal with complex elemental combinations and phases, typically detected as organic (Gobas et al., 1988), inorganic (Johnson et al., 1988) and organometallic (Craig and Brinckman, 1986) agents or analytes, clearly in relation to local interactive biota. Moreover, advances in increasing the accuracy of ultratrace molecular speciation afford assays of both the biotic (Baldi et al., 1987) and abiotic (Ballester et al., 1990) components essential to such elemental transformations and redistribution potentials, and clearly establish the critical need for both basic chemical and biotechnical appraisal of key land-sea interactions that obviously are relevant to the current issues and potential problems or solutions of future environmental controls. Another major advantage of this element-specific molecular monitoring capacity is that it provides options for adequate selection and on-line monitoring controls for technological applications of defined abiotic and biotic transformations that can supply significant materials processing, recycling and production (Olson and Brinckman, 1986), that clearly can additionally support environmental needs (Baldi et al., 1989).

1.3 Status and prospects for multielement molecular speciation

As shown in Figure 2, the modern consequence of our internationally established molecular monitoring approach permits specific elemental identification, and offers a significant prospect for both abiotic and biotic in situ evaluation of quantitated molecular topologies for key transformed chemicals in relationship to their physicochemical (Pearlm, 1986), hydrophobic (Veith et al., 1979; Hammers et al., 1982) and biochemical properties (Mogwan and Mellors, 1986). Such biophysical responses to molecular shapes have been demonstrated applicable to organic molecules such as aromatic hydrocarbons (Yalkowsky and Valvani, 1979). Our own research (Eng et al., 1987) has even more specifically focused on quantitative metalloorganic molecular topologies, characterized as molecular total surface areas (MTSA, in Å²), with which we are able to deal with both rigid or flexible carbonaceous metal clusters (Fig. 3) that must occur between bulk solvents and membrane or lipid interfaces (Brinckman et al., 1982). We subsequently demonstrated that such a realistic approach quantitatively predicts necessary aquatic solubilities (Fig. 4), as well as gas and liquid chromatographic retention or separation parameters (Fig. 5) (Tierney et al., 1988), all directly predicted by carbon moiety bonding hybridization, viz.,

\[
\ln k' = 0.243 \text{ (No. of sp}^3\text{ carbons)} - 1.15 \\
(\text{r}^2 = 0.994)
\]

\[
\ln k' = 0.231 \text{ (No. sp}^3\text{ carbons)} + 0.065 \text{ (No. sp}^2\text{ carbons)} - 1.15 \\
(\text{r}^2 = 0.971)
\]

Furthermore, these results definitely correlate membrane-water partition TSA coefficients and consequent microbiotic toxicity as represented in Figure 6 (Brinckman et al., 1988; Eng et al., 1988), even for various metals bearing isomeric carboligands (Eng et al., in press). Additional important prospects that also can aid stated environmental abiotic and biotic chemical transformations relevant to reliable land-sea monitoring, include extensive parallel opportunities to fully exploit established combinations of both abiotic and biotic.
Fig. 2 During the past two decades, the presently reliable state-of-art field and laboratory ultratrace molecular speciation measurements are achieved by on-line molecular chromatographic processes employing on-line multi-element-specific detectors, also consistent with necessary analyte extraction and derivatization as applied to both abiotic and biotic sources in all matrices.
processes in environmental media that can significantly assist crucial materials biotechnology (Brinckman and Olson, 1986), while extending improved environmental controls (Mikami and Misono, 1968; Patterson and Passino, 1989).

**Fig. 3** Determination of appropriate quantitative molecular total surface areas (QMSA), applicable to the reality of fluxional and conformational changes occurring as organic and metalloorganic species transport between solvent, gaseous, membrane, or abiotic substrates, assures most accurate applications for both physicochemical and biochemical partition and toxicity data.

2. **TRENDS AND PROSPECTS**

2.1 **Current scope/status of land-sea bioactive chemicals**

In view of the now broadly developed environmental monitoring applicable to both abiotic and biotic pathways described above (Figs. 1, 2), it now becomes essential to focus further on the crucial topics defined by the present Consultation Meeting that assure sufficient breadth of key chemical substances are carefully surveyed, reliably in terms of abiotic and biotic activity (Sigel, 1984). Table 1 offers a
Fig. 4 The molecular total surface areas calculated for a major group of tetraalkyl Main Group IVA elements representing metals, metalloids, and organics, demonstrate a remarkable linear correlation with fully independent aqueous solubility data reported over the past 40 years that depicts a solubility range over 10,000-fold.

brief but representative example of both the diverse types of organic, inorganic, and organometallic substances resolved internationally by on-site measurements cited that also suggest the nature and extent of prospective additional field and laboratory research that must define both abiotic and biotic interactions critical for natural and technological resource or products cycling. As also indicated in Table 1, biomethylation represents a major endo- and exo-cellular process.
Fig. 5 Thermodynamically controlled chromatographic separation periods, defined as retention times, ln K', are quite precisely correlated with appropriate independent molecular total surface areas calculated via reported Van der Waal data for appropriate carbon and metal bonds (here tin). Not only do both highly flexible alkyl moieties combined with rigid aromatic moieties yield the reliable correlations, but the numbers of each type of group additively predict retention times that can significantly transform many metals and metalloids which are of concern, both for health and resource reasons (Brinckman, 1985). Related biotic and abiotic transformations of chemical substances, naturally occurring or from anthropogenic sources, therefore also are included in the full picture of what land-sea chemicals we must monitor and control (Jaworski et al., 1984).

As many contributors to the current UNEP Consultation Meeting, along with numerous external colleagues, have reported, the representation of global bioreponses (Roesijadi and Spies, 1989) and accumulation (Brierley and Brierley, 1983; Ferraro et al., 1990) of chemical contaminants by aquatic biotic and abiotic transformations (Maki et al., 1980) is resolved and placed with future needs and prospects significantly by the Mediterranean Sea. Because the Mediterranean Sea represents a unique region with established
Fig. 6 The defined QM TSA methodology shown applicable to solubility and hydrophobic or membrane partitioning, here reveals the quantitative application to assay and predict biotic toxicity for both bacteria and algae, dependent upon the toxicant TSA versus realistic environmental aqueous μM concentrations. Even though TSA values for tetraorganometals may differ only by 5% (Pb versus Sn), the toxicity ratio can exceed 10-fold with the organolead...
Table 1
Examples of chemical substances released into and/or biotransformed in the Environment.

<table>
<thead>
<tr>
<th>Metal Species</th>
<th>Origin/Matrix</th>
<th>Commercial use or source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylmetals</td>
<td>Biotic, abiotic air, water soils, sediments</td>
<td>Manufacture wastes, mines</td>
<td>Brinckman, 1985</td>
</tr>
<tr>
<td>Mercury</td>
<td>Cinnabar</td>
<td>Mining waters</td>
<td>Baldi et al., 1989</td>
</tr>
<tr>
<td>Cadmium volatilization</td>
<td>Abiotic/biotic marine waters</td>
<td>Polymers, alloys ceramics, wastes</td>
<td>Huey et al., 1975</td>
</tr>
<tr>
<td>Chromium</td>
<td>Sewage</td>
<td>Tannery waters</td>
<td>Baldi et al., 1990</td>
</tr>
<tr>
<td>Organotins</td>
<td>Harbors</td>
<td>Antifoulants</td>
<td>Blair et al., 1988</td>
</tr>
<tr>
<td>Organoaarsenicals</td>
<td>Retortwater/ wastewaters</td>
<td>Oil shale Processing</td>
<td>Fish et al., 1982</td>
</tr>
<tr>
<td>Organoleads</td>
<td>Atmosphere soils, waters</td>
<td>Gasoline</td>
<td>Brinckman and Fish, 1981</td>
</tr>
<tr>
<td>Gallium, indium, thallium</td>
<td>Atmospheric particles</td>
<td>Semiconductor products, wastes</td>
<td>Fowler, in press</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organic species</th>
<th>Origin/Matrix</th>
<th>Commercial use or source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloorganics</td>
<td>Biotic/ores waters, soils</td>
<td>Metals bioprocessing</td>
<td>Brinckman and Olson, 1988a</td>
</tr>
<tr>
<td>Polychlorinated biphenyls (PCBs)</td>
<td>Seawaters</td>
<td>Industrial applications</td>
<td>Burns and Villeneuve, 1987</td>
</tr>
<tr>
<td>Hydrocarbons and halohydrocarbons</td>
<td>Riverine and sewage</td>
<td>Fuels and cleaners</td>
<td>Marchand et al., 1988</td>
</tr>
<tr>
<td>Polynuclear aromatic hydrocarbon</td>
<td>Sediments and soils</td>
<td>Fuels refining combustion</td>
<td>Means et al., 1980</td>
</tr>
</tbody>
</table>

Man-made land-based metal-containing materials transmitted to the bioactive common waters for millennia, timely establishment of necessary monitoring and management prospects meeting Mediterranean Action Plan goals (Meth, 1985) for relevant evaluation of both extant biotransmission and bioaccumulation in this long-active global promoting region has clear potential for related land-air-sea interfacial consequences influence by both abiotic and biotic pathways throughout global technological activities.
2.2 Newest approaches to needed land-sea key substance detection

The substantial data base for critical metals and/or haloorganics (Bernhard, 1978; Stoeppler et al., 1979; Baldi et al., 1983; Burns and Villeneuve, 1987; Marchand et al., 1988), and organotins (Pytiyanos and Samanidou, 1990) already reported for the Mediterranean land-sea environments, makes clear the need to strongly focus appropriate on-site abiotic and biotic elemental speciation towards generic release of both natural and anthropogenic chemical substances. Thereby, the molecular pathway and rate of important and diagnostic transformations and ultimate bioaccumulation both at marine sites and atmosphere-connected land uptake of such aquobiotransformed contaminants can be adequately assayed (Matthias et al., 1989) and altered for safer environments. Figure 7 summarizes the overall analytical and interpretive opportunities thus obtained, leading to necessary international measurement standards and quality controls and aiding necessary joint environmental management. Also notable is the fact that our demonstrated QMTSA data (Figs. 3 and 6) predicting bioresponses of microbiota, can also be applied to marine macroorganism uptake defined in comparable solvatochromic parameters (Hawker, 1990).

2.3 The long-range scope and worth of advanced molecular monitoring of both biotic an abiotic processed marine contaminants amplified by major biotechnological prospects

Our basic research activities at the National Institute of Standards and Technology (formally the National Bureau of Standards) have long sought to deliver both the needed ultratrace molecular- and element-specific measurement capability required for the transect between land, sea and atmosphere as realistic field monitoring methodologies, delivering both the purely chemical and concurrent biological routes chiefly directing amounts and paths by which known contaminants and possible new contaminants occur (Brinckman and Olson, in press). Application of our established molecular separation and metal-specific detection provided by laboratory and field-based chromatographic units coupled with appropriate detectors, henceforth has provided both on-site and laboratory intercomparisons applicable for effective environmental assay with concurrent laboratory mechanistic quantitation needed for ultimate standards production and relevant prediction prospects for contaminant impacts or range from both man-made and natural land-sea interfacial sources (Blair et al., 1988). Well-established direct emissions of such contaminants between the land-to-sea interface are also incorporated in the measurement and pathway assessments we pursue (Olson et al., 1988).

In view of the needed basic strategic materials bioprocessing studies we've conducted (Brinckman and Olson, 1988b), relevant to such land-sea contaminant transmissions and reliable in vivo monitoring (Trout et al., 1989), an even more basic aspect of biogeochemical research has both focused and fostered essential amplification of our work directed to stated objectives of this Consultation Meetings' concern for both transformation and accumulation of key chemical contaminants by aquatic abiotic and biotic processes. This condition and requirement reflects the realism invoked by appraisal of interactive modes between the common presence of many active elements influencing either biological or non-biological molecular routes.
CHEMICAL SPECIATION:

The Operational Delivery to Databases

Fig. 7 The operational utility of ultratrace molecular speciation relates the degree of molecular and elemental specificity with appropriate biotic or abiotic effects. Thereby, the combination of environmental mobilization, transport, and accumulation or deposition of critical contaminants or precursors are quantitatively defined, assuring useful regional and international environmental standards.
dictating the stated land-sea monitoring and prospective controls or improvements. Such examples of our past and latest chemical and bioprocessing technology elucidations (Brinckman and Fish, 1981; Olson et al., 1990) are reflected by the rather common influence of both toxic and essential heavy elements that dictate the modes and extent by which such contaminants issue. Long-standing international assays of the global biometabolism and consequences of methylmercury is one major case (Wood and Goldberg, 1977; Siegel and Siegel, 1983), but our results further demonstrate (Brinckman et al., 1985) that many aquatic organisms can effect such methylmercury production via indirect or extracellular paths that also promote global biometabolism of major metals and metalloids, which consequently exocellularly (Huey et al., 1974) or abiotically effect later transmethylation of adjacent or later present diverse metals (Brinckman and Olson, 1988a). Clearly, the presence of several metals with biota can thereby produce conmetallic biometabolizations.

Additionally, we demonstrate (Iverson and Brinckman, 1978) that both bacterial and algal microorganisms common to the crucial land-sea interface can also produce extracellular metabolites that via direct solid-liquid or gaseous-solid or gaseous-liquid interfaces can deliver the crucial hydrophobic but lipophilic heavy-element contaminants relevant to stated objectives. A significant example (Fig. 8) is represented by marine algal production of haloorganic molecules such as methyl iodide, which we show have remarkable capacity in both fresh or saline waters to directly dissolved common refractory ores, alloys, waste particles via carbocationic dissolution and production of volatile bioaccumulative organometals and -metalloids (Thayer et al., 1984; Thayer et al., 1987). Consequently, susceptible to natural mobilization are ancient bronzes, precious alloys, or mine ores as well as modern high technology alloys (Elderfield et al., 1990) and merit current monitoring, even in sedimentary porewaters (Lee and Weber, 1988). The independent reporting of the enormous variety of organochlorane compounds ranging from halomethanes to complex alkylaromatic halides, produced by both marine and terrestrial organisms (Paulsen, 1980), along with later consideration of such halometabolites for industrial potential (Neidiman and Peggert, 1987), quite strongly points to the realistic concurrency of required multi-elemental molecular speciation for ultimate assays and controls meeting that which must serve both stated environmental requirements while also extending relevant biotechnologies.

3. CONCLUSIONS

3.1 Reasonable prospects for coordinated environmental and biotechnical research and applications

A clearly necessary aspect of the timely, well-defined objectives of the Consultation Meeting can thus be resolved in terms of the present status of in-field, co-elemental ultratrace, molecular speciation monitoring coupled with the land-air-sea evaluation of diagnostic chemical and biotic intermediates, such as haloorganics, organometals and mobilizing ligands. Such organic-based ligands represent a key insight (Means et al., 1980) for predicting both mobilization and bioaccumulation of heavy element clusters (Hallberg et al., 1980), just as our described results on quantitative molecular
Fig. 8 Observed heterogenous reactions in water between methyl iodide and bulk metals (Sn, Fe, Pb), binary metal sulfides, ternary minerals, enargite ($\text{Cu}_3\text{AsS}_4$) and arsenopyrite (FeAsS), and estuarine sediment are compared. Speciated form of methylated products and the oxidation state of product metal ions are shown; the products in brackets quantitated by conventional atomic absorption detectors were not speciated. From the metal sulfides, dimethyl sulfide was a common coproduct sometimes with lesser amounts of dimethyl disulfide.

topology prediction of essential physicochemical and cellular toxicity properties demonstrate.

A vital prospect evolves from the fact that present international state-of-art environmental monitoring exploiting the defined ultratrace molecular speciation, additionally sustains rapidly emerging materials biotechnologies, especially as these must deal with parallel biotic and abiotic transformations or selective bioaccumulation (Tsezos, 1985) of critical and precious element clusters noted, employing analogous process monitoring (Olson et al., 1989; 1989a; Olson and Brinchman,
1989b; Thayer, 1990). Just as Figure 8 reveals, we've exploited the noted capacity of marine biota's production of methyl iodide to deliver significant waste, sediment, metals, alloys, ores bioloeaching into notable organometals and aquated metal ions which also presents a global scale for metals and metalloids transmethylation (Jewett et al., 1978). Clearly, as illustrated in Figure 9, the present and future emphasis on appropriate interdisciplinary research between environmental chemists, molecular speciation analysts, and biologists committed to broad elemental biotransformations, constitutes the basic approach that must fully exploit present and prospective findings defined by the present UNEP Consultation Meetings' focus on dangers of chemical contaminants as bioamplified in the marine environment. Thus,

Fig. 9 The ultimate outcome from a realistic appraisal of critical and crucial chemical materials undergoing both biotic and abiotic transformations in mixed land-sea-air environments, whether induced by naturally-occurring or anthropogenic sources can be reliably assayed, interpreted and quantitatively defined by mutual applications of the current and prospective state of ultratrace molecule-specific measurements discussed herein. The interplay between both basic environmental and chemical needs thus equally define future biotechnological prospects controlling global health, environment and desirable materials production.
realistic motivation for joint assembly of adequate land-sea toxic and needed substances coupled with truly reliable assays, clearly forms the profitable joint ventures for both environmental management and crustal/marine materials bioengineered technologies.

Finally, this presentation concludes with grateful recognition for site-specific monitoring, diagnostic biotic or abiotic analytes, and current or future applications relevant to the quality-assured approach globally needed, but significantly prompted with the wealth of Mediterranean Sea and States biogeochemical history.

4. REFERENCES


Brinckman, F.E. and W.P. Iverson, 1975. Chemical and bacterial cycling of heavy metals in the estuarine system. In: Marine chemistry in the coastal environment, edited by T.M. Church, Washington, DC, American Chemical Soc. Ser. 18, pp.319-342


BIOACCUMULATION OF PAHS IN SELECTED BIOTA FROM SYRIAN COASTAL WATERS

by

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Environmental Studies Laboratory
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1. INTRODUCTION

Contamination of coastal waters has a variety of negative effects on human health and on the economical activities related to marine resources.

Very little information is available concerning the level of pollutants, especially aromatic hydrocarbons (PAHs) in the Eastern Mediterranean sea water and food chain. Most of the studies generated from this area dealt with aliphatic hydrocarbons and trace metals (Aboul-Dahab and Halim, 1981; El-Sekkary, 1981; Taliadouri-Voutsinou, 1981; Demetropoulos and Loizides, 1986).

Abosamra et al, (1989) published the first data on the level of total aliphatic hydrocarbons (T.A.H.) and trace metals in sea water and some selected biota species in the Syrian part of the Mediterranean sea. Two main conclusions could be drawn from this study:

a) The levels of T.A.H. and trace metals in sea water is directly related to industrial and urban activity.

b) The levels of T.A.H. and trace metals in the selected species were in the same range found by other researchers in the Eastern Mediterranean region.

This paper deals with the bioaccumulation of PAHs in the tissues of certain marine species shown in Table 1, collected from the Syrian coastal waters in the vicinity of Lattakia (Fig. 1).

Qualitative and quantitative analyses were carried out in order to:

a) Estimate the levels of PAHs in the species tissues and compare them with others from different areas.

b) Identify the possible sources of pollution which could justify the bioaccumulation in the tissues.

c) Assess the possible transport of anthropogenic pollutants to the eastern Mediterranean region by marine currents.
Fig. 1 The Syrian coast

Table 1
Species selected for analysis.

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat</th>
<th>Main food</th>
<th>Fishing procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sardina pilchardus</td>
<td>Pelagic</td>
<td>Phyto &amp; Zooplankton</td>
<td>Gill nets</td>
</tr>
<tr>
<td>Mullus barbatus</td>
<td>Bottom</td>
<td>Bottom invertebrates</td>
<td>Bottom</td>
</tr>
<tr>
<td>Ruditapes philippinarum</td>
<td>Pelagic</td>
<td>Fish molluscs</td>
<td>Floating line</td>
</tr>
<tr>
<td>Penaeus kerathurus</td>
<td>Sandy-mud bottom</td>
<td>Zooplankton</td>
<td>Bottom trawl</td>
</tr>
<tr>
<td>Pinctada martensi</td>
<td>Rocks</td>
<td>Filter-feeding</td>
<td>Direct</td>
</tr>
</tbody>
</table>
2. SAMPLING AND ANALYTICAL PROCEDURES

After collection, the specimens were wrapped in clean aluminium foil and kept at -15°C.

The muscle tissues were separated using stainless steel instruments and homogenized. Details for the digestion of tissues (Warner, 1976) and the extraction and analysis of PAHs (Lee, 1981) are described in Fig. 2.

A PAH standard mixture with different concentrations was analysed in order to quantify the different PAHs and Unresolved Complex Mixture (U.C.M.).

3. RESULTS AND DISCUSSION

Table 2 outlines the concentrations of different PAHs found in tissue of species under study.

Different species had different concentrations of PAHs in their tissues, and the average concentrations (μg g⁻¹ fresh weight) ranged as follows:

- Naphthalene: 0.016 - 0.077
- Biphenyl: 0.045 - 0.41
- Acenaphthalene: 0.015 - 0.279
- Fluorene: 0.0035 - 0.241
- Phenanthrene: 0.012 - 0.393
- Anthracene: 0.048 - 2.279
- Pyrene: 0.081 - 0.894
- Benzo-a-pyrene: 0.108 - 2.570
- Total PAH: 0.629 - 4.241

The total PAH concentrations found in this study are lower than those indicated by Albaigés (1987) in an industrial area (33.8 μg g⁻¹ dry weight) but are of similar range to the concentrations found in non industrialized and fishing areas (3.3 and 1.9 μg g⁻¹) in Mullus barbatus.

Mullus barbatus lives in muddy and sandy bottoms and feeds on bottom invertebrates, Penaeus kerathurus has the same habitat as for Mullus barbatus but zooplankton are considered as its main food.

The two species bioconcentrate the PAHs in their tissues; the major difference is that, all PAH compounds analysed were found in all individuals of Mullus barbatus while only pyrene and B.a.p. were detected in some individuals of Penaeus kerathurus.

Sediments are known to act as a sink for various pollutants and this means that a species like Mullus barbatus is in direct contact with high concentrations since they can consume animals that are already living in the mud and possibly ingest accidentally pollutants through food intake.
Homogenated Tissues
↓
Digestion in NaOH at 90°C
For 2 Hours
with Shaking at half Time.
↓
Cooling
↓
Extraction with distilled
Diethyl ether
↓
Centrifugation for 10 min.
3000 rpm.
↓
Removal of ether and
re-extraction 3 times.
↓
Combined extracts
↓
Drying over anhydrous
Magnesium sulphate
↓
Fractionation in Sephadex 200
Column.
↓
Elution with distilled Dichloromethane

55 ml T.A.H. → 100 ml P.A.H.

Evaporation with N₂ until dryness
↓
Redissolving in 1 ml of
distilled hexane
↓
Chromatographic analysis
by H.R.G.C.

Column: SE-30 capillary column. 25 m.
Detector: FID 310°C
Injection: split - splitless system 200°C
Oven: 80°C - 1 min - 250°C / 5°C/min.
Carrier gas: Helium.

Fig. 2 Analytical procedure for the extraction of PAHs from tissues
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<th></th>
<th>Naphthalene</th>
<th>Biphenyls</th>
<th>Acenaphthylene</th>
<th>Fluorene</th>
<th>Phenanthrene</th>
<th>Anthracene</th>
<th>Pyrene</th>
<th>B.A.P.</th>
<th>Total</th>
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<td></td>
<td>(0.007)</td>
<td>(0.018)</td>
<td>(0.012)</td>
<td>(0.015)</td>
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<td>(0.010)</td>
<td>(0.014)</td>
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<td></td>
<td>(0.09)</td>
<td>(0.589)</td>
<td>(1.44)</td>
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<td>(0.04)</td>
<td>(0.039)</td>
<td>(0.162)</td>
<td>(0.052)</td>
<td>(2.79)</td>
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<td>(0.150)</td>
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<td>(0.004)</td>
<td>(4.3)</td>
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<td>nd</td>
<td>0.114</td>
<td>0.032</td>
<td>0.032</td>
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<td>0.241</td>
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<td>(0.092)</td>
<td>(0.21)</td>
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<td>(0.018)</td>
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<tr>
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<td>(0.01)</td>
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<tr>
<td>Gariba</td>
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<td>(0.007)</td>
<td>(0.0053)</td>
<td>(0.00)</td>
<td>(0.016)</td>
<td>(0.080)</td>
</tr>
</tbody>
</table>

nd = not detected

Table 2
PAH concentrations in selected organisms in µg g⁻¹ fresh weight (values between brackets: standard deviation).
The concentration of PAHs in the tissues of bivalves was not on the high side. Also some PAHs were not detected in certain individuals.

Table 2 presents a rough comparison of the concentrations exhibited by the different species studied. Generally speaking, high concentrations are associated with bottom feeders like Mullus. However, Sardina exhibited the highest concentrations in the case of pyrene and B.a.P. since it feeds on phytoplankton which is capable of concentrating organic compounds and especially some lighter fractions which float on the surface of the water. It also feeds on zooplankton which eats detritus and phytoplankton.

It is interesting to note that the most localised of all species, Pinctada martensi, which is basically sedentary and should, therefore, reflect the actual local situation showed intermediate concentration levels in comparison with the rest. But since this species is found in rocky areas where there is no fine sediment, we can only say from the present information that while our coastal waters seem to be rather uncontaminated, further studies are needed on the sediment which is very likely to be a potential source of some of these compounds.

The migratory nature of Mullus and Sardina suggests that distant point sources are very likely, particularly when we consider the higher concentrations found elsewhere.

The results from the abundant local species of fish "Gariba" are promising because this fish feeds on a variety of organisms and its widespread presence in all parts of the Syrian coast makes it a suitable candidate for further investigations particularly since it is caught both by fishermen and anglers. Its relatively cheap price makes it even more accessible to the public and this increases its importance as a species worthy of continuous investigation.

It is generally appreciated that sediment profiling for PAHs and other compounds is a necessary procedure in any pollution study. The relationships in the food web should also be considered.

The presence of the unresolved complex mixture (U.C.M.) emphasizes the petrogenic nature of the PAHs (see Figs 3 and 4). Therefore, further studies on their petrogenic origin are also of interest.

Another point that should be considered is the possibility that some species metabolize the PAHs and as a consequence the mother compounds cannot be detected (Garrigues, 1990).

Pyrene and benzo-a-pyrene were present in high concentrations in all the individuals analysed.
Fig. 3  Chromatogram of PAHs in Mullus barbatus tissues
Fig. 4 Chromatogram of PAHs in *Sardina pilchardus* tissues
4. REFERENCES


MICROBIAL TRANSFORMATION OF MERCURY

by

F. BALDI

Università di Siena, Dipartimento di Biologia Ambientale,
Via P.A. Mattioli 4, 53100 Siena, Italia

ABSTRACT

The role of bacteria in the biogeochemical cycle of mercury is crucial for the transformation of this metal in the environment. For many years it has been known that aerobic heterotrophic bacteria are resistant to mercury by virtue of two enzymes: mercury reductase and organomericcurial lyase. These enzymes convert both inorganic and organic mercury to elemental mercury. Hydrocarbons are also formed from the degradation of organomercurials. As regards anaerobic bacteria, it seems that their influence on the transformation of mercury compounds is not of genetic origin, but it is due to aspecific interactions. Ionic mercury, for example, reacts with H$_2$S, an extracellular metabolite produced by sulphate reducing bacteria (SRB), to form mercury sulphide. Several phylogenetically unrelated species of this group, also seem to be involved in the methylation of inorganic mercury. However, even though the direct microbiological formation of methylmercury is still a source of debate, it opens a valid biogeochemical approach to key biotic transformations of additional metallic contaminants.

1. INTRODUCTION

Schemes of the mercury biogeochemical cycle were first drawn up in the seventies, and today are still published with small variations from the original one. Several key points of Hg transformations have not yet been investigated enough, for instance the transformation of Hg$^0$ to oxidized chemical forms (Hg$^{+2}$, Hg$^{+3}$), and the production of dimethylmercury in the environment. Only recently have scientists become acquainted with new aspects of mercury speciations in aquatic and terrestrial environments, but the debate has shifted mainly to the detection limit of mercury compounds such as Hg(II), Hg$^0$ and CH$_3$Hg$^+$. New sophisticated methods allow us to determine Hg at picogram levels (Bloom and Fitzgerald, 1988; Bloom, 1989). However, in studying the biogeochemical cycle of mercury, the direct determination of the mercury species is important. Apparatus such as gas chromatography plus mass spectroscopy (GC-MS) or gas chromatograph in line with Fourier Transform infrared spectroscopy (GC/FTIR), which are less sensitive but accurate in the determination of the mercury species, are suitable for this type of investigations. The analytical aspect is important to avoid misinterpretation of the mercury cycle, and it is decisive in investigating the role of microorganisms in mercury transformation.

Strains of bacteria interact with mercury in a defensive way, and during their evolution they have developed different mechanisms to detoxify the cell cytoplasm of mercury. The aim of this report is to
highlight the role of bacteria in the biogeochemistry of mercury by demonstrating the production of specific biocatalysts, active to mercury species only, and investigating specific components which might be important in anoxic environments, especially as regards mercury methylation by sulfate reducing bacteria.

2. BACTERIAL TRANSFORMATION OF Hg(II) IN THE ENVIRONMENT

The mercury resistance in bacteria was first documented 30 years ago (Moore, 1960), and subsequently found to be plasmid-linked (Dycke and Richmond, 1967; Novick and Roth, 1968). The mechanism of resistance (Furukawa et al., 1969; Furukawa and Tonomura, 1972) consists of the reduction of inorganic mercury to elemental mercury (Hg⁰), a volatile and less toxic chemical form of the metal. The first strains of mercury resistant bacteria were isolated in hospital settings (Moore, 1960; Novick and Roth, 1968) and then aquatic environments (Spangler et al., 1973; Nelson and Colwell, 1975). In the Mediterranean basin, the first isolation of mercury resistant bacteria was performed in 1984 (Baldi et al., 1985; Baldi et al., 1989) in freshwater from ponds and rivers near Monte Amiata, an area well known for its ancient cinnabar deposits. About 37 strains were isolated, the six isolates of which were mercury resistant because they volatilize Hg. The rapid test for the mercury resistant bacteria was their rapid growth on agar plates amended with 5 µg ml⁻¹ HgCl₂, and subsequently (Baldi et al., 1989) with 10 µg ml⁻¹ of HgCl₂. Each isolate from the plates was also checked for mercury volatilization. This was performed by incubating the strain for a few minutes with 1 µg HgCl₂ in a liquid medium. The dense bacterial culture (1.0 Abs at 600 nm) was sealed in a test tube with a "mininert valve" cap and then 1 ml of headspace was injected with a gas sampling syringe into the atomization cell of an atomic absorption spectrophotometer (Perkin Elmer mod. 300S). The Hg⁰ concentration was determined by injecting different volumes of Hg⁰ vapour sampled above liquid metallic mercury kept at room temperature. The two tests applied to sampling in aquatic and in terrestrial environments demonstrated that all bacteria which grew well on agar plates with 10 µg ml⁻¹ of HgCl₂, were also able to reduce Hg(II) to Hg⁰. This is valid only for bacteria; molds and yeasts show different mechanisms, in which no Hg transformation is involved. Nevertherless, Neospora crassa (Iadner, 1970), has been found to methylate HgCl₂ by "incorrect" synthesis of methionine, and other species of fungi have also been reported (Vonk and Sijpesteijn, 1973) to methylate Hg. These findings have never been confirmed (Reisinger et al., 1983).

Mercury resistant bacteria (MRB) may be readily isolated in mercury polluted areas, so much so that recently we suggested the use of the percentage of MRB as an index for monitoring Hg pollution in geothermal areas (Baldi et al., in press). The distribution of total Hg content in moss leaves in a geothermal area near Siena was compared with the distribution of the percentage of MRB. The bacterial strains were isolated from the same moss sample. In monitoring Hg pollution in several geothermal areas in Tuscany, it was shown (Barkay and Olson, 1986) that the percentage of MRB is a good biomarker with different characteristics from the total Hg in mosses. The bacteria which colonize the leaf surface of mosses, are sensitive to temperature and pH changes. In addition, they only interact with available Hg, which induces the synthesis of detoxifying enzymes.
The response of the MRB community in aquatic environments towards HgCl₂ in picogram levels was recently investigated in an experimental study (Baldi et al., in preparation). After adding HgCl₂ to the water column, the percentage of MRB increased rapidly (30% of whole bacterial community), a maximum was reached in 24 hours, and was significantly correlated with the concentration of HgCl₂ (from 20 ng l⁻¹ to 2000 ng l⁻¹). These results encourage the use of this bioclipper, not only for monitoring Hg pollution, but also to check Hg availability.

2.1 Genetics of narrow-spectrum Hg resistant bacteria

The most common Hg-resistant bacteria belong to the narrow-spectrum group, and are so named because they only enzymatically convert inorganic mercury to elemental mercury. Several genes are involved in the biotransformation of inorganic mercury (mer genes), and they are often "horizontally" transferred to other species by transposons (transferable DNA). These mer genes are harboured in plasmids (extrachromosomal DNA), but in a strain of Bacillus sp, the mer operon was found in the chromosome (Wang et al., 1989).

The most studied mer gene systems were detected in two transposons: Tn501 and Tn21 which belong to the Tn3 family (Brown et al., 1980; De la Cruz and Grinsted, 1982; Brown et al., 1986; Summers, 1986). The best known mer gene is merA, which codifies for mercury reductase. Sequence analysis shows a high homology with genes codifying for glutathione reductase and lysozyme dehydrogenase. Other genes involved in mercury resistance are: gene merP, which is important for the active transport of Hg(II) into the cytoplasm; the genes merT and merC codify for two proteins of the inner cell membrane, but do not seem essential for the final transformation of Hg(II) (Summers, 1986). The gene merD codifies for a small peptide. Insertions in merD produce a sensitive phenotype (Hg⁶⁺), but only when there are many copies of this gene. Interestingly, the polypeptide expressed by the gene merD is homologous to the polypeptide from gene merR (Nucifora et al., 1989), which is the regulator of mer operon. The merR behaves as a switch: when it is on, it produces a large amount of mer proteins, when it is off, no mer proteins are synthesized. The presumed regulating protein, shows 4 cysteine residues in a peculiar site, and we think that this location might be involved in binding the effector. The effectors are Hg(II), organomercurials, and the gratuitous inducers Merbromin and Thymersal. The latter are not degraded by the mer operon products (Robinson and Tuovinen, 1984). Mercury resistance is inducible in all the aerobic heterotrophic bacteria studied so far. The only exception is the acidophilic chemolithotrophic bacterium, Thiobacillus ferroxidans, which has constitutional mercury resistance (Olson et al., 1982).

Although mercury resistance was detected for the first time in plasmids from gram-positive bacteria (Dyke and Richmond, 1967), resistance has been studied mostly in gram-negative bacteria. The mer operon from gram-negative bacteria retains 40% homology, but the DNA sequence expressing the active site of mercury reductase reaches 90% homology (Laddaga et al., 1987).
2.2 Mercury reductase

The mer operon consists of 5 genes, and codifies for the same number of proteins. Mercury reductase is believed to be formed by 564 amino acids in Tn21 and 561 amino acids in Tn501 (Summers, 1986). It was partially purified by Isaki et al. (1974) and later by others (Schottel, 1978; Fox and Walsh, 1982; Rinderle et al., 1983) from strains of Escherichia coli, Pseudomonas (Furukawa and Tonomura, 1972; Tezuka and Tonomura, 1976; 1978; Fox and Walsh, 1982) and Thiobacillus ferrooxidans (Booth and Williams, 1984). The mercury ion-reducing flavoproteins purified from different species and strains show certain similarities, including the molecular weight (Silver and Misra, 1988). The absorbance spectrum of the oxidized form shows a maximum peak at 455 nm, and after NADPH addition the maximum absorbance shifts to 540 nm. Kinetic properties of the enzyme are reported in Table 1.

Table 1
Comparison of kinetic properties of mercury reductase from different species of bacteria.

<table>
<thead>
<tr>
<th>bacteria species</th>
<th>references</th>
<th>Km HgCl₂ (µM)</th>
<th>Turnover no. (min⁻¹)</th>
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<tr>
<td>P. aeruginosa</td>
<td>Fox and Walsh (1982)</td>
<td>12</td>
<td>800</td>
</tr>
<tr>
<td>E. coli</td>
<td>Rinderle et al. (1983)</td>
<td>14</td>
<td>1044</td>
</tr>
<tr>
<td>T. ferrooxidans</td>
<td>Booth and Williams (1984)</td>
<td>8.9</td>
<td>746</td>
</tr>
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</table>

The enzyme belongs to the disulfide reductase flavoprotein class. There are also similarities between mercury reductase and other FAD-containing disulfide reductases: lipoamide dehydrogenase and glutathione reductase (Silver and Misra, 1988). The enzyme contains the co-enzyme FAD, utilizes NADPH as an electron donor, and requires an excess of exogenous thiols for its activity. The presence of thiols ensures that Hg(II) will be presented as dimercaptide to the active site of the enzyme. The overall reaction is as follows:

RS-Hg-SR + NADPH + H⁺ -----→ Hgₐ + NADP⁺ + 2 R-SH

The mechanism of Hg(II) reduction to Hgₐ suggested by Brown (1985) follows these sequential steps: a) the protein of merP gene, which occurs in the periplasmic space, reversely binds Hg(II) with two cysteine residues, and releases 2 H⁺; b) Hg(II) is then transferred from the merP protein to the cystein groups of the merT protein, which is codified by merT gene and incorporated in the cell membrane. In the meantime, the 2 H⁺ return to the previous site, and Hg(II) bound to merT now migrates towards the cytoplasm; c) Mercury reductase (merA), a cytoplasmic enzyme, is temporarily associated with the merT protein at the cell membrane. So, Hg(II) migrates from position D to other cysteine groups in position E (Fig. 1). Here it is reduced by NADPH to Hgₐ. d) Elemental mercury crosses the cell envelope by physical diffusion.
3. BACTERIAL TRANSFORMATION OF ORGANOMERCURIALS

Bacteria, also able to degrade organomercurials, are classified as broad-spectrum mercury resistant and they are less widely distributed in the environment. In two surveys for mercury resistant bacteria in the Monte Amiata region (southern Tuscany), only 7 strains out of 145 narrow-spectrum mercury resistant isolates, were also able to degrade organomercurials (Baldi et al., 1985; Baldi et al., 1989). However, in Minamata Bay, well known for methylmercury contamination since 1951 (Irukuyama, 1977), it has been found that of 1,428 strains studied, 75 were able to volatilize Hg, and 65 of these were broad-spectrum mercury resistant. In adjacent control areas, out of 3,176 strains only 54 were able to volatilize Hg and only 36 of these were broad-spectrum resistant (Nakamura et al., 1990).

In aquatic ecosystems, demethylation of methylmercury is mainly a microbial process opposing Hg methylation (Ramlal et al., 1986). In oligotrophic lakes in northern Wisconsin (USA), the demethylation process has been found in water columns, was greatest in the sediment floc and decreased slightly with sediment depth. In addition, demethylation activity was significantly correlated with the percentage of carbon in surficial sediments and was high in attached microbial communities. Seasonal variations were important in the demethylation of Hg, degradation increasing in early summer and reaching a maximum in mid-summer (Korthals and Winfrey, 1987).

Broad-spectrum mercury resistant strains can be identified by detecting Hg²⁺ from organomercurial degradation by atomic absorption spectrophotometry (Baldi et al., 1988) in the headspace above the bacterial culture, or detecting elemental mercury by X ray film (Nakamura and Nakahara, 1988). In the environment, the demethylation process is mostly detected by adding CH₃²⁰⁵Hg to sediment or water column (Ramlal et al., 1986) and after a few days of incubation, measuring radiolabelled elemental mercury.

Organomercurial degradation takes place in two different steps: first step, the cleavage of the C-Hg bond occurs producing Hg(II) and the respective hydrocarbons (methane from methylmercury, ethane from ethylmercury, etc.). In the second step Hg(II) is reduced to Hg⁰. To date, no strain has been isolated, able to transform organomercurials without volatilizing Hg. In addition, the biodegradation of organomercurials may be induced, but no other organometals can be degraded (Baldi et al., 1989). A case has been reported however, of a broad-spectrum mercury resistant strain converting a rare organostannous compound, triethyl-vinyltin, to ethylene and ethane (Walts and Walsh, 1988).

The bacterial transformation of organomercurials is so fast that in in vivo experiments, 1 µg of CH₃HgCl is completely degraded in two hours (Baldi et al., 1989). We recently suggested the use of this metabolic characteristic for determining methylmercury in biological extracts by its enzymatic derivatization from CH₃Hg⁺ to CH₄ and its successive determination by gas chromatography with flame ionization detector (GC-FID) (Baldi and Filippelli, 1991).
Fig. 1 The model for mercury resistance in bacteria. The suggested locations of merP, merT and merA gene products relative to the inner membrane of the bacterial cell are shown. The solid circles represent the paired cysteine residues to which the Hg(II) ion is suggested to bind. These cysteines are referred to by their letters (boxed) in the text. The mercury reductase protein is represented as a dimer with P2 symmetry. That mercury is shown binding to one pair of cysteines [E], rather than the others, is arbitrary. The stars represent the redox-active cysteins (and the FAD) at the active site of the reductase; the diagram does not show the possibility that these cysteines may bind Hg(II) (from Brown, 1985)
3.1 Genetics of broad-spectrum Hg resistant bacteria

Broad-spectrum mercury resistant bacteria synthesize organomercurial lyase, which is responsible for the transformation of organomercurials. The enzyme is codified by the gene merB. This gene has been studied in R31 or, located on a conjugative plasmid, and in pDU1358, a plasmid from Serratia marcescens. Metagenetic studies show two mer operons in the plasmid pDU1358 and the genetic site for ionic mercury resistance is adjacent to the site for organomercurial resistance (Griffin et al., 1987). "Oured" strains, obtained by chemical mutation, show constitutional ionic mercury resistance, but may still be induced to produce organomercurial lyase. This suggests that merB has its own promoter, which is not included in the mer operon. The plasmid pDU1358, codifying also for antibiotic resistance, contains two mer operons, one codifying for narrow spectrum mercury resistance, the other for broad-spectrum Hg resistance. Both belong to the Tn21 type transposon (without merC) and are separated by 20 Kb (Griffin et al., 1987) (Fig. 2). In the broad spectrum operon, merB shows a sequence of 639 pair DNA bases, which corresponds to a 212 amino acid polypeptide: organomercurial lyase. From other genetic studies it appears that merB is a cytoplasmic enzyme, and that it has a different origin to mer operon; the broad-spectrum operons probably evolved from other DNA insertions, harbouring the merB gene.

3.2 Organomercurial lyase

Organomercurial lyase performs the C-Hg bond cleavage in organomercurials as follows:

a) $\text{CH}_3\text{Hg}^+ \rightarrow \text{CH}_4 + \text{Hg(II)}$

b) $\text{C}_2\text{H}_5\text{Hg}^+ \rightarrow \text{C}_2\text{H}_6 + \text{Hg(II)}$

c) $\text{C}_6\text{H}_5\text{Hg}^+ \rightarrow \text{C}_6\text{H}_12 + \text{Hg(II)}$

Tezuka and Tonomura (1976; 1978) first purified the enzyme S-1 and S-2 from a Pseudomonas K-62 strain. The molecular weights of S-1 and S-2 were 19 Kda and 20 Kda respectively. The former enzyme seems tightly linked to cytochrome C1. The kinetic properties of the enzymes are reported in Table 2.

Schottel (1978) purified organomercurial lyase from the J53-1 strain of Escherichia coli. Gel filtration through Sephadex G-200 gave molecular weights for the two organomercurial degradation proteins of 22 Kda and 16.5 Kda. The kinetic properties were: $K_m = 7.7 \mu M$ and $V_{max} = 13 \text{ nmol min}^{-1} \text{ mg}^{-1}$ for methylmercury; $K_m = 0.56 \mu M$ and $V_{max} = 16 \text{ nmol min}^{-1} \text{ mg}^{-1}$ for ethylmercury. Two step reactions were observed for phenylmercury: $K_m = 0.24 \mu M$ and 26 $\mu M$, $V_{max} = 26 \text{ nmol min}^{-1} \text{ mg}^{-1}$ and 2800 $\text{nmol min}^{-1} \text{ mg}^{-1}$. The C-Hg splitting enzyme purified by Tezuka and Tonomura (1976) showed almost the same properties as found by Schottel (1978) for phenylmercury degradation, however different results were obtained by Begley et al. (1986), who also investigated other organic mercury substrates. These authors achieved an overproduction (3%) of organomercurial lyase in soluble cell proteins with a T7 promoter for the mer operon for E. coli strain J53-1. The purified enzyme is a monomer of 22,400 Dalton with no detectable cofactors or metal ions, and catalyzes the protonolysis of the C-Hg bond to the hydrocarbon and mercury ion in a wide range of organomercurial salts (Table 3).
Fig. 2 Restriction nuclease map of plasmid pDU1358 antimicrobial-agent-resistance region as determined by single- and double-restriction enzyme digestion and analysis on agarose gels. Location of the resistance determinants and regions of DNA that have been cloned into vector plasmids are indicated. Restriction nuclease sites: B, BamHI; Bg, BglII; E, EcoRI; H, HindIII; and P, PstI. AAD, aminoglycoside adenylase; APH, aminoglycoside phosphorylase; Tc, tetracycline resistance; Cm, chloramphenicol resistance. Ap, ampicillin resistance; R, T, P, A, B and D, the merR, merT, merP, merA, merB and merD genes. The orientations of the mer operons are known from the hypersensitivity of the cells with plasmids pHG108 and pHG111 and also from Southern blot analysis. The hypersensitivity establishes a transport activity that probably requires merT and merP functions. We do not know if either operon has a merC gene or whether the narrow-spectrum mer operon has a merD gene (from Griffin et al., 1987)

<table>
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<th>PhHg Vmax</th>
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<th>PCHgB Vmax</th>
<th>MeHg Km</th>
<th>MeHg Vmax</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1</td>
<td>45</td>
<td>0.68</td>
<td>78</td>
<td>0.26</td>
<td>20</td>
<td>5.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>S-2</td>
<td>250</td>
<td>20</td>
<td>180</td>
<td>3.1</td>
<td>N.A</td>
<td>N.A</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>E..J53-1</td>
<td>0.24</td>
<td>26</td>
<td>ND</td>
<td>ND</td>
<td>7.7</td>
<td>13</td>
<td>0.56</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>2800</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Km (µM); Vmax, µM min⁻¹ mg⁻¹; PhHg = phenylmercuric; PCHgB = p- chloromercurybenzoic; MeHg = methylmercury; EtHg = ethylmercury; NA = non activity; ND = non detected.
4. BACTERIAL TRANSFORMATION OF Hg SPECIES IN ANAEROBIC ENVIRONMENTS

The resistance of bacteria to mercury compounds in aerobic environments is related mainly to enzymes like mercury reductase and organomericurial lyase. There are likely other minor mercury resistances which are less efficient. In fact, from our studies, all bacteria growing well on agar media with 10 μg ml⁻¹ of HgCl₂, synthesize one or two enzymes. Other types of Hg resistance are described in the literature (Saxena et al., 1983); for example a Staphylococcus aureus strain was reported to be resistant to mercury by virtue of extracellular compound production, but the result has not been reconfirmed, and no volatilization test has been performed.

Table 3

Kinetic properties of organomercurials lyase isolated from Escherichia coli J53-1 (Begley et al., 1986).

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Vmax (mM·mg⁻¹·min⁻¹)</th>
<th>Km (mM)</th>
<th>Turnover no. (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylmercury chloride</td>
<td>0.031</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>n-butylmercury chloride</td>
<td>0.88</td>
<td>0.9</td>
<td>20.0</td>
</tr>
<tr>
<td>sec-butylmercury bromide</td>
<td>0.11</td>
<td>0.8</td>
<td>2.5</td>
</tr>
<tr>
<td>tert-butylmercury bromide</td>
<td>0.04</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>phenylmercury acetate</td>
<td>0.67</td>
<td>0.9</td>
<td>15.0</td>
</tr>
<tr>
<td>crotylmercury bromide</td>
<td>2.60</td>
<td>1.3</td>
<td>58.0</td>
</tr>
<tr>
<td>vinylmercury bromide</td>
<td>0.53</td>
<td>1.0</td>
<td>12.0</td>
</tr>
<tr>
<td>cis-2-butenylmercury chloride</td>
<td>10.70</td>
<td>3.3</td>
<td>240.0</td>
</tr>
<tr>
<td>dimethylmercury</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Other aspecific resistances have been hypothesized (Pan-Hu and Imura, 1982a; 1982b; Barkay and Olson, 1986), such as H₂S production. Rudrik et al. (1985) found a variable resistance to inorganic and organic mercury compounds in obligate anaerobic bacteria (Bacteroides ruminicola, Clostridium perfringens), isolated from clinical settings and sewage. The mercury resistance in these isolates was neither inducible nor plasmid mediated. Enzymatic mercury resistance has been detected in a facultative anaerobic strain of Yersina enterocolitica (Blachyn et al., 1983). In anaerobic bacterial communities, the transformation of Hg mainly takes place by aspecific interactions with extra and intracellular compounds. In particular, inorganic mercury is transformed to mercury sulphides or methylmercury (Furutani and Rudd, 1980; Campeau and Bartha, 1983), which are the two typical mercury species formed in anoxic environments.

4.1 Biological origin of mercury sulphide

Like other metal sulphides, mercury sulphide forms in anaerobic environments. This common Hg transformation is carried out by one of
the most common groups of bacteria: the sulphate reducing bacteria (SRB). They are so named because they enzymatically reduce the sulphate to hydrogen sulphide (H₂S) as an energy source. The copious quantity of this malodorous gas reacts with Hg(II) to give a stable form of mercury, mercury sulphide (HgS). This chemical species of mercury is visibly formed in pure cultures of Desulfovibrio desulfuricans. This bacterium grows well also in liquid Postgate's medium with sulphate sources different from FeSO₄, and after two days of incubation, H₂S is highly concentrated in the anaerobic culture. If HgCl₂ is added now to the culture, a black precipitate suddenly forms. Elemental analysis of this floc shows the presence of sulphur and Hg, together with a high content of carbon due mainly to the bacterial biomass. This aspect of mercury transformation is ignored by scientists dealing with Hg pollution, because it is believed that all Hg in the sulphide form is stable and not reactive. It is possible that, during the reaction of Hg(II) with biological H₂S, other intermediate mercury compounds form and might be responsible for further transformation, perhaps to methylmercury. It has been reported that methylmercury might form from mercury sulphide (Gillespie and Scott, 1971; Fagerstrom and Jernelov, 1971; Yamada and Tonomura, 1972b; Gilmore et al., 1988).

4.2 Methylmercury formation mediated by biological activity

The direct formation of methylmercury from Hg(II) by microbial activity is one of the hot points in the biogeochemical cycle of mercury. Are bacteria directly involved in the methylation of mercury? At the end of the sixties Jensen and Jernelov (1967) and then Wood et al. (1968) demonstrated that both in the environment and in pure cultures of the methanogenic Methanosarcina barkeri, methylmercury is formed from Hg(II). In the seventies, several authors (Vonk and Sijpesteijn, 1973; Handy and Noyes, 1975) reported that Hg methylation took place in both anaerobic and aerobic environments; however, this transformation was mainly detected in anoxic habitats. Yamada and Tonomura (1972a) demonstrated that a gram-positive, anaerobic sporeforming bacterium, Clostridium cochlearium strain T-22 was capable of producing methylmercury from many inorganic Hg salts in the presence of vitamin B₁₂. This Hg alkylation was also feasible from mercury sulphide if methyl-cobalamin (CH₃-B₁₂) was added to the microbial culture (Yamada and Tonomura, 1972b); however, the H₂S in the culture depressed the methylation activity. Co-cultures of sulphate reducing bacteria with C. cochlearium also depressed methylmercury formation, because H₂S reacting with Hg(II) led to mercury sulphide production. In the same period, Imura et al. (1972) demonstrated that inorganic mercury can be methylated chemically by CH₃-B₁₂, without bacterial activity. Thus, all the experiments conducted by adding cobamide substances to microbial cultures might bias the Hg methylation findings.

Pan-Hu and Imura (1982a; 1982b) provided convincing evidence that Hg methylating activity in a T-2C strain of C. cochlearium played an important role in Hg(II) detoxification. The strain T-2C has the peculiarity of producing vitamin B₁₂, and when Hg(II) is added to the culture, methylation occurs. T-2C is resistant to inorganic salts. The vitamin B₁₂ auxotrophic strain (mutant) obtained by chemical mutation from the parent T-2C is very sensitive to the addition of Hg(II) and does not produce methylmercury. However, both strains parent and mutant, have the same degree of resistance to methylmercury.
Campeau and Bartha (1985) demonstrated that, in anoxic estuarine sediments, sulphate reducing bacteria were the principal Hg methylators. An experiment in situ showed that the inhibition of SRB by sodium molybdate (20 mM) added to sediments, leads to a decrease in Hg methylation. The addition, instead, of 2-bromoethane sulphonate (30 mM) inhibits the methanogenes (methane-producing bacteria) and leads to an increase in Hg methylation. This process is controlled by available fermentable organic substrates and by limited amounts of sulphates. It was also demonstrated (Berman and Bartha, 1986) that in anoxic saltmarsh sediments, the resulting methylmercury levels are approximately one order of magnitude lower than those formed by biological methylation. Aerobic conditions inhibited the synthesis and promoted the destruction of methylmercury, whereas anaerobic conditions had the opposite effect (Campeau and Bartha, 1984).

The mechanism of Hg methylation was studied in a Desulfovibrio desulfuricans strain growing in the presence of radiolabelled pyruvate and serine (Berman et al., 1990). Carbon incorporation in the methylmercury moiety was consistent with methyl groups originating from the 3-C of serine. This reaction seems mediated by a Co-sirohydroxyprorphyrin (LeGall and Fauque, 1988), identified in the bacterium as total cobalamin by the simulTRAC immunodiagnostic radiodilution kit. The propagation of cobalamin by propyl iodide has been shown to inhibit transmethylation in the synthesis of methionine and in methane production. In washed and resting cells of D. desulfuricans treated for 30 min with propyl iodide, methylmercury synthesis failed, but light exposure partially restored the metabolic process. The selective inhibition of mercury methylation activity by propyl iodide and the reversibility of this inhibition by light suggests that the CH3- group is transferred to mercury ions by a methyl donor that is either closely related or identical to methylcobalamin (Berman et al., 1990). Isolation of different species of SRB from freshwater sediment, have shown that not all species are able to methylate Hg. Several of them, independent of SRB group location in the phylogenetic tree, are capable of performing the Hg alkylation (Gilmore et al., 1988).

However, Hg methylation in the environment and in pure cultures is still controversial. The techniques to detect methylmercury in microbial cultures require detailed examination. The search for greater sensitivity in analytical methods often goes to the prejudice of direct detection of the chemical forms of Hg, in microbial methylation studies indirect analysis of methylmercury is carried out. The most common technique for determining methylmercury in biological extracts, which is very efficient for fish tissues, uses a gas chromatograph equipped with an electron capture detector (GC- ECD 63Ni) (Cappon and Smith, 1977). For microbiological extracts this technique is unreliable, because other Hg species are probably removed by sequential benzene and thiosulphate extractions (Baldi et al., 1989). In addition, several peaks appear in the gas-chromatogram, which can overlap with the retention time of methylmercury. Another method makes use of the additions of 203HgCl2 to microbial cultures. After a certain period of incubation, methylmercury incorporating 203Hg is extracted by a conventional technique and then detected as total 203Hg with a scintillation counter along with background and standard samples (Furutani and Rudd, 1980).
In collaboration with M. Filippelli our laboratory has developed two specific methods: an enzymatic method based on the specific transformation of methylmercury to methane by the organomercurial lyase (Baldi and Filippelli, 1991), and a second method based on the spectrum determination of the "molecular fingerprint" obtained by a "purge and trap" with a gas-chromatograph in line with a Fourier Transform infrared spectroscopy (P/T-GC-FTIR) (Filippelli et al., in press).

Hg methylation experiments were reproduced in our laboratory with Desulfovibrio desulfuricans (kindly provided by Bartha). This strain is considered a "good Hg methylator". The results were not confirmed after several replicates, when the methylmercury determination was performed with our improved methods for the direct determination of this organometal. A large amount of Hg was extractable but at the end it was not methylmercury. Furthermore, additions of methylmercury to the D. desulfuricans led to the formation of dimethylmercury, Hg²⁻, and methane in the headspace above the culture, and a significant disappearance of methylmercury from solution. Further studies are in progress to shed light on these findings.

Hg methylation from Hg(II) is probably a more complicated transformation, not due to a simple reaction, but involving the reaction of different substrates, possibly produced by SRB metabolism, in anoxic environments.

5. REFERENCES


De la Cruz, F. and J. Grinstead, 1982. Genetic and molecular characterization of Tn21, a multiple resistance transposon from R100. J.Bacteriol., 151:222-228


Filippelli, M., F. Baldi, F.E. Brinckman and G.J. Olson, Methylmercury determination as volatile methylmercury hydride by purge and trap gas chromatography in line with Fourier Transform Infrared Spectroscopy (PT-GC/FTIR) in press


MERCURY–SELENIUM AND CADMIUM–SELENIUM RELATIONS
IN EDIBLE SPECIES OF THE NORTHERN TYRRHENIAN SEA

by

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A B S T R A C T

Commercially important edible species of marine organisms from the northern Tyrrhenian sea were analyzed for mercury, cadmium and selenium. They were: Merluccius merluccius, Eledone cirrhosa, Trisopterus minutus capelanus and Nephrops norvegicus. The flatfish Solea vulgaris and Lepidorthombus bosci were considered as well. Except in E. cirrhosa, Hg concentration increased with length, while for Cd and Se such relationship was not found. No significant Hg-Se correlation was found in any of the studied species, nor was any observed between Cd and Se, except in E. cirrhosa, in which a significant Cd-Se correlation was observed.

1. INTRODUCTION

Hg and Cd are considered as the two most toxic elements among the trace metals.

The northern Tyrrhenian sea is affected by mercury contamination, mainly because of the cinnabar anomaly of Mt. Amla and the mining activity carried out there in the past. Indeed, relevant Hg concentrations have been found in marine sediments along the coast (Renzoni et al., 1973; Baldi and Bargagli, 1982), in marine organisms in general (UNEP/FAO/WHO, 1987) and in some species of flatfish in particular (Barghigiani et al., 1986a; Barghigiani et al., 1986b).

As regards cadmium, the Mediterranean countries account for about 10% of the world's production of the metal, and in this basin anthropogenic Cd sources are present, such as metallurgical industries and plants producing pigments, stabilizers for synthetic materials such as PVC, nickel-cadmium batteries and other products. Cadmium is also found in domestic sewage (UNEP/FAO/WHO, 1989). Thus, the northern Tyrrhenian sea, because of the Metalliferous Hills, the industrial activity, and the densely populated areas such as the Arno basin, may be exposed to cadmium contamination.
Selenium, toxic at high concentrations, also plays an important role as an essential micronutrient for growth and reproduction, and seems to exert an antagonistic effect in mercury poisoning (Spallholz et al., 1981). In the literature, a linear Hg-Se correlation is reported for many marine mammals in the liver (Koeman et al., 1973; Koeman et al., 1975; Martin, 1976; Kari and Kauranen, 1978) and in the kidney (Koeman et al., 1975), while no relevant information is available on muscle. As concerns fish muscle tissue, Cappon and Smith (1981 and 1982) found no relation between Hg and Se either in marine or freshwater fish, and a non-linear correlation was also found in swordfish by Freeman et al. (1978). In freshwater fish no correlation was found by Froslie et al. (1985).

In this paper a screening is reported of Hg, Cd and Se contents in different-sized organisms of important edible species from this area, and the relation between the two metals and Se was studied.

2. MATERIALS AND METHODS

Samplings were carried out by several trawl surveys off the Tuscan coast, from the promontory of Argentario to the Gulf of Follonica, from 1985 to 1987.

The following species were studied which are considered the most important both commercially and for their abundance in the study area: Merluccius merluccius, Eledone cirrhosa, Trisopterus minutus capelanus, and Nephrops norvegicus. The flatfish Solea vulgaris and Lepidorhombus boscii were also collected.

Samples of muscle tissue were analyzed for Hg, Cd and Se after digestion with concentrated HNO₃ in a pressurized decomposition system at 120°C for 6 hours. The measurements were performed for total Hg by flameless atomic absorption spectrometry (AAS), and for the organic fraction using the method of Capelli et al. (1979).

Cadmium was measured by AAS using graphite furnace atomization. Selenium was determined by a sensitive, accurate technique based on hydride generation coupled with non-dispersive atomic fluorescence detection using hydrobromic acid-based aqueous matrices according to the procedure of D'Ulivo (1989). This technique avoids the interference of other elements and of the acid mixture. The dry weight was determined on separate samples at 60°C. Each sample was analyzed in duplicate or triplicate. The analytical procedures were tested using Certified Reference Materials DORM-1 (dogfish muscle, μg g⁻¹: 0.789±0.074 Hg; 0.086±0.012 Cd; 1.62±0.12 Se) of the National Research Council of Canada.
3. RESULTS AND DISCUSSION

All the studied organisms, with the exception of E. cirrhosa, show an increase in Hg concentration with length (Fig. 1).

What is observed in E. cirrhosa is probably due to the life cycle of the organism which is smaller than two years, and hence the low exposure difference between the specimens of different age. In this species even the smaller specimens display mercury concentrations above the value of 0.7 μg g⁻¹ fresh weight (the maximum legal limit for edible parts of marine organisms suggested by the European Community).

The highest total Hg concentrations were observed in M. merluccius. However, in all the analyzed species the Hg content of larger-sized specimens exceeds the legal limit, except for sole, probably owing to the feeding behaviour (Pellegrini and Barghigiani, 1989).

The percentage of organic to total mercury in fish was 86±7, very close to that of other fish species (Westoo, 1967) and without significant differences between different species or with regard to size. In N. norvegicus and E. cirrhosa organic Hg was not determined.

As regards Cd and Se (Figs. 2 and 3), no significant relation was found between these elements and the length of the organism in any of the studied species. Cd concentrations, except in E. cirrhosa where they were very high, were lower than that reported for other organisms and for M. merluccius collected in the Mediterranean (Bernhard, 1978; UNEP/FAO/WHO, 1989) and especially they were very low in S. vulgaris. In E. cirrhosa also Se was high ranging from 2 to 11 ppm d.w., while it was 1 to 5 ppm d.w.in the other species.

From Fig. 4 it can be seen that no Hg-Se correlation exists in muscle tissue of the studied species, probably because most of the selenium in fish muscle seems to be water soluble and does not form stable complexes with mercury which is mostly in the organic form (GESAMP, 1986). Nor was a relation observed between Cd and Se in any of the organisms except for E. cirrhosa (Fig. 5), in which a significant correlation exists with a stoichiometric ratio of 1:1 (calculated slope: 1.06±0.10).

E. cirrhosa, for Hg, Cd and Se concentrations, and for the Cd-Se relationship seems to be an interesting organism for further study.

4. ACKNOWLEDGMENTS

The project was carried out in the framework of the MED POL-Phase II programme and an MTIF contribution was received through FAO.
Fig. 1 Hg concentration (μg g⁻¹ dry weight) versus the length of the organism (mm). The horizontal line shows the limit of 0.7 μg g⁻¹ fresh weight suggested for edible parts of fish by the EC standards.
Fig. 2  Cd concentration (μg g⁻¹ dry weight) versus the length of the organism (mm)
Fig. 3  S\textsubscript{e} concentration (\textmu g g\textsuperscript{-1} dry weight) versus the length of the organism (mm)
Fig. 4  Relationship between Se and Hg concentrations (nmol g$^{-1}$ dry weight). The inner dashed lines show the 95% confidence limits for the linear regressions; the outer ones show the 95% confidence limits for the estimations.
Fig. 5 Relationship between Se and Cd concentrations (nmol g⁻¹ dry weight). The inner dashed lines show the 95% confidence limits for the linear regressions; the outer ones show the 95% confidence limits for the estimations.
5. REFERENCES


PARTITIONING OF ORGANIC MICROCONTAMINANTS BETWEEN COASTAL MARINE COMPARTMENTS

by

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ABSTRACT

A variety of organic compounds including hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), 4,4'-DDE, long-chain alkylbenzenes (LABs), polycyclic aromatic hydrocarbons (PAHs), long-chain alkyl nitriles (LANs), trialkylamines (TAMs), and organotin derivatives (tributyl- and triphenyltin) have been determined in biotic and abiotic compartments close to an urban wastewater disposal site. The field experimental partition coefficients between particulate and dissolved seawater phases (K'd), sediment and water (Kd) and biota and water (Kb) of these organic contaminants were calculated in order to assess their fate in the marine environment. Several hydroxy metabolites were identified.

1. INTRODUCTION

The marine disposal of municipal wastewater involves the release of large amounts of anthropogenic compounds into coastal environments. It is estimated that the Mediterranean sea receives about $3 \times 10^7$ tons of wastewaters per day (Osterberg and Keckes, 1977), mostly from primary treated or untreated municipal effluents. The large number of organic chemicals within the effluents that potentially may cause environmental concern and the wide variety of aquatic organisms and environmental exposure conditions, require an understanding of factors controlling concentrations in the different aquatic compartments to the extent that predictive models can be derived.

Substantial progress in all aspects made during the last decade, has shown that organic inputs are transported in the form of dissolved and particulate organic matter within the water column, where they undergo intense mixing as well as several biotic and abiotic transformation processes such as hydrolysis, biodegradation or photo-oxidation. Depending on the phase-association of the organic species, they may be remineralized, recycled into the food web, incorporated into the underlying sediments or transported towards the open sea. These processes determine the ultimate fate and impact of the organic components into the receiving system. Although, it is generally accepted that sediments constitute a sink for the more hydrophobic compounds (Förstner, 1989), a major concern exists of their possible release to the overlying waters and uptake by benthic organisms (O'Connor et al., 1983).
Knowledge on the bioavailability of such contaminants also deserves a great interest with respect to the amenability of some species to trace marine pollution. In this regard, tissue concentrations of several chemical classes have been determined in bivalves used as sentinels for monitoring marine pollution in large areas (Farrington et al., 1983). Besides the information already existing for some particular hydrophobic contaminants, namely hydrocarbons and organochlorinated pesticides, knowledge of the bioavailability and biotransformations of those associated with urban wastes is scarce.

In this paper, the partitioning of a large variety of widely distributed contaminants (Valls et al., 1990) between environmental compartments including water, sediments, benthic and pelagic biota samples collected in coastal areas and close to disposal sites will be shown.

2. EXPERIMENTAL

2.1 Sampling

Coastal sediments (0-2 cm) were collected in 1988 with a box corer in the stations indicated in Fig. 1. These include Barcelona harbour (station 1) and the vicinities of sewage (140 000 tons per year) and primarily treated wastewater disposal sites (stations 3 and 4, respectively). Seawater was collected from the surface along a transect parallel to the coast (station 2) and near the bottom (5 m) close to the two areas of study. Large volumes of surface seawater (100-1000L) were filtered (0.45 μm) and extracted with polyurethane foam, using a sampling assembly previously described (Gómez-Belinchón et al., 1988). Bottom seawater was collected using a 30 l Niskin bottle, and filtered and extracted in the same manner. Polychaeta (Prionospio cirrifera, Spiochaetopterus costarum and Capitella capitata) were isolated from the sediments by filtering through 1 mm rated pore size and transferred to glass jars on site. The jars were kept cool until return to the laboratory where organisms were sorted within 3 days of collection. Representative biota samples (mussels, crustacea and fishes) were also collected from the area of study.

2.2 Sample handling

2.2.1 Organic micropollutants

About 10 g of freeze-dried sediments were soxhlet extracted with methylene chloride-methanol mixture (2:1) for 36 h. Tissues of benthic organisms (5 g fresh wt.) were homogenized and analyzed as a composite containing at least samples from two cruises. The homogenates were transferred into centrifuge tubes and were treated with 15 ml of aqueous NaOH 6N, preextracted with methylene chloride. Tubes were closed with teflon-lined caps, shaken for several minutes and kept at 30°C for 18 h. The hydrolyzed mixture was extracted with diethylether (5x10 ml).
Organic extracts were reduced to small volume by rotary evaporation, adsorbed onto 2 g of alumina under a gentle stream of nitrogen, and transferred on top of a glass column packed with 6 g of alumina (top) and silica (bottom), both 5% deactivated. A total of seven fractions were collected by eluting with eluents of increasing polarity, as described elsewhere (Valls et al., 1990).

2.2.2 Organotins

Seawater. 1 l of filtered seawater was liquid-liquid extracted (2x60 ml) with 0.05% (w/v) tropolone in hexane. The organic extract was dried over anhydrous Na$_2$SO$_4$, rotary evaporated, and derivatized with 3 ml of 20% CH$_3$MgCl-THF in diethyl ether. The mixture was allowed to stand for 5 min and the excess reagent was destroyed with 10 ml of bidistilled water at 4°C, and 2 ml of HCl 32%. The organic phase was recovered by extraction in diethyl ether (2x10 ml). The organotin fraction was recovered by column chromatography using 1 g of 3% water deactivated neutral alumina with 10 ml of hexane.

Biota. A homogenized pool of 15-20 organisms (5g dry wt), was mixed with 25 ml of methanol and 17 ml of HCl. The mixture was allowed to stand for 10 min, saturated with NaCl and extracted by stirring with a mixture of 0.25% tropolone in diethyl ether and hexane (3:2) (3x40 ml). The organic extracts were recovered by centrifuging, and dried over Na$_2$SO$_4$. Derivatization was identical to that described formerly, but volumes of HCl and CH$_3$MgCl were increased to 3 and 4 ml, respectively. Derivatized samples were cleaned through 2 g of activated silica, eluting the organotin fraction with 50 ml of 10% diethyl ether in hexane.
2.3 Instrumental analysis

Organic fractions were analyzed in a Mega 5300 series gas chromatograph (Carlo Erba, Milan, Italy) equipped with flame ionization (FID), nitrogen-phosphorus selective (NPD), electron capture (ECD), and flame photometric (FPD) detectors. Detector temperatures were held at 370, 320, 310 and 250°C, respectively. Splitless or cold on column injection modes were performed at 300 and 95°C, respectively, by using isooctane as a solvent. Fused silica capillary columns of 25 m x 0.2 mm i.d. coated with 0.10 µm of SE-54 (HP-5) was used. The oven temperature was usually programmed from 50 to 300°C at 6°C min⁻¹.

Quantitation of individual components was performed by capillary gas chromatography (GC) using external standards. Total aromatic hydrocarbons were quantified from fraction III by UV-spectrofluorimetry (Perkin Elmer, MF3) and chrysene as external standard. (GC)-mass spectrometry (MS) confirmatory analyses were performed in a Hewlett-Packard 5985A instrument interfaced to a 9825A data system as described elsewhere (Valls et al., 1990).

The partition coefficients between particulate and dissolved phases (K'pd) were calculated from the ratio of microcontaminant concentrations in both phases on volume basis. The bioconcentration factors (Kb) were calculated as the ratio between tissue and sediment concentrations (dry wt.) in the case of Polychaeta sp., and the ratio of concentrations between tissue and the dissolved phase of seawater for the rest of biota samples.

3. RESULTS AND DISCUSSION

The distribution of selected chemical classes of organic contaminants between the marine compartments, as well as the composition of untreated wastewater is reported in Table 1. Special emphasis has been devoted to the partitioning of organic contaminants between the dissolved and particulate phases of seawater since reduction in the uptake of chemicals by fish due to the sorption on particles has been demonstrated (Schrap and Opperhuizen, 1990).

3.1 Hydrocarbons

It is estimated that municipal wastewaters constitute 25% of the total input of hydrocarbons in the Mediterranean region. The composition of the aliphatic fraction (AHs) is characterised by a variable distribution of n-alkanes according to the marine compartment, overlaying an unresolved complex mixture (UCM) (Fig. 2).

While in the water column a low carbon number preference index (CPI) and pristane-phytane ratio close the unity is consistent with a fossil origin (Tissot and Welte, 1978), in sediments as well as in some biota species, several biogenic inputs were evident. In fact, the long-chain n-alkanes with odd carbon number preference from n-C35 to n-C31, which are characteristic of epicuticular waxes of continental plants (Eglinton and Hamilton, 1967), were identified in sediments. On the other hand, several biota species (e.g. Sardinella aurita) exhibited a CPI>1 with the maximum concentration in the n-alkanes of
15 and 17 carbon numbers characteristic of a planktonic origin (Clark and Blumer, 1967). Conversely, the distribution of n-alkanes in other benthic species reflected the distribution pattern of petrogenic n-alkanes, demonstrating an uptake of fossil inputs of hydrocarbons (Fig. 2).

The quantitative distribution of the UCM between the abiotic marine compartments (Table 1), demonstrated an enrichment in the particulate matter and sediments; while in bivalves and bottom feeding species of the biotic compartments, a maximum concentration was observed in Polychaeta sp.. This trend can be due either to the different levels of exposure between species or to the different enzymatic activity, allowing the metabolism of hydrocarbons (Table 1). The bioconcentration factors (Kb) showed the highest values in mussels, and in benthic fishes (e.g. Mullus barbatus in Table 2) but they were significantly lower in the pelagic ones.

Polycyclic aromatic hydrocarbons (PAHs) constitute another class of ubiquitous contaminants in coastal areas, including fossil, pyrolytic and biogenic-diagenetic sources. Therefore, in order to distinguish among these inputs, the determination of individual components becomes necessary. The particulate phase of wastewaters is relatively enriched in PAHs (K′d=63) in comparison with superficial seawater, where the concentration levels between both phases are similar (K′d=1.3). On the other hand, the composition of PAHs in seawater is decoupled according to the different water solubility of components (Valls et al., 1990). In this sense, the more condensed PAHs (>4 aromatic rings) were preferentially associated to the particulate matter and consequently, to sediments. On the other hand, alkylated PAHs of fossil origin, which are predominant in wastewaters are concentrated in the seawater dissolved phase while parent PAHs, characteristic of pyrolytic sources, are preferentially associated to the particulate phase. Consequently, the distribution of PAHs observed in coastal sediments is characteristic of pyrolytic sources, whereas in the area of influence of the dumping site (station 3 in Fig. 1), fossil hydrocarbons predominated.

As far as the bioconcentration of PAHs in Polychaeta is concerned (Table 3), it is interesting to note the pattern exhibited by the two species. In this regard, whereas the sample containing polychaeta sp. (Prionospio cirrifera and Spiochaeotopterus costarum) exhibited a positive correlation of the parent PAHs with the Kow, in Capitella capitata the correlation was negative. Indeed, the alkylated derivatives of PAHs also presented the same trend. This opposite bioconcentration behavior might be explained on the basis of different metabolic pathways or by kinetic considerations (Fernández and Bayona, 1989).

The bioavailability of total PAHs was considered in other species (Table 2). Among them, Mytilus exhibited the highest Kb value. Other pelagic species presented the lowest values in the range from 3.6 to 3.9, which are comparable to those reported previously in fish (Karcher, 1988). This bioconcentration pattern is in contrast with the results of AIs (UCM in Table 1), which were detectable only in several benthic species.
Table 1
Distribution of organic classes of micropollutants between the marine compartments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>UCN</th>
<th>LABs</th>
<th>HCB</th>
<th>4,4'-DDE</th>
<th>PCBs*</th>
<th>PAHs**</th>
<th>LANs</th>
<th>TAHs</th>
</tr>
</thead>
<tbody>
<tr>
<td>waste water</td>
<td>t.p.***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μg l⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>particulate dissolved</td>
<td>2145±350</td>
<td>33±18</td>
<td></td>
<td>0.15±0.1</td>
<td>0.14±0.02</td>
<td>0.10±0.02</td>
<td>63±8</td>
<td>4.7±0.6</td>
<td>169±20</td>
</tr>
<tr>
<td>K'd</td>
<td>34.0</td>
<td>1.8</td>
<td></td>
<td>37.5</td>
<td>2.5</td>
<td>&gt;10</td>
<td>63</td>
<td>4.3</td>
<td>1.6</td>
</tr>
<tr>
<td>superficial seawater</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>particulate</td>
<td>1222</td>
<td>10</td>
<td></td>
<td>&lt;0.1</td>
<td>0.17</td>
<td>0.17</td>
<td>13</td>
<td>7.05</td>
<td>39.3</td>
</tr>
<tr>
<td>dissolved</td>
<td>38</td>
<td>2</td>
<td></td>
<td>&lt;0.1</td>
<td>0.07</td>
<td>0.06</td>
<td>10</td>
<td>0.22</td>
<td>4.5</td>
</tr>
<tr>
<td>K'd</td>
<td>32.1</td>
<td>5.0</td>
<td></td>
<td></td>
<td>2.4</td>
<td>2.8</td>
<td>1.3</td>
<td>32.0</td>
<td>8.7</td>
</tr>
<tr>
<td>bottom seawater</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(μg l⁻¹)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>particulate</td>
<td>49±35</td>
<td>0.6±0.5</td>
<td>1±0.5×10⁻³</td>
<td>1±3×10⁻³</td>
<td>11±3×10⁻³</td>
<td>35±10×10⁻³</td>
<td>11±4</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>particulate</td>
<td>13±2</td>
<td>6±2×10⁻²</td>
<td>0.2±0.1×10⁻³</td>
<td>4±1×10⁻³</td>
<td>6±2×10⁻³</td>
<td>4±1×10⁻³</td>
<td>0.4±0.1</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
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<td>sediments</td>
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</tr>
<tr>
<td>(μg l⁻¹)</td>
<td></td>
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<td></td>
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<td></td>
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</tr>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>(ng l⁻¹, fresh. wt.)</td>
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<tr>
<td>Polycheuta sp.</td>
<td>4</td>
<td>27230</td>
<td>715</td>
<td>1.7</td>
<td>12</td>
<td>34</td>
<td>67800</td>
<td>19</td>
<td>433</td>
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<tr>
<td>Nucroptus sp.</td>
<td>3</td>
<td>7140</td>
<td>1.7</td>
<td>172</td>
<td>359</td>
<td>679</td>
<td>&lt;0.8</td>
<td>448</td>
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</tr>
<tr>
<td>Nucroptus sp.</td>
<td>4</td>
<td>2900</td>
<td>&lt;0.1</td>
<td>31</td>
<td>91</td>
<td>930</td>
<td>&lt;0.8</td>
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<td></td>
</tr>
<tr>
<td>Mytilus galloprovincialis</td>
<td>4</td>
<td>8173</td>
<td>2950</td>
<td>0.7</td>
<td>40</td>
<td>114</td>
<td>31430</td>
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<tr>
<td>Nuculina barbatana</td>
<td>4</td>
<td>1230</td>
<td></td>
<td>96</td>
<td>298</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sardina aurita</td>
<td>4</td>
<td>1.1</td>
<td>&lt;5.0</td>
<td>0.5</td>
<td>16</td>
<td>26</td>
<td>92</td>
<td>10</td>
<td>158</td>
</tr>
<tr>
<td>Solea vulgaris</td>
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<td>&lt;1.0</td>
<td>&lt;5.0</td>
<td>0.3</td>
<td>26</td>
<td>47</td>
<td>44</td>
<td>&lt;0.8</td>
<td>157</td>
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<tr>
<td>Sepia officinalis</td>
<td>3</td>
<td>&lt;1.0</td>
<td>&lt;5.0</td>
<td>0.1</td>
<td>8</td>
<td>41</td>
<td>86</td>
<td>&lt;0.8</td>
<td>174</td>
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</table>

* Σ IUPAC No: 28, 52, 101, 118, 153, 138 and 180
** Total polycyclic aromatic hydrocarbons determined as chryene equivalents
*** Treatment plant influent
Fig. 2 Distribution of n-alkanes, the carbon number of the homologous series is indicated in the peak apex, and isoprenoids (pristane, P, phytane, Ph and squalene, S) in A: bottom seawater, B: *Macropipus depurator* and C: *Sardinella aurita*, collected in the sampling site 3 (Fig. 1).
Table 2

Field bioconcentration factors (Kb) of organic micropollutants in the coastal environment.

<table>
<thead>
<tr>
<th></th>
<th>log Kow</th>
<th>Polycheta sp.</th>
<th>Macropipus depurator</th>
<th>Mytilus galloprovincialis</th>
<th>Nucula barbatum</th>
<th>Sardinella aurita</th>
<th>Solea vulgaris</th>
<th>Sepia officinalis</th>
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<tr>
<td><strong>HYDROCARBONS</strong></td>
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<td></td>
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<tr>
<td>UCM</td>
<td>4.6-6.5</td>
<td>0.37*</td>
<td>4.88**</td>
<td>5.33**</td>
<td>4.50**</td>
<td>1.46**</td>
<td>3.96</td>
<td>3.64**</td>
</tr>
<tr>
<td>PAHs</td>
<td>3.4</td>
<td>4.83</td>
<td>6.49</td>
<td>3.89</td>
<td>3.96</td>
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<tr>
<td><strong>ORGANOCHLORINATED</strong></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<td>COMPOUNDS</td>
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<td></td>
</tr>
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<td>HCB</td>
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<td>0.17</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>4,4'-DDE</td>
<td>5.7</td>
<td>0.13</td>
<td>5.65</td>
<td>5.75</td>
<td>6.13</td>
<td>5.36</td>
<td>5.57</td>
<td>5.06</td>
</tr>
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<td>PCB 52</td>
<td>5.8</td>
<td>1.79</td>
<td>5.30</td>
<td>6.00</td>
<td>6.38</td>
<td>5.30</td>
<td>5.75</td>
<td>4.75</td>
</tr>
<tr>
<td>118</td>
<td>6.7</td>
<td>0.86</td>
<td>6.36</td>
<td>6.32</td>
<td>6.82</td>
<td>5.48</td>
<td>5.71</td>
<td>5.70</td>
</tr>
<tr>
<td>138</td>
<td>6.8</td>
<td>0.32</td>
<td>6.38</td>
<td>6.25</td>
<td>6.90</td>
<td>5.82</td>
<td>5.75</td>
<td>5.77</td>
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<tr>
<td>153</td>
<td>6.9</td>
<td>0.54</td>
<td>6.20</td>
<td>6.15</td>
<td>6.94</td>
<td>5.84</td>
<td>5.97</td>
<td>6.08</td>
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<tr>
<td>180</td>
<td>7.4</td>
<td>0.22</td>
<td>6.30</td>
<td>5.95</td>
<td>6.95</td>
<td>5.93</td>
<td>6.10</td>
<td>6.20</td>
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<td><strong>URBAN MARKERS</strong></td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>LABs</td>
<td>8.8</td>
<td>0.54</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LANs</td>
<td>0.27</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>TAMs</td>
<td>0.10</td>
<td>4.99</td>
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<td></td>
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<tr>
<td><strong>ORGANOTIN COMPOUNDS</strong></td>
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</tr>
<tr>
<td>DBT</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBT</td>
<td>3.7-3.8</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPhT</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Kb between polychaeta (dry wt.) and sediment (dry wt.)
** log Kb between biota tissue (fresh wt.) and dissolved phase seawater
Table 3

Bioconcentrations (Kb) of individual PAHs in polychaete worms.

<table>
<thead>
<tr>
<th>Compound</th>
<th>log Kow</th>
<th>Polychaete sp</th>
<th>Capitella capitata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenanthrene</td>
<td>4.6</td>
<td>5.67</td>
<td>30.66</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.5</td>
<td>6.58</td>
<td>23.60</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>5.2</td>
<td>5.74</td>
<td>12.0</td>
</tr>
<tr>
<td>Pyrene</td>
<td>5.17</td>
<td>5.22</td>
<td>13.30</td>
</tr>
<tr>
<td>7H-Benzo(a)fluorone</td>
<td>5.3-5.7</td>
<td>11.21</td>
<td>9.55</td>
</tr>
<tr>
<td>1H-Benzo(b)fluorone</td>
<td>5.7</td>
<td>21.8</td>
<td>13.20</td>
</tr>
<tr>
<td>Benzo(c)phenanthrene</td>
<td>5.8</td>
<td>15.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Triphenylene</td>
<td>5.7</td>
<td>4.94</td>
<td>2.15</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>5.8-5.9</td>
<td>9.42</td>
<td>3.64</td>
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<tr>
<td>Chrycene</td>
<td>5.8</td>
<td>14.71</td>
<td>6.22</td>
</tr>
<tr>
<td>Cl-Substituted m/z=228</td>
<td>6.0</td>
<td>11.93</td>
<td>4.0</td>
</tr>
<tr>
<td>Benzo(j)fluoranthene</td>
<td>6.4</td>
<td>8.16</td>
<td>0.57</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>6.4</td>
<td>9.07</td>
<td>1.74</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
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<td>14.1</td>
<td>1.83</td>
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<td>Benzo(e)pyrene</td>
<td>6.4</td>
<td>11.59</td>
<td>1.52</td>
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<td>Perylene</td>
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</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>6.0-6.4</td>
<td>13.78</td>
<td>0.73</td>
</tr>
</tbody>
</table>

* Composite sample containing Prionospio cirrifera and Spiochaetopterus costarum

3.2 Organochlorinated compounds

All the organochlorinated compounds determined exceeded log Kow of 5.5, which is characteristic of hydrophobic compounds. Therefore, their distribution between dissolved and particulate phases of seawater is consistent with their solubility, exhibiting K'd values ranging from 2.5 to 37.5 in seawater and wastewater, respectively. The different concentration of suspended solids as well as natural colloids could account for the differences between both water systems (Baker et al., 1986). The distribution of PCBs in sediments, enriched in the highly substituted compounds, is similar to that of the particulate phase. In this regard, the role played by organic-rich material in scavenging hydrophobic contaminants from the water column toward sediments has been extensively reported (Elder and Fowler, 1977).

In the biotic compartment, congeners 153 and 180 were the most abundant in the species examined agreeing with previous studies in fishes (Niimi and Oliver, 1989) and the highest tissue concentration of the total eight congeners analyzed were found in Macropipus and Mytilus sp. (Table 1). Generally, the PCB profiles found in benthic and pelagic organisms show some evidences of degradation. The congeners substituted in any or in a combination of 3',4,5,3',4',5' positions are more recalcitrant to metabolism as a result of blockage of adjacent carbon atoms, preventing enzymatic formation of an epoxide as the first step in metabolism (Baxter and Sutherland, 1984). The Kb values of
selected PCBs congeners (Table 2) show less interspecific variability in comparison to hydrocarbons. Generally, Kb values of PCBs increase with hydrophobicity, but Polychaeta exhibited an opposite trend, probably because Kb is referred to sediment instead of the dissolved phase of seawater considered for the other species. HCB and p,p'-DDE exhibited a similar bioconcentration behaviour to PCBs in accordance with similar Kow, being concentrated in the same species as PCBs.

3.3 Urban markers

Although several urban markers have been proposed as tracers of marine pollution (Vivian, 1986), surfactant derivatives appear to be the most useful, due to their specificity for domestic sources and the relatively high concentrations in urban wastewaters (Ishiwatari et al., 1983; Valls et al., 1989a).

The long-chain alkybenzenes (LABs) constitute a suite of 26 positional isomers derived from the phenyl substitution within the n-C10-C14 alkyl chains. They are present as traces in anionic surfactants, and since they are more persistent than their linear alkylbenzene sulphonate counterparts, the former are used as tracers of the latter. On the other hand, the long-chain alkyl nitriles (IANs) and trialkylamines (TAMs) are the corresponding markers of cationic surfactants. The former are integrated by an even-to-odd carbon number distribution, saturated and mono-unsaturated, ranging from 14 to 18 carbon numbers. The close distribution pattern between fatty acids and IANs evidences a genetic relationship. Finally, although we have identified three classes of TAMs, differentiated by the number of long-alkyl chains (C14-C18) in the molecule, those containing two long-chains are usually the ones of higher environmental relevance (Valls et al., 1989b).

In Table 1 the concentrations of LABs and TAMs are shown, in both, the sources and the receiving waters, as they are less conservative. It is significant to mention that their relative concentrations in wastewaters do not reflect the pattern of domestic use of anionic and cationic surfactants probably due to local sources of the latter.

The K'd values of IANs and TAMs between particulate and dissolved phases increased by one order of magnitude from wastewater to seawater, in contrast with hydrocarbons and organochlorinated compounds (Table 1). These can probably be explained by the different ionization extent of the nitrogen containing compounds. In the bottom seawater, only LABs could be identified, attributable to a relatively high Kd value of cationic surfactants, probably comparable to the protonated species of TAMs and IANs (Han et al., 1990).

In the biotic compartment, the distribution of urban markers was completely different according to their physico-chemical properties. LABs exhibited a similar distribution to that found in the aliphatic hydrocarbons, being bioconcentrated mostly in benthic species, TAMs were concentrated at higher trophic levels (Table 1). IANs exhibited also a bioconcentration potential but their limited environmental stability infers some restricted occurrence in the biotic compartment. It is also worth noting the high Kb values of TAMs and IANs in comparison to the quaternary ammonium compounds, where the log Kb is less than 2 (Knezovich et al., 1989), stressing the need for their monitoring in the marine environment.
3.4 Organotin compounds

Several organotin species including tributyl (TBT) and triphenyltin (TPT), as well as their degradation products (di and mono substituted derivatives) were identified in water and sediments. In this compartment, several hydroxy derivatives of TBT were also present (Tolosa et al., 1991), evidencing other biotic degradation pathways in toxic sediments, in addition to dealkylation. In fact, the ratio between TBT/DBT, which is an indicator of the kinetics of degradation, was higher in the biotic compartment than in sediments and seawater. This fact could be accounted for by a higher metabolic degradation rather than a preferential accumulation of DBT, since this possesses a higher water solubility, and hence a lower bioavailability.

The occurrence of both TBT and TPT in the coastal environment, with high concentrations in harbour samples, clearly reflects their applications as antifouling paints, although other sources (i.e. industrial) cannot be excluded.

The bioconcentration of organotin compounds has been investigated in benthic species (Table 2) being consistent with the lipophilicity of each compound; it increased from TBT to TPT. The values found in this study for TBT are comparable to those reported in other areas (Salazar and Salazar, 1988).

4. REFERENCES


BIOTIC AND ABIOTIC METHYLATION AND DEMETHYLATION
OF MERCURY IN AQUATIC ENVIRONMENTS: A REVIEW

by

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1. INTRODUCTION

The Minamata accident alerted the general public to the risks of mercury poisoning. At first it was thought that methyl mercury (MeHg) was produced in the marine environment through a conversion of inorganic Hg to MeHg because inorganic Hg was released in great amounts from the Minamata plant and because Jensen and Jernelov (1969) had discovered that inorganic Hg could be converted into MeHg and dimethyl mercury (DiMeHg) by freshwater sediments. Later, however, it became evident that very small amounts of MeHg had been released together with inorganic mercury from a factory producing organic chemicals and that this had caused the Hg poisoning. Following the publication of Jensen and Jernelov's paper many others studied the transformation of inorganic Hg into MeHg both on single microorganisms or on complex matrices such as natural sediment samples. In order to facilitate the Hg analyses and because near point sources high Hg concentrations in sediment were observed, it was customary to add to the culture medium and to the sediment samples inorganic Hg in quantities of 1 to 100 mg Hg kg⁻¹ of matrix. Many bacteria in natural population are sensitive to these high Hg concentrations and consequently the conditions of these methylation experiments were selective for Hg-resistant bacteria.

Later, after surveying many different marine regions, it became evident that another Hg hazard exists for humans. High MeHg concentrations were also found in fish and other aquatic organisms from locations far remote from any man-made source of Hg. The locations-geochemical mercury anomalies and Hg mining areas - were characteristic for high natural concentrations of inorganic Hg in soil and sediments. Especially, in old specimens of aquatic organisms located in high positions of the food chain (e.g. tuna, swordfish, marine mammals) high MeHg concentrations were observed. Persons consuming frequently these seafoods - fishermen and their families mainly - had high Hg levels in their hair and blood. Sometimes levels similar to those found in the victims of the Minamata disease.

Following the discovery of high amounts of Hg near many industries, such as chlor-alkali plants, petrochemical plants, etc., waste treatment facilities were installed or the industrial production processes were changed so that much less Hg was released into the environment. This reduction convinced many persons that the Hg hazards were overcome. However, recently new concern has arisen over high MeHg levels in fish from many Swedish and Canadian fresh water bodies and in the soil of northern European countries.
In this paper, scientific literature is reviewed for information on the origin of MeHg in environments not under stress by man-made introductions of inorganic or organic mercury. The review starts with some general concepts on the methods for MeHg determination because it is not always easy to decide if MeHg was really determined by the methods used.

2. METHODS TO DETECT ORGANIC MERCURY (METHYL MERCURY)

For the study of methylation and demethylation of mercury it is of primary importance that both specific and accurate analytical methods are available. In environmental samples MeHg is present in very low concentrations, therefore, the methods must also be very sensitive. MeHg can be determined directly with methods which identify the molecular structure of MeHg such as mass spectroscopy (MS), infrared spectroscopy (IR) or nuclear resonance spectroscopy (NMR). However, these methods are not sensitive enough to detect the often very small amounts of MeHg present in environmental samples in particular in natural waters. It is, therefore, necessary to use indirect methods which utilise the high sensitivity of the atomic absorption spectrometry (AAS) and gas chromatography (GC) to detect Hg species after they have been transformed by appropriate pretreatments to separate inorganic and organic Hg species. Because these methods do not identify the organomercurial compounds according their molecular structure. They are less specific and, therefore, it is very important that more than one indirect method is used.

2.1 Indirect determination of total organomercury compounds

Mercury determination is usually determined by cold-vapour generation followed by atomic absorption spectrometry (CVAAS) (Hatch and Ott, 1968). Since mercury in environmental samples is bound to other molecules (e.g. proteins) the mercury must be liberated from the matrix. This is done by acidic cleavage (addition of HCl, H2SO4). Inorganic mercury is determined applying the CVAAS method without digestion of the sample. Total mercury is determined after mineralization with strong acids following by CVAAS technology. Total organic mercury is obtained from the difference between the total and the inorganic mercury.

2.2 Indirect determination and species identification of organic mercury compounds

The most commonly used methods are chromatographic separations: thin layer chromatography (TLC) or GC. Westoo (1966) described the determination of methylmercury in food utilizing both TLC and GC procedures. The Westoo method requires the addition of a halogenated acid to an aliquot of the environmental sample (Westoo and Noren, 1967). This causes the alkyl mercury originally bonded to a matrix to form soluble alkyl mercury halides. These are then extracted with an organic solvent such as benzene or toluene. The extract is then purified by re-extracting the organomercurials into a aqueous solution containing a complexing agent such as thiosulphate or cysteine. During the reextraction the analyte is also concentrated to a smaller volume. From this aqueous solution the organomercurials are again extracted into an organic solvent after adding acid and copper salts in order to
liberate the mercury from the complex. Then the extract is dried with anhydrous sodium sulphate. Finally an aliquot of this extract is analyzed by GC with an electron capture detector (ECD) or by atomic spectrometric determination (AAS) (Davies, 1978; Filippelli, 1987). The GC analysis allows one to distinguish among different organic Hg compounds if the number of peaks of other substances is not excessive because the usual electron capture detector is not Hg specific. In fact the interpretation of a chromatogram with a ECD detector is not easy because the ECD is highly sensitive to halogenate impurities in solvents and reagents used which also produce peaks that can easily mask the MeHgCl peak (Fig. 1).

In environmental samples, such as soil and sediment, inorganic mercury is usually complexed with organic molecules. These Hg complexes are extractable with organic solvents. Therefore, it is necessary to liberate the mercury from the complex to avoid extraction of inorganic Hg complexed with organic Hg compounds. This can be achieved by substituting Hg ion for Cu++, Cd++ or Ag++. Cu-salts are the most common used. If one of these salts is not added during the extraction procedure Hg complexed by organic matter will be extracted and then erroneously taken for MeHg because the detection method is not MeHg specific. Great attention must be paid to this separation of MeHg from complexed Hg, especially when extracting media with high contents of organic matter such as bacteria culture media, soil samples etc. Several other problems exist in the methylmercury determination by GC. The MeHgCl easily interacts with active sites on glass and metal surface when it is injected into the GC column. It is very interesting to observe that MeHgCl has an unique behaviour in GC columns. In some GC columns the retention time of MeHg may change and the peak height can decrease considerably during repeated analysis (Longbottom et al., 1973) (Fig. 2). For this reason, the column must be treated occasionally with an injection of mercury halides to block these active sites and it is recommended to work at the lowest temperature possible. The occasional unreproducibility of the peak height is a serious problem when MeHg is present at very low levels and in complex matrices. But this GC method is very sensitive: 1 pg of Hg per injection (absolute) can be easily detected.

Recently, Bloom (1989) has developed a method that is based on the ethylation of inorganic Hg(II) and MeHg present in the sample and the detection as diethylmercury and methylethyl-Hg after chromatographic separation by atomic fluorescence. In this way the determination becomes specific for Hg species. Detection limit is 0.6 pg Hg absolute or 0.003 ng L⁻¹.

2.3 Specific determination of organomercury compounds

When samples containing unknown chemical species of mercury are examined, it is necessary for the identification of Hg species present to use techniques which are specific for the molecular structure of the mercury species under examination such as massspectrometry (MS), infrared (IR) or nuclear magnetic resonance (NMR) spectroscopy. These three analytical methods, however, are much less sensitive than the indirect methods discussed above. NMR can only be used for absolute amounts in the order of mg. Therefore, this technique can not be used on environmental samples where the concentrations are much lower. Recently the formation of MeHg hydride after reduction by NaBH₄ to
Fig. 1  Matrix effect on the gas chromatogram in methylmercury detection. (A) 3 μL of a methyl mercury standard in benzene containing 1.6 ng L⁻¹; (B) 5 μL of benzene extract after a 6820-fold preconcentration from field surface water containing 0.15 ng L⁻¹ (Lee et al., 1989)

MeHgCl was used to determine MeHg in a GC-Fourier-Transformed-IR instrument. 200 ng (absolute) are sufficient for an identification of the compound (Filippelli, pers. comm.). MS needs about 1 ng (absolute) for analysis (Johansson et al., 1970). The sensitivity of this technique is one thousand times less than GC with ECD detection. It is clear that if one wants to be sure that methylmercury is present in a sample containing unknown chemical species of Hg, only one of these three methods can be used.

3. METHYLATION AND DEMETHYLATION BY MICROORGANISMS AND IN WATER AND SEDIMENTS

Single bacteria cultures and samples containing complex matrices such as water or sediment have been shown to methylate inorganic Hg salts to MeHg or DIMEHg and demethylate organomercury compounds. The scope of these experiments was to obtain information on possible methylation of inorganic Hg and demethylation of MeHg and to identify the agents (biotic or abiotic) that may be responsible for these transformations.
Fig. 2  Chromatograms of 0.10 ng methyl mercury iodide: A: typical of good column, retention time, 2.4 min; B. example of poisoned column, retention time 4.7 min. (Longbottom et al., 1973)

Two analytical procedures (chemical and radiochemical) have been used in these experiments.

In the chemical procedure an inorganic Hg salt is added to an experimental system and the MeHg produced is determined by comparing the MeHg concentration at the beginning with that at the end of the experiment. Three problems as regards extrapolation of the results to natural environments arise from this procedure. (i) the inorganic Hg salt used in the experiments may not be the same molecular species as the Hg species present in a natural environment. Also, more than one Hg species may be present which are not in fast equilibrium with the species added. (ii) The concentration of the MeHg determined will only reflect the net concentration of methylation and demethylation, because methylation and demethylation processes occur simultaneously in most matrices. The exceptions concern biota other than microorganisms where demethylation is very slow in relation to the duration of the experiment so it can be neglected. (iii) The increase of the Hg concentration in the experimental set-up presents a third problem. In particular, in earlier experiments high amounts of Hg compounds (10 and 100 mg Hg L⁻¹) which were 2 to 6 order of magnitude higher than environmental concentrations were used.

The radiochemical procedure consists in adding a radioactive isotope (and the stable carrier) to the experimental system. Methylation has occurred if the radioactive isotope added as inorganic salt can be extracted as an organomercurial. The only other way in
which MeHg will become radioactive is through the exchange of inorganic radioactive Hg with Hg atoms in the MeHg present at the beginning of the experiment. Kudo et al. (1977) have studied isotope exchange between MeHg and HgCl₂ and vice versa. In 24 h maximal 0.5 \% isotope exchange was observed in Ottawa River water (Fig. 3). This means that over short time intervals isotope exchange is negligible, but considering the very small amounts of MeHg produced in many experiments and taking into account the long durations (weeks) of many methylation experiments, isotope exchange may become important. A radioisotope protocol can be used to determine gross methylation and demethylation rates, if the experiments are set-up to yield data (time sequences) that can be used in compartment theory, otherwise also radioisotope protocols will determine only net methylation. Adding radioisotopes to the samples, has the advantage that the concentration of inorganic Hg in the sample does not increase to high concentrations since the inorganic radioactive Hg increases only slightly the chemical Hg concentration. With the exception of water samples, the carrier contributes little to the chemical concentration. The total mercury (HgT) of water samples (in the nanogram range L⁻¹) may be increased 300 to 500 times, by the addition of the carrier, but in uncontaminated sediments, with about 100 \( \mu \text{g} \text{Hg T Kg}^{-1} \) background concentrations, the HgT concentration increases, due to carrier addition, only by about 25\%. The species distribution problem for the inorganic Hg species, however, still exists to the same extent as in the chemical procedure, i.e. the radioisotope may not distribute rapidly into all the inorganic species which it supposedly shall simulate. For the above given reasons all these investigations based on additions of stable and radioactive Hg can estimate only potential processes. Therefore, in recent papers these processes rightly have been called potential methylation/demethylation (Callister and Winfrey, 1986), the methylation/demethylation capacity or specific methylation/demethylation (Ranlal et al., 1986).

3.1 Frequency of Hg-resistant strains

Methylation processes have been studied exclusively with Hg-resistant strains. This is acceptable if one is only interested in the effects of man-made pollution sources where high Hg levels are encountered. However, MeHg is also the dominant species in biota that lives in areas far remote from man-made Hg sources. In these areas Hg-resistant bacteria are very unlikely to occur. Therefore, it seems interesting to investigate the frequency of Hg-resistant strains among the total number of bacteria isolated from the environment. Colwell et al. (1976) isolated bacteria strains from Chesapeake Bay. Most of these bacteria belonged only to seven generic groups (Fig. 4). Of these seven groups Vibrio, Pseudomonas, Achromobacter-/Alcaligenes/Acinetobacter and Cytophaga/Flavobacterium species accounted for about 70\% of the total number of bacteria strains isolated. In six groups the number of Hg-resistant strains is less than 10\% of the total number. Only in Pseudomonas spp. about 70\% are Hg-resistant species. Also the frequency of Hg-resistance was different for strains isolated from water and from sediments (Fig. 5). Baldi (1987) investigated the ratio between total and Hg-resistant strains along the course of the river Flora in the Mt. Amiata mercury geochemical anomaly and found great seasonal fluctuations. In October 1985, 37 strains were isolated and about 50\% of them were Hg-resistant. In February 1986, the total number of bacteria was about 10 times higher than in October but only 0.1\% were Hg-resistant.
Fig. 3  Isotopic mercury exchanges for various concentrations in Ottawa River water (after 24 h, concentration ratio was 1:1 for both directions) (Kudo et al., 1977)

![Graph showing isotopic mercury exchanges](image)

<table>
<thead>
<tr>
<th>GENUS</th>
<th>MERCURY RESISTANT</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acroebacter/Acaraugenes/Aceto bacter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corynebacterium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytophaga/Flavobacterium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterics</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4  Average distribution of genera in total and Hg-resistant populations in an estuarial environment (Colwell et al., 1976)

In May, no Hg-resistant bacteria could be found except in the river mouth. The frequency of Hg-resistance strains, if present, is not constant in time but may vary considerably.
3.2 Methylation

Methylation has been studied in single bacteria and fungi species and in water and sediment samples. These organisms can also carry out conversion of Hg compounds. In the early studies, large amounts of inorganic Hg salts were added to the culture medium or the sample and only the net MeHg production was determined; i.e. the difference between methylation and demethylation. In recent experiments radioisotopes have been used to try to study the methylation and demethylation processes at lower Hg concentrations separately, but also here the rates observed are between net and gross rates. The true fluxes may be determined applying compartment theory, but this has not yet been done.

In general, only relatively low yields were obtained in the methylation experiments (Table 1). Plotting the data (Fig. 6) shows that there is no general correlation between the percentage of the inorganic Hg converted and the total concentrations of added inorganic Hg. However, the data in this table give only a very approximate idea of the magnitude of methylation because if the kinetics of the MeHg production is examined (see below), it will be noticed that the MeHg production increases usually only in the first days of the experiments.

3.2.1 Single species of microorganisms

Reviewing the literature it seems that only Hg-resistant bacteria strains have been studied. Hg-resistance seems to be related to Hg methylation but, so far, it is not clear if only Hg-resistant strains produce MeHg, since no data on Hg-sensitive strains are available. Vonk
Table 1

Estimated daily yield of methyl mercury conversion in percent of the amount of inorganic mercury added in selected model experiments using chemical analytical procedure.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>HgCl₂ mg L⁻¹</th>
<th>MeHg produced % per day</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>5</td>
<td>0.001</td>
<td>Vonk &amp; Sijpsteijn, 1973</td>
</tr>
<tr>
<td><em>M. phlei</em></td>
<td>20</td>
<td>0.0003</td>
<td>Vonk &amp; Sijpsteijn, 1973</td>
</tr>
<tr>
<td><em>B. megaterium</em></td>
<td>20</td>
<td>0.006</td>
<td>Vonk &amp; Sijpsteijn, 1973</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>20</td>
<td>0.006</td>
<td>Vonk &amp; Sijpsteijn, 1973</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>20+B₁₂</td>
<td>0.004</td>
<td>Vonk &amp; Sijpsteijn, 1973</td>
</tr>
<tr>
<td><em>A. aerogenes</em></td>
<td>20</td>
<td>&lt;0.0002</td>
<td>Vonk &amp; Sijpsteijn, 1973</td>
</tr>
<tr>
<td><em>A. aerogenes</em></td>
<td>20+B₁₂</td>
<td>0.008</td>
<td>Vonk &amp; Sijpsteijn, 1973</td>
</tr>
<tr>
<td><em>C. coohlearium</em> 48 h</td>
<td>10+B₁₂</td>
<td>0</td>
<td>Pan-Hou and Imura, 1982</td>
</tr>
<tr>
<td>Methanogenic culture</td>
<td>5</td>
<td>0.001</td>
<td>Bishop &amp; Kirsch, 1972</td>
</tr>
<tr>
<td>Methanogenic culture</td>
<td>10</td>
<td>0.002</td>
<td>Bishop &amp; Kirsch, 1972</td>
</tr>
<tr>
<td>Methanogenic culture</td>
<td>0.1</td>
<td>0</td>
<td>Bishop &amp; Kirsch, 1972</td>
</tr>
<tr>
<td><strong>Freshwater Sediments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake sediments</td>
<td>0.1</td>
<td>(0.015)</td>
<td>Jensen &amp; Jerneloev, 1969</td>
</tr>
<tr>
<td>Lake sediments</td>
<td>1</td>
<td>(0.0014)</td>
<td>Jensen &amp; Jerneloev, 1969</td>
</tr>
<tr>
<td>Lake sediments</td>
<td>5</td>
<td>(0.0003)</td>
<td>Jensen &amp; Jerneloev, 1969</td>
</tr>
<tr>
<td>Lake sediments</td>
<td>10</td>
<td>(0.00015)</td>
<td>Jensen &amp; Jerneloev, 1969</td>
</tr>
<tr>
<td>Lake sediments</td>
<td>50</td>
<td>(0.00003)</td>
<td>Jensen &amp; Jerneloev, 1969</td>
</tr>
<tr>
<td>Lake sediments</td>
<td>100</td>
<td>(0.00001)</td>
<td>Jensen &amp; Jerneloev, 1969</td>
</tr>
<tr>
<td>Lake sediments</td>
<td>500</td>
<td>(0.000003)</td>
<td>Jensen &amp; Jerneloev, 1969</td>
</tr>
<tr>
<td>Lake sediments</td>
<td>1000</td>
<td>(0.000001)</td>
<td>Jensen &amp; Jerneloev, 1969</td>
</tr>
<tr>
<td>Aquarium sedim.</td>
<td>100</td>
<td>0.04</td>
<td>Jensen &amp; Jerneloev, 1969</td>
</tr>
<tr>
<td>Pond sedim. anaerobic</td>
<td>50</td>
<td>0.0038</td>
<td>Bishop &amp; Kirsch, 1972</td>
</tr>
<tr>
<td>Pond sedim. anaerobic</td>
<td>10</td>
<td>0.001</td>
<td>Bishop &amp; Kirsch, 1972</td>
</tr>
<tr>
<td>Pond sedim. anaerobic</td>
<td>.1</td>
<td>0</td>
<td>Bishop &amp; Kirsch, 1972</td>
</tr>
</tbody>
</table>

Values in brackets are estimated from figures.

DW = dry weight.

and Sijpsteijn (1973) showed that pure cultures of Hg-resistant bacteria (*P. fluorescens, M. phlei, B. megaterium, E. coli, E. coli W/B₁₂, A. aerogenes, A. aerogenes W/B₁₂*) could aerobically methylate HgCl₂ (Table 1). *A. aerogenes* and *E. coli* also methylated Hg anaerobically, but at a lower rate. Vonk and Sijpsteijn (1973) also observed methylation by yeasts (*A. niger, S. brevicaulis* (baker's yeast), but Reisinger et al. (1984) were unable to confirm methylation by *C. cerevisiae* (baker's yeast).

Landner (1971) studied the relationship between Hg methylation and resistance to inorganic Hg in *Neurospora crassa*. Mutants resistant to
more than 200 ppm inorganic Hg produced more MeHg than the original wild-type strain. The addition of 40 ppm of DL-homocysteine or L-cysteine increased methylation (Table 2). At higher concentrations (80 ppm) of DL-homocysteine less MeHg was produced, while 80 ppm of L-cysteine increase the MeHg production even further. Since methylcobalamin is not involved in the metabolism of this fungi, Linkner suggested that, here, the methylation of Hg could be regarded as an 'incorrect' synthesis of methionine.

Handy and Noyes (1975) isolated Hg-resistant strains from freshwater sediments. 14 were gram-negative short rods belonging to the genera Escherichia and Enterobacter, six were gram-positive cocci (3 Staphylococcus sp. and 3 Streptococcus sp.). These authors found so large variability in the MeHg production of a Hg-resistant A. aerogenes strain, both under aerobic and anaerobic conditions, that no difference in the aerobic or anaerobic production rate could be established. In two Hg-resistant strains isolated from water and sediments of Chesapeake Bay, Hg volatilization was plasmid-mediated (Olson et al., 1979; Barkay et al., 1979). In one strain, Hg volatilization was chromosomally mediated. All other strains tested could only reduce $\text{Hg}^{2+}$ to Hg(0). In media containing $10 \text{ mg HgCl}_2 \text{ L}^{-1}$ under aerobic conditions, 21.5 to 87.2% of the Hg was volatilized within 24 h to Hg(0); under anaerobic conditions 12.7 to 17.8% was volatilized. Mercuric reductase genetically encoded in plasmids mediated the volatilization. Six out of 24 Hg-resistant strains (the
Table 2
Concentration (ng Hg g⁻¹ cell) of methyl mercury (and SD) in mycelia after 4 weeks of growth (Landner, 1971).

<table>
<thead>
<tr>
<th></th>
<th>DL-homocysteine added</th>
<th>L-cysteine added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 ppm Hg 80 ppm Hg</td>
<td>40 ppm Hg 80 ppm Hg</td>
</tr>
<tr>
<td>Original strain</td>
<td>269±55 106±79</td>
<td>79±19 694±214</td>
</tr>
<tr>
<td>225 ppm Hg-resistant strain</td>
<td>3675±862 98±65</td>
<td>680±288 5125±1209</td>
</tr>
</tbody>
</table>

majority were *Pseudomonas*. Three of these strains contained plasmids. Only two strains could produce MeHg under either anaerobic or aerobic conditions. A strain of *C. cochlearium* that could decompose DimHg was also isolated (Pan-Hou et al., 1980). This ability was cured with acridine dye and recovered by conjugation of the cured strain with the parent strain. The cured strain then showed the ability to methylate Hg²⁺.

Blair et al. (1974) isolated seven Hg-tolerant bacteria from Chesapeake Bay. Although most of them produced only Hg(0), one obligate anaerobe strain generated both Hg(0) and CH₃Hg⁺. One of the facultative anaerobes produced both Hg(0) and CH₃Hg⁺ under anaerobic conditions but only Hg(0) under aerobic conditions. Another facultative anaerobe produced anaerobically only Hg(0). From a set of eight Hg-resistant strains isolated from the Chesapeake Bay and one strain from the Cayman Trench only two strains, one of *P. fluorescens* under aerobic and one of an enteric bacterium under anaerobic conditions could methylate Hg (Olson et al., 1979). Except for one strain, where the volatilization under anaerobic conditions was five orders of magnitude higher, total volatilization of Hg of the other 8 strains under aerobic conditions was about the same as under anaerobic conditions. 30 to 60% of the initial amount of Hg present were volatilized within 24 hours. In the following 24 hours the volatilization was one or two orders of magnitude lower. All other strains tested could transform Hg²⁺ to Hg(0). Mercuric reductase genetically encoded in plasmids mediated the volatilisation. Volatilisation was shown to be a phenomenon different from methylation.

Compeau and Bartha (1985) isolated bacteria from anoxic estuarine sediments. Of five isolated cultures only one sulphate-reducer, which was identified as a *Desulfovibrio desulfuricans*, produced MeHg it could not form MeHg from pure HgS. From a medium containing 15 μg HgCl₂ mL⁻¹ about 12 ng MeHg mL⁻¹ was produced in 48 h. This corresponds to about 0.08% per day. Most of the MeHg was produced during the first hours when the HgS concentration was low. More recently Compeau and Bartha (1987) isolated a *D. desulfuricans* from a high salinity (S = 24) environment. The growth of this strain was stimulated by higher chloride concentration. This strain could not methylate HgS. Interestingly, this strain methylated HgCl₂ only when added at inoculation. If the strain had already grown for 24 h, at which time
12.3 mg HgS mL⁻¹ had been produced, the addition of HgCl₂ did not result in a production of MeHg.

Recently more sophisticated methods have been used to study Hg methylation in bacteria species. Berman et al. (1990) showed with radio-carbon labeled serine that the methyl group of MeHg produced by Desulfovibrio desulfuricans derived from the C-3 of serine. They suggested a transmethylation through a sequential involvement of tetrahydrofolate and cobalamin or a closely related corrinoid (Fig. 7). Additional evidence for this methyl transfer was supplied by reversal of the inhibition of transmethylation to Hg by propyl iodide which is known to inhibit transmethylation in the synthesis of methionine and methane.

\[
\begin{align*}
\text{CH}_3\text{ - CO - COOH} & \quad (1) \\
\text{ATP} & \quad \text{OR} \\
\text{ATP + Pi} & \\
\text{P} & \quad \text{AMP} \\
\text{AMP + PPi} \quad & \\
\text{CH}_2\text{ = CO (P) - COOH} & \quad (2) \\
\text{HOCH}_2\text{ = CHO(P) - COOH} & \quad (3) \\
\text{(P)OCH}_2\text{ = CHO - COOH} & \quad (4) \\
\text{(P)OCH}_2\text{ = CO - COOH} & \quad (5) \\
\text{(P)OCH}_2\text{ = CH(NH)_2 - COOH} & \quad (6) \\
\text{HOCH}_2\text{ = CH(NH)_2 - COOH} & \quad (7) \\
\text{HOCH}_2\text{ = NH(NH)_2 - COOH} & \quad (8) \\
\text{5,10-C}_2\text{H}_2\text{ - THF} & \quad (9) \\
\text{5-C}_2\text{H}_3\text{ - THF} & \quad (10) \\
\text{CH}_3\text{ - CoPorph} & \quad (11) \\
\text{CH}_3\text{ - Hg}^+ & \quad (12)
\end{align*}
\]

Fig. 7 Proposed pathway of carbon in D. desulfuricans during methylation of mercury. The carbon that becomes CH₃ in methylmercury is in boldface. The enzyme proposed to transfer the C-3 group of serine to tetrahydrofolate is serine hydroxymethyl transferase (11) (Berman et al., 1990)
Selective inhibition and stimulation experiments

Recently, Compeau and Bartha (1985) used another approach to identify the microorganisms responsible for the production of MeHg. Addition of a specific inhibitor of methanogens (2-bromoethane sulfonate, BES) to anoxic estuarine sediments spiked with 75 μg Hg g⁻¹ sediment as HgCl₂ showed that MeHg production increased, while adding a specific inhibitor (sodium molybdate) for sulphate reducers decreased the methylation by more than 95%. These experiments suggest that sulphate-reducing bacteria are important MeHg methylators while methanogenic bacteria are not. Unfortunately, due to the high amounts of the Hg spikes, the conditions of these experiments also are selective for Hg-resistant strains. Maximum yields of MeHg are obtained under sulphate limiting conditions because the H₂ generated by the sulphate respiration interferes with the Hg methylation. A comparison of steam-sterilised sediments without and with an inoculate of the D. desulfiticans showed that the bacterium was able to form MeHg in the sediment. No MeHg was observed in the sterile sediment.

Kinetics of methylation

Tonomura et al. (1972) observed that in the Hg-resistant strain of C. cochlearium during the log phase of growth extracellular MeHg concentration increased up to 40 h rapidly but, thereafter, the intracellular MeHg concentration remained constant during the stationary population growth phase and the intracellular MeHg concentration increased (Fig. 8).

Influence of B₁₂

Vonk and Sijpsteijn (1973) found that in Hg-resistant A. aerogenes methylation of Hg is enhanced in the presence of B₁₂, but in the presence of 100 mg cysteine/L adding 1 mg L⁻¹ of B₁₂ did not increase MeHg formation. Experimenting under anaerobic conditions with C. cochlearium Tonomura et al. (1972) and Yamada and Tonomura (1972) observed an increase of the MeHg concentration with increasing B₁₂ additions to the culture medium (Fig. 9). A. aerogenes (Salem and Forster, 1972) and C. cochlearium (Yamada and Tonomura, 1972) can synthesise B₁₂.

Influence of organic compounds in the medium on MeHg production

The composition of the culture medium influences MeHg production. Adding 0.2% of casamino acid or 0.2% tryptase (and also 10mg HgCl₂ and, 0.5 mg B₁₂) stimulated markedly the growth of the anaerobic culture of Hg-resistant C. cochlearium, but no MeHg was produced (Yamada and Tonomura, 1972). On the other hand MeHg production was observed when the medium contained meat extract and peptone. Also glucose increases the production of MeHg.

Influence of complexing agents

Vonk and Sijpsteijn (1973) found that in Hg-resistant A. aerogenes methylation of Hg is not enhanced in the presence of B₁₂ when 100 mg cysteine L⁻¹ had been added. Yamada and Tonomura (1972) showed that adding increasing amounts of cysteine will increase methylation at lower concentrations of cysteine but decrease
Fig. 8  Formation of methylmercury in time (hours) by C. coclearium with addition of 0.5 mg B12 L⁻¹. ○ = extracellular methyl mercury; O = intracellular methyl mercury; △ = total cell counts (Tonomura et al., 1972)

Fig. 9  Effect of B₁₂ on the formation of methyl mercury (Tonomura et al., 1972)
methylolation at higher concentrations (Fig. 10). At low concentrations, cysteine probably at first decreases the toxicity of HgCl₂ and keeps the environment in a reducing state, but at higher concentrations it interferes with methylolation.

Influence of mercuric chloride

Yamada and Tonomura (1972) showed that increasing concentrations of HgCl₂ from 5 to 50 mg L⁻¹ would first slightly increase (?) the production of MeHg but at concentrations greater than 15 mg L⁻¹ reduce the MeHg production (Fig. 11).

![Graph showing effect of cysteine concentrations on the formation of MeHg](image)

Fig. 10  Effect of cysteine concentrations on the formation of MeHg (Yamada and Tonomura, 1972)

3.2.2 Water and sediment

For the interpretation of the results obtained with sediments, the observations made by Barlett et al. (1977) have to be considered. These authors found that after homogenizing (and involuntary oxygenation) the sediments the concentration of MeHg in the sediments increased for about 10 days and then decreased (Fig. 12). This increase and then decrease was also observed in sediments sterilized with a 4% v/v formaldehyde solution. The authors pointed out that this phenomenon was only observed in polluted sediments that had 5 to 8 mg Hg-T kg⁻¹ DW. In fact, they could not observe any changes in the MeHg concentrations of sediments with Hg-T levels lower than 1.5 mg Hg-T kg⁻¹ DW and suggested that the high Hg concentrations in the Hg-polluted experiments may be a requirement for the phenomenon to be observed. Nevertheless, their concentration range lies within the range used in the experiments by many authors. These results show how important adequate controls are.
Fig. 11  Effect of HgCl₂ concentration on the formation of MeHg (Yamada and Tonomura, 1972)

Fig. 12  Methylmercury concentrations in formaline-sterilized (broken line) and unsterilized (continuous line) sediments after Barlett et al., (1977)
Water column

Using radioactive Hg tracer, Callister and Winfrey (1986) could not detect methylidy in the water column over Hg-polluted river sediments. Applying the same method, Furutani and Rudi (1980) found methylation activities in 5 out of 15 trials; and Korthals and Winfrey (1987) incubating water samples aerobically for 24 h observed that methylation was low (<0.05%), but detectable in the water of an oligotrophic lake (Fig. 13). Comparing potential methylation with potential demethylation, by adding radioactive inorganic Hg and radioactive MeHg respectively, showed that in the water column the demethylation was higher than the methylation.

In oxygenated water samples Xun et al. (1987) found that measurable amounts of inorganic Hg were methylated and detectable quantities of MeHg were demethylated (Fig. 14). The methylation rate is higher during the first day than afterwards. The demethylation rate increases between the 3rd and 5th day. Methylation exceeds demethylation in the first 4 days and later demethylation exceeded methylation. These observations are difficult to interpret and their use is limited for explaining processes occurring in nature. In the natural environment methylation and demethylation occur simultaneously. If demethylation exceeds methylation then no MeHg should be found in the water column unless the MeHg formed was immediately trapped and made unavailable for demethylation. If the results of Xun et al. (1987) are extrapolated to the natural environment, then an addition of inorganic Hg salts will first stimulate methylation and then demethylation.

Influence of Hg concentration

Xun et al. (1987) showed that with increasing addition of inorganic Hg the potential methylation rate increases (Fig. 15), even at a concentration of about 65 mg Hg L⁻¹. For additions of 3.3 mg to 28 mg inorganic Hg L⁻¹ the potential yield is constant, i.e. about 0.26%/day. At an addition of about 65 mg Hg L⁻¹ the yield increases to 1.8%.

Influence of pH

Lowering the pH in unpolluted epilimnetic water samples (5 mg HgT L⁻¹) increased the methylation rates and decreased the demethylation rates (Table 3). Consequently the methylation/demethylation ratio increases markedly with decreasing pH.

3.2.2.2 Sediment

The first to show that fresh water sediments could methylate inorganic Hg salts were Jensen and Jernelöv (1969). Sediment samples have been incubated with additions of inorganic and inorganic radioactive Hg. Most authors have used batch incubations, but also sediment slurries with a continuous feed of nutrients, Hg solutions, etc. have been employed.
Fig. 13  Depth distribution of mercury methylation and demethylation at a profundal site in Lake Clara (Korthals and Winfrey, 1987)

Fig. 14  Time course of potential methylation (□) and demethylation (●) rates for epilimnetic water (Xun et al., 1987)
Kinetics of methylation

Similar to the observations made on single bacteria species and on incubated freshwater samples the methylation rate in sediments is not constant in time. The MeHg concentration in sediments from a Swedish lake showed a rapid increase until about 7 days when a plateau was reached (Jensen and Jernelöv 1969) (Fig. 16). Because the MeHg concentration reflects the balance between MeHg produced and MeHg decomposed this means that either the MeHg production ceases after 7 days or demethylation of Hg increases during the first 7 days and reaches rates similar to the rate of methylation. Also Wright and Hamilton (1982) found that in a non-aerated "flow through microbasin", after a lag period of about 1 week, the net MeHg concentration in the effluent increased to a maximum at about 40 days, then decreased to reach a level-off phase after about 90 days (Figs. 17 and 18). Similarly investigating the anaerobic MeHg transformation in Hg-polluted sediments with radioactive inorganic Hg showed that methylation first increased and then, after about 70 hours of incubation, slowed down considerably (Callister and Winfrey, 1986) (Fig. 19). The increase of net MeHg production following an addition of inorganic Hg seems to be a general pattern because it has been observed in almost all experiments. This suggests that the daily production rates of inorganic Hg salts listed in Table 1 are very likely much higher than the ones actually occurring in nature because these rates
Table 3

Effect of pH on specific rates of potential methylation (Rm) and demethylation (Rd) and net methylation (M/D) for epilimnetic water samples. (Yun et al., 1987).

<table>
<thead>
<tr>
<th>Date</th>
<th>Lake</th>
<th>pH</th>
<th>(% L⁻¹ d⁻¹ x 10⁻²)</th>
<th>(% L⁻¹ d⁻¹ x 10⁻¹)</th>
<th>M/D</th>
</tr>
</thead>
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<td>239</td>
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<td>4.19 (0.03)</td>
<td>6.05 (0.27)</td>
<td>6.93</td>
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<td>6.5</td>
<td>5.67 (0.00)</td>
<td>6.64 (0.12)</td>
<td>8.54</td>
</tr>
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<td></td>
<td>5.5</td>
<td>9.00 (0.21)</td>
<td>4.00 (0.18)</td>
<td>12.1</td>
</tr>
<tr>
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<td>239</td>
<td>7.1*</td>
<td>2.51 (0.05)</td>
<td>6.12 (0.15)</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.5</td>
<td>2.60 (0.05)</td>
<td>6.10 (0.54)</td>
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<td></td>
<td>5.9</td>
<td>3.35 (0.06)</td>
<td>5.71 (0.93)</td>
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<td></td>
<td></td>
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<td>3.51 (0.08)</td>
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<td>5.82 (0.09)</td>
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<td></td>
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<td>7.11 (0.15)</td>
<td>9.01 (1.91)</td>
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<tr>
<td></td>
<td></td>
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<td>11.16 (0.33)</td>
<td>9.36 (1.82)</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Mean rate of two samples with range in parentheses.
* Untreated samples, initial epilimnetic pH.

were calculated from the MeHg concentrations observed during the beginning of the experiments. Apparently, sampling and sample treatment stimulated the MeHg production. In this context the experience of Compeau and Bartha with spiked sediments is important. These authors noted that "concentration of MeHg approached a steady-state after 8 to 12 days of incubation. At this point, the addition of a second identical HgCl₂ spike resulted in renewed MeHg synthesis at the previous rate doubling the steady-state concentration in each case". Unfortunately the authors did not show the data. Apparently the addition of inorganic Hg stimulated methylation for a certain period.
Fig. 16  Concentration of methyl mercury in bottom sediment after addition of 10 mg Hg kg\(^{-1}\) (continuous line) and 100 mg Hg kg\(^{-1}\) (broken line) of inorganic mercury following different times of incubation (Jensen and Jernelöv, 1969)

Fig. 17  Net release of methyl mercury from Clay Lake sediment with added inorganic mercury as a function of time showing (1) lag, (2) exponential, (3) maximum, (4) decrease and (5) level-off phases (Wright and Hamilton, 1982)
Fig. 18  Net release of methyl mercury from Wabigoon River sediment with added mercury as function of time (Wright and Hamilton, 1982)

Fig. 19  Methylation and binding of 203 Hg in B-1 sediments. Error bars represent the range of triplicated values. Peptone (1 mg mL⁻¹) was added to stimulate methylation (Callister and Winfrey, 1986)
Compeau and Bartha (1984) also noted that the MeHg went through a maximum during the experiment. Excluding oxygen contamination during the sampling and preparation of anaerobic sediment samples eliminated the decline of the MeHg concentration after a maximum was reached.

Influence of mercury concentration

Bottom sediments taken from upper sediment layer (< 10 cm) of Lake Langsjon (near Stockholm) and spiked with inorganic Hg showed that the MeHg concentrations increased with increasing Hg concentration. In experiments run with additions ranging from 0.1 to 1000 μg Hg g⁻¹ sediment Jensen and Jernelov (1969) found that higher additions of inorganic Hg salts increased the total amount of MeHg produced up to a concentration of 100 μg Hg g⁻¹ sediment and then declined at higher concentrations. The relative amounts, however, declined with increasing amount added. Addition from 0.1 to 100 μg g⁻¹ sediment resulted in net yields of 0.002 to 0.15%. At 1000 μg Hg_T g⁻¹ sediment the yield was only 0.0001% (Table 1). Similar observations were made by Wright and Hamilton (1982) (Fig. 17). However, the results on the influence of increasing concentration of Hg on the net release of MeHg from slurries of a river sediment showed that the net yield is difficult to assess because it depends on the dynamics of the MeHg release (Fig. 18). After reaching a maximum at about 28 days and relative net yields of 2.5 to 9%, in the level-off phase the relative net yields are lower: 0.9 to 2%. In a lake sediment the highest concentration (42 μg Hg g⁻¹ sediment) did not result in the highest MeHg concentration and hence the relative yield was higher with the additions of 20 μg Hg g⁻¹ sediment. Also here the relative yields diminish in the level-off periods similar to those of the river sediments. This suggests that the matrix of the river sediment can bind more Hg than the matrix of the lake sediment, showing the importance of the matrix for the availability and toxicity of the inorganic Hg added. Berdievsky et al. (1979) made similar observations. Although their data are incomplete (because they did not determine MeHg in the sediments), their data, nevertheless, indicate that the total amount produced increases with increasing Hg concentration while the percentage of MeHg formed in the medium decreased with increasing Hg concentration and with time of exposure. Likewise, in marine sediment samples higher Hg additions increase the absolute MeHg concentrations (Fig. 22). Similarly, Compeau and Bartha (1984) observed with anaerobic estuarine sediments that higher amounts of Hg added increased the absolute quantities of MeHg produced but not the relative amounts. Additions of 1 to 300 μg HgCl₂ increased after 14 days the MeHg concentrations from 20 to 700 ng MeHg g⁻¹ of sediment, while the relative amounts decreased from 2.6 to 0.23%.

Influence of metabolic activities and organic enrichment

Already Jensen and Jernelov (1969) have shown that, after adding untreated natural freshwater sediments samples to autoclaved sediments, the previous sterile sediments would methylate, while sterilized sediments without added untreated sediment did not produce MeHg. Similar experiments were carried out on autoclaved and untreated sediments from a contaminated area in the Haifa Bay (Berdievsky et al., 1979). Large amounts of mercury (100 μg Hg-T L⁻¹), added under sterile conditions together with the bacteria medium to the flasks containing the sterilized sediments, did associate with the sediment.
and with the surface of the glass flasks. After reinfection with untreated sediment, MeHg was observed both under aerobic and anaerobic conditions in the medium above the sediment. However, the MeHg in the sediment was not determined and hence these results are not directly comparable with the experiments discussed previously. In order to show that bacteria were necessary to produce the MeHg Berdicevsky et al. (1979) added Hg-resistant bacteria strains to autoclaved sea water/sediment media. Autoclaved medium without bacteria served as control. Only in the media with bacteria very small amounts of MeHg could be detected: i.e. 0.01 to 0.04% of the Hg added at the beginning of the experiment. Obviously these results can only serve as a rough indication of what might happen in the environment, since the experimental system also contained, besides sediments, considerable amounts of organic substances contained in the culture medium. The reduction of the MeHg production during the experiment also indicates that the bacteria fauna changed considerably. In fact, toxic effects were observed at 1 µg Hg L⁻¹ under anaerobic conditions and at 10 µg Hg L⁻¹ under aerobic conditions.

The methylation rate was higher in sediments with higher content of organics (Olson and Cooper, 1976). After 30 days under anaerobic conditions only about 0.1% of the 100 mg HgCl₂ kg⁻¹ added to the sediment and 0.8% of the 10 mg HgCl₂ kg⁻¹ sediment were transformed into MeHg. In sediments with the lowest organic content, to which 10 mg HgCl₂ kg⁻¹ was added, the increase in the MeHg concentration was minimal (Fig. 22). Stimulation of methylation was observed after addition of protein hydrolysates to river sediments (Furutani and Rudd, 1980) and lake sediments (Wright and Hamilton, 1982). Callister and Winfrey (1986) could show that the addition of 10 to 100 mg peptone/L increased methylation rate, but the addition of various amounts of glucose or starch had no effect. Cooley (1981) determined the effects of dextrose, acetate, propionate and formic acid on anaerobic methylation. Only 0.04 µg Hg (as MeHg) L⁻¹ was formed during 6 h when no organics were added. Dextrose produced 0.44, propionate 0.21, acetate and formic acid 0.04 µg Hg (as MeHg) L⁻¹. In a batch culture increasing concentrations (0.25 g L⁻¹ to 2.54 g L⁻¹) of dextrose augmented the MeHg production after 10 h from about 0.3 µg L⁻¹ to about 1.2 µg L⁻¹. Furutani and Rudd (1980) found that the additions of Tryptic Soya Broth (TSB) increase the methylation activity in water 15 times. Wright and Hamilton (1982) observed that the addition of TSB increased the MeHg production to about 100 times over those of controls. Interesting are also their experiment, in which TSB was added during the course of the investigation. After the MeHg concentration in the effluent water had levelled off at about 110 days, an addition of TSB increased markedly the MeHg concentration in the effluent of the "microbasin" (Fig. 20). Furutani and Rudd (1980) found that the rate of oxygen consumption (microbial activity) is linearly correlated with methylation in water samples.

Influence of oxygen and redox

Several authors have investigated methylation under aerobic and anaerobic conditions. Vark and Sijpsesteijn (1973) found that under aerobic conditions E. coli and A. aerogenes produced less MeHg than under aerobic conditions. Olson and Cooper (1976) experimenting with San Francisco Bay sediments found that under anaerobic conditions, the MeHg concentration in the sediments spiked with same amount of
inorganic Hg was higher than under aerobic conditions (see Fig. 22). Autoclaved and non-autoclaved sediment samples without Hg additions served as control. The non-autoclaved sample of this sediment produced about four times more MeHg than the autoclaved sample. Of special interest is that, except for sediment A (high organic content), none of the sediments produced any MeHg without Hg additions neither under aerobic nor under anaerobic conditions and also sediment type A produced MeHg only under anaerobic conditions.

Cooley (1981) found that methylation occurred only under anaerobic conditions. Compeau and Bartha (1984) investigated the influence of the redox potential and salinity on methylation and demethylation in estuarine sediment slurries. Higher methylation was observed at Eh of -220 mV than at +110 mV (Fig. 23).

Berdichevsky et al. (1979) found that under anaerobic conditions the addition of 1 mg HgCl₂ L⁻¹ already reduced the growth of the natural population present in the sediments, while under aerobic conditions a reduction in growth was only observed at concentrations greater than 5 mg HgCl₂ L⁻¹. Callister and Winfrey (1986) and Rudd et al. (1983) found with ²⁰₃Hg that methylation in lake sediments is much favoured under anaerobic conditions.
Furutani and Rudd (1980) observed that although no radioactive MeHg was produced in the water column over anoxic sulphide containing sediment, Hg was methylated in the floc of these sediments. The MeHg found in the overlying water column originated most probably from the methylation of the sediment floc. About 2 ng MeHg L⁻¹ was found in up to 13 meters depth and then the MeHg increased rapidly to 10 to 20 ng L⁻¹ while the oxygen content decreased to zero.

It seems that no general pattern exists which favors the production of MeHg under aerobic or anaerobic conditions. As expected, many factors play a role and as long as the bacteria responsible for methylation (and demethylation) have not been identified in each case it is impossible to predict if aerobic or anaerobic conditions will produce more MeHg.

Presence of sulphide

Under anaerobic conditions HgS may be formed. Fagerstroem and Jernelov (1971) investigated if mercuric sulphide could serve as a source for the methylation of mercury. Impurity-free HgS was prepared and added, in increasing amounts, to samples of aquarium sediments which previously had shown to have a high methylation activity. For comparison, a second series of the same aquarium sediments was spiked with HgCl₂. MeHg is produced also from HgS but much higher additions (about 1000 times) are need to produce similar amounts.

However, some authors found that pure HgS cannot be methylated by pure cultures of sulphate-reducing bacteria (see page 17). Yamada and Tonomura (1972) suggested that impurities in the HgS used were methylated and not HgS itself.

It may be interesting to note that Rowland et al. (1977a) using a chemical model and Craig and Bartlett (1978) using a sediment model could show that H₂S can mobilize MeHg and convert it to DimeHg which then volatilizes from the sediment.

Selective inhibition experiments

Interesting are the results of Compeau and Bartha (1985, 1987). These authors used specific metabolic inhibitors to demonstrate that sulphate-reducing bacteria are Hg methylators in saltmarsh sediment. Unfortunately, also in these experiments high Hg concentrations (75 μg Hg g⁻¹ sediment) were used and hence the experimental conditions are again selective for Hg-resistant bacteria. Desulfovibrio desulfuricans strains isolated from these sediments could methylate about 0.1% of the HgCl₂ added. However, the full methylation potential of these strains are only obtained if sulphate is limiting and a carbon source is available because if sulphate is abundant, H₂S is generated which interferes with the Hg methylation. In fact at sulphate concentrations of normal sea water or when sulphate is added to low salinity sea water methylation is inhibited (Compeau and Bartha, 1984; 1987). However, a strain of D. desulfuricans could be isolated that methylated higher amounts of MeHg at higher chlorinity than at lower chlorinity. It seems that the production of H₂S by these bacteria in sulphate rich environments restricts the role they are playing in the generation of MeHg considerably.
Depth distribution of methylation

Callister and Winfrey (1986) studied the potential Hg methylation in the water column and in Hg-polluted sediments with $^{203}$Hg. The methylation in the water was below detection limit. In the floc above the sediment about 1% of the radioactive Hg was methylated in 4 days. The highest amounts (about 2.5%) were methylated in the top layer of the sediment and decreased to very low values at about 10 cm depth. The sediment samples were anoxic. Korthals and Winfrey (1987) studied potential methylation and potential demethylation in profundal and littoral sediments of an oligotrophic lake (Fig. 13). In the water column over profundal sediments the potential demethylation is higher than the potential methylation. In the floc both potential methylation and potential demethylation were higher than in the sediment below, and again the percentage of demethylation was higher than that of methylation. Also in the surface layer of littoral sediments potential methylation was higher than potential demethylation.

Time series

Studying the variation of methylation over about half-a-year Callister and Winfrey (1986) found in 5 occasions little variation in the water of a lake (not detected to about 0.005%/day), in the floc (n = 5) somewhat higher percentages (not detected to 0.012%/day) and in the sediments (n = 6) higher values (0.0025 to 0.045%/day). In a lake Furutani and Rudd (1980) observed during 6 months that of 14 water samples only 5 methylated, others were below detection limit. By incubating monthly for a year littoral and profundal superficial sediments of an oligotrophic lake Korthals and Winfrey (1987) found wide variations between methylation and demethylation (Fig. 21).

Influence of salinity

Blum and Bartha (1980) investigated the influence of salinity on anaerobic methylation. They found that the lowest salinity ($S = 1$) converted about 2.3% of the added Hg in 15 days while the highest salinity ($S = 30$) converted only 0.05% in the same time interval (Fig. 24). Note that the MeHg concentration decreases after 15 days respectively to 20 days. Therefore, these conversions are the maximum and may be stimulated by the experimental manipulations as mentioned above under kinetics of methylation (page 26).

Salinity depresses methylation both under aerobic and anaerobic conditions (Fig. 23). Using sediments with low salinity ($S = 4$) to which the same amount of sulphate as in high salinity sediment ($S = 25$) was added Compeau and Bartha (1984) could show that the inhibition of methylation was due to the sulphate in the sea water and probably to the $H_2S$ produced by sulphate-reducing bacteria.

3.3 Demethylation

3.3.1 Single species

Sprangler et al. (1973a, b) found that 30 bacterial cultures isolated from freshwater could aerobically degrade MeHg and 21 cultures could anaerobically degrade MeHg. Billen et al. (1974) showed that MeHg was decomposed, anaerobically and aerobically, in the presence of
Fig. 21 Seasonal variations in mercury methylation and demethylation at a littoral site (Korthals and Winfrey, 1987)
M/D: ratio between methylation and demethylation

bacterial cultures obtained from river sediments. Furakawa et al. (1969) demonstrated that a bacteria strain (Pseudomonas sp.) from soil could decompose MeHgCl to methane and Hg(0). Shariat et al. (1979) examined 40 bacterial strains isolated from soils, sediments and sewage effluent. Of these only 27 were able to tolerate the MeHg chloride concentrations of 0.37 to 2.5 mg L\(^{-1}\) (10 \(\mu\)M L\(^{-1}\)). Seven strains demethylated in 16 days over 60 percent of the MeHg (2.5 mg L\(^{-1}\)) present. \textit{D. desulfuricans} was the only anaerobe tested that had a marked demethylation ability (Table 4).

Oremland et al. (1991) studied the decomposition of radiocarbon-labelled MeHg in bacteria cultures. Demethylation of MeHg by a Hg-resistant \textit{E. coli} strain containing plasmid R831b was used as a control for the organomercurial lyase system. This coli converted \(^{14}\text{CH}_3\text{HgI}\) quantitatively to \(^{14}\text{CO}_2\). The conversion was much faster.
Fig. 22  Methyl mercury production in the San Francisco Bay in three different sediments □ and ■ 100 μg and 10 μg inorganic g⁻¹ added per g sediment under aerobic conditions; □ and ● 100 μg and 10 μg inorganic Hg added under aerobic conditions (Olson and Cooper, 1976). Chemical oxygen demand (mg g⁻¹): A:45.3; B:39.4; C:12.5
(2 times) during the first 2.5 h than later, but after 16 h all MeHg had been converted. A much lower growing methylo trophic methanogen converted $^{14}$CH$_3$HgI into $^{14}$CH$_4$ and $^{14}$CO$_2$ differently, according to the medium in which it was grown (Table 5). CH$_4$ was formed in all media but $^{14}$CO$_2$ and $^{14}$CH$_4$ only were produced in trimethylamine (TMA) and $^{14}$CH$_4$ only in DMS containing medium. Neither significant amounts of $^{14}$CO$_2$ nor $^{14}$CH$_4$ were formed when methanol was added. However, quantities detected were low and, according to the authors, could have come from impurities in the radiochemical. No demethylation was observed when Methanohalophilus oregonense were grown with TMA or Methanobacterium formicum was grown with H$_2$CO$_2$. Cultures of Desulfovibrio gigas and D. africanus produced small amounts (about 3% of the $^{14}$CH$_3$HgI added) of $^{14}$CH$_4$ after 8 days but no $^{14}$CO$_2$.

Two species of Desulfovibrio produced only traces of $^{14}$CH$_4$ from $^{14}$CH$_3$HgI. A culture of a methylo trophic methanogen formed both traces CO$_2$ and CH$_4$.

These data indicate that depending on the carbon source supplied for growth different metabolic pathways may be involved in the demethylation (see also below).
Fig. 24 Effect of salinity on the anaerobic methylation of 10 μg Hg (as HgCl₂ g⁻¹) estuarine sediment. Methyl mercury produced in μg Hg g⁻¹ dry sediment. Controls without HgCl₂ and also HgCl₂ plus 0.2% NaN₃ were included at several salinity levels but for clarity only one single control curve is shown (Blum and Bartha, 1980).

3.3.2 Water and sediment

Spangler et al. (1973b) observed that the demethylation by a mixed inoculum from sediment had a lag period of about 50 h and then MeHg was transformed into Hg(l). About 40% was transformed within 170 h. Demethylation occurs faster (about 20%) in lake sediments under aerobic than under anaerobic conditions (Ramlal et al., 1986).

Xun et al. (1987) found that demethylation of MeHg in epilimnic water samples increases approximately linearly with increasing MeHg concentration. Compeau and Bartha (1984) showed that under anaerobic conditions (Eh = -220 mV) demethylation of MeHg in a salt marsh sediment was higher at high salinity (S = 25) than at lower salinity (S = 4). Aerobic demethylation (Eh = +110 mV) at S = 25 and at S = 4 were the same as anaerobic methylation at S = 25. Xun et al. (1987) found in lake water samples that higher pH decreased demethylation. However, the changes observed were smaller than those of methylation. Similar results were observed in sediments by Ramlal et al. (1986). Oremland et al. (1991) found demethylation of MeHg in estuarine sediments to be more rapid under aerobic than anaerobic conditions. As can be seen from Table 6 in air the demethylation of radio-labelled MeHg is 63% in 24 days while anaerobic conditions yield only 35% conversion of the MeHg.
Table 4

Demethylation screening tests for selected bacteria at an initial methylmercuric chloride concentration of 2.5 mg L⁻¹ (10 μM L⁻¹) (Shariat et al., 1979).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>% CH₃HgCl reduction in 16 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serratia marcescens</td>
<td>84</td>
</tr>
<tr>
<td>Providencia sp.</td>
<td>83</td>
</tr>
<tr>
<td>Pseudomonas fluroescens</td>
<td>79</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>71</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>71</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>69</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>66</td>
</tr>
<tr>
<td>Paracolobacterum coliforme</td>
<td>59</td>
</tr>
<tr>
<td>Achromobacter pestifer</td>
<td>53</td>
</tr>
<tr>
<td>Serratia plymuthica</td>
<td>53</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>53</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>52</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>47</td>
</tr>
<tr>
<td>Flavobacterium maritumicum</td>
<td>37</td>
</tr>
<tr>
<td>Citrobacter intermedius</td>
<td>32</td>
</tr>
<tr>
<td>Desulfovibrio desulfuricans</td>
<td>32</td>
</tr>
<tr>
<td>Pseudomonas fragi</td>
<td>20</td>
</tr>
<tr>
<td>Vibrio cuneatus</td>
<td>1</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>negative</td>
</tr>
<tr>
<td>Clostridium bifermentans</td>
<td>negative</td>
</tr>
<tr>
<td>Desulfovibrio aestuarii</td>
<td>negative</td>
</tr>
<tr>
<td>Blanks:</td>
<td></td>
</tr>
<tr>
<td>Anaerobic</td>
<td>9</td>
</tr>
<tr>
<td>Aerobic</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 5

Formation of CH₄, ¹⁴CH₄, and ¹⁴CO₂ from ¹⁴CH₃HgI in cultures of the methanogen GS-16³ (Gremlana et al., 1991).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>CH₄ (μmol)</th>
<th>¹⁴CH₄</th>
<th>¹⁴CO₂</th>
<th>%b</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMA (10 mM)</td>
<td>121</td>
<td>15.5</td>
<td>13.0</td>
<td>2.9</td>
</tr>
<tr>
<td>MeOH (10 mM)</td>
<td>74</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DMS (6 mM)</td>
<td>67</td>
<td>23.5</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>Sterile (DMS)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a Incubated for 21 days with 0.7 μCi of ¹⁴CH₃HgI.

b Percent conversion of ¹⁴CH₃HgI to ¹⁴CH₄ and ¹⁴CO₂.
Formation of $^{14}$CH$_4$ and $^{14}$CO$_2$ from $^{14}$CH$_3$HgI in San Francisco Bay salt marsh sediment slurries$^a$ (Creelman et al., 1991).

<table>
<thead>
<tr>
<th>Amendment (10 mM)</th>
<th>Mean nCi of gas (±SD)$^b$</th>
<th>$^{14}$CH$_4$</th>
<th>$^{14}$CO$_2$</th>
<th>%$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>56 (20)</td>
<td>171 (24)</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Hydrogen</td>
<td>63 (33)</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>BES (10 mM)</td>
<td>56 (13)</td>
<td>151 (7)</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Molybdate (2.5 mM)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>411 (24)</td>
<td>0</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Incubated for 24 days with 0.65 μCi of $^{14}$CH$_3$HgI. All sediments were incubated under N$_2$ unless indicated otherwise.

$^b$ Results represent the mean of three samples.

$^c$ Percent conversion of $^{14}$CH$_3$HgI to $^{14}$CH$_4$ and $^{14}$CO$_2$.

(values in bracket are standard deviations)

Since no demethylation occurred in sterile sediments, microbial mediation was deduced. Because only $^{14}$CH$_4$ was formed under aerobic conditions the demethylation was thought to involve the organomercuric lyase reaction described for broad-spectrum Hg-resistant bacteria (Robinson and Tuovinen, 1984).

Under anaerobic conditions the estuarine sediments produced three times more $^{14}$CO$_2$ than $^{14}$CH$_4$ suggesting that an oxidation reaction was more important for demethylation. However, if under anaerobic conditions molybdate was used to inhibit sulphate-reducing bacteria, the production of CH$_4$ by the methanogens was stimulated, but the formation of $^{14}$CO$_2$ and $^{14}$CH$_4$ from $^{14}$C-labelled MeHg was blocked. On the other hand, when the methanogens were inhibited with BES (2-bromoethane-sulfonic acid), the formation of CH$_4$ was inhibited but not the production of $^{14}$CH$_4$ or $^{14}$CO$_2$ from $^{14}$C-labelled MeHg. This shows that the sulphate-reducers were demethylating the MeHg and the methanogens were not involved in the MeHg decomposition in estuarine sediments. In freshwater sediments the situation was different. While aerobes were the most important demethylators in estuarine sediments where they produced only $^{14}$CH$_4$ (see above), in estuarine sediments aerobes formed only small amounts of $^{14}$CH$_4$ and $^{14}$CO$_2$ (Table 7 and Table 8). In one experiment 11% of the radio-labelled MeHg was decomposed under aerobic conditions in 27 days, while under anaerobic conditions 35% was decomposed in the same period. The percentage of conversion was not very reproducible because in a second experiment 71% was decomposed under anaerobic conditions. Under anaerobic conditions BES inhibited CH$_4$ production completely, partially inhibited $^{14}$CH$_4$ and inhibited somewhat less $^{14}$CO$_2$ (Table 8). Inhibition of the sulphate-reducers with molybdate diminished the $^{14}$CO$_2$ production by 85%, but enhanced the formation of CH$_4$ and $^{14}$CH$_4$. This indicates that in freshwater sediments both sulphate-reducers and methanogens participate in the
Table 7
Formation of CH₄, ¹⁴CH₄, and ¹⁴CO₂ from ¹⁴CH₃HgI by freshwater sediment slurriesa (Greeland et al., 1991).

<table>
<thead>
<tr>
<th>Amendment</th>
<th>µmol of CH₄</th>
<th>nCi of gas (±SD)b</th>
<th>¹⁴CH₄</th>
<th>¹⁴CO₂</th>
<th>%C</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5815 (422)</td>
<td>58 (17)</td>
<td>212 (27)</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>BES (10 mM)</td>
<td>461 (19)</td>
<td>25 (3)</td>
<td>150 (15)</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Autoclaved</td>
<td>3 (0.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>4 (1)</td>
<td>64 (34)</td>
<td>41 (7)</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

a Incubated for 27 days with 1.0 µCi of ¹⁴CH₃HgI. All sediments were incubated under N₂ unless indicated otherwise.
b Results represent the mean of three samples.
c Percent conversion of ¹⁴CH₃HgI to ¹⁴CH₄ and ¹⁴CO₂.
(values in bracket are standard deviations)

Table 8
Production of CH₄, ¹⁴CH₄, and ¹⁴CO₂ from ¹⁴CH₃HgI in anaerobic freshwater sediment slurriesa (Greeland et al., 1991).

<table>
<thead>
<tr>
<th>Amendment</th>
<th>µmol of CH₄</th>
<th>nCi of gas (±SD)b</th>
<th>¹⁴CH₄</th>
<th>¹⁴CO₂</th>
<th>%C</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6364 (2995)</td>
<td>55 (9)</td>
<td>514 (80)</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>BES (20 mM)</td>
<td>278 (60)</td>
<td>25 (7)</td>
<td>456 (14)</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Molybdate (2 mM)</td>
<td>9687 (1305)</td>
<td>119 (29)</td>
<td>78 (8)</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

a Incubated for 24 days with 0.8 µCi of ¹⁴CH₃HgI under N₂
b Represents the mean of three samples.
c Percent conversion of ¹⁴CH₃HgI to ¹⁴CH₄ and ¹⁴CO₂.
(values in bracket are standard deviations)

The new findings that anaerobic demethylation in estuarine and freshwater sediments and aerobic demethylation in freshwater sediments produce CO₂ indicate a yet unknown oxidative pathway.

4. MERCURY TRANSFORMATION BY HIGHER MARINE ORGANISMS

The data on methylation in higher organisms are few. Pentreath (1976a, b) could not detect any organic radioactive Hg after plaice or the worm Neris were kept in sea water containing ²⁰³HgCl₂. Also brook trout could not methylate Hg(II) compounds, nor could their tissues or
organs (Huckabee et al., 1978). Also pure bacteria cultures isolated from the intestine of tuna did not methylate inorganic Hg (Pan-Hou and Imura, 1981) but some of these pure cultures, which had a higher Hg resistance, could demethylate MeHg.

But the indigenous microflora of isolated intestines of six fresh water fishes could methylate Hg under anaerobic conditions (Rudd et al., 1980). Pike and walleye intestine contents methylated a larger fraction of $^{203}\text{Hg}$ than those of white fish and suckers. Also in terrestrial higher organisms methylation was observed. The intestinal flora of rats, in the cecum and to lesser extent in the small intestine, can methylate HgCl$_2$ (Rowland et al., 1977b). Synthesis of the suspensions of gut content was inhibited by antibiotics or by filtration through membrane filters. Also pure cultures isolated from the gut content could methylate Hg. No methylation of inorganic Hg but demethylation of MeHg occurs through cow rumen microflora (Kozak and Forssberg, 1979). Most of the mercuric compounds passed through unchanged and only a small amount was reduced to Hg$^0$. These data show that higher organisms cannot methylate inorganic Hg but only microorganisms in the digestion system are able to produce MeHg.

The liver of marine mammals has been indicated as a site for demethylation because MeHg is present at low percentage of the total high concentrations, but experimental evidence is still missing. Also in some marine fish much lower MeHg percentages have been observed in the liver than in the muscle (Halim et al., 1986).

5. ABIOTIC AND ENZYMATIC METHYLATION AND DEMETHYLATION

For the methylation of mercury, a methyl donor is required. In the environment both natural and anthropogenic methyl donors occur. Several of these methyl donors are synthesized by organisms. Direct biological methylation (enzymatic methylation) requires the presence of an active metabolizing organism, while for indirect biological methylation (non-enzymatic methylation) and for abiotic methylation only a methyl donor is needed. Similarly, one can distinguish between the corresponding categories of demethylation. Herebelow, indirect biological and chemical reactions are discussed together.

5.1 Methylation

Several abiotic methylation mechanisms of Hg species have been reported. Photomethylation, transmethylation from metallic (trimethyl-Sn or tetramethyl-Pb, methylcobalamin, etc.) and non metallic methyl donors (e.g. humic and fulvic acids). Studies with enzymes serve as models for the intracellular and extracellular methylation by microorganisms.

5.1.1 Methylation by methylcobalamin

Methylcobalamin can be characterized as an octahedrally coordinated Co(III) species with a cobalt methyl group bond. Wood et al. (1968, 1972) had suggested this compound as a methyl donor because it can transfer methyl groups as a carbonion (CH$_3^-$), as carbonium ion (CH$_3^+$) or as radical (CH$_3^*$). Two other methylating coenzymes S-adenosylmethionine and 5-N-methyltetrahydrofolate derivatives have
been excluded by Wood et al., because they transfer carbonium ions. In their study Wood et al. (1968) found that after adding methylcobalamin to extracts of Methanobacterium omelianskii, methane is released. The overall reaction requires ATP and hydrogen as a source of electrons. R-Co-SCOH benzimidazolylycobamide is the prosthetic group. If, however, Hg^{++} is added the formation of methane is inhibited and MeHg is formed. Whether MeHg or dimethyl mercury (DiMeHg) is produced depends on the amount of Hg^{++} added. At low concentrations (0.1 µmol Hg^{++} and 5 µmol methylcobalamin) DiMeHg is formed. In fact spiking (tipping) HCl into the solution an additional formation of methane (Fig. 25) indicates that DiMeHg was produced. On the other hand the presence of high concentrations of Hg^{++}, MeHg is formed. Also Imura et al. (1971) found that if HgCl₂ solutions in phosphate buffer were equimolar or lower to methylcobalamin DiMeHg was formed. This could then be transformed into MeHg by adding further amounts of HgCl₂. Later, other authors confirmed these observations (Bertilson and Neujahr, 1971; DeSimone et al., 1973; Craig and Morton, 1978).

![Formation of methane by a methanogenic bacteria (Hg^{++}) and inhibition of methane formation by Hg^{++} (HgCl₂) and the liberation of methane after the addition of HCl (TIP HCl). Reaction contained: crude extracts 56 mg protein; 10 µmol of ATP; 500 µmol of potassium phosphate buffer, pH 7; 0.1 µmol Hg^{++} at "Hg^{2+}"; gas phase: H₂ (Wood et al., 1968)](image)

However, Wood et al. (1968) could also show that under reducing conditions (8 g of Zn dust in 150 mL of a 10 per cent w/v NH₄Cl) methylcobalamin and Hg^{++} would produce MeHg and DiMeHg without the
presence of a bacteria extract. This indicates that the sole addition of methylocobalamin is sufficient to product MeHg abiotically and in experiments in which methylocobalamin were added to cultures of microorganisms both biotic and abiotic methylation may have occur simultaneously. Complexing the HgCl₂ with different substances does not interfere with the production of MeHg. Also Craig et al. (1983) found that after incubating methylocobalamin and HgCl₂, uncomplexed and complexed with cysteine, penicillamine or methionine, MeHg is formed. De Simone et al. (1973) have studied the reactions between methylocobalamin and mercuy(II) diacetate Hg(OAc)₂. They proposed the reaction scheme shown in Figure 26. Since the different species have different UV-visible spectra, the reactions could be followed by spectrophotometry. Depending on the addition of Hg diacetate an equilibrium results between 'base-on' (species 1 in Figure 26) and 'base-off' (species 2 and 3) methylocobalamin. In the 'base-off' species the benzimidazole N is not longer bonded to the Co atom. Once species 2 or 3 are formed, an electrophilic displacement of CH₃⁻ will allow the formation of CH₃HgOAc.

![reaction scheme diagram](image)

Fig. 26 Proposed mechanisms for the mercuric acetate-methylocobalamin reaction (Thayer and Brinckman, 1982)

These findings suggest an electrophilic attack of the mercury on the methylocobalamin and the transfer of the methyl group as a carbanion.
Compeau and Bartha (1983) studied the influence of various factors on the formation of MeHg. Using the formation of aquacobalamin as an indicator for methylation, they found that while methyl transfer rates were very fast in acetate buffer (90% in 10 min), the presence of 0.1 M chloride slowed down the reaction considerably. Sulfide added (10, 50 and 100 ppm Na$_2$S) under anaerobic conditions prevented methylation of Hg completely. (Na$_2$S had no effect on the stability of MeHg nor on the stability of methylocobalamin.) Chloride depressed the formation of MeHg only slightly. Also Yamada and Yonemura (1972) have shown that no MeHg was produced from pure HgS by chemical reaction with methylocobalamin.

5.1.2 Methylation by soil components

Both humic and fulvic acids have the ability to methylate inorganic mercury. Nagase et al. (1982) investigated several factors which influence the Hg methylation by humic acids (HA): temperature, Hg concentration and HA concentration. If one attempts an extrapolation to natural environmental conditions, i.e. 20°C, 1 mg Hg L$^{-1}$ and 1 mg HA L$^{-1}$, one would obtain the following:

Starting from the influence of temperature (because, as can easily be verified, the starting conditions of the various experiments do not all give the same results) one obtains:

at 20°C 6 mg HA yield 2 µg MeHg L$^{-1}$ at a concentration of 750 mg Hg L$^{-1}$;

6 mg HA methylate 0.000 25% of the inorganic Hg present in 3 days;

1 mg HA L$^{-1}$ methylate 0.000 013% of the inorganic Hg per day; and

1 mg HA L$^{-1}$ methylates 0.004 5% of the inorganic Hg/year

This is a very small amount of MeHg indeed.

Similar results were obtained by Rogers (1976, 1977). 0.5 mg Hg(NO$_3$)$_2$/g soil were added to sand, loam and clay and the MeHg determined. Surprisingly, a comparison between autoclaved and non-autoclaved samples showed that the autoclaved samples had higher concentrations of MeHg than the non-autoclaved suggesting that a biological demethylation may have occurred in the unsterilised samples. Also, after three weeks, less MeHg was observed in the three soil types than after one week suggesting again a decomposition of MeHg. Increasing Hg(II) nitrate increased the amount of MeHg produced (Table 9). Calculating the yield of the data shows that only 0.0004 to 0.001% of the inorganic Hg added is converted to MeHg within a week. The higher additions of inorganic Hg converted somewhat higher amounts of MeHg. The highest amounts are produced by clay. Weber et al. (1985) studied the methylation of HgCl$_2$ and Hg(NO$_3$)$_2$ by soil derived fulvic acid. They found that Hg nitrate gave about six times the yield of Hg chloride and that at pH 4 about twice the yield of pH 6 was obtained. The maximum yield was 0.02% conversion of the Hg(II) added, in 200 h. Lee et al. (1985) investigated the influence of some metals on the methylation of mercury by fulvic acids. They found that Fe increases considerably the production of MeHg (Fig. 27). The highest yield (with Fe) was 0.00003% in 44 h.
Table 9

Methylmercury in soils incubated for one week with different amounts (mg Hg/50 g soil) of mercury(II) nitrate (Rogers, 1976).

<table>
<thead>
<tr>
<th>Soil</th>
<th>mg added</th>
<th>ng MeHg/50 g soil after one week</th>
</tr>
</thead>
<tbody>
<tr>
<td>sand</td>
<td>5</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>567</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>9825</td>
</tr>
<tr>
<td>loam</td>
<td>5</td>
<td>388</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>795</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>18812</td>
</tr>
<tr>
<td>clay</td>
<td>5</td>
<td>414</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1009</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25614</td>
</tr>
</tbody>
</table>

Fig. 27  Methymercury production in the presence of fulvic acid (180 mg L\(^{-1}\)) with and without the addition of Fe\(^{++}\), Fe\(^{+++}\), Cu\(^{++}\), Mn\(^{++}\) and Al\(^{+++}\) (9.4×10\(^{-5}\) mole L\(^{-1}\)) at various pH. Inorg. Hg: 20 mg L\(^{-1}\). Incubation 44 h (Lee et al., 1985)

5.1.3 Methylation by other methyl donors

Several authors have tested other methyl donors. Craig et al. (1983) detected qualitatively MeHg after incubating Hg(0) with CH\(_3\)I, however, no MeHg was produced when HgCl\(_2\) was used instead of Hg(0). Methyl iodide is produced by marine algae and its average concentration should be about 1 ng L\(^{-1}\), but it is much higher over kelp beds (Lovelock 1975). The methylation of SnS has been reported (Manders et al., 1984) as well as the formation of (CH\(_3\))\(_4\)Pb in the presence of methyl iodide. From these methylated metals transmethylation to Hg could occur. Jewett and Brinckman (1974) observed in aqueous solution of about 10\(^{-2}\)M for each reactant transmethylation from (CH\(_3\))\(_3\)Sn\(^+\) and
from \((\text{CH}_3)_3\text{Pb}^+\) to \(\text{Hg(II)}\) resulting in \(\text{CH}_3\text{Hg}^+\), but no transmethylation was obtained from \((\text{CH}_2)_2\text{Sn}^{+++}\), \(\text{CH}_3\text{TI}^+\) and \(\text{CH}_3\text{S}^+\) (below 100°C). The formation of MeHg from \((\text{CH}_3)_2\text{Sn}^+\) has a half-time of 40 min and from \((\text{CH}_3)_3\text{Pb}^+\) it is 10 min. Huey et al. (1974) produced evidence that a Sn- and Hg-tolerant strain of Pseudomonas isolated from Chesapeake Bay grown in the presence of both Hg and Sn would form MeHg. The authors suggested that the methylation of Sn is biologically mediated and the successive methylation of Hg is an abiotic transfer reaction from methyl Sn compounds. Imura et al. (1971) tested S-adenosylmethionine as a methyl donor under the same conditions used for methylation with methylcobalamin, but could not detect any MeHg. Also Reisinger et al. (1984) could not find any MeHg when Hg(II) was incubated with S-adenosylmethionine.

5.1.4 Photomethylation

Methyl mercury can be formed from Hg(II) salts and acetic acid photochemically with UV and visible light. Photomethylation using methanol, ethanol, acetic and propionic acids produced MeHg from mercuric chloride (Akagi et al., 1977). About 0.1% of the total HgCl₂ present was transformed into MeHg in 20 hours. Hayashi et al. (1977) also observed photomethylation of inorganic Hg when aliphatic amino acids were irradiated with UV light for 4 hours. The formation of MeHg was not related to the type of alkyl residues of the amino acids. Photolysis of glycine and phenylglycine did not yield alkyl-mercury compounds indicating that the formation of the MeHg was due to an apparent fragmentation of the alkyl residues of the amino acids during photolysis.

After an irradiation with a blacklight lamp (nominal emission region 310-420 nm) in the presence of acetic acid Akagi et al. (1977) could observe a solubilization of HgS and a consecutive methylation of Hg. The yield of MeHg was proportional to acetate concentration. Longer irradiation time increases the yield. Photosolubilization of HgS occurred also in water without acetic acid addition but was about one third slower (Fig. 28). No HgS solubilization nor Hg methylation was observed in the dark. In the presence of acetic acid and after 20 days of irradiation, about 100% of HgS present was methylated. Oxygen has a marked effect on the 'photomethylation' of HgS (Fig. 29). Since acetic acid is a natural metabolite (e.g. of sulphate-reducing bacteria), this reaction could occur in nature. However, chloride ions inhibit this methylation making it an unlikely process in marine and brackish environment.

5.2 Demethylation

Monomethyl mercury is chemically quite stable. After 42 hours after adding concentrated HCl, 93% of the MeHg present at the beginning of the experiment can be extracted. After 14 days 66% can be still recovered (Cerrati and Bernhard, pers. com.). The solution is, of course, sterile in concentrated HCl.

Enzymatic decomposition of MeHg can occur. E.g. Tonomura and Kanzaki (1969) showed that a cell-free extract of a Hg-resistant pseudomonad converts MeHg to Hg(0) and methane in the presence of NADPH and thioglycollate. Schottel (1978) described an organomercurial.
Fig. 28  Photo-solubilization and methylation of HgS in the presence of acetic acid (▲) photo-solubilization in water, (●) photo-solubilization in water in the presence of acidic acid, (○) photo-methylation in water + acetic acid. (Akagi et al., 1977)

Hydrolase isolated from Hg-resistant (1) E. coli which breaks the mercury-carbon bond. This enzyme is inducible by inorganic Hg and organic mercury compounds. A similar enzyme, that also came from a Hg-resistant strain (Pseudomonas), has been described by Tezuka and Tonomura (1978). Enzymes from Hg-sensitive strains seemed not to be studied. Recently Baldi and Filippelli (1991) have used a similar reaction for the determination of MeHg.

Photodegradation of MeHg, DiMeHg and methylmercury hydroxide is slow because of the low sunlight adsorption rate constants of these substances (Baughman et al., 1973). However, methylmercuric thiols (minimum photochemical half-life 46 to 120 h) and methylmercuric sulfide ion complexes (minimum photochemical half-life 0.4 h) are faster decomposed by sunlight.
Fig. 29 Atmospheric effect on the photo-methylation of HgS in aqueous solutions. Reaction system: 100 mg HgS + 5 mmol AcOH/100 mL H₂. (Akagi et al., 1977)

Under natural conditions MeHg will be present mainly as sulphur complexes which can be decomposed photochemically (Lexmond et al., 1976):

\[
\text{UV light} \\
\text{CH}_3\text{HgS}^- \quad \longrightarrow \quad \ast \text{CH}_3 + \text{HgS} \\
\text{CH}_3\text{HgSR} \quad \longrightarrow \quad \ast \text{CH}_3 + \ast \text{SR} + \text{Hg(0)}
\]

DiMeHg yields two MeHg when exposed to Hg^{++} at low pH:

\[
(\text{CH}_3)_2\text{Hg} \quad \longrightarrow \quad 2 \text{CH}_3\text{Hg}^+
\]

DiMeHg is photolysed in the atmosphere:

\[
\text{UV light} \\
(\text{CH}_3)_2\text{Hg} \quad \longrightarrow \quad 2\text{CH}_3 + \text{Hg(0)}
\]

Demethylation of MeHg when exposed to UV has also reported in this workshop (Renzoni et al., this workshop). In aqueous environment the action of UV is, of course, limited to surface layer of the water body.
Demethylation of MeHg also can occur by transmethylation because MeHg can be a methylator for other metal ions. It can methylate NaAuCl₄, Na₂PdCl₄ and K₂PtCl₄, but not (CH₃)₂SnCl₂ (Jewett and Brinkman, 1974). But these are reactions which have little significance in the environment.

6. TRUE IN SITU EXPERIMENTS ON HG TRANSFORMATIONS

Of great interest are the in situ experiments of Bothner et al. (1980). These authors placed a bell jar in a contaminated site (station 3A) and another in a relatively uncontaminated site (station 3) on the sediment surface in Bellingham Bay (Northern Puget Sound, Washington). Station 3A was situated about 100 m from the outfall of a chlor-alkali plant and station 3 at 700 m. The area of station 3A also received wastes from a sewage outfall and from a pulp and cardboard mill. At station 3 the conditions in the sediment were aerobic down to circa 20 cm. At station 3A the sediments were anaerobic, but the water circulation above the sediment surface maintained oxidizing conditions. The Hg concentrations in the sediment and the interstitial water of the station 3A were much higher than those of the uncontaminated station 3. In the experiments Hg-free air was passed through the bell jars. The different (operationally defined) forms of volatile Hg in the passing air stream and dissolved Hg in the water above the sediment were determined. In order to obtain a blank and to block the mercury evolving from the sediment, a glass plate was placed under the bell jar to isolate the water contained in the bell jar from the sediment. In both stations no volatile Hg deriving from the sediment could be detected since "blank" and "sample" gave statistically equal results. In both conditions, about 1 ng Hg h⁻¹ was carried with the air stream into the Hg traps, therefore, it is clear that the Hg was stripped from the water and did not originate in the sediments. However, determination of dissolved Hg in the sea water contained in the bell jar of station 3A showed a marked increase over the "blank". The flux from the sediment to the water above the sediment was not measurable at station 3 probably because of the small concentration difference between the Hg in the interstitial water (0.03 μg L⁻¹) and in the overlying water (0.01 μg L⁻¹). It is interesting to note that in the blank condition and when the jar was placed directly on the sediment 50 to 75% of the volatile Hg was Hg(0) and that the increase in the dissolved Hg in the bell jar at station 3A strangely had no measurable effect on the amount of volatile Hg produced, although the concentration of soluble Hg had increased from 30 to 120 ng Hg L⁻¹. From these data the authors estimated a flux of 600 ng cm⁻² year⁻¹ from the sediments to the sea water above. If one assumes a concentration of about 40 μg Hg g⁻¹ sediment then the sediment should contain about 70 μg Hg cm⁻³ (fresh weight = 0.7 dry weight; specific gravity 2.5). That means that during a year 0.8% of the Hg in the first cm of the sediment was lost to the water as soluble Hg. A second experiment, in which unfortunately the flux of volatile Hg was not determined but one bell jar was kept under oxygen limitation, showed that the concentration of dissolved Hg in sea water above the sediment increased more in the oxygen-limited conditions than in the previous ogenated arrangement. No MeHg was detected.
7. AMOUNT OF METHYL MERCURY NECESSARY TO CONTAMINATE MARINE BIOTA

The data discussed above show that only very small amounts of MeHg are produced by bacteria and abiotically. It would, therefore, be interesting to calculate how much MeHg L\(^{-1}\) is necessary to contaminate marine biota at the levels observed. Phytoplankton, the largest biomass contains about 20% MeHg of the Hg-T. Following a model calculation carried out by Topping and Windom (1981) on the amount of total mercury incorporated by phytoplankton, a similar calculation may give an idea on the amount of methyl mercury to be removed from sea water to allow phytoplankton an accumulation of 20% MeHg of Hg-T.

(a) Assuming a phytoplankton primary production of 50 g C m\(^{-2}\) year\(^{-1}\), which is about equal to a production of 100 g DW m\(^{-2}\) year\(^{-1}\) in an euphotic zone of 100 m depth, 100 g DW of phytoplankton are produced in 100 m\(^3\) of sea water. This equals a phytoplankton production of 1 mg DW litre\(^{-1}\) year\(^{-1}\).

(b) Plankton caught with small pore size plankton nets (mainly phytoplankton) contains about 100 ng Hg-T g\(^{-1}\) DW (Bernhard, 1988). Hence 1 mg DW plankton biomass accumulates about 0.1 ng Hg-T from one litre of sea water a year.

(c) Assuming that 20% of the Hg-T is MeHg then phytoplankton should accumulate 0.05 ng of MeHg litre\(^{-1}\) year\(^{-1}\) or 0.0001 ng MeHg litre\(^{-1}\) day\(^{-1}\).

For comparison, recent Hg-T concentrations in sea water in the Mediterranean areas range from about 1 to 10 ng Hg-T/litre. Certainly these 0.05 ng MeHg/liter withdrawn is an amount too small to be detectable in a field experiment with conventional techniques. The uptake of MeHg by organisms of higher trophic levels is negligible, although they reach higher Hg concentrations in their tissues than in phytoplankton because the biomass of higher trophic levels is much smaller - 10% for every increase in trophic level - than that of phytoplankton.

8. CONCLUSIONS

One of the first requisites for an investigation on the origin of MeHg is that this compound is securely identified. The unequivocal determination of MeHg is not easy. Three methods exist for a direct identification and determination of MeHg. However, these methods are not sensitive enough for the detection of the small amounts of MeHg usually found in environmental samples. On the other hand the much more sensitive indirect methods used until very recently were not MeHg specific. Hence, the reviewers could not always be certain that the MeHg reported in the experiments was really MeHg.

Methylation has been observed in various environments i.e. in the water column and sediments of freshwater bodies and in estuarine sediments. Direct evidence for methylation in the marine water column has not been reported. Single strains which are able to methylate Hg have been isolated from freshwater and estuarine sediments. With the exception of the experiments carried out with radiotracers high amounts of inorganic Hg had been added in these in vitro experiments. These
high Hg concentrations are selective for Hg-resistant microorganisms. Experimental conditions using high Hg concentrations are justified if one takes into consideration the concern about environmental Hg pollution raised by the Minamata accident and pollution by industrial point sources. The environments adjacent to these point sources are characterized by high concentrations of inorganic Hg and this, besides the ease of experimentation with high concentrations, has conditioned the experimental design of methylation and demethylation experiments. Recent concern, however, is oriented towards environments with much lower Hg concentrations. These are areas in and near natural Hg sources (geochemical anomalies), acidic lakes remote from Hg contaminating point sources and the soil of countries in northern Europe. The Hg from the geochemical anomalies cause high MeHg concentrations in marine biota of large areas of the Mediterranean and probably also in other areas. Also in Swedish lakes increased amounts of MeHg in fish are found. This raises the question: what can the methylation experiments carried out with high Hg concentrations tell us about the production of MeHg under conditions which are characterized by much lower Hg concentrations relevant for these recent concerns?

Several problems exist in extrapolating these experiences to natural environments. All authors worked either with Hg-resistant strains or under conditions selective for Hg-resistant strains.

As has been shown Hg-resistant strains possess enzyme systems induced by high Hg concentrations which enables them to carry out reactions to which the non-resistant strains, are most probably, not able.

The few data available show that the Hg-resistant strains are not the most frequent strains and, as to be expected, may not occur at all in uncontaminated or slightly contaminated environments.

On the other hand, a high percentage of total Hg in organisms is MeHg whether these come from uncontaminated or slightly contaminated sites, MeHg must also be produced without the mediation of Hg-resistant bacteria. However, to our knowledge methylation of Hg by Hg-sensitive bacteria strain has not been reported. Therefore, despite the fact that strains from a wide range of microorganisms (aerobes, obligate and facultative anaerobes) have been shown capable of in vitro methylation of Hg²⁺, but at present is it not certain if these same organisms are also those which are responsible for the methylation of Hg in vivo in uncontaminated environments or environments which do not induce Hg-resistance.

Also recent experiments on estuarine sediments with selective inhibitors for methanogens and sulphate-reducers cannot resolve the question. These experiments were carried out with high additions of inorganic Hg and in order to assess their environmental significance it would be interesting to repeat these experiments with low-level radioisotope techniques.

The kinetics of the biological methylation experiments present another problem. In nearly all experiments - on single species, water and sediments - the increase in MeHg concentration is greater at the beginning of the experiment. After some time the MeHg concentration reaches a maximum and later it diminishes. A successive addition of
inorganic Hg can stimulate a renewed increase of MeHg production although only very small amounts of the Hg initially present has been converted to MeHg. This may be due to changes in the availability of the inorganic Hg, although it has also been shown that complexed Hg can be methylated. A source of MeHg in the environment, on the other hand, is expected to produce MeHg more or less continuously with time. It seems that the manipulations necessary to set-up the experiments induce this pattern of MeHg production as has been shown for anaerobic sediments. It may also be possible that the initial formation of MeHg stimulates biological demethylation resulting in a decrease in the net production of MeHg and hence in the MeHg concentration. The determination of potential methylation and demethylation in the north American lakes has shown that the potential demethylation can be greater than the potential methylation. Unfortunately, not all authors investigated the dynamics of the distribution of the Hg chemical species during the experiments. In any case, this initial increase of MeHg concentration followed by a decrease seems to be an artefact.

The only Hg methylation experiments carried out with low Hg concentrations were conducted with radioisotopes on the water column and sediments of North American lakes. The results obtained show that methylation and demethylation occurs in the water column and in sediments of freshwater ecosystem. The "kinetic effect" was observed leaving some doubt about its importance in natural systems. Also in many cases, potential demethylation was higher than potential methylation raising the question of how MeHg can exist in the system in significant amounts. Similarly, it seems that more bacteria species are able to demethylate than to methylate. MeHg found in natural systems must somehow be "protected" from demethylating processes in order to exist. In biota higher than microorganisms, methylation does not occur and demethylation is a very slow process, but very little is known about MeHg in/on abiotic matrices.

Many parameters of methylation and demethylation by single species and different chemically undefined matrices have been carried out. Increasing salinity inhibits methylation. Higher concentrations of inorganic Hg increase the production of MeHg but decrease the percentage of of MeHg produced.

Many authors have compared aerobic with anaerobic methylation and it seems that no general pattern exists which favors the production of MeHg under aerobic or anaerobic conditions. As long as the bacteria or the processes in unspecific matrices responsible for methylation and demethylation have not been identified in each case, it is impossible to predict under which conditions MeHg will be produced.

Less information is available on abiotic methylation. Methylation with methylcobalamin has been given considerable attention, but there seem to be no consensus about the significance of this substance in actual environmental context. Methylation by fulvic and humic acids seem to be more likely to occur in the environment. If photomethylation should prove to be important it will certainly be limited to the upper layer of water bodies.
Very small amounts of MeHg are produced in the biotic and abiotic methylation experiments. Nevertheless, these amounts are sufficient to explain the high concentrations found in aquatic biota, because their biomass is so small and biota accumulates inorganic and especially methyl mercury very efficiently.

Taking into consideration that microbial mediated methylation of Hg has not been demonstrated for uncontaminated or low level contaminated environments, that the dominant form of mercury in the biota is MeHg, abiotic processes may play a greater role than previously thought.

9. REFERENCES


THE IMPACT OF SIZE ON THE BIOACCUMULATION RATE OF HEAVY METALS AND PAHS BY Mytilus galloprovincialis FROM SARONIKOS GULF

by

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ABSTRACT

Different size mussel samples from Saronikos Gulf were analysed for heavy metals and polyaromatic hydrocarbons (PAHs). The observed values were statistically treated by applying two-way analysis of variance and least significant range test. The results of this treatment showed that for Cu, Cr and Ni a negative correlation between length and body content of mussels exists. On the contrary, for PAHs the correlation was positive while in the case of Cd a clear relationship was not observed.

1. INTRODUCTION

Mussels, as well as other bivalve molluscs have become widely used as indicators of many pollutant levels in coastal waters. Among these pollutants polyaromatic hydrocarbons (PAHs) and heavy metals (HMs) are known to pose threats to public health or integrity of ecosystems. It is yet an absolute need to know, beyond the estimation of pollution levels, the distribution and biochemistry of these pollutants in coastal ecosystems if we wish to assess the magnitude and location of any threats in living organisms.

As far as the concentration of heavy metals is concerned, it was pointed out since the early 1970's (Pedersen et al., 1973 ; Romeril, 1974) that the size of shellfish has a general influence on their heavy metal content. A difference was also observed between the heavy metal content of young individuals and that of older ones even though both lived under the same environmental conditions. In addition, for the PAHs, preliminary results showed no consistent trend of the relation between shellfish body concentrations and their size (Burns and Smith, 1978).

This work, undertaken since 1988 in the framework of the MED POL programme in Greece has as its principal aim the elucidation of how the body concentrations in PAHs and heavy metals depend on mussel length.
2. METHODOLOGY

For this study a sampling station (C3) located in the inner part of Saronikos Gulf was used (Fig. 1). Generally, Saronikos Gulf is considered as one of the most polluted gulfs in Greece as it receives the untreated domestic wastes of the Athens metropolitan area, about 600 000 m³ d⁻¹ y⁻¹ (Leondiadis, pers. com.), the wastes of the biggest Greek refinery as well as the wastes of all industries located along its North West coast (Elefsis bay) where sampling station C3 is also found.

Fig. 1 Map of Saronikos Gulf showing sampling location C3

Mussel samples were collected by scuba diving from 0.5-2.0 m depth, in June 1988 which is a low reproduction period (Valli, 1971), and split into four groups according to the following length ranges: 2.0-2.5 cm, 4.0-4.45 cm, 6.0-6.5 cm and 7.0-9.0 cm.

After byssus removal, mussel samples were washed out with distilled water to remove any traces of impurity or sediment in order to avoid analytical errors (Bernhard, 1976). Three to nine homogenates
consisting of six individuals each were prepared. All samples were transported to the laboratory deep frozen (-10°C) until lyophilisation.

For heavy metal analysis, 0.5 g of dried soft tissue were digested with 5 ml of nitric acid into appropriate vessels under pressure at 120°C for 12 hours (UNEP/FAO/IAEA, 1982). The analysis of heavy metals was performed by Atomic Absorption spectroscopy with a Varian AA 157.

The concentration of PAHs was determined by UV-fluorescence spectroscopy (UNESCO, 1982), after KOH-MeOH saponification and clean-up using column chromatography.

3. RESULTS

Table 1 summarizes the measured concentrations of Cu, Cr (total), Ni and Cd as well as of PAHs in bivalve samples expressed in μg of metal per g of dry tissue (ppm). The total number of samples analysed for heavy metals was 26 and for PAHs 12.

<table>
<thead>
<tr>
<th>contaminant</th>
<th>Length (cm)</th>
<th>Cu</th>
<th>Cr</th>
<th>Cd</th>
<th>Ni</th>
<th>PAHs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0-2.5</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Avg.</td>
<td>7.33</td>
<td>4.30</td>
<td>0.47</td>
<td>7.49</td>
<td>56.45</td>
</tr>
<tr>
<td></td>
<td>Std.</td>
<td>0.42</td>
<td>1.53</td>
<td>0.49</td>
<td>0.73</td>
<td>10.50</td>
</tr>
<tr>
<td></td>
<td>Min.</td>
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<td>1.67</td>
<td>0.06</td>
<td>6.37</td>
<td>39.00</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>7.90</td>
<td>6.11</td>
<td>1.19</td>
<td>8.65</td>
<td>65.20</td>
</tr>
<tr>
<td></td>
<td>4.0-4.5</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Avg.</td>
<td>6.86</td>
<td>3.34</td>
<td>0.50</td>
<td>6.32</td>
<td>74.75</td>
</tr>
<tr>
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<td>Std.</td>
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<td>1.95</td>
<td>0.24</td>
<td>2.19</td>
<td>30.03</td>
</tr>
<tr>
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<td>Min.</td>
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<td>1.28</td>
<td>0.30</td>
<td>3.36</td>
<td>72.90</td>
</tr>
<tr>
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<td>Max.</td>
<td>10.22</td>
<td>7.28</td>
<td>1.00</td>
<td>11.15</td>
<td>80.00</td>
</tr>
<tr>
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<td>7</td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
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<td>Avg.</td>
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<td>1.00</td>
<td>5.89</td>
<td>83.73</td>
</tr>
<tr>
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<td>2.82</td>
<td>1.05</td>
<td>4.31</td>
<td>3.76</td>
</tr>
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<td>1.21</td>
<td>0.05</td>
<td>3.02</td>
<td>80.20</td>
</tr>
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<td>7.0-9.0</td>
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<td>5</td>
<td>5</td>
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<td>-</td>
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<td>1.60</td>
<td>0.55</td>
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<td>0.06</td>
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<td>Max.</td>
<td>6.11</td>
<td>2.56</td>
<td>0.97</td>
<td>4.53</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1

Mean concentrations of heavy metals and PAHs in different length ranges of mussels in μg g⁻¹ (dry weight) (St. C3, June '88).
The data of Table 1 clearly show a gradual decrease of Cu, Cr and Ni concentrations in relation to the length of bivalves but this is not true for PAHs. In the case of PAH concentrations, an increase with the bivalve size is observed. As far as the Cd concentration is concerned, it is rather difficult to detect any trend with bivalve length.

So, in order to investigate any existing relationship between the bivalves’ size and the concentration of the contaminants under study, the results were treated statistically applying single regression analysis. Since concentrations of heavy metals do not follow the normal distribution, they were transformed into the log form before applying single regression analysis.

The results of this statistical analysis are summarised in Table 2. In addition, Figs. 2 to 6 show graphically the correlation of each contaminant with length. This correlation in the case of Cu, Cr and Ni, is negative i.e. the concentration in the mussels’ body decreases as their length increases. On the other hand, even though the significance level for the existence of these correlations is lower than 5%, only in the case of Cu a considerable percentage of the results is described by the correlation equation. In the case of Ni only 35% of the results are described by the corresponding equation. The correlation between the length of the mussels and the concentration of Cd and PAHs is positive. Statistically significant results were, however, only observed for PAHs, where the relevant equation describes the 70.71% of the data.

Table 2

Statistical analysis of concentrations of heavy metals and PAHs in mussels (R= correlation coefficient, R²= coefficient of determination, F= F ratio, P=significance level).

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>R²%</th>
<th>F</th>
<th>P</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>-0.780</td>
<td>60.81</td>
<td>34.14</td>
<td>&lt;0.001</td>
<td>Linear</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>y=a+bx</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a=1.121</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b=-0.064</td>
</tr>
<tr>
<td>Cr</td>
<td>-0.408</td>
<td>16.64</td>
<td>4.79</td>
<td>0.039</td>
<td>Exponential</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>y=e^(a+bx)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a=-0.103</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b=0.102</td>
</tr>
<tr>
<td>Cd</td>
<td>+0.148</td>
<td>2.20</td>
<td>0.52</td>
<td>0.479</td>
<td>Linear</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>y=a+bx</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a=0.137</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b=0.010</td>
</tr>
<tr>
<td>Ni</td>
<td>-0.589</td>
<td>34.70</td>
<td>12.75</td>
<td>0.002</td>
<td>Reciprocal</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>1/y=a+bx</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a=0.923</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b=0.067</td>
</tr>
<tr>
<td>PAHs</td>
<td>+0.841</td>
<td>70.71</td>
<td>24.15</td>
<td>&lt;0.001</td>
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<td></td>
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<td></td>
<td>b=6.154</td>
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</tbody>
</table>
Fig. 2 Regression of Cu on length

Fig. 3 Regression of Ni on length
Fig. 4  Regression of Cr on length

Fig. 5  Regression of Cd on length
4. DISCUSSION

The influence of biological factors e.g. the size, on the bioaccumulation have been several times discussed as it constitutes the conceptual basis of any biological monitoring. It is self understood that a knowledge of all factors governing the bioaccumulation process is absolutely necessary in order to gain valuable information on relative distribution of PAHs and heavy metals in the water column or to estimate sources and types of contaminants. Also, the existing relationship between bivalves age and bioaccumulation is dependent on the nature of the metal and the organism under study (Boyden, 1977).

It is apparent from the data of Table 2 that, in the majority of the cases e.g. Cu, Cr and Ni, the existing relationship is negative. This means that the young individuals present a greater retention in heavy metals than the older ones. In fact, at least for Cu and Ni, this negative relationship has also been mentioned by other authors (Bryan, 1976; Harris et al., 1979; Boyden, 1977). This phenomenon can be related to the fact that young individuals are probably more vulnerable or they have a more intensive metabolism. On the other hand, as a consequence of the observed high dispersion of Ni, and particularly of Cr, bioaccumulation values, only a small percentage of the results are described by the established correlation equation. This is probably due to the fact that, especially in the case of Cu, slopes relating metal body content to body weight may be suppressed in a grossly contaminated environment (Boyden, 1977).
Regarding Cd concentration, no clear relationship has been established between bioaccumulation and mussels' length. The observed values present high dispersion without a defined pattern (see Fig. 5). Similar contradictory situation is also found in the literature where several authors support the view that such a correlation does not exist (Boyden, 1977), although other authors proposed a positive one (Peden et al., 1973; Ayling, 1974; Muller and Prosi, 1978; Harris et al., 1979). According to the literature, this is also true for PAHs which have no consistent trend, but our results show a positive correlation (Fig. 6), probably because the effect of PAHs bioaccumulation increases with size.

In conclusion, young individuals had higher bioaccumulation levels of Cu, Cr and Ni than the older ones, probably because they are more vulnerable or they have a more intensive metabolism; while for PAHs the opposite holds true probably because of the additive character of the effect with age. On the other hand, a definite pattern for Cd does not exist.

The above phenomenon must be considered when mussel samples are collected for monitoring purposes. The simplest solution consists of collecting always the same size of mussels; when it is not possible, conclusions could be based on statistical comparison of regression analysis and especially the intercepts.

5. REFERENCES


FATE AND AVAILABILITY OF TRACE METAL POLLUTANTS IN THE
MUSSEL Mytilus sp. UPTAKE AND DISTRIBUTION AT THE
CELLULAR AND SUBCELLULAR LEVELS

by

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ABSTRACT

Investigations were performed on the Mediterranean mussel Mytilus
sp., using secondary ion mass spectrometry (ion microscope and ion
microprobe) associated with photon microscope and X-ray spectrometry
(electron microprobe) associated with transmission electron microscope.
Uptake and distribution of stable chromium 52Cr and of radioactive
uranium 238U were investigated. The target organelles of Cr and U
accumulation were shown to be spherocrystals and lysosomes where the
metals were associated with phosphorus and trapped in an insoluble
form. Both metals were also detected from microgranules non membrane-
limited, incorporated into the byssal threads. Cr alone, was detected
in microgranules non membrane-limited, incorporated into the muscles.
The metabolism kinetics of Cr and U were compared.

1. INTRODUCTION

Many environmental chemicals may be accumulated by marine
organisms and thereby have a toxic effect which varies depending on the
concentration in which that element occurs and on its location in the
organism.

Organisms continue to survive despite their polluted environment,
due to the fact that they can tolerate the toxicity of these elements,
not only at the low concentrations which occur naturally in seawater,
but also at the higher concentrations which organisms inevitably
accumulate in their tissues.

The filter feeding bivalves such as the mussel Mytilus sp., which
lives in the Mediterranean, are of particular interest as they are well
known for their ability to reflect environmental composition of trace
metal contaminants in marine ecosystems (Bryan, 1980).

The physiological and biochemical strategies for uptake, storage
and elimination of toxic metals differ widely. Various mechanisms are
involved in these processes depending on the elements and the organism.

In order to obtain a better insight of these events, conventional
methods such as those commonly used for determining metal
concentrations in tissues are inadequate: absorbed and adsorbed
elements cannot be distinguished. Moreover, large quantities of
biological materials are needed and investigations are time consuming.
The available microanalytical techniques allow faster and better progress to determine elemental composition of marine organisms. One of their advantage is their high sensitivity.

2. MICROANALYTICAL TECHNIQUES

2.1 Secondary ion mass spectrometry

In Secondary ion mass spectrometry (SIMS), a primary ion beam is focused on the specimen surface, resulting in the production of positive and secondary negative ions from the surface layer of the tissue section. The secondary ions are extracted from the specimen and analyzed in a mass spectrometer. Based on SIMS, the analytical ion microscope was invented by Castaing and Slodzian (1962). The first biological applications were developed by Galle (1982a). The observer using the analytical ion microscope can visualize and focus on an area in a histological section of the tissue, analyze its chemical contents and photograph the microscopical distribution of any element in that area of the tissue.

There are two types of SIMS instruments. The first type is the analytical ion microscope which has a large beam to bombard an area 30-400 μm in diameter. The image of the distribution of the secondary ions is obtained with appropriate ion optics.

The second type of instrument is the ion microprobe, which scans the surface of the tissue with an ion beam measuring less than 2 μm in diameter to form an image of the tissue examined. The distribution of an ion is visualized by calibrating the instrument to accept only pulses of the particular ion chosen.

SIMS is used for the following purposes:

(i) the detection of most of the stable or radioactive isotopes of every element, with very high sensitivity of about 0.1 ppm (0.1 μg g⁻¹, D.W. tissue);

(ii) visualisation and photographic recording of the distribution of these isotopes in an area 20-250 μm in diameter with a lateral resolution of about 0.5 - 1 μm;

In association with SIMS, a processing of secondary ion microscope images has been developed by Cavellier et al. (1989). The ion images are obtained at the price of progressive erosion of the specimen by the incident ions, so that the analysis cannot be replayed and it is necessary to record the maximum amount of information during specimen erosion. This method uses a highly sensitive camera connected to a video analog-digital converter.

2.2 Electron probe X-ray microanalysis

Electron microprobe (EMP) microanalysis (Castaing, 1951) provides means for studying the local composition and structure of heterogeneous materials such as biological specimens. It does this by irradiating the sample with an electron beam focused to about 1 μm in diameter at
the surface of the sample and by measuring the characteristic X-rays emerging from the irradiated area. These X-rays allow qualitative and semi-quantitative analysis of chemical composition of precipitates or local deposits. EMP can be used in association with a scanning electron microscope or with a transmission electron microscope allowing the detection of elements at the subcellular level.

A comparison of SIMS with other microlocalization methods leads to the following conclusions. The sensitivity of detection of the SIMS is generally the greatest, compared to the other microanalytical techniques. The smallest concentration of an element that can be detected with EMP in a biological specimen ranges between 100 to 1000 ppm for most elements, whereas SIMS can detect concentrations of the order of about 0.1 ppm, D.W. tissue.

All the elements of the periodic table can be detected by SIMS whereas EMP allows detection of elements with an atomic number higher than 4. On the other hand, it is possible to visualise the ultrastructure of the specimen with EMP but not with SIMS.

Concerning the intracellular localization of radionuclides, SIMS is a method which is particularly well adapted (Chassard-Bouchaud et al., 1984). This may appear to be paradoxical in view of the fact that these radioactive elements would be expected to be easily localized by means of autoradiographic methods, which are reputed to be very sensitive. In reality, apart from the fact that microautoradiography is unable to determine with which chemical element (P, S, Ca...) these nuclides precipitate locally, the long half-lives of these elements often make this method relatively insensitive. Moreover, several months of exposure are generally required in order to have a chance of obtaining a detectable alpha desintegration. With SIMS, only a few seconds or minutes are required to obtain the image of distribution of a radionuclide.

3. SAMPLE PREPARATION

3.1 SIMS microanalysis

For SIMS, tissues were fixed chemically in Carnoy's fixative (Carnoy (1886) in Langeron (1949)), embedded in paraffin, sectioned (5 μm) and deposited on a highly pure gold specimen holder, with removal of paraffin. Semi-thin sections (2 μm) obtained from glutaraldehyde fixed samples, were also used for this technique. Alternatively, cryofixation was performed with isopropanol and then liquid nitrogen, and sections were deposited on the gold specimen holder.

3.2 EMP microanalysis

For X-ray spectrometry, samples were fixed in 2.5% glutaraldehyde in 0.2 M sodium dimethylarsenolate buffer at pH 7.0. After washing with the buffer, the samples were dehydrated and embedded in Epon. Ultrathin sections (60nm) were then deposited on copper grids and coated with carbon.

Grids to be observed by electron microscopy were stained (uranyl acetate and lead citrate) while grids to be examined with the electron microprobe were unstained.
4. RESULTS

We were interested in investigating the following elements: stable chromium and radioactive uranium. The reasons are that these elements are highly toxic and, in addition, they have not been the subject of microanalytical investigations at the structural and ultrastructural levels. The available data on Cr concentration in marine organisms shows large variability, particularly in M. edulis; measurements on whole soft tissues range between 0.3 and 49 \( \mu g \) g\(^{-1}\) D.W. (Karbe et al., 1977, Lande, 1977). Metabolism and toxicity of uranium are well documented for man (Galle, 1982b) while data is lacking concerning marine invertebrates.

Worldwide inventories of industrial discharges of Cr into the aquatic ecosystem show that anthropogenic input of Cr reaches a median value of about 142,000 tons per year in comparison to the 138,000 tons per year for lead (Nriagu and Pacyna, 1988).

Cr salts are active at low concentrations and are toxic to fish at a 0.1 \( \mu g \) g\(^{-1}\) dose. Thus investigations on these metals require specially equipped laboratories with very sensitive analytical instrumentation such as ion microscope and microprobe and electron microprobe.

On the other hand, studies of radionuclides in marine organisms have been carried out by many radioecologists because of their increasing significance for evaluating the impacts of radioactive substances released from nuclear facilities into the marine environment.

Species caught for food in surface waters may be contaminated through food chains, originating on the sea floor or at the sea surface and leading to man. It can be assumed that toxic elements concentrated in the sediment are transferred to detritivorous species, which are then eaten by crustaceans and finally ingested by man. In the same manner, mussels which are suspension feeders may be contaminated by toxicants and then finally ingested by man.

Chromium and uranium uptake by the mussel, occurs via the gills (Fig. 1) where high levels of metal were detected. Moreover, uranium uptake also occurs via the mantle and the digestive tract (Fig. 2 and 3). The main organ for uranium concentration is the digestive gland (Fig. 2), but for chromium, very low levels of this metal were detected in this gland. The kidney which had the highest chromium concentration may be considered the prime determinant site of chromium bioaccumulation; it seems that the kidney, saturated with chromium, was unable to maintain its excretion function and was adopting a storage function. Uranium excretion was not performed by the kidney.

In the organs quoted above, we were able to demonstrate that lysosomes of the digestive gland (Fig. 4), labial palps (Fig. 5 and 6), kidney (Fig. 7) and spherocysts of the kidney (Fig. 8) were the target organelles of chromium and uranium concentration. In both of these organelles chromium and uranium (Table 1) were concentrated in an insoluble form and associated with phosphorus; Cr and U phosphates were the result of acid phosphatase activity.
Fig. 1  *Mytilus sp.* U-exposed gill. Ion microscope micrograph of a semithin cross section of filaments. (A) 40 Ca+ image showing topography of the section; X 400. (B) 238 U+ image obtained from the same area as (A) showing the high U emission from bright points which correspond to lysosomes (arrows); X 400.

Fig. 2  *Mytilus sp.* U-exposed digestive gland. Ion microscope micrograph of a semithin section. (A) 40 Ca+ image showing topography of the digestive diverticula (d); X 400. (B) 238 U+ image obtained from the same area as (A) showing the high U emission from bright points which correspond to lysosomes (arrows); X 400.

Fig. 3  *Mytilus sp.* U-exposed digestive tractus. Ion microscope micrograph of a semithin section. (A) 40 Ca+ image showing topography of the section, l: lumen; X 400. (B) 238 U+ image obtained from the same area as (A) showing the high U emission from the absorptive border (arrows) and from the lumen; X 400.
Fig. 4  *Mytilus sp.* U-exposed digestive gland. Electron micrograph of an epithelial cell with a lysosome (L) containing dense microgranules and microneedles of U associated with P; (non osmicated and unstained material). Inset: X-ray emission spectra of P (Kα line) and U (Mα line) obtained from this lysosome; X 40,000.

The muscle is an important chromium storage tissue where the metal appeared as dense precipitates, non membrane-bound. Chromium and uranium were also detected from microgranules non membrane-bound, incorporated into the byssal threads (Fig. 9).

Chromium uptake by *Mytilus sp.* occurs via the gills. Contaminated water is then conveyed towards the labial palps (Fig. 5 and 6) before entering the digestive system. The main storage tissue appears to be muscle, while the digestive gland plays a very minor role in the Cr concentration. Excretion is chiefly performed by the kidney. Fig. 10 summarizes the tissue distribution of chromium.
Fig. 5  *Mytilus* sp. Cr-exposed labial palp. Ion microprobe micrographs of semithin section of filament. (A) 26 CN⁻ image showing the topography of the section: epithelial cells (ec) with cilia (c) and nuclei (n); X 2000. (B) 52 Cr⁺ image obtained from the same area as (A) showing Cr emission from small points corresponding to lysosomes (l) located along the border of the epithelial cells (ec); X 2000
Fig. 6  *Mytilus* sp. Cr-exposed labial palp. Electron micrograph of filament. Epithelial cells (ec) with cilia (c) and lysosomes (l) located along the cell border and containing Cr associated with P; X 20,000

**Table 1**

*Mytilus* sp. Electron probe X ray microanalysis. Element contents of lysosomes and spherocrystals (100 sec. counting). Means from measurements on 20 organelles from 10 individuals.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Operating conditions</th>
<th>Organelles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crystal</td>
<td>Line</td>
</tr>
<tr>
<td>uranium</td>
<td>PET</td>
<td>Mz1</td>
</tr>
<tr>
<td>phosphorus</td>
<td>TAP</td>
<td>Kz1</td>
</tr>
</tbody>
</table>
Fig. 7  *Mytilus sp.* Cr-exposed kidney. Electron micrograph of epithelial cells (ec) with microvilli (m) and lysosomes (l) containing microprecipitates of Cr associated with P. Inset: X-ray emission spectra of Cr and P (Kaline) obtained from the lysosomes; X 40,000
Fig. 8 Mytilus sp. Cr-exposed kidney. Electron micrograph showing spherocrystals (s) containing Cr associated with P and which have been extruded out of the cell. Inset: X-ray emission spectra of Cr and P (Kaline) obtained from these spherocrystals; X 54000.

Uranium uptake occurs via the gills. Contaminated water is then conveyed towards the labial palps before entering the digestive system where the digestive gland appears to be the main storage organ. Excretion is performed by the kidney. Byssal threads are also involved in chromium and uranium storage.

The macrophage amoebocytes which are distributed throughout the vascular system of the mussel are also found within the tissues. The amoebocytes are phagocytic; they play an important role in directly ingesting particles of toxic metals which are then engulfed to form heterophagolysosomes (Fig. 11 and 12).
Fig. 9  *Mytilus* sp. Cr-exposed byssal thread. Electron micrograph showing dense granules (arrows) of Cr associated with F; X 16,400

5. DISCUSSION AND CONCLUSIONS

As regards tissue and cell chemical structure, complementary results were obtained using several microanalytical methods. With SIMS we were able to obtain structural ion images with a very high sensitivity. At the ultrastructural level, the electron microprobe allowed determination of the precise location of elements and their chemical form. In our previous studies (Chassard-Bouchaud, 1987; 1988; 1989) we were able to demonstrate how SIMS and EMP can provide useful information in biological and marine pollution research.

Our purpose was, indeed, to try to understand how mineral toxicants can affect marine organisms, how these elements are taken up from the sea water, in which tissues they are bioaccumulated and finally, how they are excreted and how organisms can possibly detoxify.

The digestive gland is commonly known to be the main organ of metal concentration in the mussel; this was demonstrated for uranium. But it appears from our present data that chromium behaves toxicologically in a different manner, since the digestive gland is the organ which exhibits one of the lowest Cr levels.

The investigations at the ultrastructural level reveal two main features:

(i) when toxicants are trapped into special organelles such as spherocrystals or into membrane-limited lysosomes, they play a defensive role by preventing the diffusion of the toxic metal throughout the cell. They cannot induce any cell damage and they are generally extruded toward the external environment;
Fig. 10  *Mytilus edulis*. Chromium exposed mussels. Tissue distribution of chromium (Cr), phosphorus (P) and sulfur (S) obtained by X-ray microanalysis (electron microprobe). G: gill. LP: labial palp. DG: digestive gland. K: kidney. B: byssal threads. M: muscle. Metals were detected from lysosomes of gill, labial palp, digestive gland and kidney and from non membrane limited granules of byssus and muscle. Graphs represent means and standard deviation from the measurement of 20 organelles from 10 individuals.

(ii) when toxicants are scattered in the cytoplasm, and not located in membrane-limited organelles, such as chromium and uranium in the byssus and chromium in the muscle, they are likely to provoke injuries in the tissue. Moreover, they cannot be extruded from the cells where they stay indefinitely.

As regards the byssal threads, several authors (Coombs and Keller, 1981) question whether the metals would be either adsorbed or complexed directly from the surrounding waters and incorporated within the tissues. Our data obtained by microanalytical imaging techniques clearly support the incorporation of chromium within the byssal threads of the mussel.
Fig. 11  *Mytilus sp.* Cr-exposed mantle. Ion microscope micrographs of a semithin section. (A) 40 Ca⁺ image showing topography of the section with a microphage amoebocyte (m). (B) 52 Cr⁺ image showing the high Cr emission from the macrophage (m); X 400

An excretion role for the byssus has been suggested (George et al., 1976), involving transfer from the other tissues to the byssus via the hemolymph; therefore the byssal threads may constitute a significant pathway for the elimination of toxic metals. This process would provide a simple and convenient method for monitoring longer environmental changes in metal toxicants.

Much more basic research on pollution-related topics is necessary before a reliable understanding of processes dealing with man-made pollutants can be achieved. Therefore, in our field of research, we may monitor pollution using mussels investigated by several microanalytical techniques. Thereby, we may provide rapidly useful information for the diagnosis and possibly the control of environmental problems caused by the contamination of coastal waters with trace metal toxicants.

6. ACKNOWLEDGEMENTS

This work was executed in the framework of the MED POL programme and a MED TRUST FUND contribution was received through the Food and Agriculture Organization of the United Nations (contracts FRA/24-G and PRA/39-G). Electron Microscopy was performed in the Laboratoire de Microscopie Electronique appliquée à la Biologie, 105 Bd Raspail, F-75006 Paris, France.
Fig. 12 *Mytilus* sp. Cr-exposed macrophage amoebocyte. Electron micrograph showing heterogenous content of the cell with many heterophagolysosomes (arrows); X 32,000
7. REFERENCES


DISTRIBUTION AND FATE OF TBT AND ITS DEGRADATION PRODUCTS IN THE LA SPEZIA GULF

by

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ABSTRACT

Tributyltin and its degradation products are well known contaminants recently introduced into the marine environment. Data on water, sediment and mussel samples collected in the civil and military harbours located in the La Spezia Gulf (Italy) are presented together with some considerations on possible degradation pathways and accumulation mechanisms in sediments and mussels.

1. INTRODUCTION

Tributyltin (TBT) contamination in the marine environment has been well documented as early as the 70's. TBT replaced then, copper salts as the active ingredient in antifouling paints, due to its higher toxicity to fouling organisms (Evans and Hill, 1983). This introduction has resulted in deleterious biological effects (Maguire, 1987): high TBT water concentrations caused mortality in shellfish and microalgae but also very low TBT concentrations (ng l⁻¹ levels) caused sublethal effects such as poor growth rates and low reproduction success in a wide range of marine organisms (Alzieu et al., 1982; Waldock and Thain, 1983; Stroemgren and Bongard, 1987). Among these, Pacific oysters (Crassostrea gigas) and Blue mussels (Mytilus edulis) showed to be able to accumulate large amounts of TBT (Waldock and Thain, 1983; Laughlin et al., 1986a; Zuolian and Jensen, 1989). The toxicity of TBT and the high bioconcentration factors (BCF) found for these species resulted in a production decline in some shell fisheries with great economic consequences (Alzieu, 1990).

In water, TBT is easily degraded (by chemical, biological or UV mechanisms) down to inorganic tin by a stepwise debutylation process forming the less substituted intermediate compounds dibutyltin (DBT) and monobutyltin (MBT) (Blunden et al., 1984; Maguire et al., 1983; Maguire et al., 1984). Trisubstituted organotin compounds show a maximum toxicity and therefore their degradation is a mechanism of detoxification.

During 1989, water, sediment and mussel samples were collected in the La Spezia Gulf during two sampling cruises in order to determine the concentrations of TBT and its degradation products. TBT data relative to the first sampling (August, 1989) showed low concentrations in water (Chiavarini et al., 1991) and very high concentrations in mussels. In this paper, data from the second sampling cruise, where sediments were also analysed (January, 1990) are presented.
2. EXPERIMENTAL

2.1 Sampling

2.1.1 Sampling area

Fig. 1 is the map of the sampling area with the sampling points indicated.

The Gulf of La Spezia lies between the Tino Island (lat. 44° 01′33″N - long. 09° 51′02″E) and the Corvo Cape, surrounded by mountains that constitute a secondary branch of the Appennines. The internal part of the Gulf, the roadstead, is protected by an artificial dyke (2200 m length). There exist two accesses into the roadstead, the Passo di Levante (200 m width) and the Passo di Ponente (400 m width) that is the main way for the maritime traffic through a 14 m deep canal. The mean depth of the basin is 13 m and the surface is 12 km². In the Gulf of La Spezia there is also a military harbour, near the Arsenal, in the western part joined to the Gulf by two canals (120 and 50 m wide).

Fig. 1  Map showing the sampling points in the La Spezia Gulf
The prevalent wind is from E-SE and does not cause perturbation of the sea in the inner part of the Gulf, behind the dyke, where sea currents have only moderate speeds with mean values of 5.5 cm/s and rarely reach 15 cm/s. The prevalent direction is from East to West. Outside the Gulf, the currents are generally parallel to the coast (direction NW) with a surface speed of about 25 cm/s, sometimes reaching 100 cm/s during winter time (Boniforti, 1988).

2.1.2 Sampling procedures

Two samples were collected from each sampling point: one in the superficial layer and the other at a depth varying from 5 to 10 meters. The samples were filtered as soon as possible through 0.4 µm polycarbonate filters. The sampling apparatus was a Teflon coated PVC 30 liters bottle with a remote-controlled stopper (Sartorius). The salinity of the water samples was ca. 38 while pH values ranged from 7.95 to 8.25. The sample was split into two 1 l aliquots for duplicate analyses.

Sediment cores were collected at points A1, A3, A5, B1, B2, B4, CE2 and CW2 and squeezed as soon as possible. The sediment samples were homogenized, freezeed and stored at -20°C until analysis. An aliquot was dried to determine the water content.

Mussel samples were collected from points CE0, CE1, CE2, CW0, CW1, CW2 and B4 where a mussel farm is located. Samples from this latter point were collected by farm personnel. The other samples were collected at the bottom of the rocks on the coast in front of the sampling points using a stainless steel rake with a net. The mussels were separated from the shells, stored at -20°C, freeze-dried and then homogenized.

2.2 Analytical procedures

2.2.1 Water samples

The sample (1 l) was transferred to a cylindrical separatory funnel and tripropyltin chloride (in methanolic solution) was added as an internal standard up to a concentration of 50 ng l⁻¹ (as Sn). The following volumes were added to the solution in the same order as specified: 15 ml of hexane:isooctane (4:1); 4 ml of a 4% solution of sodium borohydride in 1% NaOH; 2 ml of 1M HCl.

The solution was moderately shaken and left undisturbed for at least 15 minutes. The water phase was transferred in a second separatory funnel and again treated as above. The organic phases were passed through anhydrous sodium sulfate and concentrated down to 0.5 ml by a gentle stream of nitrogen.

2 µl were injected into a GC using the following chromatographic conditions:

Varian Vista 6000 gas-chromatograph
FPD detector (operated without filter)
carrier gas flow (He): 9 cc min⁻¹
flame gases: Air(1): 200 cc min⁻¹;
Hydrogen (1): 120 cc min⁻¹; Hydrogen(2): 80 cc min⁻¹
temperature programme: 80°C x 1', 10°C min⁻¹ to 280°C
injector temperature: 240°C
detector temperature: 240°C
column: megabore DB-1 (0.53 mm i.d.; 1.5 μm film thickness;
30 m length

The instrumental detection limit both for TBT and DBT is 8 pg
injected (corresponding to 2 ng l⁻¹ if the final solution is evaporated
down to 0.5 ml). The CVs were evaluated both in distilled water and
artificial sea water spiked with two different amounts of TBT and DBT
(20 ng l⁻¹ and 200 ng l⁻¹). The results based on 15 replicate
analyses ranged from 3.7% to 7.5%.

2.2.2 Mussel and sediment samples

100-200 mg (dry weight) of sample were placed in a Pyrex vial (230
ml), then the internal standard (Tripropyltin chloride 200 ng in
methanolic solution) and 15 ml of a 0.25% methanolic Tropolone solution
were added. The vial, capped with a teflon-lined screw cap, was
placed in a ultrasonic bath for 15 minutes at 35°C.

The procedure is repeated 2 times collecting the supernatant,
after centrifugation, and transferring it to a 250 ml cylindrical
separatory funnel which contains 100 ml of a 25% w/w NaCl solution.
Extraction is performed adding two times 15 ml of dichloromethane.
The organic phases are collected through anhydrous sodium sulfate and
concentrated down to a few mls by a gentle stream of nitrogen and
transferred to a reaction vial. Two mls of isooctane were added and
the solution was reduced to approximately 0.5 ml. One ml of 2 M
ethereal solution of penty Imagnesium bromide was added. The vial is
capped and the reaction is allowed to proceed at least for 20 minutes
shaking from time to time, then the excess reagent is destroyed by
carefully adding 1 M hydrochloric acid.

The organic layer is removed by a Pasteur pipette and put on the
top of a mini silica-gel column (300 mg in a Pasteur pipette) eluting
with 4 ml of a hexane/benzene 1:1 mixture for clean-up. The solution
was finally reduced to an exact volume (usually 1 ml) and 2 μl were
injected for gas-chromatography.

The detection limit is 50 ng g⁻¹ (dry wt) for a 100 mg sample
weight.

3. RESULTS AND DISCUSSION

TBT inputs in the La Spezia Gulf are exclusively due to maritime
traffic and to dockyard activities. The power plant situated near A2
sampling point stopped using TBT as antifouling in cooling pipes in
1985 because of the Ministerial Act which prohibits the discharge of
organotins in areas with mariculture activities.

Table 1 shows concentration data in water from all sampling
points and Table 2 data in water, sediment and mussels from five
sampling points (the samples were collected in January 1990; all the
concentrations are expressed as Sn content).
Table 1
Concentrations in water (ng 1⁻¹ as Sn).

<table>
<thead>
<tr>
<th>Sampling point (depth in m)</th>
<th>TBT</th>
<th>DBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 surface</td>
<td>11</td>
<td>86</td>
</tr>
<tr>
<td>A1 deep (5.1)</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>A2 surface</td>
<td>11</td>
<td>96</td>
</tr>
<tr>
<td>A2 deep (5.1)</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>A3 surface</td>
<td>21</td>
<td>111</td>
</tr>
<tr>
<td>A3 deep (5.1)</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>A4 surface</td>
<td>16</td>
<td>97</td>
</tr>
<tr>
<td>A4 deep (5.1)</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>A5 surface</td>
<td>19</td>
<td>123</td>
</tr>
<tr>
<td>A5 deep (5.1)</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>B1 surface</td>
<td>50</td>
<td>145</td>
</tr>
<tr>
<td>B1 deep (5.1)</td>
<td>12</td>
<td>104</td>
</tr>
<tr>
<td>B4 surface</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>B4 deep (6.1)</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>CW2 surface</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>CW2 deep (7)</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>CE2 surface</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>CE2 deep (6.1)</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

In Table 2, sampling points A1 and A5 are representative of the inner part of the harbour, B4 has been chosen for the presence of the mussel farm, and CE2 and CW2 are representative of the Gulf outside the dyke. In the same table, bioconcentration factors (BCF) for mussels and accumulation factors (ACF) for sediments are reported. BCFs are calculated as the ratio between the concentration in mussels (ng kg⁻¹) and the concentration in water (ng 1⁻¹). The concept of accumulation factor instead of distribution or partitioning factor is used because the ratio between the concentration in the sediment (ng kg⁻¹) and the concentration in water (ng 1⁻¹) at the surface and at 5m deep (and not at the water-sediment interface) is calculated. The differences in density among the different matrices were not considered.

MBT water concentrations are not reported because the analyses were performed with the method described in section 2.2.1.

As it can be seen from Table 1 DBT surface levels are significantly higher than the deep water ones, whereas TBT levels are comparable. DBT surface levels are higher than TBT levels by as much as an order of magnitude; TBT and DBT deep water levels are comparable. These findings could possibly be explained by a higher TBT degradation rate in surface than in deep water. Two routes of degradation are generally considered: photolysis and biodegradation. Photolysis is always effective only in the surface layer because it becomes negligible at depths greater than 10-20 cm. Biodegradation seems to be actually more effective in the surface; this is supported by the
### Table 2

Water, mussel and sediment concentrations (as Sn).

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Water (surface) (ng l⁻¹)</th>
<th>Water (5m depth) (ng l⁻¹)</th>
<th>Mussels ng g⁻¹ dry weight</th>
<th>Bioconcentration factor</th>
<th>Sediments ng g⁻¹ dry weight</th>
<th>Accumulation factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>B4</td>
<td>TBT 15 DBT 85 MBT -</td>
<td>TBT 22 DBT 14 MBT -</td>
<td>2940 1290 400</td>
<td>130000-200000 15000-90000</td>
<td>400 60 60</td>
<td>18000-26000 650-4200</td>
</tr>
<tr>
<td>A1</td>
<td>TBT 11 DBT 86 MBT -</td>
<td>TBT 13 DBT 9 MBT -</td>
<td>550 110 140</td>
<td>42000-50000 1300-12500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>TBT 19 DBT 123 MBT -</td>
<td>TBT 12 DBT 16 MBT -</td>
<td>350 220 180</td>
<td>18400-29000 1800-14700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE2</td>
<td>TBT 10 DBT 5 MBT -</td>
<td>TBT 7 DBT 3 MBT -</td>
<td>1010 180 &lt;50</td>
<td>100000-150000 40000-60000</td>
<td>580 &lt;50  &lt;50</td>
<td>58000-83000 &lt;50 &lt;50</td>
</tr>
<tr>
<td>CW2</td>
<td>TBT 10 DBT 6 MBT -</td>
<td>TBT 6 DBT 4 MBT -</td>
<td>2270 570 190</td>
<td>230000-380000 100000-140000</td>
<td>750 &lt;50  &lt;50</td>
<td>75000-125000 &lt;50 &lt;50</td>
</tr>
</tbody>
</table>
results of a microbiological study in the area which show a higher bacterial population in surface water (Cironi et al., unpublished results).

The data in Table 2 show very high concentration factors for mussels and sediments that range, for TBT, from 100,000 to 380,000 in mussels and from 18,000 to 125,000 in sediments; for DBT, the values range from 15,000 to 140,000 in mussels and from 650 to 14,700 in sediments. These values are much higher than those expected from theoretical calculations based only on partition coefficients (Laughlin et al., 1986b). However, they fit very well with data from field experiments and with the general assumption that BCFs increase as the TBT water concentration levels decrease (Zuolian and Jensen, 1989).

It is important to note that TBT concentrations comparable to those found in the La Spezia mussels have been shown to produce some adverse effects on mussels in some growth experiments (Page and Widdows, 1990). No adverse effects have been observed by the La Spezia mussel farm up to day.

The data for water, mussels and sediments show that, in water, DBT is higher than TBT, while in mussels and sediments it is the opposite. If DBT is accumulated at the same rate as TBT, it would be expected that DBT concentrations in sediments and mussels are higher than those of TBT. On the contrary, the hypothesis that TBT is the only accumulable species and that DBT and MBT are produced only by TBT degradation in mussels and sediments, could explain the different TBT/DBT ratios in water versus sediment and mussels.

Furthermore, the concentration factors for both TBT and DBT are higher in mussels than in sediments, but, while the ratio mussels/sediment concentration is 3 - 5 for TBT, it rises up to 23 for DBT. The explanation can be found in the fact that in mussels the primary degradation product is DBT while in sediments it is MBT, which, because of its hydrophobicity, may enter the water column in a short time after its formation (Seligman et al., 1990). Data presented in Table 2 seem to confirm this hypothesis. This suggests two different degradation mechanisms in mussels and sediments. Both these mechanisms are characterized by slow kinetics as compared to that in water.

Lastly it should be underlined that different analytical procedures performed on the same sample can sometimes lead to different interpretation as far as speciation is concerned. Thus, when considering the environmental fate of these compounds one should take into account the possible undesired variations which occur in samples.

Unpublished data from analyses performed in our laboratory pointed out that sample pretreatment and storage conditions can promote degradation phenomena changing the TBT/DBT/MBT ratios in mussels and sediments.

In particular, the sample drying and storage temperatures are critical. In fact, comparison of the results obtained on wet samples and on samples dried at 40°C for ten days showed degradation phenomena of different extent from sample to sample. In some cases there were no significant differences. In some other cases the total butyltins
content (TBT + DBT + MBT) remained almost constant but TBT content decreased while DBT and MBT increased accordingly. Finally, some samples loose a large part of their organotin content. These differences between samples could depend on the different chemical composition of the sediment as regards the thermic degradation and on the bacteria, if significantly present, as regards the microbiological degradation.

The freeze-drying procedure, mentioned in the literature, does not seem to show these problems. However, freeze-dried mussel samples showed analogous degradation problems if preserved at room temperature; on the other hand, samples stored at -20°C did not show any modification in organotin speciation. Studies on TBT degradation in mussels and sediments should therefore take into account that changes in the TBT speciation can occur because of sample treatment.

4. REFERENCES


Evans, C.J. and R. Hill, 1983. Organotin-based antifouling systems. Reviews on Si, Ge, Sn and Pb compounds, 7:57-125


SURFACE ACTIVE SUBSTANCES AND PROCESSES AT
NATURAL PHASE BOUNDARIES

by

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ABSTRACT

The paper reviews the determination and characterization of
surface active substances in marine, freshwater and estuarine samples.
Absorption at natural phase boundaries, and possible interactions
between surface active substances and other ions and molecules in the
bulk phase and in the interfacial region is discussed at model and
natural phase boundaries.

1. INTRODUCTION

Adsorption processes at phase boundaries have an important role in
the biogeochemical cycle of elements in natural aquatic systems
(Parks, 1975; Stumm, 1987). The exchange of substances between water
and atmosphere is influenced by mass-transport processes in the
interfacial region at the air-water boundary. It is dependent on the
chemical composition and physico-chemical properties of the naturally
occurring surface films, which consist of dissolved and particulate,
surface active organic molecules (Hunter and Liss, 1982). The
elimination processes for most trace elements from the bulk water are
regulated by adsorption on particles and scavenging mechanisms
(Balistrieri et al., 1981). The weathering of rocks and mineral
dissolution processes are ruled by surface reactions and mediated by
organic molecules that are present (Stumm and Furrer, 1987).
Adsorption of organic substances at solid particles has a complex
influence on the particle-particle interactions in establishing
colloidal stability in aqueous systems.

Surface films at the air-water interface and organic coatings at
the mineral surface are formed by sorption of organic surface active
material from the aqueous phase. The physico-chemical properties of
the adsorbable organic substances in various natural waters and their
possible role in processes at natural phase boundaries have been
extensively studied at the Center for Marine Research Zagreb. Part of
this work is summarized and presented here.

2. SURFACE ACTIVE SUBSTANCES IN NATURAL WATERS

Organic matter in the sea originates from several internal and
external sources including excretion by plants and animals, bacterial
decomposition, autolysis of dead organisms, inputs by rivers and
effluents and from the atmosphere. Natural organic matter in aquatic
media is a complex mixture of substances such as polysacharides,
proteins, peptides, lipids and humic substances. Humic material is
itself a mixture of polymers of a wide range of molecular weights. Besides naturally occurring substances, various artificial compounds are introduced in natural waters as a result of human activities.

Organic compounds with surface active properties (about 70% of total organic carbon) show a tendency to be concentrated by adsorption processes at the natural phase boundaries of water with the atmosphere, and with solid particles such as sediment, and biota.

Surfactant activity of seawater and surface microlayer samples in the Adriatic Sea have been measured by two electrochemical methods. Method A: polarographic maximum (Zvonaric et al., 1973; Zutic et al., 1981) and Method B: capacity current measurement by a.c. polarography (Cosovic and Vojvodic, 1982).

No pretreatment of water samples, such as filtration and pre-concentration, is needed. Surfactant activity is expressed in terms of surfactant equivalents of the nonionic surfactant Triton-X-100 in mg dm\(^{-3}\) (Cosovic et al., 1985).

Method A is comparatively sensitive to all classes of surfactants and represents a measure of total surfactant activity, whilst method B is specifically very sensitive to hydrophobic molecules and corresponds to hydrophobic surfactant activity. Systematic comparison of dissolved organic carbon (DOC) and surfactant activity of marine and estuarine samples measured by method A has shown a linear relationship between the two quantities that can be expressed as 1 SA (mg dm\(^{-3}\)) = 1.3 DOC(mg dm\(^{-3}\)) (Hunter and Liss, 1981). Methods A and B correlate in freshwater samples, while in marine samples poor or no correlation was found.

Concentrations of surface active material in the sea are generally lower than in open fresh waters. The concentration ranges of surfactant activity values of different natural waters, which were obtained on the basis of electrochemical measurements of a large number of samples, are given in Table 1.

Table 1

Surfactant-activity values (equivalent Triton-X-100 in mg dm\(^{-3}\)) of different natural waters as obtained by two electrochemical methods.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water</td>
<td>1-10</td>
<td>0.05-1</td>
</tr>
<tr>
<td>Ground water</td>
<td>0.2-2</td>
<td>0.01-0.2</td>
</tr>
<tr>
<td>Seawater</td>
<td>0.2-2</td>
<td>0.01-0.2</td>
</tr>
<tr>
<td>Sea surface microlayer</td>
<td>1-3</td>
<td>0.1-2</td>
</tr>
</tbody>
</table>

The main source of natural surface active substances in the sea is the excretion of phytoplankton species. The surfactant activity values of bulk seawater samples show seasonal variations that have the same trend as the variations of biological activity of the sea. Higher
surfactant activity values, usually from spring to autumn, correspond to seasons of higher biological activity, while the lowest values are obtained in the winter season and coincide with the period of the lowest biological activity (Zutic et al., 1981; Cosovic and Vojvodic, 1982; Cosovic et al., 1985). The most remarkable peaks of the surfactant activity values measured in the North Adriatic coincided with intensive phytoplankton blooms (Zutic et al., 1981).

Laboratory experiments with different batch cultures of marine phytoplankton showed that healthy cells excrete surface active substances during the growth. The content and composition of organic matter showed differences between different species, especially between diatoms and flagellates.

Direct measurements of surface active substances in marine samples and in phytoplankton cultures without any pretreatment of the samples and comparison with data obtained with filtered or gently centrifuged samples demonstrated that there is a high loss of organic material heterodispersed fraction through filtration for selected samples (Cosovic and Vojvodic, 1987). The samples were compared with different model surfactants and were found to possess similar adsorption characteristics to hydrophobic, fatty surfactants.

Recently, surface active substances were determined during the evolution of a phytoplankton bloom in a model plankton ecosystem (Plavsic et al., 1990). It was found that a large amount of the surface active substances, predominantly of the humic (fulvic) type, was present from the beginning of the experiment and partly masked the effects of organic substances released by phytoplankton. The transformations of the organic matter during the two week experiment were obvious, especially the dynamic process of formation and elimination of the heterodispersed fraction of the surface active material. The most probable explanation is that during the plankton bloom different organic substances are excreted, a process which changes with time and is superimposed on the already present large amount of surface active substances which are of humic or fulvic type. The heterodispersed fraction is either formed because of salt effect on coagulation of humic material (S=26%) or is a contribution of the presence of excreted surface active material.

3. ADSORPTION OF HYDROPHOBIC FRACTION OF ORGANIC MATTER AT NATURAL PHASE BOUNDARIES

Surface active substances have a characteristic molecular structure consisting of structural groups that have very little attraction for the water phase (hydrophobic groups), together with groups that have a strong attraction for water (hydrophilic groups). The molecule at the surface is oriented with the hydrophilic part in the aqueous phase and its hydrophobic part away from it. Hydrophilic interactions depend on the presence of such functional groups as -OH, -NH₂, -COO⁻ and -NH₂CH-O, as well as ionic groups which determine electrostatic hydration. Hydrophobic and electrostatic interactions are of great importance in determining the surface excess of solute species at the air/water, water/mineral and biological interfaces.
3.1 Air/seawater interface

The air-water interface, covering 71 percent of the Earth's surface, has specific chemical, biological and physical properties that govern exchange processes between the atmosphere and the ocean and cause interfacial fractionation of trace elements. Air-sea interfacial solutions have characteristically high concentration of trace metals, microorganisms and organic substances relative to bulk solutions. Historically, studies on the chemical composition and physicochemical properties of the sea surface film started during the 1960s and since then a number of research and review papers were devoted to this interesting and important problem (MacIntyre, 1974; Wangersky, 1976; Lion and Leckie, 1981; Hunter and Liss, 1982; Williams et al., 1986; and others). Naturally occurring sea surface films or microlayers may vary in thickness from tens of Angstroms to tens of micrometers. Enrichment of dissolved organic carbon (DOC) and particulate organic carbon (POC) is generally found in the microlayer samples, taken by different sampling devices (100-300 μm thick), relative to the adjacent subsurface water.

Because of hydrophobic interaction it is expected that hydrophobic organic solutes are more enriched than total DOC in the surface film. The electrochemical method based on the measurement of adsorption effects at the mercury electrode surface was used for the determination of the hydrophobic surfactant activity of organic constituents of the bulk phase as well as of interfacial microlayer samples (Cosovic and Vojvodic, 1989). A number of surface microlayer samples and the corresponding subsurface water from the Adriatic sea have been analyzed and enrichment factors were calculated for total surfactant activity and hydrophobic surfactant activity. As shown in Table 2, in comparison with the behaviour of total surfactants and dissolved organic matter, the hydrophobic surfactants were found to possess several times higher enrichment factors at the air/sea interface. However, the enrichment of hydrophobic surfactants at the air/sea interface is much more like that of POC. In filtered samples, the enrichment factors of hydrophobic surfactants are usually lower than in untreated samples. In situations where surfactant activity measurements indicate a predominance of polar polymeric material, the enrichment factors are considerably lower and the influence of filtration is less visible (Marty et al., 1988).

3.2 The organic layer at the halocline in a stratified estuary

In a stratified estuary, as in the case of the Karstic Estuary of the Krka River in Yugoslavia, the mixing region of fresh and saline waters along the depth profile is limited to a highly compressed layer with a salinity gradient of 30 per 20 cm (Zutic and Legovic, 1988). A typical distribution of hydrophobic surface active substances along the depth profile in the Krka Estuary is presented in Fig. 1. It is evident that there is enrichment of surface active substances at the halocline with respect to both the upper freshwater or brackish layer and underlying saline water. Typical values of the hydrophobic surfactant activity at the depth profile, starting from the sea/air interface down to 6 m in depth are given in Table 3. At the halocline the enrichment factors for total surfactant activity and for dissolved organic carbon are lower than for hydrophobic surfactant activity similar to the behaviour at the air/sea interface (Cauwet, 1991).
Table 2

Enrichment factors in the microlayers with respect to the subjacent water for total and hydrophobic surfactant activities (SA) and DOC and POC (from Cosovic and Vojvodic, 1989).

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Location</th>
<th>Total SA</th>
<th>Enrichment factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hydrophobic SA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DOC</td>
</tr>
<tr>
<td>Cosovic et al. (1985)</td>
<td>Adriatic Sea</td>
<td>1.1-8</td>
<td>2.1-100</td>
</tr>
<tr>
<td>Marty et al. (1988)</td>
<td>Northern Adriatic Sea</td>
<td>1.1-1.4</td>
<td>1.6-2.2</td>
</tr>
<tr>
<td>Williams (1967)</td>
<td>Coastal and off-shore Peru</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Williams et al. (1986)</td>
<td>Gulf of California</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>West coast of Baja California</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Mean values

 Generally, we can conclude that because of preferential adsorption of hydrophobic organic substances a fractionation of organic matter occurs in the interfacial region at natural phase boundaries, resulting in a different organic chemical composition in the interfacial layers from that in the aqueous bulk phase. This fact has to be taken into account for possible interactions of adsorbed organic layers with other substances and pollutants and the consequences for their fate in the aquatic environment.

4. INTERACTION OF SURFACE ACTIVE SUBSTANCES WITH OTHER MICROCONSTITUENTS AND POLLUTANTS

The fate of many trace substances in natural waters depends on the interaction with present organic matter. These interactions can occur in the bulk solution, as well as in the interfacial layer at phase boundaries. Organic substances can exhibit various properties such as complexation ability towards metals, solubilization of readily soluble organic and inorganic substances, influence on surface tension, photochemical properties, etc. Organic substances adsorb at mineral particles, modify physicochemical properties of the surface and affect mass and charge transfer processes in the interfacial layer.
Fig. 1 Vertical profile of salinity (o-o-o-) and surfactant activity values (●●●●) in a stratified estuary (Krka River Estuary, April 1985). Surfactant activity measured by a.c. polarography.

Very useful information on the structure of the adsorbed layer itself and possible interactions with other ions and molecules can be obtained from electrochemical investigations at the model electrode/solution interfaces. In the adsorption study at electrodes, the charge and surface potential of the electrode are adjustable by the applied excitation signal independently of the pH of the solution. The adsorbed layer of organic molecules generally presents a barrier to the transport of ions and electrons at the interface. This was demonstrated for synthetic compounds, like commercial detergents (Kozarac et al., 1986). For natural surface active substances, for
Table 3
Salinity and hydrophobic surfactant activity values at the vertical profile in the stratified Krka River Estuary in May 1986 (from Cosovic and Vojvodic, 1989).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Salinity</th>
<th>Hydrophobic SA (mg Triton-X-100 dm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface film (150 µm)</td>
<td>2.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Subsurface (0.5 m)</td>
<td>3.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Interface at the halocline (2.2 m)</td>
<td>21.9</td>
<td>0.15</td>
</tr>
<tr>
<td>Bulk (6 m)</td>
<td>36.9</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Example, biopolymers (proteins) and geopolymers (humic substances), it was found that the pH of the solution has a dominant effect on the structure of the adsorbed layer as well as on the interactions with other ions and molecules (Kozarac et al., 1986; Plavsic and Cosovic, 1989; Gasparovic, 1990). At natural pH values (7-8), natural polymers are negatively charged and therefore have attractive interaction with positively charged cations as shown for cadmium, while there was observed repulsive interaction with negatively charged ions, like nitrophenolate. In acidic solution, the polymers become protonated and thus uncharged. The consequence is that adsorbed layers of natural polymers upon acidification show similar effect on charge transfer processes of other ions and molecules like neutral synthetic surfactants (Plavsic and Cosovic, in press).

Adsorption at natural phase boundaries from the complex mixture of organic substances depends on the qualitative and quantitative composition of the mixture, absorbability of the components and the time period of adsorption. For example, synthetic surfactants occur in the marine environment together with much higher concentrations of natural surface active molecules. Reactivity of adsorbable species and synergistic effects in the mixture are very important. Small amounts of strongly adsorbable species, especially of low molecular weight, may have tremendous effect on the properties of the adsorbed layer. Five percent of nonionic surfactant Triton-X-100 in the model mixture with humic material (47.5%) and polysaccharide Dextran (47.5%) showed dominating influence on the properties of the adsorbed layer for transport of cadmium ions (Cosovic, 1990).

An extensive study of the interaction of cadmium and copper with surface active organic matter and complexing ligands, released by marine phytoplankton Dunaliella tertiolecta, demonstrated that strong interaction of excreted organic substances with copper occurs in the bulk phase and with cadmium in the interfacial region (Kozarac et al., 1989). It was also observed that the content and composition of
organic matter and adsorption and complexation properties towards cadmium and copper are different in stress conditions during growth (deficiency of essential metals in our experiment). A qualitatively similar behaviour has been observed in natural samples of estuarine waters, indicating the existence of stressed conditions during the mixing of fresh and saline waters.

5. ACKNOWLEDGEMENTS

The financial support of the Authority for Scientific Research of Croatia, the National Institute of Standards and Technology, Washington, DC, and UNEP (Mediterranean Trust Fund) research grant is gratefully acknowledged.

6. REFERENCES


POLYCHLORINATED BIPHENYLS IN WATER, SUSPENDED PARTICULATE MATTER AND ZOOPLANKTON FROM THE OPEN ADRIATIC SEA

by

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ABSTRACT

Polychlorinated biphenyls (PCBs) were determined in seawater, suspended particulate matter and zooplankton samples collected from the open Adriatic Sea during several oceanographic cruises carried out between 1979 and 1989. PCB concentrations are reported on a volume, dry weight and lipid weight basis. Distribution coefficients of $10^4$ - $10^5$ on dry weight and $10^6$ on lipid weight, consistent with an equilibrium partitioning model of PCBs between water and abiotic and biotic suspended phases, have been calculated.

1. INTRODUCTION

This paper presents a summary of the mean PCB levels in water, suspended particulate matter and zooplankton from the open Adriatic Sea. These studies were intended to provide a data base to assess the distribution and the impact of these chemicals on the marine ecosystem and to obtain some quantitative information on the partitioning of the PCBs between suspended phases and water for the low levels expected in the open Adriatic Sea.

The samples were collected during several cruises carried out in the North and Central Adriatic (Fig. 1) between 1979 and 1989 in the framework of the Yugoslav-Italian Adriatic Scientific Co-operative Program (ASCOP).

2. MATERIALS AND METHODS

The sampling procedure adopted in the ASCOP cruises entailed the collection of water from 0.5 - 1.0 m depth using a 10 dm$^3$ glass bottle placed in a heavy holder, which was deployed just before arrival on station. During each cruise, 6 - 13 water samples of 20 dm$^3$ each were taken and filtered on board through Gelman A/E glass fiber filters (1 μm pore size) in a closed system to prevent sample contamination.

15 dm$^3$ of filtered water were then extracted twice with a total of 600 cm$^3$ n-hexane. The extracts, to which anhydrous sodium sulphate was added, were taken to the laboratory for analysis.

The filters, to which 2-3 g of anhydrous sodium sulphate was added, were stored at -20°C and subsequently Soxhlet-extracted for eight hours with n-hexane.
Fig. 1 Sampling stations in the North and Central Adriatic Sea
Large zooplankton samples were collected by sub-surface tows using a WP-2 net (200 μm mesh size, 75 cm mouth diameter). The samples were stored in glass jars and kept frozen until the time of freeze-drying. The wet to dry weight ratio was recorded. An aliquot of 3–5 g of freeze-dried zooplankton sample was Soxhlet extracted for eight hours with n-hexane.

For all extraction procedures, the hexane extracts were concentrated in a rotary evaporator to about 5 cm$^3$ and shaken with concentrated sulfuric acid to remove interfering co-extracted substances (Murphy, 1972). The extracts were eluted on a silica gel microcolumn (Palmer and Villeneuve, 1980) collecting a series of fractions (containing different chlorinated hydrocarbons) which were concentrated to 0.5–1.0 cm$^3$ prior to their analysis with a Carlo Erba FV 2351 gas chromatograph equipped with a N$_2$-63 ECD. The identification of PCBs was based on their appearance in the appropriate silica gel column fraction and on their retention time on two GLC columns (200 cm long x 0.4 cm i.d.; 5% DC-200 on Gas Chrom Q BW-DMCS and 5% QF-1 on Chromosorb W AW-DMCS).

PCBs were quantified by measuring the height of the four–five major peaks eluting later than pp'DDE (5 to 7 chlorine atoms PCB congeners) and by comparing their responses to reference standards Aroclor 1254 and 1260.

On separate filters, the weight of the suspended particulate matter was determined after drying at 105°C to constant weight.

3. RESULTS AND DISCUSSION

The method of analysis adopted for ASCOP cruises, based on hexane extraction of 15 dm$^3$ of filtered sea water, was tested for recovery and precision. The recovery from four water samples, to which 2 ng dm$^{-3}$ of PCB Aroclor 1254 were added, was 89 ± 8%. The estimated detection limit was 0.2 ng dm$^{-3}$.

According to earlier investigations (Pavlou and Dexter, 1979; Fossato et al., 1982; Duinker, 1986), the residue quantities were calculated on a volume and dry weight basis; when appropriate, as it is the case of zooplankton, concentrations were also normalized to lipid weight (Clayton et al., 1977; Fossato et al., 1982).

A presentation of PCB data for the period 1979-1985, together with supporting hydrographic and biological measurements, is available elsewhere (Campesan et al., 1989; Fossato et al., 1989). Relevant results are summarized below.

Plots of mean PCB concentrations on a per volume basis versus time in the Adriatic Sea are shown in Fig. 2. Within the uncertainty of the data, it cannot be concluded that the levels have changed significantly over a ten-year period despite regulatory attempts to restrict the discharge of PCBs in the area. In this respect, the relatively low PCB values found in October-November 1989 may be attributed, at least in part, to the reduced freshwater inflow in the sea due to an exceptionally dry season.
Fig. 2 Plots of the mean PCB concentrations vs. time for samples of water and SPM from the open Adriatic Sea. Whole water is dissolved plus particulate fraction; water is dissolved fraction; SPM is particulate fraction. Standard deviation includes spatial variability and analytical uncertainty.
Table 1 shows the PCB mean concentrations in whole water, the percentage of PCBs accumulated on SPM and the abundance of SPM in the open Adriatic Sea. The concentration of PCBs dissolved in surface waters is relatively uniform within the region, although slightly lower values were generally found along transect 6 (Central Adriatic). In contrast, significant gradients with PCB concentrations decreasing eastward and southward were observed, when total water concentrations of PCBs (dissolved plus particulate fraction) were considered. These distributions clearly match those of the SPM which actually accumulates an important fraction of PCBs.

Linear, exponential and multiplicative relationships were tested to correlate the percent of PCBs sorbed on SPM (P) with the concentration of suspended matter [SPM] in mg dm$^{-3}$; the best relationship is mathematically described by the multiplicative function $P = a [SPM]^b$ plotted in Fig. 3. The correlation allows an estimation of the relative contribution of suspension and solution to the total concentration of PCBs per unit volume.

Note that in this discussion we have not considered the PCB fraction associated with zooplankton because its contribution is small; although the PCB values on dry weight for zooplankton were similar to the ones for SPM, the dry mass concentrations of the former were usually two orders of magnitude lower than the corresponding SPM values.

An interesting relation was also found between the content of PCBs in SPM (expressed in ng g$^{-1}$ dry wt.) and the concentration of suspended matter [SPM] (expressed in mg dm$^{-3}$). The relationship is described by the multiplicative function $[PCB] = a [SPM]^b$ plotted in Fig. 4. It appears that higher PCB content values were found for several samples with low SPM concentration, typically below 2 mg dm$^{-3}$. A similar relation was found by Duinker (1986) in several estuaries and offshore regions of the North Sea and was explained as the increased sorption properties of particles with smaller size and larger specific surface area.

Table 1

Total PCB load in the water (dissolved plus particulate fraction), percent of PCBs sorbed on SPM and concentration of SPM.

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Samples No.</th>
<th>Total PCB load in the water (ng dm$^{-3}$)</th>
<th>PCBs on SPM (%)</th>
<th>SPM (mg dm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct/Nov. 1979</td>
<td>7</td>
<td>1.2 ± 0.2</td>
<td>45.0 ± 6.2</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>November 1980</td>
<td>6</td>
<td>1.9 ± 0.3</td>
<td>57.5 ± 11.2</td>
<td>7.4 ± 0.6</td>
</tr>
<tr>
<td>May 1983</td>
<td>13</td>
<td>2.1 ± 1.1</td>
<td>42.1 ± 13.1</td>
<td>8.9 ± 6.8</td>
</tr>
<tr>
<td>Aug/Sept. 1983</td>
<td>12</td>
<td>1.7 ± 0.4</td>
<td>45.6 ± 5.6</td>
<td>5.5 ± 3.0</td>
</tr>
<tr>
<td>August 1984</td>
<td>13</td>
<td>1.3 ± 0.2</td>
<td>24.3 ± 7.7</td>
<td>2.2 ± 2.2</td>
</tr>
<tr>
<td>Oct/Nov. 1989</td>
<td>13</td>
<td>0.9 ± 0.4</td>
<td>47.9 ± 4.4</td>
<td>3.3 ± 1.2</td>
</tr>
</tbody>
</table>
Fig. 3 Percent of PCBs sorbed on SPM per unit volume in relation to the suspended matter concentration [SPM]

\[ P = a [\text{SPM}]^b \]
\[ a = 3.21 (\pm 0.07) \]
\[ b = 0.34 (\pm 0.04) \]
\[ r = 0.70 \]
\[ n = 63 \]

Fig. 4 PCB content in SPM in relation to the suspended matter concentration [SPM]

\[ [\text{PCB}] = a [\text{SPM}]^b \]
\[ a = 5.36 (\pm 0.10) \]
\[ b = -0.34 (\pm 0.06) \]
\[ r = -0.58 \]
\[ n = 63 \]
The concept of equilibrium partitioning, developed by various groups (Neely et al., 1974; Chiu et al., 1977; Clayton et al., 1977; Karickhoff et al., 1979; Brownwell and Farrington, 1985) was applied to the distribution of PCBs within abiotic and biotic components in the Adriatic environment. This approach implies some approximation, since a differentiation of physico-chemical properties exists between PCB congeners according to their degree of chlorination. The distribution coefficients Kd, defined as the concentration ratio of PCBs between SPM (ng kg\(^{-1}\) dry wt.) and water (ng dm\(^{-3}\)), have been calculated and reported in Table 2. For comparison purposes, we refer to the regional Kd values for SPM and water in Puget Sound (Pavlou and Dexter, 1979) and the North Sea (Duinker, 1986). Kd values increase from 4.36 to 9.67x10\(^4\) in Puget Sound and from 4.8 to 26x10\(^4\) in the North Sea according to the number of chlorine atoms (3 to 7) of PCB constituents. Earlier studies on PCBs in water and SPM of the Po River Delta (North Adriatic) gave the following average Kd values: 23.2x10\(^4\) in August 1979 and 6.0x10\(^4\) in September 1980 (Fossato et al., 1982). While there are large uncertainties associated with the data, the distribution coefficients are reasonably similar over the range of spatial and temporal regimes encountered in these investigations; they are consistent also with Kd values predicted either from octanol-water partition coefficients or aqueous solubilities of PCBs (Chiu et al., 1979; Brownwell and Farrington, 1985).

Table 2

Concentrations of PCBs dissolved in seawater and sorbed on SPM, and distribution coefficients between water and suspended matter on a dry weight basis. Means and standard deviations.

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Samples No.</th>
<th>water SPM ng dm(^{-3})</th>
<th>SPM ng g(^{-1})</th>
<th>Kd x10(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct./Nov. 1979</td>
<td>7</td>
<td>0.6 ± 0.2</td>
<td>80 ± 15</td>
<td>13.2 ± 3.7</td>
</tr>
<tr>
<td>November 1980</td>
<td>6</td>
<td>0.8 ± 0.2</td>
<td>148 ± 42</td>
<td>20.0 ± 8.2</td>
</tr>
<tr>
<td>May 1983</td>
<td>13</td>
<td>1.1 ± 0.6</td>
<td>110 ± 45</td>
<td>10.6 ± 4.3</td>
</tr>
<tr>
<td>Aug./Sept. 1983</td>
<td>12</td>
<td>0.9 ± 0.2</td>
<td>160 ± 51</td>
<td>17.7 ± 5.2</td>
</tr>
<tr>
<td>August 1984</td>
<td>13</td>
<td>1.0 ± 0.1</td>
<td>205 ± 96</td>
<td>21.7 ± 10.2</td>
</tr>
<tr>
<td>Oct./Nov. 1989</td>
<td>13</td>
<td>0.5 ± 0.2</td>
<td>142 ± 47</td>
<td>33.6 ± 16.1</td>
</tr>
</tbody>
</table>

Since PCBs are lipophilic compounds, it was felt appropriate to use lipid as a normalization parameter for biota. Residue concentrations and distribution coefficients for zooplankton of the open Adriatic Sea, calculated both on a dry weight and lipid basis, are shown in Table 3. Although PCB levels in zooplankton have been reported for several areas in the world, the general absence of simultaneous measurements of PCBs in water does not permit direct comparison of distribution coefficients. The most pertinent data were reported by Clayton et al. (1977), who found regional mean Kd values between 0.90 and 2.41x10\(^6\) on lipid weight for zooplankton in Puget Sound. Again, Fossato et al. (1982) reported Kd values of 5.7-22.7x10\(^4\)
on dry weight and of 1.3–5.8x10^6 on lipid weight for net zooplankton of the Po River Delta. Direct comparison is possible also with Kd values calculated from physico-chemical properties of PCBs (Chiou et al., 1979; Ernst, 1985). Within an uncertainty of one order of magnitude, Kd values appear uniform over a wide range of spatial and temporal regimes, species composition and lipid content of the organisms.

Table 3

Concentrations of PCBs in zooplankton and distribution coefficients between water and zooplankton on dry weight and lipid weight. Means and standard deviations. (Values in parentheses are the number of samples).

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>ng g⁻¹ dry wt.</th>
<th>Kd x10⁴</th>
<th>µg g⁻¹ lipid wt.</th>
<th>Kd x10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct/Nov. 1979 (7)</td>
<td>45 ± 17</td>
<td>7.3 ± 2.6</td>
<td>4.6 ± 2.3</td>
<td>7.2 ± 3.2</td>
</tr>
<tr>
<td>Nov. 1980 (6)</td>
<td>91 ± 28</td>
<td>12.7 ± 3.2</td>
<td>6.0 ± 3.6</td>
<td>9.1 ± 3.3</td>
</tr>
<tr>
<td>May 1983 (12)</td>
<td>169 ± 123</td>
<td>16.9 ± 14.4</td>
<td>2.9 ± 2.0</td>
<td>2.9 ± 2.3</td>
</tr>
<tr>
<td>Aug/Sept. 1983 (11)</td>
<td>145 ± 19</td>
<td>16.1 ± 5.0</td>
<td>4.7 ± 2.3</td>
<td>5.5 ± 3.4</td>
</tr>
<tr>
<td>Oct/Nov. 1989 (6)</td>
<td>30 ± 8</td>
<td>6.5 ± 1.7</td>
<td>1.1 ± 0.4</td>
<td>2.4 ± 0.6</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

The contribution of particulate forms to the total PCB concentration per unit volume appears to be significant in the open Adriatic waters, due to the abundance of SPM and the high affinity of PCBs for abiotic and biotic suspended phases.

Levels of PCBs in SPM and zooplankton are similar in terms of dry weight concentrations; however the PCB fraction associated with zooplankton is usually small, owing to the low mass weight of this component.

Distribution coefficients of 10⁴–10⁵ on a dry weight and 10⁶ on a lipid weight basis agree with values reported in literature for suspended matter and zooplankton and are consistent with an equilibrium partitioning model.

5. REFERENCES


Palmroth, K.H. and J.P. Villeneuve, 1980. Outline of the method to be used for the determination of chlorinated hydrocarbons in sea water. In: Workshop on the intercalibration of sampling procedures of the IOC/WMO/UNEP pilot project on monitoring background levels of selected pollutants in open-ocean waters (Bermuda, 11-26 January 1980). IOC Workshop Report No. 25:25-55

DISTRIBUTION AND BIODISTRIBUTION OF AROMATIC COMPOUNDS IN COASTAL MEDITERRANEAN ECOSYSTEMS

by

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ABSTRACT

Polycyclic Aromatic Hydrocarbon distribution was determined in sediments, and Mixed Function Oxygenases (MFO) enzyme activities were measured in microsomes from either whole mussels (Mytilus galloprovincialis) or cobler's livers (Serranus cabrilla) or Posidonia oceanica white tissues, in coastal locations of the French Riviera and Corsica during 3 oceanographic cruises in 1987-1988.

Benzo(a)pyrene Monoxygenase (BaP-MO) enzyme activities in mussel and EthoxyResorufin-O-Deethylase (EROD) measured in fish were strongly correlated to PAH concentration (log) in sediments. The first results for Cinnamate-4-Hydroxylase in Posidonia showed significant differences related to PAH contamination level. The increase in MFO activities measured in Corsica in the summer of 1988 indicated a recent weak oil spill which was also confirmed by PAH molecular weight distribution.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are commonly ubiquitous contaminants in marine ecosystems. While a biogenic origin is the diagenetic transformation of organic matter derived from living organisms, these compounds are mainly from anthropogenic sources. They originate from combustion sources (industrial activities, automobile exhaust, waste waters) or from petroleum spills, and enter the marine environment via road water runoff, river transport and atmospheric pathways (Neff, 1979; Varanasi, 1989).

Many biochemical techniques have been developed during the last decade for the detection of the impact of aromatic compounds on marine organisms, a major one using mixed function oxygenase enzymes (MFO). These enzymes have iron-containing hemoproteins (P-450) as terminal oxidase (Blumberg, 1978) which can be induced by liposoluble compounds such as drugs and xenobiotics of major environmental interest. Changes in MFO activities are generally expected to be quite sensitive and occur even at low concentrations before physiological damage. Organic contaminants, including PAH, polychlorinated biphenyls and dioxins, are effective in inducing MFO in fish (Narbonne and Gallis, 1979; Narbonne et al., 1987; Stegeman et al., 1988) and as recently observed in
molluscs (Suteau et al., 1988; Livingstone et al., 1989) and in plants (Saladin et al., 1986). Hepatic MFO induction in fish has been used on many occasions as a monitoring tool (Payne et al., 1987). Measurements of related cytochrome P-450 enzymes in molluscs show responses which can be related to pollutant distribution. However, the use of molluscs for field experiments was actually supported by a better knowledge of xenobiotic biochemistry in these animals (Livingstone et al., 1989). As recently observed, plants contain inducible MFO enzymes and this has expanded their utility as monitoring organisms.

Field evaluation is now essential for the validation of any technique proposed as a monitoring tool. The purpose of this study is to evaluate the relationship between MFO enzyme activities in some marine organisms (fish, mussel and plant) and the level of chemical pollution (chiefly polyaromatic hydrocarbons PAH) measured in sediments collected from selected sites of the French Mediterranean coast.

2. MATERIALS AND METHODS

2.1 Sampling sites

The sampling cruises took place in 1987 (summer # 1) and in 1988 (summer # 2 and winter # 3) along the French Riviera and the West coast of Corsica aboard the Research Vessel "Winnaretta Singer" (Oceanographic Institute of Monaco). The sampling stations are shown in Fig. 1. Some are characterized by high PAH concentrations (Marseille, Toulon) whereas other locations are quite unpolluted (Corsica sites).

2.2 Chemical measurements

Bottom sediments were sampled by skin divers from a depth of about 20 m. Only the superficial layer (about 2 cm) was collected and frozen aboard. The procedure for the extraction of the organic matter involving Soxhlet extraction was explained in detail by Garrigues et al. (1987). Aromatic compounds were determined in the total organic extract by High Performance Liquid Chromatography (HPLC) with fluorescence detection as reported previously (Socol et al., 1986). The so-called PAH content represents the total concentration of 12 priority pollutant unsubstituted PAHs.

2.3 Biochemical measurements

2.3.1 Fishes

The oomber (Serranus cabrilla) was caught by fishing (five samples at each site). This species was chosen since it is a hermaphrodite and thus eliminates possible variations due to sex.

The microsomal fractions were prepared as follows: on board, the livers were sliced and rapidly homogenized (pool of 2 or 3 fish livers) in a cold volume of Tris 10 mM, sucrose 250 mM buffer pH 7.4 containing protease inhibitors and 20% (V/V) glycerol. The homogenates were frozen and stored in liquid nitrogen. In the laboratory, the homogenates were centrifuged at 10,000 g for 15 mn and the supernatant collected and centrifuged at 105,000 g for 60 mn. The microsomal pellets were suspended in a Tris 10 mM sucrose 250 mM buffer at pH 7.4.
Fig. 1 Locations of the coastal sampling sites in the Mediterranean sea
Table 1

PAH content in sediments and NFO enzyme activities measured in marine organisms collected during oceanographic cruises along the French Riviera and on the west coast of Corsica (Standard Error means (SEM) are reported in brackets).

<table>
<thead>
<tr>
<th>Sampling station</th>
<th>Oceanographic cruise</th>
<th>PAH in sediment (ng g⁻¹)</th>
<th>ERGD in fish (pmole/mn/mg P)</th>
<th>BAPMO in mussel (pmole/mn/mg P)</th>
<th>CH4H in Posidonia (pmole/mn/ml MP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carteau (Pos)</td>
<td>2</td>
<td>1726(45)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Le Pellanier</td>
<td>1</td>
<td>1582(44)</td>
<td>82(17)</td>
<td>51.0(4.3)</td>
<td></td>
</tr>
<tr>
<td>Lighthouse (Marseille)</td>
<td>2</td>
<td>489(15)</td>
<td>232(46)</td>
<td>51.2(2.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>103(8)</td>
<td>335(81)</td>
<td>22.5(2.3)</td>
<td>16.5(2.5)</td>
</tr>
<tr>
<td>Lazaret Bay (Toulon)</td>
<td>1</td>
<td>8525(410)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3874(86)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>912(26)</td>
<td>477(134)</td>
<td>44.0(4.2)</td>
<td>12.2(2.3)</td>
</tr>
<tr>
<td>Porquerolles</td>
<td>3</td>
<td>58.4(4.8)</td>
<td>311(70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>La Fourniquge (Cannes)</td>
<td>1</td>
<td>835(33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lighthouse (Cannes)</td>
<td>2</td>
<td>697(17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St Marguerite (Cannes)</td>
<td>3</td>
<td>393(35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>433(19)</td>
<td>86(22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>661(25)</td>
<td>320(70)</td>
<td>47.8(8.4)</td>
<td></td>
</tr>
<tr>
<td>Roquebrune Bay</td>
<td>1</td>
<td>4093(135)</td>
<td>83(10)</td>
<td>54.1(7.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1835(27)</td>
<td>342(41)</td>
<td>48.8(4.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1860(45)</td>
<td>440(124)</td>
<td>50.7(5.8)</td>
<td>22.2(4.1)</td>
</tr>
<tr>
<td>Canelle (Corsica)</td>
<td>1</td>
<td>24(3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galeria (Corsica)</td>
<td>1</td>
<td>30.2(1.7)</td>
<td></td>
<td>29.6(2.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.8(0.4)</td>
<td>465(109)</td>
<td>41.8(12.3)</td>
<td></td>
</tr>
<tr>
<td>Staraos (Corsica)</td>
<td>1</td>
<td>53.5(2.0)</td>
<td>54(19)</td>
<td>34.5(5.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.9(0.2)</td>
<td>424(28)</td>
<td>102.8(10.9)</td>
<td></td>
</tr>
<tr>
<td>Scandola-Marine Park (Corsica)</td>
<td>2</td>
<td>3.3(0.2)</td>
<td>625(122)</td>
<td>41.8(6.9)</td>
<td></td>
</tr>
</tbody>
</table>
The stability of hepatic microsomal and cytosolic activities was investigated in order to develop a standardized freezing and storing procedure to maintain the enzyme optimum levels. EthoxyResorufin-O-Deethylase (EROD) activity was measured fluorimetrically as described by Burke and Mayer (1974). Protein content was quantified by the method of Lowry et al. (1951). All analyses were performed in duplicate and were run at 30°C. The enzyme reactions were performed under conditions of maximal velocity and were linear with time and protein concentration. EROD activity was expressed in pmol/min/mg of total protein.

2.3.2 Mussels

The mussels (Mytilus galloprovincialis) were collected by skin divers. Each sample was composed of a pool of 1 male and 1 female. Ten samples were collected at each station for biochemical measurements. On board, adductor and mantle retractor muscles, foot, byssus and crystalline style were removed. The rest of the mollusc was rinsed in buffer, damp-dried and frozen in liquid nitrogen.

In the laboratory, the samples were homogenized and Benzo(a)PyreneMonoxygenase (BaPmo) was determined on the microsomal fraction as previously reported (Suteau et al., 1988). Results are expressed in pmol/min/mg of total protein.

2.3.3 Posidonia

Posidonia oceanica was collected by skin divers. Life white tissues (25 g fresh weight) were washed several times with distilled water and reduced in powder with liquid nitrogen in a mortar. Microsomes were prepared as previously described (Salanit et al., 1988), except that tissues were homogenized with an Ultra Turrax in 60 ml of 0.25 M Tris-HCl buffer (pH 8.4). Microsomal cinnamic acid 4-Hydroxylase (CA4H) activity was measured using the radiochemical method already described (Salanit et al., 1986). Activities were expressed in pmole/min/ml microsomal preparation.

3. RESULTS

3.1 Chemical indices

Results of the PAH analysis in sediment samples are presented in Table 1. The fluctuation in PAH contents in each of the sampling sites was considerable. Samples from certain highly industrialized urban centres (such as Toulon and Carteau) were very contaminated (PAH up to 8 μg g⁻¹) compared with other French coastal environments in the Mediterranean or the west Atlantic (Garrigues et al., 1987). Sampling stations in Corsica appear to be good baseline sites, as they are only slightly contaminated (less than 0.05 μg g⁻¹). The source of PAH in the studied coastal locations is essentially anthropogenic as evidenced by the PAH distribution criteria (Scolto et al., 1986) and will be discussed below.
3.2 Biochemical indices

3.2.1 Fishes

Fishes were not present in high polluted areas. Thus, the results are limited to medium and low polluted sites. EROD activities measured in Serranus cabrilla caught during the first oceanographic cruise were very low but correlated to the pollution gradient \(r=0.717\) (Fig. 2). However, the use of glycerol and EMSF in the medium for liver homogenization leads to obtain higher values for EROD activities.

The EROD activities measured in combers from the continental sites were correlated with the log of PAH content in sediments both for cruise # 2 \(r=0.803\) and cruise # 3 \(r=0.934\). However, the values obtained for Corsica sites during cruise # 2 were higher than those obtained in the French Riviera stations. Seasonal changes in EROD induction have been found:

- between different sites: 2.3 fold difference between the lowest polluted site (Porquerolles) and the highest contaminated area (Roquebrune) in summer (cruise # 2). In winter, (cruise # 3) this difference was only 1.4.

- within each station: 2 fold increase between summer and winter in slightly polluted areas (Porquerolles) and only 1.3 fold increase in the most polluted station (Roquebrune) where the fishes were caught.

3.2.3 Mussels

Consistent responses occurred in measurements of BaPMD activities in microsomal fractions of whole mussels. About 2.5 fold increase was seen between slightly polluted sites (Corsica cruise # 1) and highly contaminated stations (Lazaret Bay, cruise # 1). The values measured in the Corsica areas during cruise # 2 were high, while the PAH contents measured in sediment were very low. However, all the other values were strongly correlated to the log of PAH content in sediment \(r=0.921\). The same relation was found during cruises # 1, # 2, and # 3 as indicated in Fig. 3.

3.2.3 Posidonia

Measurements of MFO activity in Posidonia were carried out during cruise # 3. A 2 fold increase was seen between slightly polluted sites (Porquerolles) and highly polluted areas (Roquebrune) along the French Riviera coast. The five results obtained were correlated \(r=0.700\) linearly with the PAH content in sediments.

4. DISCUSSION

4.1 PAH Content

The source of PAH for sediments collected in 1987 is essentially anthropogenic as evidenced by the PAH pattern. As it can be seen in Fig. 4(a) low molecular weight compounds (3 and 4 aromatic rings from phenanthrene to chrysene) have the same relative importance as those of
Fig. 2 Correlation curve between EROD activity in fish and PAH content in sediments. (J-S 87=June-Sept. 87, cruise #1; J-S 88=June-Sept. 88, cruise #2; N 88=Nov. 88, cruise #3)

higher molecular weight compounds (5 and 6 aromatic rings from perylene to indenopyrene). The ratio of selected isomeric parent compound, such as phenanthrene/anthracene (value less than 10) and fluoranthene/pyrene (value about 1) indicate clearly a PAH contamination due to high temperature combustion processes (Scollo, 1986). On the contrary, a complete different PAH pattern was observed in Corsica sediments (Fig. 5) collected in the summer of 1988 from three island locations (Galeria, Stareso, Scandola): small importance of high molecular weight compounds, phenanthrene/anthracene ratio about 50, fluoranthene/pyrene ratio about 0.3. These observations suggest a petroleum input as clearly evidenced when compared to the PAH pattern obtained on petroleum products (Scollo, 1986; Wise et al., 1988).

Fig. 6 shows the relationship between the two pre-cited isomeric compound ratios for several sediments.
Fig. 3 Correlation curve between BaPMD activity in mussels and PAH content in sediments (notations as in Fig. 2)

4.2 Fish

EROD activities determined in the combers collected during cruise # 2 (by using buffer for homogenization containing glycerol and PMSF) and cruise # 3 (175 to 625 pmol/min/mg prot.) can be compared to the values measured in marine fish collected in open waters (Platichthys flesus: 129 to 547 pmol/min/mg prot., Stegeman et al., 1988; Fundulus heteroclitus: 135 to 185 pmol/min/mg prot., Kloppeer-Sams and Stegeman, 1988).

Our results show seasonal variations in EROD activities from the same station but also in magnitude of response to contamination. Seasonal changes in MFO activities have been demonstrated in a variety of fish species either from the north west Atlantic (Payne and Fancey, 1982) or from semitropical areas (James and Little, 1981) or from the Mediterranean sea (Lafaurie et al., 1990).

It was reported (Payne and Fancey, 1982) that seasonal differences in MFO activity may be related to the reproductive state and induction in the liver tissues of spawning capelin and cod fish. Payne et al. (1987) suggested that seasonality in the abundance of chemical
Fig. 4 PAH distribution in two selected sediments from (a) Lazaret Bay and (b) Galeria (Corsica) during summer 1987 (cruise # 1); Phe: phenanthrene, An: anthracene, Flt: fluoranthene, Pyr: pyrene, BaA: benzo(a)anthracene, Chr: chrysene, Per: perylene, BkF: benzo(k)fluoranthene, BaP: benzo(a)pyrene, dBahA: dibenz(a,h)anthracene, BghiP: benzo(g,h,i)perylene, InP: Indeno-pyrene
Fig. 5 PAH distribution in a selected Corsica sediment (Galeria) collected in the summer of 1988 (cruise # 2). Compound abbreviations as in Fig. 4

inducers could also play a role in MFO seasonal variations. In our conditions there were no significant changes in PAH pollution between summer and winter (cruises # 2 and # 3). However, temperature can be expected to play a role in the kinetics of pollutants in animal systems (Payne, 1984). The activities measured in fish from the Corsica sites were very high and not related to the level of PAH in sediments. We suspect the presence of a recent petrogenic contamination as evidenced by PAH profiles discussed in the previous paragraph. Due to the water residence time, hydrocarbons reach sediment after some time resulting in the specific PAH pattern observed in Corsica summer 1988 sediments.

4.3 Mussel

Generally, it would seem that, compared to fish, mussels are relatively insensitive to induction (Payne, 1977). However, recent studies (Livingstone, 1988; Suteau et al., 1988) have reported that consistent responses (2 fold magnitude) occurred in measurements of MFO activities in molluscs in mesocosm experiments. Marked elevation in cytochrome P-450 levels was found in mussels collected in hydrocarbon polluted bays in Brittany (Gilewicz et al., 1984). Our results in the Mediterranean sea are consistent with these studies and pointed out a high degree of correlation between MFO activities and PAH pollution gradient. Previous results from our laboratory (Suteau et al., 1985)
showed that the natural variability of PAH-metabolizing enzymes from *Mytilus galoprovincialis* in the Atlantic Coast was about 1.5 fold. No significant seasonal changes were found in mussels from Mediterranean Sea. We can note that as for fish, the three Corsica locations (cruise #2) exhibit high values of enzyme activities not related to PAH content in sediments.

4.4 *Posidonia*

In recent years the metabolism of xenobiotics in plants has received more attention. Many of the environmental chemicals are presumably biotransformed by plants in a manner similar to that of the mammalian microsomal activation system (Higashi, 1985).

Frear et al. (1969) confirmed the presence of mixed function oxidases in the extract from etiolated cotton seedling hypocotyls. The studies of Russel (1971) have shown the involvement of Cytochrome P-450 in the conversion of trans-cinnamate to 4-hydroxycinnamate (CMH) in pea seedling. Reichhart et al. (1980) reported that this enzyme activity was enhanced in higher plants by cytochrome P-450 inducers.

Fig. 6 Plot of specific PAH ratios for several sediments from various sampling cruises. Note the deviation of 1988 sediments from Corsica Island.
such as phenobarbital, ethanol and herbicides. Our results indicated that CA4H can be measured in marine plants such as Posidonia and a significant difference was obtained between slightly polluted sites and highly polluted stations. However, a very significant relationship between MFO activity in plant and pollution gradient remains to be validated in future field studies.

5. CONCLUSIONS

EROD measurements in common perch Serranus cabrilla liver and BaP4MO activity in whole mussel Mytilus galloprovincialis gave sensitive response to PAH pollution gradient over broad geographical areas as indicated by the PAH content in sediments. Moreover, the magnitude of MFO induction was nearly the same in these two species. The strong induction of MFO activities found in Corsica sites during cruise # 2 demonstrated that this biochemical index is of sufficient sensitivity to discriminate point pollutions chiefly in low polluted areas. Our results clearly show that different species from a marine ecosystem (vertebrates, invertebrates and plants) are sensitive to PAH gradient pollution. Moreover, specific aromatic compound ratios demonstrate to be very useful in the identification of contamination sources in the studied sediments.

Thus, field trials with a number of species have now established that the induction of MFO enzymes can be useful as a monitoring tool to assess unpairoed quality of sea water.

6. ACKNOWLEDGEMENTS

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7. REFERENCES


Frear, D.S., H.R. Swanson and F.S. Tanaka, 1969. N-demethylation of substituted 3-phenyl-1-methylureas isolation and characterization of a microsomal mixed function oxidase from cotton, Phytochemistry, 8:2157-2169


Higashi, K., 1985. Microsomal cytochrome P-450 in higher plant, GAM monograph on Cancer Research, 30:49-66


Russel, D.W., 1971. The metabolism of aromatic compounds in higher plants X. Properties of the cinnamic acid 4-hydroxylase of pea seedlings and some aspects of its metabolic and developmental control, J.Biol.Chem., 246:3870-3878

Salaün, J.P., A. Simon and F. Durst, 1986. Specific induction of lauric acid hydroxylase by clofibrate, diethylhexyl-phthalate and 2,4-dichlorophenoxyacetic acid in higher plants, Lipids, 21:776-779


DISTRIBUTION, ACCUMULATION AND TRANSFORMATION OF ORGANIC CONTAMINANTS IN AN EASTERN MEDITERRANEAN COASTAL AREA

by

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ABSTRACT

This study represents an attempt to define the fate of a group of hydrophobic contaminants in an oligotrophic system by assessing their introduction into the environment, their subsequent distribution within and between environmental compartments, and their degradation and modification within these compartments. The results demonstrate the role of physico-chemical factors in the distribution of the contaminants, as well as that of biological factors (ecological and physiological) in their incorporation and modification within the biotic phase.

1. INTRODUCTION

The Eastern Mediterranean Sea has been demonstrated to be subject to contamination by oil-derived compounds, arising principally from the activities of tanker traffic and other shipping using these waters (IOC, 1981; UNEP/IOC, 1988). The location of the Island of Crete (Fig. 1), may be expected, therefore, to render it susceptible to such contamination via weather-mediated long-range transport, in addition to that arising locally. The unique biological and physico-chemical conditions which exist in the area of Crete's coastal waters make it an important area for contaminant fate studies. In particular, the oligotrophic nature of its waters and lack of autochthonous industrial inputs is in contrast to the Western and Central Mediterranean areas which have formed the basis for the majority of such studies to date.

This study, therefore, is aimed at assessing the fate of hydrocarbons entering the waters, sediments and organisms present in the Port of Heraklion, and the wider area of the adjoining Bay of Heraklion.

The city of Heraklion, while not supporting large-scale industry, is the centre of highest population on the Island (150,000) and its Port serves both small-scale pleasure and fishing craft and larger passenger and cargo vessels. In addition, the majority of the city's untreated waste-waters enter the Port and its adjoining coastline via a series of outfalls which terminate at the water-line.
A preliminary assessment of the sources of major inputs to the area of the Port and Bay was made on the basis of accepted criteria for the identification of biogenic and anthropogenic compounds (Blumer et al., 1971; Brassell et al., 1977; Douglas et al., 1981; Voudrias and Smith, 1986) and followed by a study of the nature of uptake and deposition in organisms and sediments from these areas (Fig. 2).

2. MATERIALS AND METHODS

Organisms were selected in order to provide a cross-section of trophic levels as well as comparison with existing data on Mediterranean pollution. Thus, molluscs (Mytilus galloprovincialis, Thais haemastoma and Patella coerulea) as well as a crustacean (Pachygrapsus marmoratus) and fish species (Mullus surmuletus, Chelon labrosus and Boops boops) are represented.

All solvents used were pesticide grade (Merck, FRG), while cellulose extraction thimbles (Schleicher and Schuell, FRG), anhydrous sodium sulphate (Merck), Florisil 60-100 mesh (Fluka), Silica gel 70-100 mesh (Merck) and cotton wool were pre-extracted in dichloromethane prior to use.
Fig. 2(a) Heraklion Bay sediment sample sites and Mullus trawl locations (T1 and T6)

2(b) Heraklion Port sample locations. Pc= Patella, Mg= Mytilus, Pm= Pachygrapsus, Cl= Chelon, Bb= Boops, S1, S2, S3 = Sediment grab sample sites
Sodium sulphate was activated at 400°C for 4 hrs. All glassware and utensils were washed and rinsed with nitric/sulphuric acid prior to use.

2.1 Sample processing

2.1.1 Waters/Waste-Waters

Subsurface water samples (1-2 m) from Heraklion Bay were taken using the research vessel M.V. Philia at a depth of 30 m. Samples were collected in acid-washed glass containers and refrigerated until analysed. Port waters were sampled by hand and treated as above. Waste-waters were taken by hand from an outfall entering the area of the Port and refrigerated in glass containers containing formalin until analysed.

2.1.2 Sediments

Marine sediments from Heraklion Port and Bay were taken from the centre of a Smith-McIntyre grab deployed from the vessel M.V. Philia. Samples representing 0-2 cm depth were placed in foil-lined containers and stored at -30°C. The samples were freeze-dried after hand homogenisation and extracted as described above.

2.1.3 Biota

Collection

Three molluscs, one crustacean and three fishes were collected for analysis. The limpet *Patella coerulea*, the gastropod *Thais haemastoma* and the mussel *Mytilus galloprovincialis* were collected by hand using a stainless steel diver’s knife. The crab *Pachygrapsus marmoratus* was collected using a handnet. Samples were placed in bags or glass jars for transportation to the laboratory. After washing with distilled water to remove particles etc. the samples were dried on paper towels, placed in glass jars and frozen at -30°C prior to analysis.

The fish *Mullus surmuletus* were obtained by trawling at 30-50 m depth in the Bay of Heraklion. The fish were placed in bags and stored at -30°C until analysed. Port fish (*Boops boops* and *Chelon labrosus*) were obtained by line fishing and treated as above.

Preparation

*Patella coerulea*, *Mytilus galloprovincialis* and *Thais haemastoma* were individually weighed, measured and shelled. *Pachygrapsus marmoratus* individuals were weighed and measured before removal of the carapace. Fish samples were rinsed thoroughly with distilled water, the skin discarded and muscle tissues removed for analysis. Pooled samples were homogenised and freeze-dried, or weighed and dried with sodium sulphate before extraction.

2.2 Extraction and quantitation

2.2.1 Sediments and tissues

Sediments and biological tissues were treated according to Berthou
et al. (1984). After Soxhlet extraction with dichloromethane, the concentrated extracts were cleaned on a Florosil column using pentane. Subsequent separation was effected on silica with aliphatics eluting in hexane and aromatics in hexane/dichloromethane.

2.2.2 Waters, waste-waters and air filters

Water samples (20 litres) were passed through 0.45 µm filters. The aqueous fraction was liquid-liquid extracted, while particulates were Soxhlet extracted with dichloromethane. Separation and purification were carried out according to Peltzer et al. (1984). Aliphatics were eluted on silica with hexane, while aromatics were removed using hexane/toluene. After Soxhlet extraction with dichloromethane, air filter extracts were treated as for waters. Waste-water subsamples (1 litre) were liquid-liquid extracted with dichloromethane and the evaporated extracts separated and purified as above.

2.2.3 Quantitation

Quantitation of sample extracts was carried out by the addition of internal standards (chlorohexadecane and hexamethylbenzene). Periodic extraction blanks were analysed to ensure the absence of contamination. All results were corrected for column retention and extraction efficiency.

2.3 Gas chromatography and GC-MS

Gas Chromatography was carried out using a Hewlett Packard HP 5890 operated with a flame ionization detector (FID). A capillary column with SE-52 stationary phase was used for quantitative determinations. Samples of 1-2 µl were injected in splitless injection mode. The temperature programme consisted of injection at 70°C (held for 1 minute) with split valve opening after 30 secs. Split ratio was 1/30 with a flow rate of 50 ml min^{-1}.

After solvent elution, the temperature was increased to 290°C at a rate of 10°C min^{-1} for 10 mins., followed by 5°C min^{-1}. Hydrogen carrier gas was used at a back pressure of 0.8 atm. Retention factor for the compounds was calculated to be 1.2. Integration and data handling were performed using HP Chemstation.

Mass spectrometric determinations were carried out using a Finnegan Model 4000, with an INCOSS 200 data system. A Carlo Erba 4160 gas chromatograph with a Grob-type split-splitless injector, with SE-54 fused silica capillary column (25 m x 0.25 mm) coupled directly to the ion source by a fused silica capillary was employed for GC-MS determinations. A helium carrier gas with back pressure of 0.8 atm. was used. The electron impact ionisation mode conditions were: Ionis. energy 70 eV, Ionis. source temp. 250°C, mass range 45-590 m/z or in the selected ion monitoring mode for PAH, scan time 1.9/sec with electron multiplier voltage 1700 V.

Chromatographic conditions for GC-MS were as given for GC above.
3. RESULTS AND DISCUSSION

In view of the aims of the research, the assessment of environmental samples was carried out with respect firstly, to known sources of contamination (such as atmospheric fallout and waste-water inputs), and secondly to other matrices where contaminant source was likely to be less clearly discernable.

3.1 Atmospheric particulates

The results of air filter analyses (Fig. 3) are characterised by the presence of both an unresolved complex mixture (UCM) with a strong high molecular weight component (nC25-36), and an n-alkane profile dominant in odd-numbered compounds. While the incomplete combustion of fuels may lead to such a UCM profile, the n-alkane distribution is indicative of a contribution from both marine algal (the low molecular weight odd-numbered homologues) and terrigenous higher plant (nC25-31) sources. A combined input from anthropogenic, petroleum-derived and biogenic marine aerosol-derived sources is therefore implied. However, while atmospheric transport of contaminants between land and marine compartments is implied by these results, levels of hydrocarbons entering coastal waters by this route should be low relative to those stemming from other sources such as waste-waters. Further analyses and experiments on bulk deposition of contaminants by this route are planned.

A further indication of the presence of combustion products in air particles is provided by the presence of PAH. The series of PAH identified from filter samples (Fig. 3) implicates vehicle emission products as a likely source, however, other factors such as long-range transport from forest fires must also be considered. Comparison with PAH in Bay sediments (and indeno[1,2,3-cd]pyrene/benzo[ghi]perylene ratios) give further evidence for this route in coastal contamination (Randahl et al., 1984).

3.1.1 Waste-Waters

Distribution of aliphatic hydrocarbons in waste-waters can be seen to comprise a clear anthropogenic high molecular weight UCM on which a predominantly low molecular weight n-alkane profile is superimposed (Fig. 4). As demonstrated by previous studies (Zurcher et al., 1980) such a profile matches that of motorway runoff, as well as being similar to that seen in the air particulates from Heraklion (Stephanou, in press). The striking similarity of this distribution to that of a used engine (sump or crankcase) oil (Voudrias and Smith, 1986) however, provides a more likely source for this contamination given the climatic features of the region at the time of sampling. The observed levels (0.80 mg l\(^{-1}\) total aliphatics) represent a significant localised input which would be expected to produce tangible effects in the receiving system.

Bearing in mind the above data on inputs from the atmosphere and urban wastes, the nature of hydrocarbon contaminants in receiving waters, sediments and associated biota may be assessed in relation to autochthonous biogenic (marine algal), allochthonous biogenic (possible marine algal and terrigenous higher plant), and anthropogenic inputs of a variety of crude, refined and combusted petroleum products. The
Fig. 3 Aliphatic and aromatic air filter data
Fig. 4 Port water and waste-water chromatograms. X = possible co-elution
complexity and variety of both the possible inputs and their environmental fates makes definitive source determination difficult, however, the physico-chemical processes operating on key environmental compartments (such as the particulate phase of the water column) may be used in conjunction with data on contaminant distributions to provide an insight into the nature of the dominant contaminant/environment interactions operating to distribute and transform contaminants within and between environmental compartments.

3.1.2 Waters

Analysis of the aqueous and particulate fractions of waters from within Heraklion Harbour and the wider area of Heraklion Bay (Fig. 5 and Table 1) demonstrates the importance of suspended particulate material in effecting the partitioning of hydrophobic contaminants in the aquatic environment and hence their importance in abiotic/biotic interactions. In accordance with results from previous studies (Saliot et al., 1985; Gomez-Belinchon et al., 1988) levels are higher in the particulate than the dissolved phase with more evidence of biogenic input than the dissolved phase, which is strongly petrogenic in its n-alkane and UCM profiles. The dominance of the high molecular weight component in the aqueous phase of these samples may result from the inclusion of the surface microlayer in the sample.

The importance of the role of suspended particulates in determining the partitioning and fate of hydrophobic organic contaminants is perhaps emphasised in such oligotrophic waters.

The presence of a large amount of biological material (phytoplankton and zooplankton) in particulates has frequently been shown to influence contaminant fate. Parameters such as rate of removal of contaminants from water column to sediments by incorporation in zooplankton faecal material, adsorption and transfer of contaminants as well as (in the case of rapidly degraded compounds such as the n-alkanes) alteration within the water-column exhibit strong seasonality in response to these factors (Osterroth and Smetacek, 1980; Burns and Villeneuve, 1983).

Although further studies are needed to elucidate the effects and flux of these complex processes, available data on organic carbon, ATP, chlorophyllous pigments and sediment particle size distributions within the Bay of Heraklion (Eletheriou, 1990) are consistent with a trend in distribution of hydrocarbons in surficial sediments in conjunction with such biological material.

For example, maximum concentrations occur at 30 m depth across the Bay, with transect H3 (coincident with the city of Heraklion and it's Port as shown in Fig. 2) showing highest levels of both n-alkanes and unresolvables.

Thus the combination of physico-chemical processes acting on particles such as spatial redistribution by hydrodynamic factors, as well as phase boundary properties (eg. adsorption/desorption of hydrophobic contaminants) operate to determine bioavailability, while the ultimate fate of the compound within the biological compartment is dependant on factors such as ecology and physiology of the organism.
Fig. 5 Heraklion Bay water samples. P:P = Pristane/Phytane, CPI = Carbon preference index, TRH = Total resolvable hydrocarbons
Table 1
Summary of Hydrocarbon Data 1988-90

HERAKLION PORT

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>TRA</th>
<th>UCM</th>
<th>TOTAL ALIPHATICS</th>
<th>TOTAL AROMATICS</th>
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<td>SEDIMENT (0-3cm)</td>
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<tr>
<td>WATER</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>8.0</td>
<td>20.3</td>
<td>1.0</td>
</tr>
<tr>
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<td>21.5</td>
<td>28.7</td>
<td>-</td>
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<tr>
<td>WASTE-WATER</td>
<td>202</td>
<td>380</td>
<td>793</td>
<td>533</td>
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</table>

BIOTA

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<th>Species</th>
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<th>TOTAL AROMATICS</th>
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<tr>
<td>M. galloprovincialis</td>
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<td>70-188</td>
<td>95-294</td>
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<tr>
<td>P. maronatus</td>
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<td>-</td>
<td>-</td>
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</tr>
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<td>B. boops</td>
<td>8.5</td>
<td>62.5</td>
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</table>

HERAKLION BAY

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<th>SEDIMENT (0-2 cm)</th>
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<th>TOTAL ALIPHATICS</th>
<th>TOTAL AROMATICS</th>
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<td>1.9</td>
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<tr>
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</tr>
<tr>
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<td>1.2</td>
<td>3.4</td>
<td>0.8</td>
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<td>Particulates</td>
<td>1.0</td>
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<td>9.3</td>
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BIOTA

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<th>Species</th>
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<th>UCM</th>
<th>TOTAL ALIPHATICS</th>
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<td>122</td>
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<tr>
<td>M. surmuletus</td>
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<td>36</td>
<td>57.5</td>
<td>9.4</td>
</tr>
<tr>
<td>T. heamastoma</td>
<td>55.0</td>
<td>-</td>
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</tbody>
</table>

* Water and Waste-Water values given as ug/L

Sediment and Biota values as ug/g (Dw.t.)

TRA = Total Resolvable Aliphatics
UCM = Unresolved Complex Mixture

3.1.3 Biota

The consequences (in terms of bioavailability) of the physico-chemical partitioning of contaminants between aqueous and particulate phases may be demonstrated by the results obtained from the analysis of tissues from the mussel (Mytilus galloprovincialis) and the marine limpet (Patella coerules) shown in Fig. 6. Aliphatic
Fig. 6 Port sediment and biota chromatograms. X = unknown
hydrocarbon distribution in *Mytilus* demonstrates uptake of predominantly low molecular weight compounds (i.e. those eluting before C25) while that of *Patella* is similar to waste-water and sump oil distributions. *Mytilus*, a filter-feeding bivalve effectively sieves particulates from the water-column. Studies such as that of Salio et al., (1985) have demonstrated the preferential partitioning of low molecular weight contaminants onto small particulate size fractions, hence the enhanced uptake of lower molecular weight compounds seen in *Mytilus* may result from such physiological factors. However, since the present study based the criterion of dissolved and particulate components on separation by 0.45 μm filter, such contaminant/particle size associated differences may only be inferred.

The highly anthropogenic UCM distribution observed in *Patella* may also derive largely from the feeding mechanism of this organism. As a micro-algal grazer, *Patella* is in close association with surfaces and hence, contaminants which are preferentially adsorbed to surfaces (and also more strongly lipophilic) are likely to be available to this organism.

As mentioned above one consequence of aqueous/particulate partitioning of contaminants in the water-column is their removal to sediments. Comparison of data from particulate and sediment samples from corresponding areas (Figs. 4 and 6) shows the extent of this relationship. An important feature related to both water-column processes and the sedimentary load of contaminants is their relative significance to pelagic and benthic macrofauna. Many studies on the uptake and removal of hydrocarbons in marine organisms have identified direct uptake from the water-column as the dominant route in most situations (Lee, et al., 1972; Oppenhuizen, in press) on the basis of the slow rate of desorption of contaminants from sediments and food particles in the intestine. In this respect the results of analysis of fish tissues from both the Port and Bay (Fig. 7) are interesting, particularly in the distribution of high molecular weight n-alkanes in muscle tissues.

*Mullus surmuletus* is a benthic feeding fish which is hence in close association with bottom sediments in the Bay of Heraklion. The n-alkane profile (a reflection also of the UCM distribution for this organism) is bimodal with evidence of terrigenous, biogenic input, a profile closely resembling that of sediments taken from the same area. *Chelon labrosus* (a semi-pelagic species inhabiting the Port) shows a contaminant profile differing in its n-alkane and UCM distributions mainly in the content of higher molecular weight compounds. While this species, by its ecology, is likely to have less contact with bottom sediments than *Mullus*, all individuals analysed had gut contents comprising sedimentary material and hence a contribution from intestinal uptake of ingested contaminants cannot be ruled out. Finally, the distribution in *Boops boops* (a pelagic species, also inhabiting the Port) has a clear predominance of low molecular weight compounds. While these results may indicate the presence of uptake via the digestive tract in benthic-feeding species, it is also possible that differences in the levels and distribution of these contaminants described are the result of a differential distribution in the water column. Thus bottom waters containing a contaminant profile more closely mimicking that of the upper sediment layer may allow direct uptake from the water column to occur with the observed results.
Fig. 7 Port and Bay fish n-alkane values. P:P = Pristane/Phytane ratio, CPI = Carbon preference index, UCM = Unresolved complex mixture, TRH = Total resolvable hydrocarbons.
Further assessment is required, however, if a full explanation of these differences in contaminant distributions is to be made.

3.1.4 Aromatic compounds

In addition to the above data on aliphatic hydrocarbons, samples were also analysed for the presence of aromatics. Results of PAH analysis from atmospheric particulates revealed the presence of compounds indicative of a combustion source (Fig. 3). PAH in organisms sampled from both the Port and Bay, however show a PAH content likely to stem from a fuel-oil rather than combustion source (Fig. 8). The reasons for these differences remain unclear and further investigation of samples from these areas is under way, however it is possible that, as for aliphatic hydrocarbon distribution in *Mytilus*, particle size mediated partitioning is demonstrated.

GC/MS analysis of samples also reveals the presence of aromatic contaminants likely to stem from the presence of Linear Alkyl benzene Sulphonates (anionic detergents widely used in commercial and domestic products).

The compounds identified are believed to be desulphonation metabolites of LAS (Eganhouse et al., 1983), the presence of which in the marine environment can be linked to introduction via waste-waters (Takada and Ishitari, 1987). The contaminants identified from environmental samples in this study contain lateral alkyl chains from C11 to C14. The m/z91 mass chromatogram of a *Mytilus* sample is shown in Fig. 8. These compounds are also present in samples of Port sediment and particulates from the Port water samples. Thus introduction, transfer and transformation of these compounds (at least within the Port area) is demonstrated and their wider distribution in both biotic and abiotic phases should be assessed.

4. CONCLUSIONS

The data obtained by this study allows the identification of major hydrocarbon contaminant sources affecting the coastal environment of the Bay of Heraklion and the Port of Heraklion itself. In addition, data indicating the nature of physico-chemical environment/contaminant interactions and their influence on bioavailability, transfer and transformation of contaminants within and between biotic and abiotic phases highlight the necessity for further research into these phenomena.

5. ACKNOWLEDGEMENTS

This study was supported by a UNEP/FAO MED Trust Fund contribution and the Ministry of Urbanism, Environment and Public Works of Greece.
Fig. 8 Single ion monitoring (SIM) trace of alkylated benzenes in mussel (M. galloprovincialis) - Heraklion Port

6. REFERENCES


**Posidonia oceanica**: UPTAKE AND MOBILIZATION OF MERCURY IN THE MEDITERRANEAN BASIN

by

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**ABSTRACT**

In studying the biogeochemical cycle of mercury in the Mediterranean basin the capability of the marine phanerogam *Posidonia oceanica* and of its epiphytes to uptake and accumulate the metal has been demonstrated.

In this work a Hg-roots/Hg-sediment correlation has been observed, which shows that the uptake of the metal occurs mainly through the root system, even if it is not possible to exclude that the metal can also be taken up from the water by the leaves.

We have observed also a Hg-epiphyte/Hg-leaf correlation probably due to a limited form of parasitism between the epiphytes and their substrate.

Taking into account the high primary productivity (21 ton/ha/yr) and the large extension (50,000 sq. km) of *Posidonia oceanica* prairies in the Mediterranean basin, the total mercury amount (9.5 ton/yr) mobilized from the sediment to the aquatic environment has been evaluated.

1. **INTRODUCTION**

As a part of the study of the biogeochemical cycle of mercury in the Mediterranean basin, research has been carried out on the role of *Posidonia oceanica* prairies in the mobilization of mercury from the sediment to the marine environment.

The marine phanerogam *Posidonia oceanica* forms large prairies which represent a dynamic substrate that exceeds the area of the sediment surface several times over and allows settlement of epiphyte organisms.

The importance of *Posidonia oceanica* in the Mediterranean basin is reflected by the large amount of literature dealing with its growth and production (Pirc, 1985; Whittmann, 1984). In fact, in this basin, *Posidonia oceanica* prairies display a productivity comparable to that of the European forests, of the cereal cultivations and of the large Atlantic meadows of seaweed (Boudouresque and Meinesz, 1982).
Posidonia oceanica prairies form a nearly continuous belt along the two basins of the Mediterranean; to the West they disappear near the strait of Gibraltar and to the East they are absent from the sea of Marmara and from the Black sea. They extend exclusively from a few meters from the coastal belt up to 30-40 m depth, occupying an area of approximately 50,000 square kilometers.

The high productivity makes the plant important for the food web, especially by releasing vast quantities of detrital leaves, which are deposited and consumed directly in the prairies or are exported into adjacent ecosystems (Ott and Maurer, 1977; Novak, 1982). The productivity values are slightly different for the various Mediterranean areas observed due to the high sensitivity of the plant to changing local and temporal factors of its environment (Giaccone et al., 1981). According to studies reported by Boudouresque et Meinesz (1982) a considerable contribution to the productivity is due not only to the leaves but also to the epiphyte communities. The primary productivity value of the marine plant (Table 2) for the Port-Cros bay (France) has been considered as representative of the mean value of the plant productivity in the Mediterranean.

Taking into account the high values of mercury concentration in the coastal Mediterranean sediment (Bargagli et al., 1988), particular attention was devoted to the interaction between the sediment and this benthic organism. In some studies the capability of Posidonia oceanica to uptake mercury from the sediment and to accumulate the metal in its various organs was evidenced (Augier et al., 1977; Maserti et al., 1988; Sanchiz et al., 1990).

2. EXPERIMENTAL

The Posidonia oceanica plants were collected, together with their sediment and water, at a depth of 8-10m from 5 sampling areas selected on the basis of the sediment mercury content; the sampling took place in the summer of 1988 and 1989. Sampling sites 1-4 are located in the Tyrrenhenian sea (Tuscan Archipelago), whereas sampling site 5 is located in front of a chlor-alkali plant (R. Solvay, Livorno).

After sampling, the leaves were detached from the rhizomes and roots and were examined separately. Each leaf was subdivided into two parts: the photosynthesizing part and the basal part. The green part was then scraped in such a way as to collect the epiphytic component.

While in previous work (Maserti et al., 1988) mercury concentrations were expressed on a fresh weight basis, the use of dry weight has given more comparable and reproducible results.

Samples were oven-dried at 40°C for 72 hours or using a microwave oven (80 W for 20 min). Both methods have given comparable results. Each sample (1 g, d.w.) was mineralized with a mixture of 5 ml HNO₃ and 5 ml H₂SO₄ in a reflux system at 120°C for 60 min.

Sediment samples (sand or sandy clay) were sieved and 1 g, d.w. of the fraction <250 μm was mineralized with a mixture of 5 ml HNO₃, 3 ml H₂SO₄ and 2 ml HCl under reflux at 120°C for 60 min.
All mercury determinations were done using atomic absorption spectroscopy.

The water samples were filtered and treated according to the methodology described elsewhere (Seritti et al., 1980). Mercury determination was achieved by atomic fluorescence spectroscopy (Ferrara et al., 1980).

From each area studied, 5 samples of plant, sediment and water were collected and analysed separately.

3. RESULTS AND DISCUSSION

Table 1 shows the average mercury concentration values and the relative standard deviation in the marine phanerogam and its epiphytes, as well as in the surrounding environment. The reported data represent the mean value of mercury concentration measured during three years of sampling. The following observations can be made as far as the data reported in Table 1 are concerned:

- as already pointed out in a previous paper, the organs of Posidonia oceanica which are in close contact with the sediment, present the highest mercury levels which can be correlated with the concentration of the metal in the sediment.

- the mercury levels in the green and basal part of the leaf appear to be dependent on the mercury levels in the sediment.

- the mercury concentration in the epiphytes is of the same order of magnitude as that determined in the leaves.

- the dissolved mercury levels in sea water are similar in the different areas examined; highest values have been observed in sampling area 5, which is located in the surrounding of a chlor-alkali plant.

Taking into account the correlation between mercury levels in sediment and in Posidonia oceanica roots, and the fact that dissolved mercury concentration in sea water in all examined areas are relatively comparable, it seems that the uptake of the mercury in Posidonia oceanica occurs mainly through the root system. This is probably the primary pathway of mercury, but it is not possible to exclude that the metal can also be taken up from the water at lower rate by the leaves.

The similar behaviour of mercury concentration observable in the epiphytes and in the leaves was at first found to be surprising; indeed it was logical to assume that the mercury levels in the epiphytes organisms would be closely related to those in water, and hence comparable between them in the different sampling areas.

The mercury content in the epiphytes may be explained by the following considerations:
Table 1

Mercury concentration in water (D=dissolved in ng l⁻¹, P=particulate in ng l⁻¹), sediment (µg g⁻¹ d.w.) and Posidonia oceanica (µg g⁻¹ d.w.).

<table>
<thead>
<tr>
<th>Sampling area</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiphytes</td>
<td>0.06</td>
<td>0.05</td>
<td>0.02</td>
<td>0.08</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.05</td>
</tr>
<tr>
<td>Leaf's green part</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.10</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.06</td>
</tr>
<tr>
<td>Leaf's basal part</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>0.08</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.04</td>
</tr>
<tr>
<td>Scales</td>
<td>0.02</td>
<td>0.05</td>
<td>0.04</td>
<td>0.10</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.01</td>
<td>±0.03</td>
<td>±0.05</td>
</tr>
<tr>
<td>Rhizome</td>
<td>0.02</td>
<td>0.03</td>
<td>0.06</td>
<td>0.11</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.03</td>
</tr>
<tr>
<td>Root</td>
<td>0.02</td>
<td>0.03</td>
<td>0.07</td>
<td>0.18</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.03</td>
</tr>
<tr>
<td>Sediment</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
<td>0.24</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.04</td>
<td>±0.08</td>
</tr>
<tr>
<td>(D)</td>
<td>2.5</td>
<td>4.2</td>
<td>3.5</td>
<td>3.8</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>±0.5</td>
<td>±0.6</td>
<td>±0.4</td>
<td>±0.5</td>
<td>±0.3</td>
</tr>
<tr>
<td>Water (P)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.2</td>
<td>±0.9</td>
</tr>
</tbody>
</table>

- A limited form of parasitism may exist between the epiphyte organisms and the leaves of Posidonia oceanica. From some investigation (McRoy et al., 1973; Fesli and Saggiomo, 1981; Libes, 1984) it appears that, as the epiphyte grows, it anchors itself more and more firmly to the leaf until it partially penetrates into the tissues of the host. This might generate a physiological exchange between epiphyte and leaf.

- The levels of dissolved mercury concentration in sea water could influence the mercury concentration in the epiphyte community.

- Particulate matter present in suspension in the water may adhere mechanically to the epiphyte organisms. In this case, the amount of mercury measured would represent the sum of the fraction accumulated by the organisms and that which mechanically adheres to the epiphyte felt.
As present it not possible to distinguish between the two components; the hypotheses presented above may also exist at the same time.

However, in assessing the contribution of the epiphyte community to the mercury cycle, it is only necessary to know the total amount of mercury mobilized by these organisms in the marine environment. In order to evaluate the mercury mobilized by Posidonia oceanica prairies from the sediment in the Mediterranean basin it is necessary to know their primary productivity and the average mercury concentration in the plant itself, in the epiphytes and in the coastal sediments.

It is well known that the coastal sediments of the Mediterranean basin present higher mercury concentration levels than those measured in deep water (Bargagli et al., 1988). This fact is a consequence of the large geochemical anomalies, mainly consisting of cinnabar deposits, that make the sediment of the continental shelf rich in mercury. The terrigenous fluvial supply and industrial discharges also contribute to increasing the mercury levels in the coastal sediment. An average mercury value of 0.2 μg g\(^{-1}\) d.w. can be considered as typical for the Mediterranean coastal sediment. This values is similar to that observed in sampling area 4; taking into account the mercury levels in the marine phanerogam collected in this area, the extent of the Posidonia oceanica prairies and their primary productivity values, it was calculated that an amount of 5.9 ton/year of mercury is mobilized by the leaves and 3.6 ton/year by the epiphytes (Table 2).

The total quantity of 9.5 ton/year of mercury mobilized in the entire Mediterranean basin from the Posidonia oceanica prairies is about a quarter of that which is exchanged through the Strait of Gibraltar, assuming a difference of mercury concentration between the outflowing Mediterranean and inflowing Atlantic water of 1 ng l\(^{-1}\) (Ferrara and Maserti, 1988).

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Productivity values of Posidonia oceanica prairies; mercury concentration in the plant and epiphyte for a sediment mercury concentration of 0.2 μg g(^{-1}) d.w.; amount of mercury mobilized by an hectare of prairie in a year; total amount of mercury mobilized in the Mediterranean basin.</td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td><strong>Productivity</strong> Kg d.w. h(^{-1}) year(^{-1})</td>
</tr>
<tr>
<td>Leaves</td>
</tr>
<tr>
<td>Epiphytes</td>
</tr>
</tbody>
</table>
4. CONCLUSIONS

The marine phanerogam Posidonia oceanica is capable of uptaking and accumulating mercury; this occurs chiefly through the interaction between the root system and the sediment. The mercury concentration in the epiphytes felt is correlated with that in the plants. This result seems to demonstrate some form of parasitism between epiphyte organisms and leaves.

It is estimated that an amount of the order of 9.5 ton/year of mercury is mobilised in the Mediterranean basin by the Posidonia oceanica prairies. This confirms the importance of the study of the interaction between sediments and benthic organisms that live in mercury-rich areas such as the coastal ones. The far-reaching implications of this phenomenon could play an important role in the biogeochemical cycle of mercury in the Mediterranean basin.

5. ACKNOWLEDGEMENTS

This work was partially supported through the MED Trust Fund (Project FAO/MEDPOL/ITA/104-K).

6. REFERENCES


Boudouresque, Ch.F. and A. Meinesz, 1982. Découverte de l’herbier de Posidonia. Cahier Parc National de Port Cros, no. 4: 1-80


THE BIOACCUMULATION OF HEAVY METALS AND PETROLEUM HYDROCARBONS IN MUSSELS FROM POLLUTED AND UNPOLLUTED AREAS OF SARONIKOS GULF

by

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²National Centre for Marine Research, Aghios Kosmas, Hellinikon, Athens 166 04, Greece

ABSTRACT

Petroleum hydrocarbon and heavy metal pollution in the coastal waters of the Saronikos Gulf, adjacent to the city of Athens in Greece, was monitored by analysis of mussels (Mytilus galloprovincialis). The concentration of polycyclic aromatic hydrocarbons (PAHs) and Cu, Cr, Ni, and Cd in the soft tissue of the mussels was determined, after wet digestion, by UV-Fluorescence and Atomic Absorption Spectroscopy.

Mussels were collected at three sampling sites at well defined distances from the main point pollution sources. Statistical treatment of the measured concentrations of PAHs and heavy metals was performed by analysis of variance. As far as the Cu, Cr and PAH concentrations are concerned, a gradient from North to South of the Gulf is observed. In addition, the concentrations are strongly affected by the presence of a local source near Aegina island. Ni concentration presents a uniform distribution.

1. INTRODUCTION

A polluted environment, particularly with respect to PAHs and heavy metals, poses serious problems to public health and ecosystems. Bivalves, filter feeder organisms, are considered sentinel organisms in monitoring the acute and chronic environmental pollution (Kidder, 1977). It does seem that molluscs reflect the environmental metal regime and that their tissues reach equilibrium with the external media (Boyden, 1977). This relationship has already been demonstrated for macro-algae (Bryan and Hummerstone, 1973) and is the conceptual basis of biological monitoring. The use of bivalves as pollution indicators of petroleum hydrocarbons depends on the ability of the organism to reflect the composition of its environment, although the exact relationship between concentration in bivalves and concentration in surrounding ecosystems is, as yet, unknown.

The results presented here were derived from a study on the assessment of the sources of PAHs and heavy metals and their concentration in bivalves; temporal and spatial distribution as well as bioaccumulation factors were also determined since 1988.
2. METHODOLOGY

The presence of natural bivalve populations (*Mytilus galloprovincialis*) as well as the relative location of sampling stations to the point pollution sources in Saronikos Gulf [e.g. the sewage outfall of Athens metropolitan area, refineries and other industrial outfalls (see Fig. 1)] were the main factors on which the choice of sampling stations was based.

As the age and consequently the length of mussels is a significant factor for bioaccumulation, all results refer to individuals with a defined length range (2.0 to 4.5 cm).

As far as the determination of PAHs and heavy metal concentrations is concerned, the recommended by IOC and UNEP techniques were followed (UNESCO, 1982; UNEP/FAO/IAEA, 1982). Mussel samples, collected by scuba diving from a depth of 0.5 - 2.0 m, after byssus removal, were washed out with distilled water to remove any traces of impurity or sediment in order to avoid analytical errors (Bernhard, 1976). Composite samples containing the soft parts of 6 to 10 individuals were prepared. All samples were immediately frozen and kept at -10°C until freeze drying and homogenization.

For the determination of PAH concentrations a quantity of freeze dried homogenate, was centrifuged with 15 ml 6N NaOH aq. solution. The centrifuge tubes were maintained at 25°C for 18 hours. The mixture was extracted with 3 x 15 ml of ethyl ether and the combined extracts, dissolved in n-hexane, were chromatographed from an alumina (deactivated with 5% water) column. Four fractions were collected and their fluorescence intensity was measured using a MFP 44A spectrofluorimeter.

The determination of heavy metals was made by digesting approximately 0.5 g of dried soft tissue with 5 ml nitric acid into appropriate vessels, under pressure at 120°C, for 12 hours. A Varian AA 157 Atomic Absorption Spectrophotometer was used.

3. RESULTS

Table 1 summarizes the results obtained for the concentrations of Cu, Cr(total), Cd and Ni in 145 bivalve samples as well as the concentrations of PAHs in 61 samples. The number of analysed replicants from each station, the average value as well as the standard deviation are also included in Table 1. The last column of this table includes the calculated bioaccumulation factor "\( K_{Bcf} \)" expressed as mg g\(^{-1}\) of lipids assuming a mean lipid content of mussels of the order of 15% dry weight (Burns and Smith, 1981). It is preferable to express the bioaccumulation factor on a lipid basis as the concentrations of hydrocarbons in mussels are more consistent when expressed on a lipid basis regardless of season (Smith and Burns, 1978). Also, variation in results seems to be reduced by expressing them on a lipid basis. It is worth noting that the different observed maxima of uptake (\( C_{\text{max}} \)) in populations of oysters with different lipid content (exposed to same
hydrocarbon concentration) appear equal if the results are expressed on the basis of uptake per gram of body lipids (Stegeman and Teal, 1973).

The highest concentrations for Cu and Cr were observed at station C_8A while for Cd and PAHs at stations C_{10} and C_3 respectively. On the contrary, a uniform spatial distribution was noted as far as the concentration of Ni is concerned (Table 1).

The results were statistically treated using One-Way analysis of variance (ANOVA) and the Least Significant Difference (LSD) test to investigate if the determined differences in concentrations of PAHs as well as of heavy metals (HMs) in bivalves in all three sampling stations were statistically significant or not. HM values, as they do not follow the normal distribution pattern, they were transformed into log form. The results of this statistical treatment are found in Table 2. Figures 2 to 6 represent the confidence intervals of the means of HM and PAH values from which the existing differences among
Table 1

Average (Avg), standard deviation (Std) and range of heavy metal and hydrocarbon concentrations in mussels, expressed in μg g⁻¹ dry weight (K_Bcf5 = bioaccumulation factor of PAHs on a lipid basis).

<table>
<thead>
<tr>
<th>HM / PAHs</th>
<th>Cu</th>
<th>Cr</th>
<th>Cd</th>
<th>Ni</th>
<th>PAHs</th>
<th>K_Bcf5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>66</td>
<td>68</td>
<td>68</td>
<td>68</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>6.94</td>
<td>3.02</td>
<td>0.65</td>
<td>6.38</td>
<td>44.84</td>
<td></td>
</tr>
<tr>
<td>Std.</td>
<td>1.67</td>
<td>1.82</td>
<td>0.48</td>
<td>2.20</td>
<td>21.48</td>
<td></td>
</tr>
<tr>
<td>Min.</td>
<td>2.32</td>
<td>0.40</td>
<td>0.05</td>
<td>3.20</td>
<td>11.80</td>
<td></td>
</tr>
<tr>
<td>Max.</td>
<td>10.52</td>
<td>9.37</td>
<td>2.05</td>
<td>14.27</td>
<td>80.00</td>
<td></td>
</tr>
<tr>
<td>C₃</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.20x10⁴</td>
</tr>
<tr>
<td>n</td>
<td>56</td>
<td>55</td>
<td>55</td>
<td>56</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>7.11</td>
<td>5.89</td>
<td>1.07</td>
<td>7.27</td>
<td>39.23</td>
<td></td>
</tr>
<tr>
<td>Std.</td>
<td>2.05</td>
<td>2.15</td>
<td>0.58</td>
<td>2.27</td>
<td>16.24</td>
<td></td>
</tr>
<tr>
<td>Min.</td>
<td>1.00</td>
<td>1.90</td>
<td>0.06</td>
<td>3.00</td>
<td>7.29</td>
<td></td>
</tr>
<tr>
<td>Max.</td>
<td>10.77</td>
<td>13.64</td>
<td>2.41</td>
<td>14.42</td>
<td>63.10</td>
<td></td>
</tr>
<tr>
<td>C₈A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.36x10⁵</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>19</td>
<td>21</td>
<td>21</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>4.92</td>
<td>3.88</td>
<td>1.42</td>
<td>7.70</td>
<td>26.24</td>
<td></td>
</tr>
<tr>
<td>Std.</td>
<td>1.62</td>
<td>2.45</td>
<td>0.78</td>
<td>3.90</td>
<td>23.26</td>
<td></td>
</tr>
<tr>
<td>Min.</td>
<td>2.18</td>
<td>0.56</td>
<td>0.26</td>
<td>2.80</td>
<td>8.50</td>
<td></td>
</tr>
<tr>
<td>Max.</td>
<td>8.49</td>
<td>8.30</td>
<td>2.73</td>
<td>14.91</td>
<td>123.20</td>
<td></td>
</tr>
<tr>
<td>C₁₀</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.25.10⁵</td>
</tr>
</tbody>
</table>

Table 2

Statistical analysis (ANOVA) of HM and PAH bioaccumulation data (F = F ratio, P = significant level, L.S.D. = Least Significant Difference homogeneous groups).

<table>
<thead>
<tr>
<th>contaminant</th>
<th>F</th>
<th>P</th>
<th>L.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>11.587</td>
<td>&lt; 0.001</td>
<td>C₁₀ &lt; C₃ + C₈A</td>
</tr>
<tr>
<td>Cr</td>
<td>34.051</td>
<td>&lt; 0.001</td>
<td>C₃ + C₁₀ &lt; C₈A</td>
</tr>
<tr>
<td>Ni</td>
<td>1.917</td>
<td>0.1508</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>15.498</td>
<td>&lt; 0.001</td>
<td>C₃ &lt; C₈A + C₁₀</td>
</tr>
<tr>
<td>PAHs</td>
<td>7.140</td>
<td>0.002</td>
<td>C₁₀ &lt; C₈A + C₃</td>
</tr>
</tbody>
</table>

The sampling stations are clearly shown. A statistical significant difference on the 95% level for the estimated concentrations of PAHs and heavy metals in bivalves, except for Ni, is apparent from the data in Table 2 and Figs. 2 to 6.
Fig. 2 Confidence intervals of Cu means in mussels collected at three stations of Saronikos Gulf

Fig. 3 Confidence intervals of Cr means in mussels collected at three stations of Saronikos Gulf
Fig. 4  Confidence intervals of Ni means in mussels collected at three stations of Saronikos Gulf

Fig. 5  Confidence intervals of Cd means in mussels collected at three stations of Saronikos Gulf
Fig. 6 Confidence intervals of PAH means in mussels collected at three stations of Saronikos Gulf

4. CONCLUSIONS

Among the studied HMs, copper and chromium show similarities in distribution and characterize the pollutant load of the Athens metropolitan area sewage system wastes (Leondiadis, pers. com.). The concentrations of these metals at the sampling station C3A, rather close to the central sewage outfall, were found to be higher than those at C10 located further away (near Aegina Island) from the source of pollution. This observation supports the view, also expressed by other authors (Leondiadis, pers. com.), that the greater part of wastes from the Athens sewage outfall as well as of the industrial wastes of the region are transferred South into the open Saronikos Gulf creating a concentration gradient from North to South. On the other hand, a smaller part of the waste load is going up to the Elefsis strait, a fact supported, also, by the observed lower concentrations of copper and chromium in this area.

The pattern for copper and chromium also holds for cadmium. However, the concentrations of Cd at station C10 are remarkably higher, two times at least, than those at station C3. This fact is hard to explain since there are no pollution sources on the island except for some domestic effluents.

The distributions of the nickel concentrations in bivalves is rather uniform and is independent of the load from the Athens sewage outfall.
The concentrations of PAHs decrease gradually from the northern part of the Gulf, where the refineries are located, to the southern. This pattern is also supported by the $K_{BCF}$ values estimated for the three sampling stations. Additionally, these estimated $K_{BCF}$ values give us a relatively clear indication of the degree of pollution in the region. These values are found to be in the middle to the lower range of those values cited in the literature (Burns and Smith, 1981); in addition, it is well known that higher $K_{BCF}$ values are observed in an environment with lower hydrocarbon concentrations and therefore it can be assumed that the Saronikos Gulf environment is moderately polluted with an additional pollution point source to the refinery waste outfall, where the higher PAH concentrations were found.

5. REFERENCES


TRACE METAL ACCUMULATION IN MOLLUSCS: THE EFFECTS OF VARIABLES AND VARIABILITY ON SAMPLING STRATEGIES

by

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ABSTRACT

Factors affecting the trace metal content of mussels are discussed. These factors include size, season, temperature, salinity, location, and metal levels in the water. Inadequate control for the effects of these parameters during and after sampling can lead to erroneous results and can compromise the applicability of mussels in contaminant monitoring programmes.

Trace element concentrations in mussels tend not to be normally distributed and a log-transformation is usually required if data are to be used in statistical analyses - whether in testing for differences between sites or in testing for temporal change at a single site.

The implications of inherent variability i.e. variability remaining in a dataset after all confounding factors have apparently been accounted for, is discussed. Such variability can be quite significant and it varies from metal to metal.

This document is intended to promote discussion on how monitoring programmes can be formulated so as to minimise the effects of biological variation when designing and implementing sampling protocols for trace metals. The conclusions are valid, not only in molluscs but are applicable to other sentinel organisms.

1. INTRODUCTION

A common objective of many monitoring programmes is the measurement of change in ecosystems. A second objective, that of controlling the change, requires a sound understanding of how the ecosystem functions and how it responds to changes in any of the governing variables driving it.

In this context, the use of bioindicators can offer many advantages over the use of water and sediment analysis. The concept of bioindicators is not new. Fishermen have used them for thousands of years - even if only empirically. The complex cocktails with which many ecosystems are now being insulted require that we extract, often literally, rather more information from bioindicators than did our predecessors.

The use of bioindicators and problems associated with their use in aquatic monitoring programmes have been discussed by many authors, notably Phillips (1980). Marine mussels, Mytilus sp. have found
particular favour with scientists testing for changing pollutant levels and are an important feature of many monitoring programmes. The application of marine mussels in this field has continued for more than fifteen years, encouraged by the impetus and the results of the U.S. Mussel Watch (Goldberg, 1975; Goldberg et al., 1983).

Advantages of using mussels to monitor pollutant levels include (a) ease of analysis (since they concentrate pollutants in the soft tissues and shells), (b) the obtention of integrated records (since the levels of contaminants in the animals do not fluctuate as widely and as quickly as those in the surrounding water and tend to persist after the original source of contamination has disappeared), (c) the levels in the bioindicator can be better used to estimate contaminant effects than can the levels in the water, (d) estimates of food-chain transfer to predators, including man, can be made directly, (e) their sessile nature allows longterm field studies, including transplantation experiments, to be completed, (f) their reasonably long lifespan allows trends in pollutant levels to be identified and (g) their widespread distribution permits comparisons to be made between different locations.

Having noted the above, it then has to be admitted that it is optimistic. In the real world many problems need to be resolved before mussels can be considered as adequate bioindicators for contaminants in marine ecosystems. Many of the problems are site-specific - one does not always find mussels thriving where one would wish, for example. The responses of mussels to changing environmental and contaminant conditions need to be identified before an interpretation is ever made of the contaminant levels in the tissues. Such responses can also be site-specific or they may be more general. Even then, mussels may not be the correct choice for a particular monitoring programme. The reader is referred to the essay by White (1984) for an alternative evaluation of the use and abuse of mussels in marine monitoring programmes.

Trace element concentrations in marine molluscs can be modulated by a host of factors - both environmental and biological. Considering the genetic diversity, and the subsequent phenotypic variability, of natural assemblages of organisms in the environment, it is not surprising that metal concentrations in the organisms should also vary significantly between individuals. Although methods exist to reduce the manifold effects of these factors in monitoring programmes e.g. pooling of samples, often, when all other factors have been apparently accounted for, there remains some unexplained variability in the data. This has been termed 'inherent' variability and is probably due to modulating factors - either environmental or biological - which have yet to be identified.

In this document, the effects of environmental factors on metal levels in mussels are summarised. Biological factors, particularly animal size, modulating metal levels are also examined, and the implications of biological variation on the selection of adequate samples for analysis are explored.
2. ENVIRONMENTAL FACTORS

The effects of environmental factors on trace metal accumulation in molluscs are important since the broad spectrum of biological responses to environmental factors is a major determinant of the variability of the final parameter which one wishes to measure - in this case contaminant concentrations in the tissues. Furthermore, it is often difficult to separate the effects of environmental from biological factors since the organism is presumably adapted to the environment in which it is found and the times spent feeding, respiring, maturing etc. will depend on food supply, currents, daily exposure etc. Behavioral responses to environmental changes can also influence metal accumulation. Although environmental factors are site-specific, generalisations can often be made which apply to any site e.g. seasonal effects. The more important environmental factors modulating contaminant concentrations in mussels are discussed briefly below.

2.1 Water and sediment quality

Bioclipicators are not necessarily used as a substitute for analysis of metals in the water or sediments and it would be a mistake to assume that the levels of metals in the tissues should reflect the total concentrations in the water. Rather, the levels of contaminants in the tissues should reflect the levels of bioavailable species of the contaminants in the sediment or water. Copper is a good example, where the CuCl\textsuperscript{-} ion is the toxic chemical species and the other forms of copper in seawater are irrelevant. Another example is cobalt. Co-cobalamin is accumulated about three times as much by clams as is ionic cobalt even though it may represent only a small fraction of the total cobalt in the water (Lowman and Ting, 1983). Keckes et al. (1967) reported that ruthenium chloro-complexes are accumulated more rapidly by mussels than are ruthenium nitroso-complexes. Keckes et al. (1969) showed that anionic forms of ruthenium are accumulated to a significantly greater extent by mussels than are cationic and neutral forms.

Water and sediment composition are important not only in determining the physico-chemical forms of trace metals but also in determining the health of the organism, the availability of nutrients (and thus, indirectly, the growth rate and sexual maturation of the animal) and the competition for uptake with other metals. Particle composition and size distributions can affect the fractions of metals distributed between aqueous and particulate phases (IAEA, 1975) as well as the quantities of material filtered by mussels (Bayne, 1976). The organic carbon content of the sediment and the suspended particulate matter can also influence feeding, growth and metal accumulation patterns (Newell, 1970).

2.2 Location

The location of a sampling site is important for several reasons such as distance from sources of contamination, depth in the water column and position on the shoreline.
There have been many reports showing that, as one would expect, contaminant levels in bioindicators and in water and sediments, decrease with increasing distance from sources of contamination e.g. silver (Martin et al., 1988), zinc and cadmium (Phillips, 1976a).

Position of mussels in the water column has been shown by Fischer (1983) to significantly influence cadmium accumulation due to differences in food availability. Phillips (1976a) also reported significant water column effects for zinc, cadmium and lead in mussels. Kamimura (1980) reported that, in cultured oysters, copper and zinc levels in the tissues tended to decrease with depth whereas the concentrations of the two metals increased with depth. He attributed this discrepancy to differing qualities and quantities of available food at each depth examined.

Tidal coverage influences metal accumulation by limiting the periods in which mussels are submerged and in contact with contaminated water. Secondary effects, such as decreased growth, can also be expected due to diminished availability of nutrients and the requirements for anaerobic respiration during periods of emersion.

2.3 Season

It is generally accepted that seasonal effects can be detected in the concentrations of trace metals in molluscs (Phillips, 1980; Boyden and Phillips, 1981). Seasonal effects can be accounted for by annual patterns of food availability, of pollutant input, and, in coastal areas, of land drainage (Hsu and Wang, 1979). Secondary factors, such as salinity changes following heavy rainfall, and temperature, can also be important. Other factors operating in this context include changes in body weight of most animals during the year (with the deposition of reserves in late summer and their catabolism in winter) and the sexual cycle. It has been argued that, although changes in metal concentration may occur, it is not necessarily so for metal content (Phillips, 1976a). Boalch et al. (1981) concluded that even though dramatic changes in concentrations were noted in late summer, no seasonal trend in Cd, Co, Cu, Pb, Mn, Hg, Ni and Zn content of mussels could be discerned.

Seasonality is often dependent on the metal. Hsu and Wang (1979) reported seasonality for Fe, Cu, Pb, and Ni in oysters and clams but not for Cd, Hg and Zn. Popham and D'Auria (1982) reported seasonality in mussels for Pb, Zn, Cu and Fe but not for Mn. Frazier (1975) observed seasonal changes in oysters for all five of the above metals. Dahlgaard (1986) noted seasonal changes in the long-term loss rates of radionuclides in mussels in the Baltic sea.

2.4 Temperature

Accumulation of metals in aquatic animals, including mussels, has been reported to be positively correlated with temperature - probably due to increased filtration rates and metabolic activities (Phillips, 1976a; Nakahara et al., 1977). Radionuclide loss rates in mussels under Mediterranean conditions reported by Nolan and Dahlgaard (1991) are similar to those reported by Dahlgaard (1981) for mussels in the North Sea at ambient temperatures about 10°C lower. Dahlgaard (1986) concluded that temperature played only a minor role in the seasonal effect which he noted for radionuclide loss rates in mussels.
2.5 Salinity

Rates of accumulation of Co, Zn, Cs and Ag in Mytilus edulis are lower under Mediterranean conditions (S=37) (Nolan and Dahlgard, 1991) than rates at the lower salinities found in the Baltic Sea (S=6 - 16) and in Danish coastal waters (Dahlgard, 1981, 1986). It is likely that the observed differences are due to both the increased rate of ion-exchange occurring at gill and mantle surfaces and the decreased competition of metals with other ions for available binding sites at the lower salinities of the Baltic. Salinity effects on metal uptake by Mytilus edulis have been documented by Phillips (1976b) and Dahlgard (1981). Significant salinity-temperature interactions during accumulation of radionuclides have been reported by Dahlgard (1981). Generally, as salinity decreases, metal accumulation increases.

2.6 Influence of other metals

Popham and D'Auria (1982) reported inter-elemental effects between copper, iron and zinc whereby accumulation of the former metal was facilitated by the presence of the other two in the water. Phillips (1976a, 1980) also discusses metal interactions during accumulation by mussels. Metals may interact directly, by competing for binding sites or transport pathways or, indirectly, by altering the health of the animal. Toxic levels of any one metal tend to diminish the ability of the animal to regulate others.

3. BIOLOGICAL FACTORS

3.1 Age and size

Size and, since they are usually highly correlated, age, are perhaps the best-known of the intrinsic variables governing metal content of molluscs.

Boyden (1974), on the basis of the exponent of the power function, identified three types of relationships between trace metal concentration in molluscs and body weight. First, where element content is proportional to about 0.75 power of body weight. This relationship held for copper in all species which he examined, and for cadmium, iron, lead and zinc in most species examined. Such an exponent probably indicates a close association between the accumulation of the metal and the metabolic rate of the animal. Larger animals would have greater metal contents than smaller animals but would have lower concentrations in the tissues. The second type of relationship, where the exponent approximates unity, and where the trace metal content is directly related to body weight (and metal concentration is thus independent of body weight), was found for nickel in all species examined, for lead and zinc in M. mercenaria, iron and zinc in V. decussata and for cadmium in M. edulis. A third pattern, seen only for cadmium in limpets, was where the trace metal content was directly proportional to the square of the body weight (the exponent of the power function was about 2).

Brix and Lyngby (1985) also reported three types of relationships between metal concentrations and animal size for Mytilus edulis in Danish Coastal Waters. They reported that (i) the concentration of Cr
in soft tissue and of Cd, Hg and Zn in the shells decreased significantly with size, (ii) the concentrations of Hg and Pb in the soft tissues increased significantly with size and (iii) the concentrations of Cd, Cu and Zn in the soft tissues, and of Cu and Pb in the shells, were independent of body size.

There is one report presented at this meeting (Catsiki et al., 1991) on the effect of size (shell length) on Cu, Cr, Cd and Ni in Mytilus galloprovincialis from Saronikos Gulf in Greece. Although the authors did not use power functions to fit the data, it is clear that Cu, Cr and Ni concentrations decrease with increasing size whereas cadmium concentrations are independent of shell length. Interestingly, PAH displays a radically different behaviour to that of the metals with concentrations increasing with size (Catsiki et al., 1991).

Figure 1(b-f) shows the results of laboratory experiments where Mytilus edulis were exposed, under Mediterranean conditions, to radiotracers of Co, Zn, Ru, Cs and Ag for eight days and then allowed to depurate overnight in flowing uncontaminated seawater (from Nolan and Dahlgaard, 1991). The contents of the metals in the animals (shell plus soft tissues) are proportional to body weight and can be divided into two classes on the basis of the exponent of the power function. The essential metals Co and Zn, and Cs (an analogue of the essential metal K) have exponents of 0.8 – similar to the exponent relating respiration to body weight (Zeuthen, 1953; Jorgenson, 1986; Fenchel, 1987). Regressions against log body length are also significant but are not an improvement on the use of log body weight. Contrary to this, the surface reactive metals, Ru and Ag, have exponents which are not significantly different from 0.66 which is the ratio of surface area to volume in solids.

Size-effects on trace metal contents of aquatic organisms could be due to changes in habitat, feeding modes and maturation. It is accepted that many biological processes are directly dependent on the size of the organism (Fenchel, 1987) and that, typically, respiration is proportional to body weight to the power of 0.81 and excretion is proportional to body weight to the power of 0.72 (Jorgenson, 1986). It has been reported that in Mytilus the rates of filtration, growth, feeding and oxygen consumption (among other processes) are a power function of body weight (Bayne, 1976). If accumulation and/or loss of a substance is linked to any of the above processes then it is reasonable to predict that the levels of that substance in the organism would also be a function of body weight and the accumulation of trace metals would be satisfactorily described in almost all cases by a power function of metal content or concentration on body weight (Content = a * Weight^b). Boyden (1974, 1977) and Phillips (1980) showed that, in general, the metal contents of aquatic molluscs are indeed a power function of body weight but that the exponent could vary with season and location not only for different metals but also among animal species and within populations of the same species.

Size effects on loss rates have been reported for zinc in snails (Mishima and Ogum, 1983) with small animals losing zinc more quickly than larger ones. In some experiments which followed the accumulation experiments described by Nolan and Dahlgaard (1991), no size effects were noted on loss rates of Co, Zn, Ru, Cs and Ag and no difference was
Fig. 1 Regression of loge shell length and of loge wholebody weight (g) for 48 mussels exposed for 8 days to five radionuclides, followed by overnight loss in flowing uncontaminated water. The radionuclide regression lines shown include 95% confidence intervals on the means: (a) background, (b) 80Co, (c) 65Zn, (d) 106Ru, (e) 134Cs, (f) 137I. (From Nolan and Dahlgard, 1991)
found between the biological half-lives of the radiotracers in mussels in the environment and mussels maintained in the laboratory (Nolan and Dahlgaard, 1991).

3.2 Sex

Although Watling and Watling (1976) reported that trace metal content of bivalves can be partly dependent on sex, there have been few other reports where this has been documented - indicating either that the effect is not significant or that few investigators have bothered to control for this parameter.

3.3 Maturation

Cossa et al. (1979) investigated the relationships between cadmium concentration and body weight in mussels in the St. Lawrence estuary and discovered that the regression coefficients became considerably more variable when the animals matured. They attributed this to biochemical changes occurring during the sexual cycle, e.g. gonad development, and proposed that only immature mussels be used in monitoring schemes. Although Phillips (1976a) attributed seasonal fluctuations in cadmium content of mussels at a site in Australia to changes in tissue weights during the reproductive cycle, Cossa et al. (1979) reported that the anomaly persisted after this factor was accounted for. Fischer (1983) showed that cadmium content of Mytilus is dependent on spawning (among other factors). Since cadmium is unusual among trace metals in that no uniform trend exists relating body weight to metal concentrations in the animal, it is not clear whether the proposal of Cossa et al. should also apply to other metals. As discussed above, changes in metal concentrations due to seasonal variations in the condition of the animals should be considered whenever temporal comparisons are made.

3.4 Distributions of metals in populations

Frequency distributions of contaminants in animal populations in relation to sampling procedures have been discussed by Eberhardt (1975). It is generally recognised that such distributions are rarely normally-shaped and tend to be skewed to the right. Log-normal distributions are quite common for metals in marine biota (Taguchi et al., 1979; Shimizu and Tsuji, 1980; Lobel et al., 1982; Wright et al., 1985; Lobel, 1987). Even when confounding effects such as size, season, location and sex are eliminated, the log-normal distribution often remains. Although the gamma distribution can also be found in environmental samples, Eberhardt and Gilbert (1973) (cited by Eberhardt, 1975) found that it may not matter much, for some situations at least, which distribution is assumed. In sampling to estimate a total quantity, such as a metal inventory in an estuary, the shape of the frequency distribution is not important but if any statistics are to be made on the data - such as testing for change or testing for differences between sites - then a log-transformation is important (Eberhardt, 1975). As a corollary, for log-normal data, a mean and standard deviation cannot be given and results should be expressed as geometric means (anti-log of the mean of the log-transformed data) multiplied or divided by the antilog of the standard deviation of the log-transformed data (Elliott, 1977; Hansen and Abraham, 1983).
Figure 2 shows frequency distributions of radiotracer activity of Co, Zn, Cs and Ag in *Mytilus edulis* exposed to the metals in the laboratory for eight days followed by overnight depuration in flowing uncontaminated water (Nolan and Dahlgaard, 1991). The mussels had been selected beforehand to be as close to 40 mm length as possible. For comparison, frequency distributions of shell length and total body weight are also shown. Length and weight are adequately treated as being normally distributed but the radiotracer contents are all skewed to the right and are best described by lognormal distributions. There is considerable spread of the data around the mean values. There is a 4% coefficient of variation (CV, defined as the standard deviation expressed as a percentage of the mean) for length which is a direct consequence of the way in which the mussels were sorted and selected and the 16% CV recorded for weight is typical of a normally distributed population parameter. The coefficients of variation for the radiotracer contents (before log-transformation) vary from 29% for $^{65}$Zn to 87% for $^{60}$Co. $^{134}$Cs and $^{110}$Ag have intermediate values of 39% and 54% respectively. The appropriateness of the distributions used may be confirmed by inspection of Figure 3 where probit plots of length, total weight and the radiotracer contents (on a log-scale) of the animals are shown. For comparison, a plot of $^{60}$Co on a linear scale is also shown. $^{60}$Co does not behave ideally - exhibiting a bimodal tendency - but the use of a log-scale is a significant improvement over a linear one. The variability of the radiotracer content data after log-transformation is so improved that, for Zn and Ru, they are as good as the data for shell length - the original criterion by which the animals were selected.

3.5 Inherent variability

Even when all other factors have apparently been accounted for there usually remains a residual variability in datasets of trace metal levels in populations. This variability, termed 'inherent variability' has been noted in the Mussel Watch (National Academy of Sciences, 1980) and has been discussed for zinc in *Mytilus* by Lobel (1987).

The CV is a good indicator of variability in a dataset. It is a useful tool when sample sizes required to detect change or differences need to be estimated since it is independent of the absolute levels of contaminants present in a sample. Eberhardt (1975) reported that CVs for many radionuclides can be expected to lie in the range of 10% - 40%. Lobel (1987) showed that variability of zinc concentrations in mussel kidneys (CV = 74%) were responsible for the CV of 44% observed for the whole soft tissues. When tissues other than the kidney were analysed, CVs of 15% to 28% were obtained. Lobel et al. (1982) reported CVs ranging from 11% (for iron in *Littorina littorea*) to 48% (for Zn in *Mytilus edulis*) in a survey of Zn, Cu and Fe contents of four mollusc species from the north-east coast of England.

For the mussels in Figure 2, selected so as to be as homogeneous as possible, the uptake of radiotracers over eight days in laboratory aquaria can vary by as much as an order of magnitude during uptake, particularly on the shells (which were clean and relatively smooth). CVs for radiotracers in the soft tissues (after log-transformation) were 15% for weight, 11% for Co, 14% for Zn, 15% for Cs and 18% for Ag. These values represent a homogeneous population of mussels, after identical exposures to the radiotracers. The CVs increased by a factor
Fig. 2 Frequency distributions of shell length (mm), total body-weight (g), and content of $^{60}$Co, $^{65}$Zn, $^{134}$Cs and $^{110}$Ag (kBq) in 159 mussels exposed to the radiotracers for eight days followed by overnight loss in flowing uncontaminated seawater (data from Nolan and Dahlgaard, 1991)
Fig. 3 Probit plots of shell length (mm), total body weight (g) and total content of $^{60}\text{Co}$ (Bq) (linear X-axes) and of total content of $^{60}\text{Co}$, $^{65}\text{Zn}$, $^{106}\text{Ru}$, $^{134}\text{Cs}$ and $^{110}\text{Ag}$ (Bq) (logarithmic X-axes) in 159 mussels (from Nolan and Dahlgard, 1991)
of two after the mussels were transplanted to an uncontaminated site and differences in excretion rates, in growth rates etc. became manifest.

The CVs for shells can be considerably greater than those of the soft tissues (Nolan and Dahlggaard, 1991). In natural populations of mussels each shell could be considered as being a micro-ecosystem, sustaining populations of bacteria, barnacles and other invertebrates, and attached byssal threads. Natural populations of mussels would have shells of greater specific surface area than those used by Nolan and Dahlggaard (1991) due to variations in annual growth rates, weathering etc. Therefore large sample sizes are required when using shells as environmental monitors, and care should be exercised in the interpretation of the results.

4. SAMPLE SIZE

Selection of the correct sample size requires not only a knowledge of what information is required (e.g. what level of confidence is needed) but also an understanding of the behaviour in the population of the test substance being sampled for (e.g. what is the CV of the test substance). If one knows the underlying frequency distribution and the CV of the contaminant of interest, then it is possible to estimate, in advance, the sample size required to detect change or differences using widely available statistical formulae. These methods have been explained by Eberhardt (1975).

In Table 1 are reported the estimated sample sizes required to detect changes in radiotracer content of 10%, 20% and 50% in a population of mussels with CVs for each radiotracer similar to those reported by Nolan and Dahlggaard (1991) and discussed here. Sample sizes of up to hundreds of mussels would be required to detect small changes but as few as 20 animals would be needed to detect changes of 20% for Zn and Ru and of 50% for the other three radiotracers. The CV can vary widely for different contaminants. For example, nine times as many mussels would need to be analysed to detect (with a confidence limit of 95%) a 10% or even a 20% change in Co content as would need be for Zn (Table 1) – all other confounding factors being accounted for. It is evident that if sampling is performed for a contaminant with a large CV, e.g. Co, then other contaminants with lower CVs will have been adequately treated.

Eberhardt (1975) has discussed the effect of the magnitude of the CV upon the advisability of pooling samples for analysis. Pooling samples will allow a mean (arithmetic) contaminant concentration to be determined. For many applications, e.g. where contaminants are log-normally distributed, a geometric mean is required. Eberhardt concluded that, when the CV is reasonably small, then pooling does not have much effect, but that when the CV is large, a considerable bias may be introduced to the data.
Table 1

Sample sizes required to detect changes (with 95% confidence) in the radiotracer content in a population of *Mytilus edulis* of homogeneous shell length. The calculations are based on the variability of the log-transformed wholebody radiotracer contents of mussels as discussed in the text. Also given are the probabilities of committing a Type II error when testing for differences in radiotracer content of 50% if sample sizes of 5, 10 and 15 mussels were to be selected and if the probability of a Type I error were to be set at 5%.
(Data from Nolan and Dahlgard, 1991).

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Sample Size Required to Detect Charge of</th>
<th>% Probability of Type II Error with Sample Size</th>
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<tbody>
<tr>
<td></td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td>$^{60}$Co</td>
<td>375</td>
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</tr>
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<td>$^{65}$Zn</td>
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<td>$^{134}$Cs</td>
<td>184</td>
<td>50</td>
</tr>
<tr>
<td>$^{110}$Ag</td>
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<td>64</td>
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5. SAMPLING DESIGN

In well-designed monitoring schemes the effects of confounding environmental and biological factors are minimised by careful design of sampling strategies. Mussels should be in good condition when sampled and should be suitable for the sites being examined. At the IAEA Marine Laboratory (IML), researchers have conducted several surveys of trace metal and organic contamination in mussels on the Mediterranean coasts of France and Italy (Fowler and Oregioni, 1976; Marchand et al., 1976; Mee, personal communication). One of the biggest problems in these surveys was finding sufficient quantities of material at the different locations surveyed. Often, populations of mussels are present at a site which are not in good condition or which are too small to facilitate comparisons with samples from other sites.

Sampling should be performed on at least one occasion in each season over an annual cycle so that seasonal effects may be discerned. Thereafter, for annual surveys, sampling should be completed at the same time of the year. Whenever practical, animals should be randomly sampled at a site - taking account of the position on the shoreline and/or the depth in the water column. It is useful to make notes of sampling sites, depths, position on shoreline etc. when sampling. When it was decided at the IML to repeat, more than ten years later, the surveys of Fowler and Oregioni (1976) and of Marchand et al. (1976) it was fortunate that the same staff were available to perform sample collection, as had participated in the original surveys. It cannot be assumed that the such serendipity will accompany the next survey.
It is important, if the data are to be used in any comparisons, temporal or spatial, that the underlying frequency distribution of the contaminant in a physically homogeneous group of mussels be determined. Although it is probable, it cannot be assumed that distributions are log-normal. Identification of the distribution should also result in a determination of the CV of the contaminant in the population sampled. Once this has been performed it is a simple exercise to determine the sample sizes required to detect differences. The power of the statistical tests to be used in testing for differences should also be noted at this stage - after determining probable errors in measurements due to sample handling and analysis. There is little point in choosing a rigorous test to detect small differences when such differences can be confounded by imprecision later in the analytical train, although biological variability tends to be much higher than analytical variability.

There are two reasons why individual animals should be analysed separately. The first is that more statistics can be gleaned from the data to facilitate comparisons with other surveys. The second is that the contaminant concentrations can be correlated with other factors such as shell length and body weight. Individual analyses are not always practicable or economic. Where the CV is low, then pooling of samples should not introduce bias into calculations (Eberhardt, 1975). Similarly, where contaminant concentrations are independent of body weight then pooling of different sized animals will not affect the results. Unfortunately, these cases are exceptions and the vast majority of instances, pooling will introduce bias.

Animal size is an important factor to consider during the design of sampling strategies for monitoring programmes. A 10% increase in shell length can result in a 33% increase in body weight and a 26% increase in radiotracer content (from Figure 1 for Co, Zn and Cs). Without careful control of the size of the animals sampled, it would be possible to detect only gross perturbations of contaminant content between any two samplings of the animals being monitored. It is unfortunate that many locations will not sustain sufficient population numbers to permit a sufficiently large sample within a small size-range for adequate detection capability (Table 1).

This dilemma and methods to surmount it are discussed by Phillips (1980). He outlined the following alternatives during sampling: (a) the effects of size are ignored, (b) similar-sized animals are taken from all locations, (c) a large size range is selected and analysed in bulk, and (d) a large size range is analysed individually and a size-concentration regression constructed for each contaminant. The first alternative is not acceptable because of the very large size effects discussed above. The second alternative is acceptable if a sufficiently small size range is chosen. In the Mussel Watch (Goldberg et al., 1983) a size range of 50 - 80 mm shell length was proposed. Rough calculations reveal that over such a size range, concentrations can vary by as much as 100% and only gross perturbations could be detected in such a sampling programme. A smaller size range would be more desirable but mussels in a given size range may not be present at all locations sampled. The third alternative, pooling samples, will also generate error, especially if the CV is large for some contaminants. The final alternative, and the most expensive in terms of labour and in analyses performed, is to generate individual
regressions for all contaminants. This would also generate a vast quantity of useful scientific information but is probably not economic. Phillips (1980) concluded that the second alternative, with as narrow a size range as possible, was the optimal strategy.

Use of softparts in monitoring is preferable to the use of shells since the data are less variable and more meaningful biologically. Shells are useless for monitoring of many contaminants (Brix and Lyngby, 1985) even though they have their proponents (Koide et al., 1982). The advantages of using wholebody analysis include less sample handling and thus less chance of sample contamination. Among the disadvantages of wholebody analysis is that assumptions must be made about the distribution of contaminants in the animals and that the large variability of contaminants binding to the shell can obscure data for the soft tissues.

6. CONCLUSIONS

Considerable resources, both budgetary and personnel can, and have been mobilised, to limit the effects of errors when determining the degree of contamination of water, sediments and biota in the Mediterranean. Without adequate consideration of potential sources of variation during sampling, these resources will essentially have been wasted, technological advances improving analytical sensitivity and accuracy will have been negated and the sampling programme itself will have been jeopardised.

Careful examination of the underlying frequency distributions and of the variability of contaminants in mussel populations should be performed at the planning stages of a monitoring programme so that optimal sample sizes can be estimated in a preliminary programme.

Animals selected should be as physically homogeneous as possible and should be collected from locations with a view to minimising the effects of environmental variables on the biology of the mussels. Where possible, individual animals should be analysed and the size-concentration relationships of each contaminant elucidated. Pooling may be performed of animals from a narrow size range if the CV of the contaminant is not large.

7. ACKNOWLEDGEMENTS

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8. REFERENCES


FORMATION OF ORGANOCHLORINE COMPOUNDS DURING
CHLORINATION OF SEAWATER IN POWER PLANT
COOLING SYSTEMS: A MUTAGENIC ASSESSMENT

by

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ABSTRACT

Samples of Mediterranean sea water taken at Tel Aviv were chlorinated in the laboratory at various chlorine concentrations. Samples of power plant cooling sea water from the Reading power station were also collected. The organic components of these samples were isolated and concentrated using an Amberlite XAD-2/XAD-8 column, eluted with 95% ethanol. The ethanol extracts were assayed with the four Ames Salmonella typhimurium strains TA 98, TA 100, TA 102 and TA 104. The mutagenicity was correlated with the formation of volatile and non-volatile organochlorine compounds, as determined from gas chromatography (GC) and Total Organic Halogens (TOX) analysis, respectively.

All the tester strains gave positive dose-response curves with chlorinated sea water, with greater numbers of revertants in larger extract volumes. Statistically significant mutagenicity was found with all four strains in seawater chlorinated with 10 mg l⁻¹ chlorine in equivalents of 100-250 ml water. However, seawater chlorinated with 2 mg l⁻¹ chlorine only showed significant mutagenicity with the TA 98 and TA 104 strains. Chlorinated sea water discharged from the Reading power station only exhibited mutagenicity with strains TA 98 and TA 102, and not with TA 100 and TA 104. This may be explained in terms of the volatile organochlorine compounds, such as trihalomethanes (THM), which are present in seawater chlorinated in the laboratory, but absent in the Reading power station effluents, presumably as a result of evaporation due to the heat generated in the latter. In general, good correlation between mutagenicity and the formation of halo-organic compounds was found. However, due to the high concentrations of bromide (60 mg l⁻¹) in sea water, most of these compounds were in the brominated, rather than the chlorinated, form. The unexpectedly high concentrations of nonvolatile organochloride compounds found in chlorinated seawater may also be due to the presence of the bromide in seawater, which stimulates the production of halogenated organic products following bromine formation via the chlorine-mediated oxidation of bromide to bromine.

1. INTRODUCTION

Sea water is used in power plant cooling systems, particularly along the eastern shores of the Mediterranean. Chlorine is routinely
added to such water as an antifoulant in order to prevent accumulation of algae and other organic deposits in the pipes. This cooling water, beside domestic sewage, and chlorinated water from other sources, all containing large quantities of organochlorine compounds, are released into the Mediterranean Sea. Since the biodegradation of some of these organochlorines, including toxic and mutagenic compounds, is very slow, its cumulative effects on marine ecosystems, and, eventually, on human health, via entry into the food chains, are likely to be considerable.

Data collected in the United States over a decade ago indicated that the annual discharge of chlorine from Maryland into the Chesapeake Bay reached some 12,000,000 kg and a further 1,000,000 kg from municipal treatment plants and power plants, respectively (Davis and Middaugh, 1978). Jolley et al. (1978) suggested that at least 3% of this material in various halogenated forms persists for long periods in marine ecosystems.

Most of the research concerning the chemical and biological effects of chlorine in water has been carried out in fresh water. The chemistry of chlorine in marine water is rendered more complex by its specific composition and properties:

- ionic strength (µ) 0.7 molar,
- pH 8, alkalinity 2 x 10^{-3}M,
- [Br^-] about 60 mg l^{-1},
- [NH_4^+] in the range of 1-50 µM.

Thus, at an ionic strength of 0.7, 20°C, and pH 7.54, 90% hypochlorite ions and 10% hypochlorous acid are present at equilibrium (Morris, 1966). Chloramine levels are low since sea water contains very little ammonia. However, the high bromide content of sea water accounts for the main differences in halogen chemistry in marine, as compared to fresh water. Among the products of the reaction of chlorine with bromine are various brominated species (including Br_2, HBrO, BrO^- and BrO_3^-) as well as interhalogen compounds. The latter react even more rapidly than chlorine with aromatic compounds and olefins (Gutfann, 1968). In addition, whereas monochloramines predominate over dichloramines, greater amounts of the dibromamines, which react very readily with organic materials (Cromer et al., 1978) but are less toxic than the chloramines, are formed in the sea water.

The extensive effects of chlorination on marine ecosystems are well established (Davis and Middaugh, 1978). Thus, an 83% decrease in growth rates was observed in phytoplankton cultured in chlorinated water taken from the cooling system of a nuclear-generating plant on Long Island Sound in the United States (Carpenter et al., 1972). Hirayama and Hirano (1970) found that after chlorination the photosynthetic activity of Skeletonema costatum was completely inhibited within 10 minutes. The metabolism and growth rates of marine phytoplankton exposed to chlorine were drastically decreased (Gentile et al., 1973). Several reports describe the toxic effects of chlorinated seawater on fish and other estuarine organisms, including L. xanthurus (Servizi and Martens, 1974), Pleurodeles platessa (Anderson, 1974), silversides (Menidia menidia), mummichogs (Fundulus heteroclitus) and hog chokers (Trinectes maculatus) (Meldrim et al., 1974; Meldrim and Povea, 1977).
As far as mutagenicity is concerned, much research has been carried out on the mutagenic effects of various organic and inorganic chemical compounds, focusing particularly on heavy metals and their derivatives. However, very little attention has been directed toward the halo-organic compounds that are discharged into the sea from power plant cooling systems, as well as from municipal wastewater and effluents. This is of particular importance since many halogenated organic compounds have been shown to be mutagenic, teratogenic, and even carcinogenic (Alavanja et al., 1978; Cheh et al., 1980; Maruoka and Yamanaka, 1980; Meier et al., 1983). It is also suggested, and indeed confirmed in this study, that the high bromide levels in sea water may result in the formation of bromoorganic compounds, which are known to be more potent mutagens than the corresponding chloro-analogues. This investigation therefore sought to assess the mutagenicity of sea water following chlorination. Toward this end, chlorination of marine water samples at various chlorine concentrations was carried out. The mutagenicity of laboratory-chlorinated sea water samples was compared to that of effluents from the Reading power station near Tel-Aviv. These mutagenic evaluations were compared to the results obtained for the production of volatile and non-volatile halo-organic compounds.

2. EXPERIMENTAL

Trihalomethane (THM) measurements

100 ml samples of seawater were introduced into 100-ml volumetric flasks without headspace, and chlorinated with 2, 5 and 10 mg l⁻¹ of chlorine. After 24 h at 20°C in the dark, the samples were extracted with 1 ml of n-hexane, and the THMs determined by gas chromatography employing an electron capture detector, against an internal standard of 1,1,1,2-tetrachloroethane. A more detailed description of the analytical procedure was given elsewhere (Rav-Acha et al., 1985). Typical chromatograms are shown in Fig. 1.

Total organochlorogen (TOX) measurements

TOX was measured by a modification of the methods of Dressman (1977) and Takahashi et al. (1981). After acidification to pH 4, the water was poured into a sample reservoir and then forced through a granular active carbon (GAC) column under N₂ pressure. The GAC columns (8 mm x 8 cm) were packed with 3 g of granular active carbon 80-140 mesh, which had been pretreated by refluxing with 5% HNO₃, and rinsed with water until neutral pH was reached. Then, 200 ml of 0.1 M KNO₃ were passed through the column to remove inorganic Cl⁻, the carbon being transferred into a quartz boat. It was then placed in the lower section of a quartz pyrolysis tube, in which the temperature was gradually increased from 100°C to 900°C. The upper section was maintained at a constant temperature of 100°C. Oxygen and water vapor were passed through the pyrolysis tube at a rate of 200 ml min⁻¹ and the hydrolysisate collected via a condenser.

The halide concentrations were determined by two different methods: (a) specific halide electrodes, and (b) potentiometric titration with AgNO₃ in 75% acetone (Prokopov, 1978).
Fig. 1 A typical chromatogram of THMs in chlorinated sea water

Recovery of organic materials for mutagenic assessment

Water samples were acidified to pH 4.0 by adding acetic acid and forced under pressure through a combined Amberlite XAD-2/XAD-8 resin column (12 x 1.5 cm, precleaned with soxhlet in acetone and methanol for 48 h). Following the adsorption step, the column was washed with 1 of distilled water, dried with nitrogen gas, and eluted with 100 ml of ethanol. The eluants were concentrated to volumes of 5 ml by evaporation and used directly in the Ames tests. This method of recovery is a modification of a similar method described by Ringhand et al. (1987).

Testing Ames strains

Tester strains of *Salmonella typhimurium* TA 98, TA 100, TA 102 and
TA 104 from Dr. B.N. Ames, Biochemistry Department, University of California at Berkeley, were despatched on discs enclosed in plastic sheeting saturated with a nutrient broth culture and soft agar in order to prevent drying. Upon arrival, the TA 98, TA 100, and TA 104 strains were streaked onto ampicillin plates, and strain TA 102 onto ampicillin/tetracycline plates. The discs were placed in sterile broth left to incubate overnight at 37°C. Following preincubation, all the strains were grown on nutrient broth (Oxoid No. 2) for 24 h and tested for genetic markers.

The various strains differ in their sensitivity to various chemical and physical factors. The TA 98 strain is sensitive to frameshift mutation, and TA 100 to base-pair substitution (Maron and Ames, 1983). The advanced strains TA 102 and TA 104 are particularly sensitive to oxidative mutagens and some physical factors. Thus, TA 102 is sensitive to hydroperoxides, X-rays and UV radiation (Levin et al., 1982), and TA 104 is sensitive to quinones and some other oxidative compounds (Chesis et al., 1984).

Testing for genetic markers

Histidine requirement

The culture was streaked on minimal plates either with or without histidine (0.1 ml of sterile 0.1 D,L-histidine spread on the agar surface). The control plates contained biotin, but no histidine. The strains used in the experiments only grew on plates containing histidine.

Rfa mutations

Strains with the deep rough trait (Rfa) were tested for crystal violet sensitivity (Ames, 1973). A sterile filter paper disc containing crystal violet was placed on a nutrient agar Petri dish containing 0.1 ml of nutrient broth culture and tested in a thin overlay of top agar. After 12 hours of incubation, a clear zone of inhibition around the disc indicated the Rfa mutation, which allows the entry of larger molecules, such as crystal violet, which inhibit growth, into the bacteria. This phenomenon was not observed in wild-type strains of E. coli, which were impermeable to crystal violet.

UV sensitivity

UVR-B detection (inability to grow without biotin in the presence of ultraviolet radiation) was assessed by cross-streaking the culture on to the nutrient agar plate, and irradiating 50% of the streak for 8 seconds with a germicidal lamp. Following incubation at 37°C for 12 to 24 hours, the UVR-B strains only grew on the non-irradiated side of the plate while the strain TA 102, which has the wild-type excision repair enzyme, grew on both sides of the plate.

Positive controls

Positive controls were used to test for mutations in all the tester strains, according to the established methods. Methyl methane sulfonate was used for the TA 104 strain, cumene hydroperoxide for TA 102, sodium azide for TA 100, and NPD (n-nitro-O-phenilenediamine) for
Fig. 2 Dose-response curve for TA 104 with methyl methane sulfonate (MMS)

TA 98. The number of revertants was measured versus the concentration of the control mutagen for each strain. The dose-response curves obtained for the four strains are presented in Figs 2-5. Toxic effects at elevated concentrations in the positive controls were observed for TA 104 and TA 102.

Calculation of mutagenicities

Mutagenicities are calculated from the mutagenicity ratio (MR), the ratio between the number of mutant colonies on the test plate and the number of spontaneous mutants. In order to account for possible effects of the concentration procedure, the spontaneous mutation rate for each strain was determined from the mean number of colonies on two plates, one loaded with distilled water, and the other with sea water, which were subjected to the same concentration process. The results were plotted as dose-response curves to determine whether the mutagenicity was linearly related to the sample concentration. The linear regressions and the correlation coefficient (r) were also determined for these plots.
Fig. 3 Dose-response curve for TA 102 with cumene hydroperoxide

Cumene Hydroperoxide (μg)

No. of revertants x 10^-3/plate
Fig. 4 Dose-response curve for TA 100 with sodium azide

Statistical evaluation

The statistical significance of the mutations in the test plates, as compared to the number of spontaneous mutations, and of the r values for the test and control plates was assessed using the Student's t-test.

3. RESULTS AND DISCUSSION

3.1 THM formation and total organohalogen content (TOX)

The THM levels found at various chlorine concentrations are summarized in Table 1. Apparently, the THM levels rise with increasing levels of chlorine between 2 and 5 mg l\(^{-1}\), but not between 5 and 10 mg l\(^{-1}\). This can be explained in terms of the demand of sea water for chlorine, which is about 2 mg l\(^{-1}\), which means that higher
concentrations of chlorine do not lead to further reactions. This finding implies that about 2 mg l\(^{-1}\) chlorine is sufficient to react with all the organics present in sea water. Therefore, increasing the chlorine above this level produces no further rise in THM concentrations. A typical THM chromatogram is presented in Fig. 1. Bromoform and dibromochloromethane are the main THM constituents in all sea water samples.

The preponderance of bromoform over the other THMs may be explained by the hypothesis that bromide, which occurs in relatively high concentrations in sea water, is readily oxidized by chlorine to form bromine (Oliver, 1980), which then reacts with aquatic humic materials faster than chlorine to produce bromoform compounds (Rook, 1975; Rav-Acha et al., 1985).

With respect to the non-volatile organohalide compounds, the preliminary results indicate that 600 \(\mu g\) l\(^{-1}\) of total organohalogenated compounds (TOX as \(X^-\)) are produced during chlorination of sea water.
Table 1

THM levels (µg l⁻¹) in sea water chlorinated at various chlorine concentrations and in Reading power station effluents.

<table>
<thead>
<tr>
<th>Sample</th>
<th>CHCl₃</th>
<th>CHCl₂Br</th>
<th>CHBr₂Cl</th>
<th>CHBr₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated sea water</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sea water chlorinated with 2 mg l⁻¹ Cl₂</td>
<td>-</td>
<td>0.017</td>
<td>1.12</td>
<td>37.68</td>
</tr>
<tr>
<td>Sea water chlorinated with 5 mg l⁻¹ Cl₂</td>
<td>0.05</td>
<td>0.10</td>
<td>4.35</td>
<td>70.24</td>
</tr>
<tr>
<td>Sea water chlorinated with 10 mg l⁻¹ Cl₂</td>
<td>0.52</td>
<td>0.05</td>
<td>4.10</td>
<td>59.45</td>
</tr>
<tr>
<td>Effluent from the Reading power station</td>
<td>-</td>
<td>0.05</td>
<td>0.05</td>
<td>5.00</td>
</tr>
</tbody>
</table>

with 4 mg⁻¹ chlorine. Taking into account that only 2.0 mg l⁻¹ of chlorine is consumed by sea water, TOX production is about 30%, which is much higher than the estimate of Jolley et al. (1978). This may be due to the presence of bromide in sea water which leads to the production of organohalide compounds following bromine formation.

3.2 Mutagenicity of chlorinated sea water

Sea water, sampled near the intake of the Reading Power Station at Tel-Aviv, was divided into two portions: one was chlorinated and the other was left untreated. The samples were concentrated, as described in the Experimental section, by passing them through XAD-2/XAD-8 columns. The column was then washed with distilled water to remove the salts, and the organic sea water component then eluted with 95% ethanol, which is known to be an appropriate solvent for mutagenic assessment (Maron et al., 1981). The ethanol extracts were directly used in the mutagenic assays with strains TA 98, TA 100, TA 102, AND TA 104.

All the assays were carried out in triplicate, at three or more sample concentrations. Metabolic activation was not used because direct-acting mutagens have been reported to account for a major part of the mutagenic activity in water (Gentile et al., 1973; McLean, 1973). Distilled water was added to some of the samples derived from 10 l volumes prior to incubation in order to avoid problems of toxicity to the tester bacteria, which are probably the result of the concentration of impurities in the ethanol extract during the evaporation step. In
the mutagenicity tests with TA 98, both regular and diluted samples were used. The results of the mutagenicity assessments with tester strains TA 104, TA 102, TA 100 and TA 98 are presented in Tables 2-7 and Figs 6-9.

Table 2

Mutagenicity with strain TA 104.

Spontaneous mutations = 479 ± 61.
The statistically significant mutation rates are typed in bold face.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Vol. 95% ethanol extract/plate (μl)</th>
<th>Equiv. vol. water prior to concentration (ml)</th>
<th>No. mutant colonies per plate</th>
<th>Mutation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5</td>
<td>7.5</td>
<td>552±67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>45</td>
<td>465±5.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>75</td>
<td>407±68</td>
<td></td>
</tr>
<tr>
<td>Sea water</td>
<td>5</td>
<td>20</td>
<td>436±46.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>525±3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>488±3</td>
<td></td>
</tr>
<tr>
<td>Sea water chlorinated with 10 mg l⁻¹ Cl₂</td>
<td>5</td>
<td>20</td>
<td>408±14</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>40</td>
<td>582±78.5</td>
<td>1.22</td>
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<td>20</td>
<td>80</td>
<td>537±46.7</td>
<td>1.12</td>
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<td></td>
<td>30</td>
<td>120</td>
<td>723±10</td>
<td>1.51</td>
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<td>40</td>
<td>160</td>
<td>873±37.5</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>880±180</td>
<td>1.84</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>240</td>
<td>1023±45</td>
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<td></td>
<td>75</td>
<td>300</td>
<td>718±49.5</td>
<td>1.50</td>
</tr>
<tr>
<td>Sea water chlorinated with 5 mg l⁻¹ Cl₂</td>
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<td>20</td>
<td>739±43.8</td>
<td>1.54</td>
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<td>30</td>
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<td>651±55</td>
<td>1.36</td>
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<td>40</td>
<td>160</td>
<td>573±15</td>
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<td></td>
<td>50</td>
<td>200</td>
<td>539±76.4</td>
<td>1.13</td>
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<td></td>
<td>75</td>
<td>300</td>
<td>605±8.5</td>
<td>1.26</td>
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<td>Sea water chlorinated with 2 mg l⁻¹ Cl₂</td>
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<td>20</td>
<td>679±144</td>
<td>1.42</td>
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<td>683±61.5</td>
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<td>621±119</td>
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<td>688±51</td>
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<td>300</td>
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<td>Effluent from the Reading power station</td>
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<td>484±5.7</td>
<td>1.01</td>
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<td>1.01</td>
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<td>30</td>
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<td>441±62</td>
<td>0.92</td>
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<td>400±40.3</td>
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<td>50</td>
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<td>429±24</td>
<td>0.90</td>
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<td>240</td>
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</tr>
<tr>
<td></td>
<td>75</td>
<td>300</td>
<td>385±42.4</td>
<td>0.80</td>
</tr>
</tbody>
</table>
Table 3

Mutagenicity with strain TA 102.

Spontaneous mutations = 357 ± 48.
The statistically significant mutation rates are typed in bold face.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vol. 95% ethanol extract/plate (µl)</th>
<th>Equiv. vol. water prior to concentration (ml)</th>
<th>No. mutant colonies per plate</th>
<th>Mutation Ratio</th>
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<td>Distilled water</td>
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<td>45</td>
<td>375 ± 28.3</td>
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<td></td>
<td>50</td>
<td>75</td>
<td>413 ± 44.5</td>
<td></td>
</tr>
<tr>
<td>Sea water</td>
<td>5</td>
<td>20</td>
<td>304 ± 39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>338 ± 34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>368 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>Sea water chlorinated with</td>
<td>5</td>
<td>20</td>
<td>399 ± 3</td>
<td>1.12</td>
</tr>
<tr>
<td>10 mg l⁻¹ Cl₂</td>
<td>10</td>
<td>40</td>
<td>413 ± 5.7</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>80</td>
<td>483 ± 11</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>505 ± 119</td>
<td>1.41</td>
</tr>
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<td>40</td>
<td>160</td>
<td>562 ± 15.5</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>649 ± 21.2</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>240</td>
<td>581 ± 60.6</td>
<td>1.63</td>
</tr>
<tr>
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<td>75</td>
<td>300</td>
<td>416 ± 20</td>
<td>1.17</td>
</tr>
<tr>
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<td>5</td>
<td>20</td>
<td>443 ± 3.5</td>
<td>1.24</td>
</tr>
<tr>
<td>5 mg l⁻¹ Cl₂</td>
<td>30</td>
<td>120</td>
<td>424 ± 4</td>
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</tr>
<tr>
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<td>160</td>
<td>452 ± 32.5</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
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<td>200</td>
<td>456 ± 1.5</td>
<td>1.28</td>
</tr>
<tr>
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<td>240</td>
<td>401 ± 37</td>
<td>1.12</td>
</tr>
<tr>
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<td>75</td>
<td>300</td>
<td>358 ± 8.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Sea water chlorinated with</td>
<td>5</td>
<td>20</td>
<td>384 ± 22.5</td>
<td>1.08</td>
</tr>
<tr>
<td>2 mg l⁻¹ Cl₂</td>
<td>30</td>
<td>120</td>
<td>437 ± 3</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>160</td>
<td>321 ± 28.3</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>50</td>
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<td>340 ± 24</td>
<td>0.95</td>
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<tr>
<td></td>
<td>60</td>
<td>240</td>
<td>330 ± 28.3</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>300</td>
<td>327 ± 21.2</td>
<td>0.92</td>
</tr>
<tr>
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<td>5</td>
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<td>370 ± 10</td>
<td>1.04</td>
</tr>
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<td>power station</td>
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<td>397 ± 62.2</td>
<td>1.11</td>
</tr>
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<td>120</td>
<td>561 ± 15.5</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>160</td>
<td>561 ± 1.53</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>580 ± 4</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
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<td>240</td>
<td>583 ± 61</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>300</td>
<td>589 ± 12.7</td>
<td>1.65</td>
</tr>
</tbody>
</table>
Table 4
Mutagenicity with strain TA 100.

Samples of 20 l were used.
Spontaneous mutations = 91 ± 20.
The statistically significant mutation rates are typed in bold face.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vol. 95% ethanol extract/plate (μl)</th>
<th>Equiv. vol. water prior to concentration (ml)</th>
<th>No. mutant colonies per plate</th>
<th>Mutation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5</td>
<td>7.5</td>
<td>108±12</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>45</td>
<td>102±4</td>
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<tr>
<td></td>
<td>50</td>
<td>75</td>
<td>79±7</td>
<td>1.00</td>
</tr>
<tr>
<td>Sea water</td>
<td>5</td>
<td>20</td>
<td>78±3.5</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>67±14</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>113±12</td>
<td>1.00</td>
</tr>
<tr>
<td>Sea water chlorinated with 10 mg l⁻¹ Cl₂</td>
<td>5</td>
<td>20</td>
<td>111±3.5</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>40</td>
<td>91±21.2</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>80</td>
<td>105±1.5</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>86.5±19</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>160</td>
<td>toxic to bacteria</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>240</td>
<td></td>
<td>0.95</td>
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<tr>
<td></td>
<td>75</td>
<td>300</td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>Sea water chlorinated with 5 mg l⁻¹ Cl₂</td>
<td>5</td>
<td>20</td>
<td>110±10.6</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>83±10.6</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>160</td>
<td>95±21.2</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>95±19</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>240</td>
<td>toxic to bacteria</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>300</td>
<td></td>
<td>1.15</td>
</tr>
<tr>
<td>Sea water chlorinated with 2 mg l⁻¹ Cl₂</td>
<td>5</td>
<td>20</td>
<td>105±10.6</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>77±18.4</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>160</td>
<td>101±21.2</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>108±23.3</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>240</td>
<td>toxic to bacteria</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>300</td>
<td></td>
<td>1.15</td>
</tr>
<tr>
<td>Effluent from the Reading power station</td>
<td>5</td>
<td>20</td>
<td>90±8.5</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>80</td>
<td>110±1.5</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>89±10</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>160</td>
<td>85±13.5</td>
<td>1.15</td>
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<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>toxic</td>
<td>1.15</td>
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<tr>
<td></td>
<td>60</td>
<td>240</td>
<td></td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>300</td>
<td></td>
<td>1.15</td>
</tr>
</tbody>
</table>
Table 5
Mutagenicity with strain TA 100.

Samples of 10 l were used.
Spontaneous mutations = 80 ± 14.5.
The statistically significant mutation rates are typed in bold face.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vol. 95% ethanol extract/plate (µl)</th>
<th>Equiv. vol. water prior to concentration (ml)</th>
<th>No. mutant colonies per plate</th>
<th>Mutation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5</td>
<td>7.5</td>
<td>100±3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>45</td>
<td>76.5±20.5</td>
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<tr>
<td></td>
<td>50</td>
<td>75</td>
<td>85.5±3.5</td>
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<tr>
<td></td>
<td>75</td>
<td>112.5</td>
<td>81.5±19</td>
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<td>Sea water</td>
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<td>16</td>
<td>86.5±29</td>
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</tr>
<tr>
<td></td>
<td>30</td>
<td>95</td>
<td>68.5±2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>158.5</td>
<td>74.5±19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>237.5</td>
<td>68.5±1.5</td>
<td></td>
</tr>
<tr>
<td>Sea water chlorinated</td>
<td>5</td>
<td>11</td>
<td>85±7</td>
<td>1.06</td>
</tr>
<tr>
<td>with 10 mg l⁻¹ Cl₂</td>
<td>30</td>
<td>66.5</td>
<td>97.5±16.3</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>89</td>
<td>115±14</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>111</td>
<td>133.5±153</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>133.5</td>
<td>107±11.3</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>*75+300 H₂O</td>
<td>167</td>
<td>141±42.5</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>*100+400 H₂O</td>
<td>222</td>
<td>135±42.5</td>
<td>1.69</td>
</tr>
</tbody>
</table>

* In these samples, the organic phase was diluted with water prior to preincubation

Mutations ratios (MRs) exceeding 2 are generally considered to be significant even without carrying out statistical tests. However, McCann et al. (1984), who tested some 450 compounds for mutagenicity by the Ames method, concluded that MRs below 2 may also be significant if several determinations are carried out for each sample so that the standard deviations for the number of colonies per plate is low. These researchers also pointed out that if the mutagenicity was solely based on MR determinations, there is a tendency to demand higher MRs for strains displaying low spontaneous mutation rates. They also regarded linearity of the dose-response curves of the number of colonies versus increasing sample concentration as an important criterion for mutagenicity. Generally, the dose-response curve is linear up to a certain concentration, after which a plateau is observed, and then a fall in mutagenicity, reflecting toxicity to the tester bacteria. Thus, linear dose-response plots with statistically significant regression curves can also be considered as good indicators of mutagenicity. In this study, samples giving results which were statistically significant as evaluated by the Student's t test for means, as compared to the number of spontaneous mutations in the same strain, were considered to be mutagenic. By this method, an MR as low as 1.3 was taken as
mutagenic in certain cases, i.e., if the standard deviation was relatively low. In parallel, the linearity of the dose-response curve was also evaluated in terms of the regression curve and the statistical significance of r, the correlation coefficient, with respect to that of a straight line. If both these indices were significant, the sample was clearly mutagenic.

Table 6

Mutagenicity with strain TA 98.

Spontaneous mutations = 22 ± 6.3
The statistically significant mutation rates are shown in bold.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vol. 95% ethanol Extract/plate (µl)</th>
<th>Equiv. vol. water prior to concentration (ml)</th>
<th>No. mutant colonies per plate</th>
<th>Mutation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5</td>
<td>7.5</td>
<td>20±8.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>45</td>
<td>15±0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>75</td>
<td>24±11.3</td>
<td></td>
</tr>
<tr>
<td>Sea water</td>
<td>5</td>
<td>20</td>
<td>22.5±3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>26±4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>26.5±2</td>
<td></td>
</tr>
<tr>
<td>Sea water chlorinated with 10 mg l⁻¹ Cl₂</td>
<td>5</td>
<td>20</td>
<td>20.5±1.5</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>40</td>
<td>32.5±5</td>
<td>1.48</td>
</tr>
<tr>
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<td>20</td>
<td>80</td>
<td>22.5±2</td>
<td>1.02</td>
</tr>
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<td></td>
<td>30</td>
<td>120</td>
<td>38.5±12</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>160</td>
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<td>0.82</td>
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<td>200</td>
<td>16±4</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>240</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>300</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Effluent from the Reading power station</td>
<td>5</td>
<td>20</td>
<td>10.5±4</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>80</td>
<td>23±3</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>31±3.5</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>160</td>
<td>36±5.7</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>200</td>
<td>30±5.7</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>240</td>
<td>21.5±4</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>300</td>
<td>31.5±6.4</td>
<td>1.43</td>
</tr>
</tbody>
</table>

Following chlorination with 10 mg l⁻¹ chlorine, sea water exhibited statistically significant mutagenicity with all the strains of bacteria tested, as shown in Tables 2, 5 and 7 for samples derived from 120-300 ml, 80-240 ml, 90-220 ml and 120-200 ml sea water, respectively. However, for chlorination of sea water with 2 mg l⁻¹ chlorine, the tests with the strains TA 104 and TA 98 gave positive mutagenicity (Tables 2 and 7) for sample volumes derived from 20-240 ml and 120-200 ml, respectively, whereas strain TA 102 only indicated mutagenicity following chlorination in the presence of 5 mg l⁻¹ chlorine of samples derived from 20-200 ml sea water (Table 3).
Table 7
Mutagenicity with strain TA 98.

These samples were diluted with distilled water prior to preincubation. Spontaneous mutations = 22 ± 4.2. The statistically significant mutation rates are typed in bold face.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vol. 95% eth. extr. per plate (µL)</th>
<th>Dil. vol. (ml)</th>
<th>Equiv. vol. water prior to conc. (ml)</th>
<th>No. mutant colonies per plate</th>
<th>Mutation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5</td>
<td>30</td>
<td>7.5</td>
<td>45</td>
<td>21±5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>45</td>
<td>75</td>
<td>22.5±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26±1.5</td>
</tr>
<tr>
<td>Sea water</td>
<td>30</td>
<td>150</td>
<td>120</td>
<td></td>
<td>19±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>160</td>
<td></td>
<td>18±10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>200</td>
<td></td>
<td>24.5±8.5</td>
</tr>
<tr>
<td>Sea water chlorinated with 10 mg 1⁻¹ Cl₂</td>
<td>30</td>
<td>150</td>
<td>120</td>
<td></td>
<td>45.5±7</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>160</td>
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<tr>
<td></td>
<td></td>
<td>50</td>
<td>200</td>
<td></td>
<td>35±8.5</td>
</tr>
<tr>
<td>Sea water chlorinated with 5 mg 1⁻¹ Cl₂</td>
<td>30</td>
<td>150</td>
<td>120</td>
<td></td>
<td>47±12.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>160</td>
<td></td>
<td>42±8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>200</td>
<td></td>
<td>52±8.5</td>
</tr>
<tr>
<td>Sea water chlorinated with 2 mg 1⁻¹ Cl₂</td>
<td>30</td>
<td>150</td>
<td>120</td>
<td></td>
<td>44±17</td>
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<td>50</td>
<td>200</td>
<td></td>
<td>51±12.7</td>
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<tr>
<td>Effluent from Reading power station</td>
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<td>120</td>
<td></td>
<td>30±1.5</td>
</tr>
<tr>
<td></td>
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<td>40</td>
<td>160</td>
<td></td>
<td>36.5±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>200</td>
<td></td>
<td>40.5±4</td>
</tr>
</tbody>
</table>

Reading power station effluent only displayed mutagenicity with the strains TA 102 in samples derived from 120-300 ml (Table 3) and TA 98 in samples from 120-200 ml (Table 7). However, with TA 104, the findings with Reading power station effluent were similar to those obtained with unchlorinated sea water. These results are not in consistent since the sensitivity to mutagenic compounds differs among the various strains. Possibly, TA 104 is particularly sensitive to certain volatile compounds, such as trihalomethanes, in which case, a low mutagenicity would be obtained for Reading power station effluent since the volatile phase is removed from the sea water during the passage through the hot pipes of the power plant cooling system.
Fig. 6 Mutagenicity with TA 104 of sea water [untreated (--0--), or treated with 2 mg $1^{-1}$ Cl$_2$ (-Δ-) or 10 mg $1^{-1}$ Cl$_2$ (-0-) and effluent from the Reading power station (▲)]

In sea water chlorinated with 10 mg $1^{-1}$ chlorine, the dose-response curves are all linear at non-toxic concentrations, conforming to the equation: $a + bx$ (where, $a$ is the number of spontaneous mutants for the particular strain, and $b$ is the sensitivity of the strain to revertants).

All the linear regression plots give significant values of $r$ ($p < 0.05$). Thus, sea water chlorinated with 10 mg $1^{-1}$ chlorine clearly exhibits mutagenicity. The dose-response curves for sea water samples chlorinated with 2 mg $1^{-1}$ chlorine are linear with strains TA 98 and TA 104. However, $r$ is only significant with the former strain, which clearly indicates mutagenicity.

Two strains, TA 98 and TA 102, gave linear dose-response curves and significant linear regressions ($p < 0.05$) with Reading effluent samples.
Fig. 7 Mutagenicity with TA 102 of sea water [untreated (---○---), or treated with 10 mg 1\(^{-1}\) Cl\(_2\) (---○---)] and effluent from the Reading power station (---▲---)

In most of the samples, the mutagenicity fell after reaching a maximum, presumably due to the toxicity to the bacteria of larger samples and possibly also of concentrated impurities from the ethanol phase. These data clearly demonstrate that mutagenic compounds are formed during the chlorination of sea water under laboratory conditions. It was generally possible to detect mutagenicity for volumes of 100-200 cc sea water following chlorination. Although these compounds have not yet been characterized, the mutagenicity may be due to haloorganic materials formed during chlorination. Although some haloorganics (such as THM) are volatile, most of these materials are non-volatile and non-biodegradable and therefore remain in the sea water for long periods (Gehrs and Jolley, 1975). Several of these compounds, such as 5-chlorouracil and 5-chlorocytosine, formed in the reaction between the pyrimidines, uracil and cytosine, and hypochloric acid, are known mutagens. This study also demonstrated the presence of THMs in sea water, which are also established mutagens or carcinogens. However, these compounds only represent the tip of the iceberg as far as haloorganic materials are concerned. The formation of bromoform and other bromoorganic derivatives in chlorinated sea water, which are also mutagenic, was also shown. Such mutagenicity would be expected to have adverse effects on marine bioecosystems, which depend on sea water for nutrients, and on humans, via fish and other sea foods. It would seem pertinent to carry out special tests on oysters, which derive food by straining vast quantities of sea water, and, thereby presumably accumulate mutagenic compounds, which, in turn, might be detrimental to
Fig. 8 Mutagenicity with TA 100 of sea water untreated (---0---), or treated with 10 mg l$^{-1}$ Cl$_2$ (---0---)

humans. Power stations use chlorine levels of about 2mg l$^{-1}$. Although some of the haloorganic materials evaporate due to the heat, the tests carried out with the TA 98 and TA 102 strains still indicate mutagenicity.

This is the first comprehensive study indicating the presence of mutagenic halo-organic compounds in chlorinated sea water. Data along similar lines at other locations, would justify a search for alternative oxidants for sea water, such as chlorine dioxide (Guttman-Bass et al., 1987) or ozone. The absence of mutagenicity in sea water sampled at sites far away from sewage drainage points and power stations is presumably due to dilution effects or to removal of the hazardous materials via precipitation, adsorption, evaporation and other chemical and physical processes. In the long term, the Mediterranean Sea, which is almost a closed basin, might prove to be an exception to this general rule so that the cumulative effects of mutagenic haloorganic materials clearly deserve further consideration.
Fig. 9 Mutagenicity with TA 98 of sea water [untreated (-- - --), or treated with 2 mg l\(^{-1}\) Cl\(_2\) (--Δ--), or 10 mg l\(^{-1}\) Cl\(_2\) (--○--) and effluent from the Reading power station (--▲--)]

4. SUMMARY AND CONCLUSIONS

Many power stations, particularly along the Mediterranean coast, use sea water for cooling. This water, which is chlorinated with 2 mg l\(^{-1}\) chlorine in order to prevent accumulation of algae and other organic matter in the pipes, is eventually discharged into the sea. Chlorinated waste water is also allowed to drain into the sea. According to one estimate based on data collected in Maryland in the United States, at least 3% of this chlorine forms stable halo-organic compounds, which persist in the sea water for long periods (Jolley et al., 1978). The adverse and toxic effects of high chlorine levels on marine ecosystems have been well documented in many parts of the world (James, 1967; Hirayama and Hirano, 1970; Carpenter et al., 1972; McLean, 1973; Gentile et al., 1973; Anderson, 1974; Serviszi and Martens, 1974; Davis and Midkauth, 1978).
This study compared the chlorine demands and mutagenicities of laboratory-chlorinated samples of Mediterranean sea water samples and effluent from the Reading power station in Tel-Aviv. The Ames method was used to measure mutagenicity in the concentrated samples. These data were correlated with the THM content as determined by gas chromatography. The chlorine demand, which was found to be 2.0 mg l⁻¹, occurred in two phases, a fast, and a slow one, which seem to correspond to reactions of the inorganic and organic phases, respectively. Mutagenicity was demonstrated in 80–300 ml laboratory-chlorinated sea water. The high THM content in chlorinated, as compared to regular sea water, indicates that the mutagenicity may be attributed to haloorganic materials, of which the THMs only account for a small volatile portion. Whereas fresh water following chlorination primarily contains chloroform, bromoform and dibromochloromethane are the major THMs in chlorinated sea water. These data are in accord with previous studies (Carpenter and Macalady, 1978) and are consistent with the known high bromine content of sea water.

Although there are differences in the haloorganic compounds formed following by chlorination of sea water and fresh water, sea water clearly exhibited mutagenicity. The Reading power station effluent samples also displayed mutagenicity in the Ames test with the TA 98 and TA 102 strains, although mutagenicity was not detected with the TA 100 and TA 104 strains, both of which detected mutagenicity in laboratory-chlorinated sea water. THM content in Reading effluent was also found to be very low in these experiments, which may be due to the evaporation from these samples of THMs, to which these strains may be more sensitive.

5. REFERENCES


METHYLmercury IN DEEP-SEA ORGANISMS
FROM THE MEDITERRANEAN

by

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ABSTRACT

Data on mercury levels in biocindicators (Norwegian lobster, Nephrops norvegicus L. and red shrimp, Aristaeus antennatus Risso) from different sites of the Mediterranean are reported. The role of methylmercury in determining the total mercury content in these organisms is discussed. Results from a small-scale photolysis experiment for methylmercury under outdoor conditions are given. The relation between the Mediterranean thermal anomaly and the high concentration of mercury in deep-sea organisms, together with the potential of photolysis in reducing methylmercury net production in surface or shallow waters is discussed.

1. INTRODUCTION

The elevated levels of mercury in organisms caught in the Mediterranean as compared to representatives of the same species from the Atlantic (Thibaud, 1971; Bernhard and Renzoni, 1977; Baldi et al., 1979; Renzoni et al., 1979; Hornung et al., 1980; Leonzio et al., 1981; Barghigiani et al., 1986; Thibaud, 1986; UNEP/FAO/WHO, 1987), have given rise to several hypotheses, including that of the geochemical anomaly of the Mediterranean basin, located on a "mercury belt" (Jonasson and Boyle, 1971). A recent hypothesis (Bacci, 1989) is based on the influence of temperature on the methylmercury net production and on the capacity of sunlight to break down methylmercury molecules.

In the biogeochemical cycle of mercury, a very important role is played by methylisation reactions mainly occurring in marine sediments by both biological (Jensen and Jernelöv, 1969; Berman and Bartha, 1986) and chemical processes (Nagase et al., 1982). The reaction rate is affected by Hg2+ concentrations in the sediments as well as the temperature; this last parameter particularly influences the metabolic activity of methylating organisms (Jernelöv and Asèll, 1975; Biscogni and Lawrence, 1975; Shin and Krenkel, 1976; Nagase et al., 1982; Wright and Hamilton, 1982).

Comparative studies of levels of mercury in the waters of the Atlantic and the Mediterranean have shown no great differences, with a tendency of Atlantic waters to be higher in mercury than Mediterranean waters (Copin-Montégut et al., 1986; Ferrara and Maserti, 1986; Ferrara et al., 1986; Ferrara and Maserti, 1988).
Mercury levels in open sea sediments of the Atlantic and the Mediterranean are comparable (Aston et al., 1986; Bargagli et al., 1988).

The deep-water temperatures of the Atlantic and the Mediterranean are very different. The latter is characterised by a thermal anomaly. In the Mediterranean basin, the water temperature below 200 m is ca. 13°C regardless of depth or season, while in the Atlantic there is a gradient from ca. 13°C at the surface to 4°C at 1,000 m and around 0°C at the bottom. The mercury methylation rate in sediments is typically low (i.e. 0.1-1% per year of the inorganic mercury present in the accessible sediment; Ziegler and Krenkel, 1975). However, methyl mercury production may increase by a factor of 2-3 with an increase in temperature from 4 to 13°C (Shin and Krenkel, 1976; Nagase et al., 1982). According to Bacci (1989) this could explain the high levels found in top-predators, like tuna (Thibaud, 1971; Renzoni et al., 1979), and in some deep-sea organisms, like the Norwegian lobster (Leonzio et al., 1981). The decrease in mercury concentration with the decrease of depth of fishing ground, from the epibathial zone (i.e. 200 m) to shallow waters, observed in some fish (Hornung et al., 1980; Leonzio et al., 1981; Barghigiani et al., 1986) may be explained by photolytic decomposition of organic mercury (Bacci, 1989), leading to mercury levels in marine organisms which probably are still anomalous, but remarkably lower than those found in animals from deeper environments. The mean dissociation energy of the carbon-mercury bond in methymercury is 29 kcal mole\(^{-1}\) (Mortimer, 1962), and sunlight at the wavelength of 400 nm contains more than 70 kcal mole\(^{-1}\) of photons (Roberts et al., 1981). In shallow waters, methymercury may undergo photodegradation with the formation of ionic inorganic mercury compounds and then elemental mercury. This form tends to partition in the air, due to its high air/water dimensionless equilibrium partition coefficient, \(K_{aw}\) (0.32 at 20°C; Sanemasa, 1975). This process is probably the main route of mercury depletion of marine waters (Fitzgerald, 1986).

With the aim of improving our present knowledge on the mercury distribution in the Mediterranean, samples of marine crustaceans caught in different areas were analysed for total and methymercury.

A small scale photolysis experiment was also carried out to verify the stability of methymercury under simulated "natural" conditions.

2. MATERIALS AND METHODS

For the photolysis experiment, glass vials containing 20 ml of an aqueous solution of methymercury chloride at 50 ng ml\(^{-1}\), as mercury, were used; the vials were placed outdoors on a wooden support facing south for 57 days in the summer of 1989 (temperature range: 15-30°C); controls were kept in the dark at 40°C for the same period. The vials were sealed with Parafilm rather than flame, so as to avoid UV exposure which could even destroy all the methyl mercury in solution.
Samples of Norwegian lobsters (*Nephrops norvegicus* L.), were caught at different depths in the epibathial zone (below the mixing layer) from 200 to 500 m (sampling areas C, D, and E in Fig. 1; sampling stations 5, 7, and 8 in Fig. 2C, D, E). Data from previous studies (Leonzi et al., 1981) on Norwegian lobsters collected in sampling area C (Fig. 1; Fig. 2C; sampling stations 3, 4, 6) were also considered. *Nephrops norvegicus* L. is usually found in the epibathial zone (200-500 m), but in the Northern Adriatic it has adapted to shallower depths. It was therefore also possible to select a shallow-water site (42 m; station 9; Fig. 2E) for the collection of the Norwegian lobster.

An additional study was performed with another crustacean species, the red shrimp *Aristeus antennatus* Risso, that was collected at ca. 300 m in sampling area A (Fig. 1; station 1, Fig. 2A) and sampling area B (Fig. 1; sampling station 2, Fig. 2B).

2.1 Analytical methods

2.1.1 Water for photolysis experiment

The content of inorganic mercury produced by the photolysis of methylmercury in the vials was detected by atomic absorption spectrophotometry (AAS; Perkin-Elmer 2280), after direct reduction to elemental mercury with an aqueous solution of SnCl₂ (10%), NH₂OH.HCl (6%), NaCl (6%) and H₂SO₄ (1 N) and air current stripping.

2.1.2 Organisms

For total mercury, muscle tissue samples of Norwegian lobster and red shrimp (1-1.5 g, wet weight) were decomposed with concentrated HNO₃ (2.5 ml) under pressure in teflon vessels at 130°C, according to Stoepler and Backhaus (1978). Homogeneous sub-samples were utilised for the measurement of the water content (24 h, 105°C). Total mercury was detected by AAS after reduction, as in the water samples.

For the measurement of methylmercury, homogeneous samples (ca. 2 g, wet weight) were hydrolyzed with an acid solution: methylmercury was extracted into an organic solvent, purified by the addition of cysteine solution, re-extracted into organic solvent and then measured by gas chromatography according to Horvat et al. (1990). A Perkin-Elmer F-22 gas chromatograph with ECD and a 1 m x 2 mm (i.d.) borosilicate glass-column packed with 5% Carbowax 20 M on 100/120 Supelcoport was used. The carrier gas was argon-methane 95/5%; flows 90 and 40 (as scavenger) ml min⁻¹; injector, oven and detector temperatures were 190, 180 and 280°C respectively.

The precision of total mercury and methyl mercury analytical methods, measured on 6 homogeneous replicates of the same sample was 9.2 and 13.6% respectively, as coefficient of variation. The accuracy was tested in two intercalibration exercises.
Fig. 1 Sampling areas. A and B for red shrimp (*Aristeus antennatus*); C, D, E, for Norwegian lobster (*Nephrops norvegicus*).

3. RESULTS AND DISCUSSION

Under the simulated environmental conditions, photolysis was found to follow first-order kinetics (Fig. 3) with a $t_1$ of 63 days. The energy of solar radiation at the sea surface is probably higher than in the present experimental situation. Hence, the photolysis of methylmercury is likely to occur under natural conditions in surface waters and other environmental compartments reached by solar radiation.

In deep waters, photolysis cannot occur, yet the net production of methylmercury may be higher than in shallow waters. It was assumed that the net production of methylmercury in the depths of the Mediterranean was the same in different areas for a time scale of 10-20 years.

The relative homogeneity and stability of the Mediterranean Sea can be explained in terms of the mass balance of mercury (Fig. 4). The essential input data used were as follows: Mediterranean water volume $4.5 \times 10^{15}$ m$^3$ with a total mercury concentration of 1 ng 1$^{-1}$ (or 1 $\mu$g m$^{-3}$) gives a total mercury content of Mediterranean waters of $4.5 \times 10^{15}$ $\mu$g, or 4500 t; Mediterranean sediment surface area was assumed to be equal to the surface area of the sea ($3 \times 10^{12}$ m$^2$), with an accessible depth of 0.05 m (sediment volume $1.5 \times 10^{11}$ m$^3$) and a mercury concentration of 0.1 $\mu$g g$^{-1}$ (0.15 g m$^{-3}$, assuming a sediment density of 1.5 t m$^{-3}$), the total quantity of mercury in the accessible sediments of the Mediterranean can be estimated at $2.25 \times 10^{10}$ g, or 22500 t.
Fig. 2 Sampling stations
Fig. 3 Kinetics of methylmercury photolysis in aqueous solution under outdoor conditions. MeHg indicates methylmercury chloride.

Fig. 4 Mass balance of mercury in the Mediterranean (from Bacci, 1989; modified)
The sedimentation rate of particulate matter was taken as 0.02 cm
yr\(^{-1}\) (Keller and Lambert, 1972), with a mercury concentration of 0.1 \(\mu g\)
g\(^{-1}\) (0.15 g m\(^{-3}\), assuming a density of 1.5 t m\(^{-3}\)) and the
remobilization rate of mercury from the sediment was assumed to be 10% of
the mercury sedimentation rate (mercury sedimentation is 90 t yr\(^{-1}\),
remobilization 10 and sediment burial 80 t yr\(^{-1}\)). The quantity of
suspended matter in the Mediterranean is 0.5 mg l\(^{-1}\) (Emelyanov and
Shimkus, 1972) with a mercury concentration of 0.1 mg kg\(^{-1}\) (Fowler,
1986); therefore the mercury quantity in this compartment is 225 t.
Fish and shellfish can be estimated to be 0.1 mg l\(^{-1}\), with an average
concentration of mercury of 0.3 mg kg\(^{-1}\); mercury content in fish and
shellfish can be estimated at 135 t. Riverine and direct inputs can be
estimated at 30 t yr\(^{-1}\) while the contribution of the Atlantic waters is
50 t yr\(^{-1}\), assuming that the mercury concentration is 2 ng l\(^{-1}\) in the
Atlantic and 1 ng l\(^{-1}\) in the Mediterranean (Bacci, 1989). Deposition
and volatilization rates are estimated at 150 t yr\(^{-1}\) and the output of
mercury by fisheries at 0.3 t yr\(^{-1}\), as previously discussed by Bacci
(1989).

Referring to Fig. 4, anthropogenic sources of mercury probably do
not produce a significant change in the overall system, although
locally there may be heavy and long-lasting contamination (Renzoni et
al., 1973; Bacci et al., 1986).

The residence time of mercury in the Mediterranean is of the order
of 100 years and the system seems to be near to a steady state, so that
mercury data from Norwegian lobsters caught and analysed late in the
'70s (Leonzio et al., 1981) in off-shore sites can be used together
with new data, as an indication of mercury levels in deep environments.

Levels of total mercury in Norwegian lobster, from different
sampling sites (200-500 m deep) in the Adriatic and Tyrrenian are
given in Figs. 5-10. The data of Figs. 5, 6 and 8 is from Leonzio et
al. (1981); all other data for Norwegian lobster is from the present
study. Total mercury concentrations are plotted against body weight,
since mercury accumulates with exposure time; separate trends are
obtained for male and female, as previously stated (Leonzio et al.,
1981), due to their different growth rates (Farmer, 1973). After
normalization for sex and body weight, the data of Figs. 5-10 is quite
homogeneous: for instance a male Norwegian lobster weighing 50 g has a
total mercury content of 870, 830, 870, 1170, 530 and 765 ng Hg g\(^{-1}\) wet
weight (water content = 75%) in sampling stations 3, 4, 5, 6, 7 and 8
respectively (Fig. 2C, D, E).

In the Adriatic, Norwegian lobsters were caught even at a depth of
40-45 m (i.e. in the mixing layer, where bottom waters can periodically
reach the sea surface). These specimens contain less mercury that those
found in deeper environments (Fig. 11).

The total mercury accumulation plots in both female and male
specimens from deep and shallow-water environments are shown in Fig.
12.

In order to obtain additional information on mercury levels in
organisms from the deep Mediterranean, red shrimp (Aristeus antennatus
Risso) females were collected from the Lion Gulf (sampling station 1;
Fig. 2A) and the Gulf of Genoa (Ligurian Sea; sampling station 2;
Fig. 5 Total mercury concentration in the muscle of *Nephrops norvegicus* L. from sampling station 3 plotted against body weight; data from Leonzio et al. (1981)

\[ Y = 275.4 + 19.8X \]
\[ r = 0.770 \]
\[ n = 44 \]

\[ Y = 430.7 + 8.8X \]
\[ r = 0.886 \]
\[ n = 45 \]

Fig. 6 Total mercury concentration in the muscle of *Nephrops norvegicus* L. from sampling station 4 plotted against body weight; data from Leonzio et al. (1981)

\[ Y = 0.3 + 19.1X \]
\[ r = 0.761 \]
\[ n = 10 \]

\[ Y = 452.6 + 7.6X \]
\[ r = 0.471 \]
\[ n = 10 \]
Fig. 7 Total mercury concentration in the muscle of Nephrops norvegicus L. from sampling station 5 plotted against body weight; data from present study.

Fig. 8 Total mercury concentration in the muscle of Nephrops norvegicus L. from sampling station 6 plotted against body weight; data from Leonzio et al. (1981).
Fig. 2B). The results are given in Fig. 13. Mercury concentrations range from 320 to 1700 ng g⁻¹ wet weight (water content = 70%), increasing with body weight, with no significant differences between the two sampling areas. These concentrations are more similar to those found in Norwegian lobsters from the deep-sea than to those observed in lobsters from shallow waters.

Methylmercury determination was carried out in 55 randomly chosen samples of Norwegian lobster previously analysed for total mercury. The results (Fig. 14) indicate that most of the metal is in the organic form, particularly when the concentration of total mercury is high. The x axis intercept (77 ng g⁻¹, wet weight, water content = 75%; standard error of the x axis intercept = 24 ng g⁻¹ wet weight) indicates a background level for inorganic mercury of 50-100 ng g⁻¹ wet weight.

The gradient of the slope in Fig. 14 is close to one, indicating that for an increase of one ng g⁻¹ in the total mercury, the level of methylmercury also increases by about one ng g⁻¹. This result, which is similar to the findings of Capelli et al. (1987) in the muscle tissue of Sarda sarda, confirms the essential role of methylmercury in bioconcentration phenomena.

As far as the ratio of methylmercury to total mercury in Norwegian lobster is concerned, it can be seen that when the concentration of total mercury is high (i.e. 500 or more ng g⁻¹), methylmercury is the major component, while when total mercury is low (i.e. 100-200 ng g⁻¹), the inorganic mercury fraction may be significant. Other organic forms of mercury seem to be negligible.

Fig. 9 Total mercury concentration in the muscle of Nephrops norvegicus L. from sampling station 7 plotted against body weight; data from present study.
Fig. 10 Total mercury concentration in the muscle of *Nephrops norvegicus* L. from sampling station 8 plotted against body weight; data from present study.

Y = 99.1 + 23.5X
r = 0.867
n = 11

Y = 290.1 + 9.5X
r = 0.874
n = 10

Fig. 11 Total mercury concentration in the muscle of *Nephrops norvegicus* L. from sampling station 9 plotted against body weight; data from present study.

Y = -69.7 + 8.0X
r = 0.854
n = 7

Y = 166.4 + 2.1X
r = 0.624
n = 22
Fig. 12 Total mercury accumulation trends in female (A, C) and male (B, D) specimens of *Nephrops norvegicus* L. from deep (A, B) and shallow water environments (C, D).

Fig. 13 Accumulation of total mercury in the muscle of female *Aristeus antennatus* Risso from the Lion (★) and Genoa Gulfs (★).
Fig. 14 Correlation between methylmercury (MeHg) and total mercury concentrations in the muscle of *Nephrops norvegicus*

4. CONCLUSIONS

The results obtained confirm the previously observed phenomenon where mercury levels in bio-indicators increase with depth in the range of 30-340 m (Hornung et al., 1980; Leonzio et al., 1981; Bargigiani et al., 1986). The relative homogeneity in the findings as regards the Norwegian lobster and the red shrimp from epibathial environments (200-500 m) seems to indicate that the levels of mercury in the deep Mediterranean are relatively non-site specific and similar, over a time scale of 10-20 years. The role of methylmercury as a major component in the total mercury found in marine organisms when the total mercury is "high" (i.e. 500 ng g⁻¹ or more) is confirmed. The small-scale photolysis experiment under outdoor conditions demonstrates the possibility of breakdown of the C-Hg bond in nature, especially in aquatic environments. All these facts, together with the low mercury and methylmercury concentrations found in Norwegian lobsters from north Adriatic shallow waters, seem to support the hypothesis that the Mediterranean mercury anomaly in epibathial environments is related to the thermal anomaly (i.e. high temperature in deep-environments leading to high microbial activity and methylmercury production), while in the mixing layer (i.e. depth less than 100 m), where the water has the possibility of interacting with sunlight, the photolytic breakdown of the C-Hg bond in the methylmercury molecule significantly reduces net methylmercury production, leading to mercury levels in fish which probably are still anomalous (Baldi et al., 1979), but lower than those found in animals from deeper environments.
5. REFERENCES


IMPORTANCE OF GELATINOUS PLANKTON ORGANISMS IN THE STORAGE AND TRANSFER OF TRACE METALS IN THE NORTH-WESTERN MEDITERRANEAN

by

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1. INTRODUCTION

Gelatinous macroplankton constitute an important part of the plankton in oceanic and coastal waters during various times of the year (Alldredge and Madin, 1982). These organisms, at the base of the food web, may significantly mediate the transport and the cycle of metals and radionuclides in oceanic waters (Gorsky et al., 1984; Krishnaswami et al., 1985).

This study is concerned with trace metal (Cd, Cu, Pb and Zn) concentrations in gelatinous macroplankton. Various factors were taken into consideration such as the taxonomy of the studied species, the sampling location and the sampling period.

2. MATERIALS AND METHODS

Most of the samples were collected 500 m from the coast at station B (43°41'10"N, 7°19'E) at the entrance of the Bay of Villefranche-sur-mer at different periods of the year between 1987 and 1990. Some organisms were sampled at two stations on the Nice-Calvi (Corsica) transect. The first station was located in the Ligurian current (St. L1: 43°38'8"N, 7°23'4"E, May 1989) 5 km from the coast, the second station (station L2: 43°24'8"; 7°52'E, March 1988 and September 89) was situated in the hydrological front of the Ligurian Sea ca 50 km from the coast, but was not in the Ligurian current.

A sampling depth varying from 1 to 10 m was chosen: a) to avoid possible surface contamination; b) to obtain a large number of samples and c) to limit damage to delicate organisms.

After collection, animals were isolated at room temperature, and maintained in an acid-washed aquarium filled with filtered seawater so that they could empty their stomach content. For salps: Pegea confoederata and Salpa maxima, when possible, the viscera (nucleus) were carefully separated with a teflon-coated spatula, otherwise, the fecal pellets were left to settle down in the aquarium where they were later collected. The samples, which often contained a relatively large number of individuals of one species (especially for salps) were identified. Then, they were very quickly rinsed with distilled water and stored frozen in plastic vials.
Before analysis, samples were thawed and then digested with concentrated nitric acid (65%) (Merck Suprapur) in a microwave oven MDQ810. Metal concentrations were determined by atomic absorption spectrophotometry (Philips Pye Unicam SP9) with flame for copper and zinc and with graphite furnace for cadmium and lead (Philips PU 9095 video furnace). Deuterium background correction was used. The analytical procedure was checked regularly using standard reference material (lobster hepatopancreas TORT-1) provided by the NRC of Canada.

3. RESULTS

The taxonomy of the collected animals is presented in Table 1.

Table 1

<table>
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<th>PHYLUM</th>
<th>CLASS</th>
<th>ORDER</th>
<th>SPECIES</th>
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<td>Nuda</td>
<td>Beroida</td>
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</table>

Tables 2, 3, 4 and 5 give trace metal concentrations in Cnidaria, Ctenophora, Mollusca and Tunicata, respectively.

A great number of specimens of Velella velella was collected at station B as well as at station L1 and L2. Table 2 indicates that Velella velella generally presents low concentrations in trace metals. Nevertheless, the samples collected at L1 have a mean cadmium concentration 0.8±0.3 µg g⁻¹ (n=22) higher than that found at station B: 0.3±0.1 µg g⁻¹ (n=20, t-test significant at p <0.001) and than that found at station L2: 0.4±0.2 µg g⁻¹ (n=8, t-test significant at p <0.005). This result may reflect differences in the metal concentrations in surface waters as Velella velella is floating at the surface of water. Station L1 is situated in the Ligurian current which carries along dissolved or particulate pollutant metals from Genoa and LaSpezia areas whereas station B located at the entrance of the Bay of Villefranche, may not be influenced by this current and station L2 is far from the coast (ca 50 km). As regards lead concentrations, they tend to be higher at B (0.3 µg g⁻¹) than at station L1 (0.1 µg g⁻¹), but the means are not significantly different.
Table 2

Trace metal concentrations (expressed as µg metal g⁻¹ dry weight) in *Veelalva veelalva* (Cnidaria, Chondrophora).

<table>
<thead>
<tr>
<th>Sampling date and location</th>
<th>Cadmium</th>
<th>Copper</th>
<th>Lead</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.05.89-St. B (n = 20)</td>
<td>0.3±0.1</td>
<td>2.4±0.8</td>
<td>0.3±0.3</td>
<td>35±9</td>
</tr>
<tr>
<td>16.05.89-St. L¹ (n = 22)</td>
<td>0.8±0.3</td>
<td>2.9±1.3</td>
<td>0.1±0.1</td>
<td>30±6</td>
</tr>
<tr>
<td>15.09.89-St. L² (n = 8)</td>
<td>0.4±0.2</td>
<td>2.1±0.2</td>
<td></td>
<td>43±11</td>
</tr>
<tr>
<td>Mean ± 1 S.D. (n = 50)</td>
<td>0.6±0.3</td>
<td>2.6±1.0</td>
<td>0.2±0.2</td>
<td>34±9</td>
</tr>
<tr>
<td>Concentration range</td>
<td>0.2-1.6</td>
<td>1.6-7.6</td>
<td>0.1-1.6</td>
<td>19-70</td>
</tr>
</tbody>
</table>

Table 3

Trace metal concentrations (expressed as µg metal g⁻¹ dry weight) in *Beroe ovata* (Ctenophora, Beroida).

<table>
<thead>
<tr>
<th>Sampling date and location</th>
<th>Cadmium</th>
<th>Copper</th>
<th>Lead</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.03.88-St. B (n=15)</td>
<td>0.3±0.2</td>
<td>3.1±1.1</td>
<td></td>
<td>49±37</td>
</tr>
<tr>
<td>Concentration range</td>
<td>0.1-0.6</td>
<td>1.5-5.8</td>
<td></td>
<td>18-128</td>
</tr>
</tbody>
</table>

Roméo *et al.* (1985) found significantly higher lead concentrations in copepods and in mixed gelatinous macroplankton organisms collected at station B than at open sea stations. This phenomenon was ascribed by the authors to contamination of coastal waters by atmospheric lead coming from car exhaust pipes.

The Ctenophora *Beroe ovata* (Table 3), collected at station B during the spring, do not show significant differences in trace metal concentrations with the Cnidaria *Veelalva veelalva*. Metal concentrations are variable and seem to be dependent on the size of *Beroe ovata*, thus allometric relationships could be calculated. Trace metal contents (expressed as µg metal) per individual were plotted versus weight (expressed as g of dry weight) as power functions $C = aW^b$. The most significant relationships were found for zinc ($b = 0.514$, $a = 38$, $r = 0.906$ at $p < 0.0001$) and for copper ($b = 0.759$, $a = 2.9$, $r = 0.873$ at $p < 0.0001$). Trace metal concentration related to $b < 0.77$ power of body weight indicates that small individuals contain higher concentrations of metals than large individuals (Boyden, 1977). This may be partially
attributable to the greater metabolic rate per biomass of smaller individuals or to an uptake of metals preferentially done by absorption.

Table 4

Trace metal concentrations (expressed as μg metal g⁻¹ dry weight) in Mollusca.

<table>
<thead>
<tr>
<th>Sampling date and location</th>
<th>Cadmium</th>
<th>Copper</th>
<th>Lead</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cavolinia inflexa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.03.88-St. L₂ (n = 2)</td>
<td>2.4</td>
<td>3.6</td>
<td>----</td>
<td>184</td>
</tr>
<tr>
<td>14.04.89-St. B (n = 2)</td>
<td>0.4</td>
<td>1.4</td>
<td>3.0</td>
<td>81</td>
</tr>
<tr>
<td>16.05.89-St. L₁ (n = 3)</td>
<td>1.1</td>
<td>1.3</td>
<td>3.0</td>
<td>52</td>
</tr>
<tr>
<td><strong>Clio pyramidata</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.03.88-St. L₂ (n = 3)</td>
<td>3.1±0.6</td>
<td>1.8±0.2</td>
<td>3.0±0.1</td>
<td>96±17</td>
</tr>
<tr>
<td><strong>Pneumodermopsis canephora</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.03.88-St. L₂ (n = 1)</td>
<td>3.9</td>
<td>8.7</td>
<td>----</td>
<td>325</td>
</tr>
<tr>
<td>14.04.89-St. B (n = 2)</td>
<td>1.2</td>
<td>4.6</td>
<td>3.0</td>
<td>106</td>
</tr>
<tr>
<td>16.05.89-St. L₁ (n = 4)</td>
<td>1.5±0.2</td>
<td>3.3±1.0</td>
<td>3.0±0.1</td>
<td>109±61</td>
</tr>
<tr>
<td>Concentration range in Mollusca</td>
<td>0.4-6.9</td>
<td>1.3-9.4</td>
<td>2.8-3.2</td>
<td>52-325</td>
</tr>
</tbody>
</table>

Compared to Cnidaria and Otenophora, planktonic Mollusca (Table 4), which are filter-feeding organisms (Thecosomata) and suspension-feeding organisms (Gymnosomata), present higher concentrations in cadmium, lead and zinc whereas copper is not significantly higher. Mollusca generally present high trace metal concentrations.

The most significant features in trace metal concentrations of salps (Table 5) are the elevated values found either in the visceras ("nucleus") or in the fecal pellets compared to that found in the animals.

For Pegea confederata, fecal pellets are richer (factor 5 to 7) in cadmium, copper and zinc than the animals themselves.

For Salpa maxima, collected in March 88 at station L₂, one single sample of fecal pellets could be analyzed which renders any interpretation difficult, whereas in January 90 at station B, more samples of S. maxima and of fecal pellets were available. As a result,
copper and zinc were shown to be particularly concentrated in the fecal pellets (factors 8 and 2.5 respectively, t tests significant at p <0.001) compared to the animals.

Krishnaswami et al. (1985) found that the concentrations of Fe, Al, Th isotopes and $^{210}$Pb in fecal pellets were about one order of magnitude higher than those in salp body, whereas Ca, Cu, Zn, Mn and Po were higher by factors of about 2-5. This is in good agreement with our results.

The cadmium and copper concentrations in the digestive apparatus (nucleus) of Salpa maxima were significantly higher (factors 5 and 3 respectively, t tests significant at p <0.001) than in the animals. For zinc, the difference is not significant. Nuclei were analyzed together with the tunic which surrounded them. Tunicin, the main component of the tunic, is a form of cellulose, specific to Tunicata (Madin et al., 1981). Nuclei (digestive apparatus) were full of phytoplankton.

Salps are herbivores and feed on diatoms, flagellates and other phytoplankton species (Baconnot, 1971; Alldredge and Madin, 1982). Metal concentrations in nuclei of S. maxima (Table 5) are comparable to those found in phytoplankton (Roméo and Nicolas, 1986).

The lower concentrations observed in the nuclei compared to those observed in the fecal pellets (particularly significant in the case of copper, t= 5.18 p <0.0001; less significant in the case of zinc) may be explained by the presence of tunicin kept around the nuclei. Analyses performed on tunicin, collected in some samples of Salpa maxima, show very low concentrations of copper (2 μg g$^{-1}$) and zinc (40 μg g$^{-1}$).

In Table 6 a comparison is made of the metal concentrations in Salps and in their faeces (this paper) to the levels of the four metals in the water in the area where the organisms were collected (Nicolas, pers. communication), in phytoplankton on which salps feed (Roméo and Nicolas, 1986) and in particulate material collected in a sediment trap, placed at a depth of 200 m in a Mediterranean coastal area (Roméo et al., 1988).

Table 6 shows that phytoplankton concentrates the four metals from sea water. Metal concentrations in salp faeces are of the same order of magnitude as those in phytoplankton and in particulate material, except lead concentration which seems to be higher in the particulate material collected in the trap.

While falling down, fecal pellets fractionate, one part comes back to the biological cycle via the food web while another part is transported to depth. This last phenomenon was observed for Chernobyl fallout by Fowler et al. (1987): some of the radionuclides were removed from Mediterranean surface waters and transported to 200 m in a few days by zooplankton grazing and by the subsequent zooplankton (and especially copepod) fecal pellet production. The importance of salp particulate products in the cycling of chemical pollutants must be emphasized since Bruland and Silver (1981) reported that the extraordinarily high sinking rates of fecal pellets of salps indicates that these tunicates may be disproportionately important in the flux of biogenic materials during periods when they form dense population blooms.
<table>
<thead>
<tr>
<th>Sampling date and location</th>
<th>Cadmium</th>
<th>Copper</th>
<th>Lead</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fege confoederata</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.05.89-St. L₁ (n = 5)</td>
<td>0.3±0.0</td>
<td>11.9±1.3</td>
<td>4.5±2.5</td>
<td>51±4</td>
</tr>
<tr>
<td>Fecal pellets of P. confoederata</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.05.89-St. L₁ (n = 3)</td>
<td>2.1±0.0</td>
<td>59.3±18.1</td>
<td>—</td>
<td>352±25</td>
</tr>
<tr>
<td>Thalia democatica</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.04.89-St. B (n = 2)</td>
<td>0.1</td>
<td>6.4</td>
<td>2.0</td>
<td>32</td>
</tr>
<tr>
<td>10.05.89-St. B (n = 1)</td>
<td>0.3</td>
<td>26.0</td>
<td>0.5</td>
<td>48</td>
</tr>
<tr>
<td>Salpa maxima</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.03.88-St. L₂ (n = 3)</td>
<td>1.7±1.4</td>
<td>2.5±1.0</td>
<td>—</td>
<td>20±8</td>
</tr>
<tr>
<td>Fecal pellets of S. maxima</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.03.88-St. L₂ (n = 1)</td>
<td>4.3</td>
<td>15.0</td>
<td>—</td>
<td>118</td>
</tr>
<tr>
<td>Salpa maxima</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.01.90-St. B (n = 7)</td>
<td>0.3±0.2</td>
<td>5.9±1.7</td>
<td>—</td>
<td>83±35</td>
</tr>
<tr>
<td>Nucleus of S. maxima</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.01.90-St. B (n = 6)</td>
<td>1.4±0.3</td>
<td>18.3±5.9</td>
<td>22.9±24.2</td>
<td>125±37</td>
</tr>
<tr>
<td>Fecal pellets of S. maxima</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.01.90-St. B (n = 7)</td>
<td>0.7±0.6</td>
<td>45.8±11.7</td>
<td>65.9±16.5</td>
<td>216±66</td>
</tr>
<tr>
<td>Salpa fusiformis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.03.88-St. L₂ (n = 1)</td>
<td>0.3</td>
<td>8.5</td>
<td>1.7</td>
<td>10</td>
</tr>
<tr>
<td>Thiesa punctata</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.03.88-St. L₂ (n = 4)</td>
<td>0.7±0.2</td>
<td>2.3±0.3</td>
<td>—</td>
<td>8±3</td>
</tr>
<tr>
<td>Concentration range in Salpida</td>
<td>0.1-3.4</td>
<td>0.1-26.0</td>
<td>0.5-6.4</td>
<td>12-117</td>
</tr>
</tbody>
</table>

As a conclusion, we may stress the importance of gelatinous organisms in the transport of trace metals in the sea. The metal concentrations in these organisms may reflect the concentrations in the water, these organisms may accumulate trace metals by adsorption, the herbivore salps may transport heavy metals by a high production of fecal pellets.
Table 6

Trace metal concentrations (expressed as μg metal g⁻¹ dry weight) in different compartments of the ecosystem.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Cadmium</th>
<th>Copper</th>
<th>Lead</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea water (ng.l⁻¹)</td>
<td>5</td>
<td>100</td>
<td>40</td>
<td>200</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>1.7</td>
<td>34</td>
<td>16.3</td>
<td>262</td>
</tr>
<tr>
<td>Salps (S. maxima)</td>
<td>0.3</td>
<td>5.9</td>
<td>___</td>
<td>83</td>
</tr>
<tr>
<td>Faeces of (S. maxima)</td>
<td>0.7</td>
<td>45.8</td>
<td>65.9</td>
<td>216</td>
</tr>
<tr>
<td>Particulate material</td>
<td>0.68</td>
<td>50</td>
<td>292</td>
<td>150</td>
</tr>
</tbody>
</table>

3. ACKNOWLEDGEMENTS

This work was supported partially through the MED Trust Fund (FAO Research Project FRA/40-K).

4. REFERENCES


MICROCOSM EXPERIMENTS ON THE ACCUMULATION AND RELEASE OF
TRACE METALS (Zn, Cu, Cd) BY MACROALGAE (Ulva lactuca)

by

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Panepistimiopolis, Kouponia
157 01 Athens, Greece

ABSTRACT

The effect of seaweeds on the transfer of metals from seawater to
sediments in coastal polluted areas is one of the least studied
biogeochemical processes.

For the better understanding of this mechanism, the uptake, accumulation and release of trace metals by a cosmopolitan green macroalgal species, Ulva lactuca, was studied by employing four microcosms installed within the Gulf of Elefsis, Greece.

Mature individuals of Ulva lactuca were placed in the microcosms where the metal concentrations were adjusted to high levels, 10 to 500 times higher than those determined in the natural environment of the area. All other factors (T, S, pH and dissolved oxygen) were kept identical with those of the surrounding environment. Trace metals in the dissolved and particulate phase and in the algal tissues, as well as nutrient concentrations in the water were determined every three days. The metal release was studied by determining the metal content in individuals regularly removed and stored for 3, 6 and 15 days outside the microcosms.

The most important findings of these experiments show that:

a) a good correlation exists between the metal accumulated in the
algal tissues and the time of exposure, for metal concentrations
ranging for Cu between 30 to 130 µg l⁻¹ (20 to 100 times higher
than those in the environment) and for Zn between 400 to 2000 µg
l⁻¹ (50 to 250 times higher than those in the environment). Cadmium exhibits the same tendency only for metal concentrations ca. 150 µg l⁻¹ or some 300 times higher than those in the environment.

b) the ability of the Ulva lactuca algae to accumulate metals
decreases as the metal concentration in the microcosm increases.
Therefore, the values of the accumulation ratio (Cu/1va/Cwater)
declines with increasing dissolved metal concentrations in the
microcosm.

c) there is a good correlation between the metal release rate from
the algal tissues and its initial concentration in them. (Algae
with higher metal content tend to loose it faster than those
having a lower metal content.)
d) in the microcosms having the highest metal concentrations, lethal effects have been observed within the first two weeks.

e) dead individuals of *Ulva lactuca* and the particulate matter derived from their degradation, release some dissolved trace metal but also retain significant amounts which are deposited rapidly to the sea bottom sediments.

1. INTRODUCTION

Various species of the green algae *Ulva* are commonly found in polluted and eutrophicated coastal marine areas.

In sea waters with high nutrient concentrations, *Ulva lactuca* exhibits an increased growth rate and an elevated mineral nutrient content in its tissues (Ho, 1981).

There are also many indications that *Ulva lactuca* accumulates heavy metals when it is exposed to high dissolved metal concentrations (Wong et al., 1982). The uptake rate of heavy metals and the correlation between the metal content of the algal tissue and the ambient concentration is not yet well understood. However, it is apparent that it could play a significant role in the food chain but also in the biological and physicochemical transfer of metals from seawater to sediments.

The present paper includes parts of an ongoing study which has as its principal aim the investigation of the behaviour of the cosmopolitan green alga *Ulva lactuca* L. under different concentrations of the trace metals Zn, Cu and Cd, and the examination of the correlation between the trace metal concentrations in the seawater and in the algal tissues. Such correlations concerning Zn have been reported by Scoullos et al. (1989) for *Ulva lactuca* regularly collected from a polluted site near Piraeus. Similar results were found for several other metals at the same site in the Gulf of Elefsis (Scoullos et al., unpublished data).

One of the major methodological problems in correlation studies carried out in natural systems (where accumulation is a crucial factor regulating the metal content of the plant tissues while the metal concentration in sea waters fluctuates widely) is to define, for a given moment, which water concentrations should be considered and correlated with the tissue ones, if the uptake rate and mechanism are not known.

Experimental laboratory models quite often fail to reproduce all physicochemical conditions (eg. daily fluctuations of light, temperature etc) which are critical in regulating the biochemistry of the species, to the extent that the final results cannot be used for direct interpretation of field findings.

In order to overcome these problems a series of experiments was designed and carried out during August 1987, in the Gulf of Elefsis, Greece, employing four microcosms, made of polyethylene. The microcosms were installed in a shallow embayment within the gulf. Free floating, mature individuals of *Ulva lactuca* were used. In three
microcosms (A, B and C) trace metal concentrations were adjusted to high levels by spiking the seawater with the appropriate quantities of ZnCl₂, CuCl₂ and CdCl₂. The initial concentrations obtained were 25, 50, 250 times for Zn, 10, 20, 100 times for Cu and 30, 70, 400 times for Cd. In the fourth, reference microcosm (D), the concentrations of the trace elements were kept at about the same levels with those of the local environment (E). All other factors (T, S, pH and DO) were kept identical with those of the natural environment of the area. However, the existence of the microcosms themselves had some impact on the conditions. Nutrients have shown a slight increase within the microcosms and in microcosm (C), with the highest metal concentrations, biochemical reactions have resulted in indirect changes of pH and DO.

The accumulation of the trace metals was studied by determining the metal content of the algal tissues every three days. The metal release from the algal tissues was also studied by determining their metal content after removing the algae from the microcosm and storing them in the neighbouring natural environment for 3, 6 and 15 days.

2. MATERIALS AND METHODS

The microcosms having a diameter of 0.7 m and length 1.5 m were made exclusively out of polyethylene and plastic parts (see Caberi, 1990) and were placed in a protected site 100 m from the locality sampled during the first phase of our studies (1985-1986), in order to obtain the maximum comparability.

The metal concentrations in each microcosm sampled every three days are given in Table 1 for the period of one month.

One of the interesting phenomena observed, was the drastic and rapid reduction (within three days) of the high trace metal concentrations adjusted in the system. It is noteworthy that the "fixed" concentrations (b) given in Table 1, are the measured actual values at the moment of every new addition, after thorough mixing. The value (a) in Table 1, corresponds to the concentration after three days, before the new addition of metal salts. The impressive reduction is due only partly to the uptake of metals by the algae. Apparently significant metal quantities were removed by adsorption and absorption of metals on the plastic walls of the microcosm and particles scavenging the metal to the bottom. To some extent, metal losses were due to unavoidable exchange of small, but significant, quantities of water from inside and outside the microcosm. The latter was particularly apparent during the late afternoon hours of the maximum water temperature, when the restricted in the microcosm water mass often had temperatures at its surface by 0.2-0.5°C higher than the surrounding water, leading to a differential expansion of the water column, followed accordingly, by decantation of water.

The opposite phenomenon (intrusion of water into the microcosms) is assumed to have taken place during the early morning hours. However, considering their final volume, it became evident that the microcosms had a small net loss of water. This explains also the fact that in the reference microcosm (D), which was installed in the immediate vicinity of the other three ones and also in the "natural"
Table 1
Dissolved Zn, Cu and Cd concentrations in the microcosms (A), (B), (C1), (D) and the surrounding natural environment (E), during the period studied.

<table>
<thead>
<tr>
<th>Sampling Days</th>
<th>Sample</th>
<th>Microcosm A</th>
<th>Microcosm B</th>
<th>Microcosm C1</th>
<th>Microcosm D</th>
<th>Environment (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Zn µg l⁻¹</td>
<td>Cu µg l⁻¹</td>
<td>Cd µg l⁻¹</td>
<td>Zn µg l⁻¹</td>
<td>Cu µg l⁻¹</td>
</tr>
<tr>
<td>1</td>
<td>a</td>
<td>7.5</td>
<td>1.4</td>
<td>0.8</td>
<td>7.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>784.8</td>
<td>58.4</td>
<td>78.7</td>
<td>1976.4</td>
<td>168.8</td>
</tr>
<tr>
<td>3</td>
<td>a</td>
<td>6.9</td>
<td>1.7</td>
<td>0.5</td>
<td>5.5</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>111.8</td>
<td>15.3</td>
<td>13.5</td>
<td>254.5</td>
<td>15.6</td>
</tr>
<tr>
<td>6</td>
<td>a</td>
<td>7.9</td>
<td>0.4</td>
<td>0.2</td>
<td>9.4</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>136.3</td>
<td>13.6</td>
<td>3.9</td>
<td>205.8</td>
<td>20.4</td>
</tr>
<tr>
<td>9</td>
<td>a</td>
<td>11.8</td>
<td>3.6</td>
<td>0.5</td>
<td>4.7</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>101.2</td>
<td>10.5</td>
<td>3.6</td>
<td>255.5</td>
<td>22.7</td>
</tr>
<tr>
<td>12</td>
<td>a</td>
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environment (E) of the restricted area surrounding them, the metal concentrations were considerably elevated in comparison with reference samples of the area, totally unaffected by the experiment.

Seawater samples were collected from the microcosms as well as from the surrounding environment and analysed for nutrients and trace metals every three days. Dissolved oxygen, pH and temperature were measured in situ, twice every day.

The seawater samples were filtered through 0.45 μm Millipore membrane filters and then passed through ion-exchange resin CHELEX 100, using a modification (Scoullos and Dassenakis, 1984) of the Riley and Taylor (1968) method. The recovery efficiency of the resin was determined by using the method of standard addition of heavy metals in seawater samples. For a mixture of HNO₃ 2N and HCl 1N (1:1), the recovery was found to be 98% for Zn and 95% for Cu.

Dissolved Zn, Cu and Cd were measured by flameless AAS. Filters were digested by concentrated HNO₃, 1:1, at 200°C, for the determination of the concentrations of trace metals of the particulate matter. In the filtered seawater samples the nutrient concentrations were also determined by methods described in Strickland and Parsons (1972).

Algal samples were cleaned from epiphytes and washed with redistilled water. Then a piece of algal tissue of exactly 100 cm² was cut from each sample, was dried at 55°C, weighed and digested by concentrated HNO₃ 1:1, at 200°C.

The digested subsamples were filtered and were used for the determination of the concentration of trace elements in the algal tissue. For the trace metal analyses a Perkin Elmer 5000 Atomic Absorption Spectrophotometer equipped with graphite furnace, was employed. All operations were carried out using exclusively plastic equipment with particular precautions and all the laboratory work was carried out in a clean-box, in order to prevent any contamination.

3. RESULTS AND DISCUSSION

The first noteworthy observation that one should make, is that the use of microcosms, despite several practical, technical difficulties, has been very successful in securing a satisfactory reproducibility of the natural conditions. The minor differentiations concerning temperature and other physicochemical parameters are positive for the experiment. For instance, the nutrient concentrations were kept at the same levels in all microcosms, but in few cases higher levels than those of the local natural environment were recorded, attributed to decomposition of dead algal cells.

In the microcosm with the highest metal concentrations (C1), lethal effects have been observed within the first fifteen days of the exercise despite the rapid decrease of the initially fixed metal concentrations. This means that the algae cannot tolerate even fluxes of very high concentrations (max. for Cd 980.6 μg l⁻¹, for Zn 7857.7 μg l⁻¹ and for Cu 522.6 μg l⁻¹). Muller and Payer (1980), Ray et al. (1980) and De Filippis et al. (1981) have reported, on the basis of
laboratory experiments, that heavy metals are enzyme inhibitors with poisoning effects at very high concentrations. Haritonidis et al. (1983) have reported lethal phenomena for Ulva lactuca when accumulation exceeds 1000 nmol g\(^{-1}\) dw for Cd and 6500 nmol g\(^{-1}\) dw for Zn. Copper is even more drastic. As a result, pH and DO values in microcosm (C) were lower than those in the other microcosms and the surrounding natural environment. The experiment in the microcosm indicates that similar phenomena are to be expected in nature. Relatively large quantities of particulate matter were readily produced, derived mainly from the algal degradation. Its content in trace metals was very high, considerably higher than the content of the algal tissues, indicating that the produced organic matter acts as a scavenger of metal ions (see Table 2). Similar conclusions were reached by using different methodologies by De Filippis et al. (1981) and Haritonidis et al. (1983) who have reported that Zn and Cd respectively tend to accumulate more rapidly in the dead algal cells. The experiment was repeated in our microcosm for a second time for another fifteen days (see C2).

The high metal content of biogenetically produced particles, quite common in restricted and shallow coastal embayments and estuaries of the Mediterranean, where Ulva lactuca and other green algae cover extensive zones, indicate that the role of algae for the effective binding and transport of metals from the seawater to the sediments is a mechanism which should be given more attention and weight. Ulva could play the role of a biological filter and a "trap" of metals with both beneficial and detrimental effects for the ecosystems. Beneficial for the offshore ones but detrimental, for at least a very narrow restricted coastal zone, where these algae are found in large quantities often in piles.

The accumulation of Zn, Cu and Cd in the Ulva lactuca is indicated in Figures 1, 2 and 3, by considering the metal content of the dry tissue throughout the experiment. It becomes immediately apparent from the figures, that the most significant total metal accumulation was observed in the microcosms (C1) and (C2), which had the highest metal concentrations in their water. In general, the highest total, integrated metal uptake by algae was found in the microcosms with the highest dissolved metal concentrations.

In microcosms such as (B), (C1) and (C2), the correlation between the metal content of the algal tissue and the time of exposure seems to be better. This is most clearly indicated by considering the accumulation ratio, if we calculate the latter by dividing the metal content in the algal tissue collected during eg. the sixth day (D\(_6\)) with the metal concentration adjusted during the previous sampling (the third day D\(_3\)). Such a correlation (see figure 4) practically signifies the ability of Ulva lactuca to accumulate and retain high levels of Zn, if the element becomes available in high concentrations, even in fluxes. Although this method of calculating the accumulation ratio does not present accurately the relationship between the metal available to the algae and the metal content in their tissues, we believe that it could reflect in a better way the bio-physico-chemical mechanisms of metal accumulation than the use of values recorded the same day in both water and algal samples.
Fig. 1 Accumulation of Zn in the tissues of Ulva lactuca, expressed as metal content (μg Zn g⁻¹ dw) of algae, collected from the microcosms A (△), B (▲), C1 (□) and C2 (■), every three days.

However, it should be clarified that the ability of the Ulva lactuca algae to accumulate metals decreases as the metal concentration in the microcosm increases. Therefore, the values of the accumulation ratio (Cuyla/Cwater) decline with increasing dissolved metal concentrations in the microcosm. This is in agreement with the findings of Markham et al. (1980) who have noticed that the "accumulation factor", what is called accumulation ratio in this paper, is higher in lower environmental concentrations.

The tendency described for Zn and Cu was not follow by Cd. The Cd concentration of the tissues remained relatively constant and low in the microcosms having dissolved metal concentrations 20-100 times higher than those of the environment. This phenomenon might be explained by the fact that Zn and Cd have similar physicochemical properties and it has been suggested that they exhibit a biologically antagonistic behaviour (Schroeder et al., 1967). The two metals share the ability to combine with sulphhydryl groups of binding proteins (Bryan, 1969; Markham et al., 1980; Ray et al., 1980; De Filippis et al., 1981). Therefore active ligand places saturated by Zn are not available for complexation with Cd.
Table 2
Concentrations of Zn, Cu and Cd in selected samples of algal tissues and particles.

<table>
<thead>
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<th>Algal tissues</th>
<th>Particles</th>
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<td>Cu $\mu$g l$^{-1}$</td>
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</tbody>
</table>

Fig. 2 Accumulation of Cu in the tissues of *Ulva lactuca*, expressed as metal content ($\mu$g Cu g$^{-1}$ dw) of algae, collected from the microcosms A (△), B (▲), C1 (□) and C2 (■), every three days.
Fig. 3 Accumulation of Cd in the tissues of *Ulva lactuca*, expressed as metal content (μg Cd g⁻¹ dw) of algae, collected from the microcosms A (△), B (▲), C1 (□) and C2 (■), every three days.

Interesting were also the findings concerning the release of the accumulated metals. It was observed that *Ulva lactuca* individuals kept for 3, 6 or 15 days in the microcosm (A), where the metal concentrations were comparatively the lowest ones (by 30 times for Cd, 25 times for Zn and 10 times for Cu, higher than those of the environment) tend to lose within the first three days almost the entire quantity of Zn and Cd accumulated, when they are returned to the natural environment. In fact, the Cd and Zn content of the "recovered" algae was similar to that of atoms which were never exposed to the high metal concentrations of microcosm (A). This was not that clear for Cu for the algae of microcosm (A).

In general, however, and for all the three metals studied, there is a tendency for rapid release of metal from the most heavily laden with metals tissues. In fact, the release from plants kept in the microcosms (C1) and (C2), which had dissolved metal concentrations 250 times, 100 times and 400 times higher than the natural environment for Zn, Cu and Cd, respectively, offers the most impressive cases (Figs. 5, 6 and 7).
Fig. 4 The increase of the accumulation ration \((\text{Cu}^{\text{Ulva}}/\text{Cu}^{\text{Water}})\) of Zn in Ulva lactuca as a function of the time of exposure, in the microcosm C1 (△) and C2 (▲)

It was observed that after returning the tissues to the natural environment for 6 days, the metal content was dramatically decreasing but it remained approximately five times higher than the content of the reference, natural samples. The fact that Ulva lactuca retained appreciable percentage of the metal accumulated under high metal stress, combined with the fact that it is a free floating species, indicates its potential role for another type of transport of metals. The latter accumulated in plant tissues could follow the passive movement of the algae. This means that metals could be released or deposited at a locality different from the initial point of discharge of metals (eg. a sewage or an industrial outfall) if large quantities of floating algae are involved, a case identified in the Gulf of Elefsis.

4. CONCLUSIONS

The study of uptake, accumulation and release of trace metals by a cosmopolitan green macroalgal species, Ulva lactuca L., by employing four microcosms installed within the Gulf of Elefsis, Greece, and by monitoring them every day for the physicochemical parameters and every three days for the behaviour of the algae and the metal content of the
Fig. 5 Release of Zn from the tissues of Ulva lactuca algae returned to the natural environment. The algae used had been subjected to different metal stress, kept for 3 (△) and 6 (▲) days in the microcosm C1 and 9 (□) days in the microcosm C2.

system, has produced a number of interesting results. First of all, it has shown that the microcosms provide a valuable "intermediate" between the field studies and the laboratory experiments, which could allow for direct interpretation of the in situ findings.

It became evident that the species in the natural system cannot tolerate very high metal concentrations, even if the latter come in fluxes. In the microcosm (C1), reaching even periodically, every three days, maximum metal concentrations (up to 7857.7 μg l⁻¹ for Zn, 522.6 ppb for Cu and 980.6 ppb for Cd), lethal effects have been observed within the first two weeks.

The dead individuals of Ulva lactuca and the particulates, readily produced as a result of their degradation, retain significant amounts of trace metals (considerably higher than the living tissues) and are deposited rapidly to the sea bottom transferring the metal from the water column to the sediments. This mechanism is particularly important for eutrophicated polluted areas.
Fig. 6 Release of Cu from the tissues of Ulva lactuca algae returned to the natural environment. The algae used had been subjected to different metal stress, kept for 3 (△) and 6 (▲) days in the microcosm C1 and 9 (□) days in the microcosm C2.

Good correlations were identified between the metal content in the algal tissues and (a) the time of exposure and (b) the dissolved metal in the water of the various microcosms for metal concentrations ranging for Cu between 30 and 130 μg l⁻¹, for Zn between 400 and 2000 μg l⁻¹ and for Cd only when the concentration is relatively very high (ca. 150 μg l⁻¹).

Although the totally retained metal is higher for algae subjected to high metal stress, the ability of the Ulva lactuca algae to accumulate metals decreases as the metal concentration in the microcosm increases. Therefore the values of the accumulation ratio (Cu/Ala/Cu) declines with increasing dissolved metal concentrations in the microcosms.

A good correlation was also found between the metal release rate from the algal tissues and its initial concentration in them. Algae with higher metal content tend to lose it faster than those having lower metal content.
Fig. 7 Release of Cd from the tissues of Ulva lactuca algae returned to the natural environment. The algae used had been subjected to different metal stress, kept for 3 (Δ) and 6 (▲) days in the microcosm C1 and 9 (□) days in the microcosm C2.

The release of metal seems to be almost completely reversible for algae subjected to a relatively moderate metal stress. This is not the case for the algal tissues kept in the microcosm with very high trace metal concentrations. These tissues released rapidly the most important part of the metal accumulated but retained also an appreciable percentage of metal. This might be due to the metal retained within relatively stable metal organic complexes, the organic ligands of which are decomposition products of dead algal cells, still connected to the algal tissues.

The results indicate the potentially very important role of Ulva lactuca and similar algae in the mobilisation, fixation and deposition of trace metals in polluted, restricted coastal zones, particularly in the Mediterranean, where tides are insignificant and newly deposited particles could accumulate effectively in sites where floating algae are transported.
5. REFERENCES


Caberi, H., 1990. Uptake and release of heavy metals (Zn, Cu, Cd) by the green alga *Ulva lactuca*. Dipl. in Oceanography thesis, University of Athens (Greece), 92 p.


OCCURRENCE AND FATE OF METHYLtin SPECIES IN THE AQUATIC ENVIRONMENT

by

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ABSTRACT

Methyltin and butyltin species were determined in water, sediments and organisms from the NE Mediterranean and also in the waters of the Marmara and southern Black seas. The methyltin findings were examined to assess its distributional pattern in different aquatic environments. Possible methylation pathways were proposed, considering the reaction mechanisms given for abiotic/biotic methylation processes under laboratory-simulated oxidizing/reducing conditions. Trimethyltin (TMT) is the predominant form in unpolluted sea waters and sediments, while methylation reactions in the inorganic and organic tin-polluted sea waters, sediments, lake and river waters follow a different mechanism to yield monomethyltin (MMT) and dimethyltin (DMT) predominantly. In the unpolluted-anoxic sediment of Sanich Inlet no DMT was observed while MMT and TMT existed only at measurable levels (ng g⁻¹), which confirms the results of model studies. Methyltin distributions in the tin-polluted aquatic environments are generally consistent with distributions produced abiotically under simulated reducing conditions. Model studies demonstrate that TMT exists only when MMT and/or DMT are available at comparable levels with TMT, indicating a sequential type of methylation reaction whereas inorganic tin in the unpolluted sea waters and oxic sediments is methylated mainly to TMT, most probably by a different biomediated reaction mechanism in nature.

1. INTRODUCTION

In recent decades, the worldwide production and consequent use of organotin compounds as heat and light stabilizers, biocides and fungicides has increased significantly (UNEP/FAO/WHO/IAEA, 1989). The known toxic potential of organotins together with the development of sensitive analytical techniques for the quantitative speciation of organotin compounds at trace levels in environmental samples (Braman and Tompkins, 1979; Hodge et al., 1979) has caused increasing concern about the distribution, fate and impact of biomethylated tins and butyltins of anthropogenic origin in the environment. An interest has also been shown in studying the biotic/abiotic methylation reactions, the degradation rates and ultimate products of man-made butyl- and phenyltin compounds under laboratory simulated environmental conditions. Reviews of the various aspects of phenyltin compounds, tributyltin and its derivatives as well as of the inorganic and methylated forms of tin can be found in UNEP/FAO/WHO/IAEA (1989),
Oceans'87 (1987) and in the Proceedings of the 3rd International Organotin Symposium (1990). However, only limited data on methyltins in water, sediments and biota are available in the literature due to their low levels in the aquatic environment.

It has been shown that tin in various oxidation states (0, II, IV) and methyltin (MT) compounds can be methylated by both abiotic and biotic pathways to yield different methyltin compounds, depending upon the redox potential of the studied environments (Ridley et al., 1977; Guard et al., 1981; Hallas et al., 1982; Rapsomanikis and Weber, 1985; Craig and Rapsomanikis, 1985; Donard and Weber, 1988). The results of these studies are reviewed briefly in the present paper in order to suggest possible methylation pathways for methyltin species determined in waters, organisms and sediments. The text also comprises a detailed discussion of methyltin distributions (including a brief evaluation of butyltin species) observed in the waters, sediments and organisms from different environments, particularly from the Turkish coastal waters and in the water column of the Marmara sea.

2. MATERIALS AND METHODS

The water, sediment and organism samples collected from the coastal waters of Turkey were analyzed by hydride derivatization-AAS technique after the adequate pretreatment of samples (Tugrul et al., 1983; Tugrul et al., 1983a; Yemenicioglu et al., 1985; Kubilay, 1989). The method adopted for methyltin (MT) analysis was slightly modified for the determination of butyltin (BT) compounds. The sampling locations along the Turkish coastal waters and in the sea of Marmara are illustrated in Fig. 1. The measurement techniques followed by other groups can be found in the articles cited.

2.1 Comments on methodology used

The alkyltin measurements in water have been carried out without filtering the samples. The data given in the text, therefore represent the total concentrations of hydride reducible (or recoverable) inorganic and organic tin compounds in the subsurface waters of the sites studied. Valkirs et al. (1985) and Johnson et al. (1987) have shown that the BT concentrations in the particulate fraction (>1 μm) represent a small percentage of the BT detected in the water samples, indicating only a minor contribution to the spatial distribution of BT in seawater. So far, no study has been performed to determine the MT concentrations in the suspended solids in water. However, Donard and Weber (1985) have demonstrated that the adsorption behaviours of methyltin compounds under laboratory-simulated estuarine conditions not only differ from each other but also from the behaviour of copper and cadmium ions in similar environments, predicting high MMT and, to a lesser extent, DMT removals by particulate matter to the sediment layer in estuarine environments whereas TMT remains predominantly in the dissolved phase. Hodge et al. (1979) also showed that naturally-produced MMT was precipitated almost completely from lake water together with inorganic tin whereas most DMT stayed in solution. The inorganic tin concentrations in the text represent the sum of Sn(II) and Sn(IV), since the methods used, particularly hydride-generation technique, are not generally adequate for the simultaneous speciation of Sn(II) and Sn(IV) in water (Brinckman et al., 1981; Tugrul et al.,
Fig. 1 Sampling locations in the Mediterranean, Marmara and Black sea

1983a). The alkyltin measurements performed by different groups on sediments and organisms represent the water or weak acid-soluble and hydride reducible (or total extractable) fractions of alkyltins existing in the samples.

The inorganic and organic tin data reviewed here were obtained by various groups, using such different extraction and measurement techniques, as hydride generation and solvent extraction-alkylation coupled to AA, GC, GC-MS, or GC-MS methods, which were capable of separating and determining the principal species of tin quantitatively in water. However, any given analytical method followed might not be adequate for determining all methyl- and butyl-tin compounds simultaneously. For instance, Chau et al. (1982) developed an extraction-alkylation and subsequent GC-MS method to determine MT species in natural waters. Consequently, they demonstrated that the recovery of trimethyltin (TMT) standards added to lake water was always less than 80%, indicating that the behaviour of TMT molecules in water, is very likely to be different to that of MMT and DMT in natural water so that TMT is not easily extracted into the organic solvent used. Therefore, the recovery level to be expected for naturally-produced TMT in water might be relatively low. Moreover, it was noted that organic pollutants (e.g. oil and grease) in water can give rise to drastic decreases in the sensitivity of the HG-AA technique (Valkirs et al., 1985). Consequently, as will be pointed out in the environmental results section, it is rather difficult to assess the spatial distribution pattern of tin species from the data of different investigators due to the differences in sample storage, extraction and measurement methods that have been adopted and the lack of intercomparison studies necessary to assess the validity of tin measurements in water, sediment and organism samples. A recent
international intercomparison study carried out by the participation of four laboratories has shown that butyltin (and probably methyltin) measurements, especially of polluted samples, need further development (Kubilay et al., 1990). Nevertheless, the relative abundances of BT compounds in the same samples as determined by all four groups were similar. Therefore, in the present review, the relative distributions of tin species will especially be taken into account to investigate the occurrence and fate of organotin compounds in different aquatic environments.

3. RESULTS AND DISCUSSION

3.1 Methyltin species in the Marmara sea Basin

The Marmara sea, situated between 40°15’-41° N latitude and 27°-29°30’ E longitude, communicates with the Black sea through the narrow Strait of Bosphorus to the northeast and with the Aegean sea through the Strait of Dardanelles to its southwest. Due to continuous water exchanges between the Aegean and the Black seas through the two straits, the sea of Marmara has a permanently stratified water body with the less saline waters of Black sea origin in the surface layer (0-20m) and the salty waters of Mediterranean origin in the lower layer (Unlalata and Ozsoy, 1986). As can be seen from the salinity and temperature profiles displayed in Fig. 2, the biochemical properties of this sea are dominated by the inflowing waters of the Aegean and Black sea and the vertical mixing at the halocline. Apart from natural processes, the biochemical characteristics of the Marmara sea are affected by substantial amounts of organic and inorganic pollutants from domestic and industrial sources, influencing algal production and oxygen depletion rates in the lower layer. The vertical distribution of other parameters related to algal production and organic matter degradation are also illustrated in Fig. 2. These depth profiles show that the algal production is limited to the upper 25 meters of the sea. Because of the presence of a permanent halocline, the salty waters below 30 m always have an oxygen deficiency, with a significant decline down to 300 m, depending on the sinking rate of organic matter from the productive surface layers and on the residence time of the basin waters.

The methyl- and monobutyltin (MBT) measurements in the Marmara sea were carried out in July, September and December 1988, and in February 1989. Briefly, the spatial and temporal distribution patterns of methyl- and monobutyltin compounds in the Marmara sea did not permit us to draw any general conclusions on their abundances at varying water depths of the Marmara or to assess the significance of physical and biochemical processes on the vertical distribution of alkyltin compounds. Thus, mono-, di-, trimethyltin and monobutyltin species, ranging in concentration between 8-90, 2-27, 70-410 and 19-32 ng l⁻¹, respectively, were recorded in the July samples from depths of 50-100 meters whereas their concentrations in the samples from other depths were at undetectable or trace levels (see Fig. 3). The surface waters of the Marmara Sea were more productive in July and February than in September and December. Methyltin compounds were mostly detected in these productive months. In February, trimethyltin was ubiquitous and the predominant methyltin species down to 100 m depth (see Fig. 2), indicating the importance of microorganism activities on the
Fig. 2 Vertical variations of DMT, TMT, total Hg, dissolved oxygen (DO), temperature, salinity, fluorescing material (HM), nutrients and total dissolved organic carbon (TOC) in the basin water of the Marmara, February, 1989.
biomethylation of tin in the water column. This finding is in agreement with the MT results obtained in the coastal waters of the Black sea and the Mediterranean. In July, the salty waters of the Dardanelles contained only MMT while in the water column of the Bosphorus MT predominated (see Fig. 3), thus indicating the existence of different occurrence and transformation pathways.

The residence time of the subhalocline waters of the Marmara has been estimated to be about 6-7 years (Unlüata and Ozsoy, 1986) while the half-life of man-made MBT in sea water is of the order of weeks. Therefore, one does not expect to have a homogeneous monobutyltin (MBT) distribution in the Marmara basin waters. The patchy existence of MBT in the lower layer waters of the Marmara and its occasional detection in the two strait waters indicates not only intermittent BT input from the Aegean and Black sea but also direct discharge of butyltin compounds from different sources (e.g. inputs from waste waters, shipping activities, rivers, run-off and atmosphere) into the surface waters of the Marmara. The occurrence of MBT and methylin (MT) species detected occasionally at intermediate water depths, particularly in the depth range of 30-150 meters which coincides with the oxygen-gradient depths, suggests that the MBT and some of the methylin compounds observed at these depths originate from dissolution and decomposition of the sinking detritus, which carries butyl- and methylin compounds actively and passively from the productive surface layers to the subhalocline waters. Donard and Weber (1985) have shown that humic substances associated with particulate matter have a high tendency to remove methylin species (and most probably butyltin compounds) from solution. The MBT detected rarely in the deep waters of the Marmara sea might therefore have originated from the sediments.

The vertical distribution of fluorescing material, dissolved organic carbon, total mercury and dissolved inorganic nutrients were examined to investigate their correlation with the depth profiles of alkyltins (see Fig. 2). However, no definite relationship indicative of the occurrence and transport pathways of the alkyltin compounds in the relatively less contaminated Marmara basin waters, was observed. Nevertheless, we suggest that methylation to yield TMT predominantly takes place in the depth range of 50-150 meters where the majority of fast sinking organic detritus is oxidized or dissolved by bacteria, some of which are capable of methylating inorganic tin in the ambient waters or associated with particulate matter. Moreover, some DO gradients observed in the subhalocline waters lying between 50-250 meters, indicate the existence of very thin layers of redox gradient in the intermediate waters of Marmara sea in which Sn(IV) can be reduced to Sn(II), being the first step of abiotic (and biotic) methylation (Ridley et al., 1977; Rapsomanikis and Weber, 1985; Donard and Weber, 1988). If all the TMT determined in the intermediate depths of the Marmara sea had been carried down from upper layers we should not have only measured lower TMT concentrations with respect to those measured in the upper layers but should also have detected MMT and DMT, the degradation products of TMT.
Fig. 3 Vertical distribution of methyltin concentrations in the waters of the Marmara sea and the two straits, July 1988
3.2 Methylltins in the Mediterranean and other seas

Table 1 shows the concentrations of methyl- and butyltin species measured in the subsurface waters of Turkish harbours, marinas, estuaries, coastal and open seas from the locations displayed in Fig. 1. Some of these data have been published elsewhere (Kubilay et al., 1990). As seen from the table, TMT predominated in the methyltin-containing subsurface waters of both unpolluted and butyltin-polluted seas. In relatively productive and less contaminated seas, e.g. Black sea coastal waters and the region within the Rhodes cyclonic gyre formed permanently between Cyprus and Rhodes Island in the eastern Mediterranean (Ozsoy et al., 1989), TMT concentrations were much greater than those of MMT and DMT. Furthermore, no positive correlation was observed between the methyltin and man-made butyltin concentrations measured in the sea waters. Methyltin species were not recorded in most of the subsurface water samples containing butyltin compounds. Therefore, these results have not been included in Table 1.

The results of the present study clearly indicate that the sole existence of butyltin compounds in sea water, ranging from some tens to some hundreds of ng per litre, generally does not give rise to the natural occurrence of methyltins at ng per litre level in marine environments. Biochemical methylation reactions need the existence of adequate environmental conditions, such as sufficient concentrations of tin, available carbonium and/or carbonion donors, optimum water temperature, other abiotic/biotic methylating agents, etc.

The results of those tin speciation studies which have appeared in the literature and involve both inorganic tin and methyltin species in the same sample, are compiled in Table 2. If the data contained inorganic tin but noted undetectable MT concentrations, they were excluded and not considered when discussing methyltin distributions.

The tin data presented in Table 2 indicate that, in tin-modified waters, the biomediated methylation reactions proceed to yield MMT and DMT predominantly. A good example of this is the Bay of Iskenderun which receives continuously a significant amount of inorganic tin from the steel and fertilizer complexes located on the eastern part of the bay (Yemenicioglu et al., 1987). The level of inorganic tin measured in the waste waters of these complexes ranged from 15 to 1100 ng l⁻¹. Even higher values were recorded in the surface waters of the Iskenderun Bay (see Table 2). According to Papsomanikis and Weber (1985), Sn(II) is methylated abiotically to MMT predominantly under reducing conditions if carbonium ions are available, while methylation yields DMT and TMT if only carbonion donors are available. Considering the MT products of these model experiments, one suggests that, in the inorganic tin-polluted surface waters of the Iskenderun Bay, tin is reduced to or discharged in Sn(II) form and then methylated by biomediated chemical reactions to produce MMT and DMT predominantly. The occasional disappearance of MMT in the polluted subsurface waters of Iskenderun bay can be attributed to the tendency of MMT to be strongly adsorbed on fast-sinking particulate matter of various origins in the bay waters (Hodges et al., 1979; Tugrul et al., 1983a; Donard and Weber, 1985) as well as to change in the bio-mediated reaction mechanisms.
Table 1
Concentrations of butyl- and methyltins measured in the coastal waters of Turkey (as alkyltin chloride, ng l⁻¹).

<table>
<thead>
<tr>
<th>Sampling Site</th>
<th>Sampling Station</th>
<th>Sampling Date</th>
<th>MBT</th>
<th>DBT</th>
<th>TBT</th>
<th>MMT</th>
<th>DMT</th>
<th>TMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marmara Sea</td>
<td>July 1988</td>
<td>nd-37</td>
<td>-</td>
<td>-</td>
<td>nd-48</td>
<td>nd-14</td>
<td>nd-10</td>
<td>nd-50</td>
</tr>
<tr>
<td></td>
<td>Sep. 1988</td>
<td>nd-17</td>
<td>-</td>
<td>-</td>
<td>nd-28</td>
<td>nd-55</td>
<td>nd-16</td>
<td>nd-10</td>
</tr>
<tr>
<td></td>
<td>Dec. 1988</td>
<td>nd</td>
<td>-</td>
<td>-</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Dardanelles</td>
<td>July 1988</td>
<td>nd</td>
<td>-</td>
<td>-</td>
<td>17-63</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Bosphorus</td>
<td>July 1988</td>
<td>2.7-7</td>
<td>-</td>
<td>-</td>
<td>nd-1.1</td>
<td>nd-2.2</td>
<td>nd-15</td>
<td></td>
</tr>
<tr>
<td>Black Sea</td>
<td>Sept. 1988</td>
<td>nd-35</td>
<td>-</td>
<td>-</td>
<td>nd-5.1</td>
<td>nd-80</td>
<td>nd-35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jan. 1989</td>
<td>nd</td>
<td>-</td>
<td>-</td>
<td>nd</td>
<td>nd</td>
<td>nd-12</td>
<td></td>
</tr>
<tr>
<td>Iskenderun</td>
<td>H4 May 1988</td>
<td>8</td>
<td>56</td>
<td>83</td>
<td>nd</td>
<td>nd</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>Harbour</td>
<td>Nov. 1988</td>
<td>8</td>
<td>23</td>
<td>31</td>
<td>nd</td>
<td>nd</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feb. 1989</td>
<td>27</td>
<td>7</td>
<td>32</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Marsin</td>
<td>H1 Nov. 1988</td>
<td>12</td>
<td>44</td>
<td>31</td>
<td>nd</td>
<td>nd</td>
<td>39.7</td>
<td></td>
</tr>
<tr>
<td>Harbour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Botas</td>
<td>H2 May 1988</td>
<td>140</td>
<td>nd</td>
<td>9</td>
<td>nd</td>
<td>nd</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nov. 1988</td>
<td>311</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>Isdemir</td>
<td>H3 Nov. 1988</td>
<td>633</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>March 1989</td>
<td>77</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Antalya</td>
<td>M2 Feb. 1989</td>
<td>102</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Marina</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Göksu Delta</td>
<td>(D3) Oct. 1988</td>
<td>212</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feb. 1989</td>
<td>268</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>R2 July 1988</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Stations</td>
<td>R1 Oct. 1988</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>18.0</td>
<td>129.2</td>
<td></td>
</tr>
</tbody>
</table>

nd: not detected (below the detection limits of the applied method)

In the San Diego Bay which receives butyltins mainly from antifoulants on the hulls of boats of different sizes, only MMT and DMT were detected by Hodge et al. (1979). However, Brannon and Tompkins (1979) determined the three forms of MT (the DMT form predominantly), in concentrations ranging between a few ng and some tens of ng per litre, in the bay waters of the United States. Both groups used the same hydride generation method to determine tin species in water.
Table 2
Methylnit concentration in saline, lake, river and rain waters
(ng l⁻¹ as Sn).

<table>
<thead>
<tr>
<th></th>
<th>Sn(IV)</th>
<th>Me₃SN</th>
<th>Me₂Sn</th>
<th>Me₃Sn</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saline water</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bay waters</td>
<td>2-62</td>
<td>nd-15</td>
<td>0.7-7</td>
<td>nd-1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(54-73%)</td>
<td>(&lt;18%)</td>
<td>(8-39%)</td>
<td>(&lt;20%)</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>nd-207</td>
<td>nd-1.1</td>
<td>0.6-1.7</td>
<td>0.6-0.95</td>
<td>1</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>(10-54%)</td>
<td>(17-44%)</td>
<td>(24-40%)</td>
<td>(12-34%)</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>6-38</td>
<td>1-4</td>
<td>5-15</td>
<td>nd</td>
<td>2</td>
</tr>
<tr>
<td><strong>Iskenderun bay:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July, 1982</td>
<td>11-175</td>
<td>0.6-83</td>
<td>4-18.3</td>
<td>nd</td>
<td>3</td>
</tr>
<tr>
<td>Aug., 1982</td>
<td>26-7710</td>
<td>5.5-10</td>
<td>5-12</td>
<td>nd</td>
<td>3</td>
</tr>
<tr>
<td>Sep., 1982</td>
<td>219-237</td>
<td>nd</td>
<td>9-12.5</td>
<td>nd</td>
<td>3,4</td>
</tr>
<tr>
<td>Nov., 1982</td>
<td>13-207</td>
<td>nd</td>
<td>0.5-1.4</td>
<td>nd</td>
<td>3,4</td>
</tr>
<tr>
<td><strong>Estuarine waters</strong></td>
<td>3-20</td>
<td>nd-8</td>
<td>0.9-3.3</td>
<td>nd-1.1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(27-88%)</td>
<td>(low-66%)</td>
<td>(7-71%)</td>
<td>(low-29%)</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>1-4</td>
<td>nd-0.1</td>
<td>0.5-1.2</td>
<td>nd-0.5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Lamas Estuary</strong></td>
<td>4.1-5.5</td>
<td>0.6-2.2</td>
<td>nd-0.6</td>
<td>nd</td>
<td>3,4</td>
</tr>
<tr>
<td>Göksu &quot;</td>
<td>3.2-27</td>
<td>nd</td>
<td>0.6-1.5</td>
<td>nd</td>
<td>3</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>12-49</td>
<td>1.5-4.8</td>
<td>2.3-20.5</td>
<td>0.9-1.5</td>
<td>3</td>
</tr>
<tr>
<td>Lamas &quot;</td>
<td>9-15</td>
<td>nd</td>
<td>0.9-3.6</td>
<td>nd</td>
<td>3</td>
</tr>
<tr>
<td>Ceyhan &quot;</td>
<td>24-123</td>
<td>12-16</td>
<td>13-21</td>
<td>nd</td>
<td>3</td>
</tr>
<tr>
<td><strong>Lake water</strong></td>
<td>3-10</td>
<td>nd-1</td>
<td>0.56-1.2</td>
<td>3.3-7.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(46-65%)</td>
<td>(max.5%)</td>
<td>(6-8%)</td>
<td>(28-46%)</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>2.4-3.5</td>
<td>2.6-4.5</td>
<td>nd-5</td>
<td>nd-1.3</td>
<td>1</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>(47%)</td>
<td>(31-52%)</td>
<td>(37%)</td>
<td>(low-8.9%)</td>
<td></td>
</tr>
<tr>
<td>Canadian Lakes</td>
<td>84-490</td>
<td>3-9</td>
<td>nd-31</td>
<td>nd</td>
<td>2</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>40-2500</td>
<td>110-530</td>
<td>20-210</td>
<td>nd</td>
<td>6</td>
</tr>
<tr>
<td>Canadian Harbours</td>
<td>130-980</td>
<td>130-1220</td>
<td>100-400</td>
<td>nd</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>80-2100</td>
<td>170-1200</td>
<td>40-400</td>
<td>nd</td>
<td>6</td>
</tr>
<tr>
<td><strong>River water</strong></td>
<td>nd-4.1</td>
<td>0.5-12</td>
<td>0.4-7.5</td>
<td>nd-1.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(17-52%)</td>
<td>(19-76%)</td>
<td>(5-37%)</td>
<td>(low-8.9%)</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>1-25</td>
<td>nd-0.5</td>
<td>nd-0.4</td>
<td>nd-0.3</td>
<td>5</td>
</tr>
<tr>
<td>Canadian Rivers</td>
<td>10-120</td>
<td>280-680</td>
<td>30-210</td>
<td>nd</td>
<td>6</td>
</tr>
<tr>
<td>Mississippi &quot;</td>
<td>128</td>
<td>1.3</td>
<td>3</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>Rhine &quot;</td>
<td>18</td>
<td>70</td>
<td>210</td>
<td>1.5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Rain water</strong></td>
<td>3-41</td>
<td>0.6-22</td>
<td>nd-1.9</td>
<td>nd-0.61</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(2-92%)</td>
<td>(low-20%)</td>
<td>(low-7.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>1-3</td>
<td>nd-5</td>
<td>nd</td>
<td>nd</td>
<td>4</td>
</tr>
</tbody>
</table>

1: Braman and Tompkins (1979); 2: Hodge et al. (1979);
3: Yenemicioglu et al. (1985); 4: Tugrul et al. (1983a);
5: Byrd and Andreac (1982); 6: Maguire et al. (1982);
7: Chau et al. (1982);
As seen from Table 2, the methylated forms of tin in estuarine waters are predominantly dimethyl- and, to a lesser extent, monomethyltin species. The rare appearance of MMT in these waters can be attributed to its removal by fast sedimentation in these relatively shallow waters (Donard and Weber, 1985). The TMT determined at trace levels in the surface waters may be produced in the water itself or released from the sediment layer, since TMT synthesized in the surface sediments tends to diffuse into overlying waters due to its low rate of adsorption by particulate matter even in the dynamic estuarine conditions (Donard and Weber, 1985).

The tin speciation studies have revealed that the production of methyltins in both polluted and unpolluted sea waters is not only dominated by the continuous tin input but also by other abiotic/biotic reaction conditions to produce measurable methyltin species.

3.3 Lake water

The patterns of the methyltin distribution in the waters of various lakes are different. TMT was the predominant form in some of the US lakes, while it was not detected in the Canadian lakes (see Table 2). These findings indicate that biomethylation reaction in the lake waters may have different mechanisms, induced by various microbial activities and physicochemical properties of the lakes. In the tin-contaminated Canadian lakes and harbour waters, and in the Lake Michigan, only MMT and DMT were observed (Hodge et al., 1979; Maguire et al., 1982). The MMT and DMT values determined by extraction-alkylation coupled with GC-AA measurement technique in the Canadian lake waters were significantly higher than in the lake Michigan waters measured by HG-AA technique. Maguire et al. (1982) suggested that the high MMT and DMT concentrations recorded in some unpolluted Canadian lakes, which do not receive waste waters directly, very likely resulted from atmospheric input. If this argument is valid the MMT and DMT concentrations determined in the US lakes by Braman and Tompkins (1979) should have been at levels comparable with those in the Canadian lakes.

3.4 River water

The distribution of methyltin species in river waters show a pattern similar to that observed in the inorganic tin-polluted bay, estuarine and lake waters (Table 2). The greatest MMT and DMT concentrations were seen in the Canadian rivers as in the case of the lakes (Maguire et al., 1982). The MT species concentrations measured by Braman and Tompkins (1979), and Byrd and Andreae (1982) in the US and European rivers were generally low. Only the polluted waters of the Rhine river contained relatively high concentrations of MMT and DMT, 70 and 210 ng l⁻¹, respectively. Braman and Tompkins (1979) and Maguire et al. (1982) observed that the majority of dissolved tin in the river waters was transported in the butylated and methylated forms to the receiving lake and coastal sea waters whereas Byrd and Andreae (1982) detected low MT concentrations in unpolluted river waters compared to the inorganic tin levels.
3.5 Rain water

The limited MT data available for rain water indicate that the atmospheric transport of methylated tin compounds may be of remarkable importance on the global cycle of tin. The sources of methyltins in rain water might be the volatile tin species (e.g. stannane, methylstannanes, tetramethyltin) produced in and released from soil and coastal marine environments (Brinkman et al., 1981; Jackson et al., 1982). Such volatile tin species were also produced by model experiments under reducing conditions (Donard and Weber, 1988; Rapsomanikis and Weber, 1985).

3.6 Sediment

The results of alkyltin measurements carried out by Tugrul et al. (1983a) and Kubilay et al. (1990) on sediment samples from the coastal waters, harbours and river deltas are compiled in Table 3. Tugrul et al. (1983a) have also determined the cold 6N HCl acid extractable inorganic tin in the samples. As seen from Fig. 4 there is no positive relationship between the inorganic tin and the total recoverable methyltin levels in the sediments. On the contrary, the total concentration of water extractable methyltins measured in the unpolluted sediments exceeded the levels in the tin-polluted sediments. Moreover, the methyltin distribution appeared to change in different sediment environments. In general, MTM was the predominant form in most of the unpolluted oxic-coastal and delta sediments. One should note that the ratios of Me₂/M₃ and Me₃/M₄ in the four oxic-delta sediments sampled from the same areas in 1988, are consistent with those of Tugrul et al. (1983a) displayed in Table 3.

The methyltin production and the consequent distributions in the inorganic tin-polluted Iskenderun Bay generated a similar pattern in both sea water and sediment, except that MTM was detected in the sediments, in lower concentrations than MMT and DMT. MTM is released more easily from sediment to the overlying water than MMT and DMT, thus its concentrations may be less than those of MMT and DMT in sediments if there exist continuous MMT and DMT sources from the surface waters through the sinking particulate matter and if the tin methylation in sediment follows a sequential type of reaction pathway to produce MMT, DMT and MTM in decreasing concentrations.

In the butyltin-polluted San Diego Bay and in Mersin Harbour, biomethylation reaction mechanisms in the polluted sediments tend to yield the mono- and dimethyltin predominantly. The relatively low ratio of (MT)/(t-Sn) observed in the polluted sediments (Tugrul et al., 1983a) strongly predicts that the MMT and, to a lesser extent, DMT transport to the sea bottom may be of great importance to the fate of methyltin species in the sediments of polluted shallow waters. The release of MTM from the sediments to the overlying water column should also be taken into account when studying the occurrence and fate of methyltins in coastal waters, and transport of tin to open waters.

Another remarkable finding from the sediment analyses is that the anoxic-unpolluted Saanich Inlet sediment contained MMT and MTM only. DMT was not detected in the same core sample. The deep waters of Saanich Inlet were found to be generally anoxic throughout the year (Murray et al., 1978). In reducing water environments, inorganic tin
Table 3
Concentrations of methylnitins and monobutyltin in the sediments from Turkish coastal waters and in the core samples from other seas (ng g⁻¹ dry weight as alkyltin chloride).

<table>
<thead>
<tr>
<th>Station</th>
<th>Sampling date</th>
<th>MeSnCl₂</th>
<th>Me₂SnCl₂</th>
<th>Me₃SnCl</th>
<th>BuSnCl₃</th>
<th>Me/Me₃</th>
<th>Me₂/Me₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manavgat (D2)</td>
<td>1988</td>
<td>15</td>
<td>36</td>
<td>143</td>
<td>100</td>
<td>0.10</td>
<td>0.25</td>
</tr>
<tr>
<td>Esen (D1)</td>
<td>1988</td>
<td>38</td>
<td>58</td>
<td>107</td>
<td>260</td>
<td>0.35</td>
<td>0.54</td>
</tr>
<tr>
<td>Seyhan (D4)</td>
<td>1988</td>
<td>65</td>
<td>81</td>
<td>159</td>
<td>100</td>
<td>0.41</td>
<td>0.51</td>
</tr>
<tr>
<td>Seyhan (D4)</td>
<td>1980</td>
<td>nd</td>
<td>1.3</td>
<td>3.9</td>
<td>na</td>
<td>&lt;0.1</td>
<td>0.33</td>
</tr>
<tr>
<td>Göksu (D3)</td>
<td>1988</td>
<td>375</td>
<td>477</td>
<td>1696</td>
<td>980</td>
<td>0.22</td>
<td>0.28</td>
</tr>
<tr>
<td>Göksu (D3)</td>
<td>1980</td>
<td>nd-0.7</td>
<td>0.6</td>
<td>1.6</td>
<td>na</td>
<td>&lt;0.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Iskenderun Bay</td>
<td>1980</td>
<td>nd</td>
<td>nd</td>
<td>0.35</td>
<td>na</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>Iskend. Bay</td>
<td>1982</td>
<td>0.6</td>
<td>2.4</td>
<td>0.5</td>
<td>na</td>
<td>1.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Iskend. Bay</td>
<td>1982</td>
<td>0.2</td>
<td>1.3</td>
<td>0.1</td>
<td>na</td>
<td>2.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Iskend. Bay</td>
<td>1982</td>
<td>0.2</td>
<td>0.9</td>
<td>0.1</td>
<td>na</td>
<td>2.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Tasucu Bay</td>
<td>1980</td>
<td>0.1</td>
<td>0.2</td>
<td>1.0</td>
<td>na</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Akkuyu</td>
<td>1980</td>
<td>nd-0.3</td>
<td>8.1</td>
<td>11.8</td>
<td>na</td>
<td>&lt;0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Lamas</td>
<td>1980</td>
<td>nd</td>
<td>nd-1.8</td>
<td>4.4</td>
<td>na</td>
<td>&lt;0.1</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Mersin</td>
<td>1980</td>
<td>nd</td>
<td>2.6</td>
<td>8.6</td>
<td>na</td>
<td>&lt;0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Mersin Harb.</td>
<td>1980</td>
<td>7.5</td>
<td>2.1</td>
<td>0.5</td>
<td>na</td>
<td>25.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Mersin Harb.</td>
<td>1980</td>
<td>0.2</td>
<td>0.5</td>
<td>0.1</td>
<td>na</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>San Diego Bay</td>
<td>1981</td>
<td>2.3</td>
<td>2.3</td>
<td>nd-0.2</td>
<td>na</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Baltic Sea</td>
<td>-</td>
<td>0.5</td>
<td>0.3</td>
<td>nd</td>
<td>na</td>
<td>&gt;5</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Chesapeake Bay</td>
<td>-</td>
<td>0.6</td>
<td>nd</td>
<td>nd</td>
<td>na</td>
<td>&gt;6</td>
<td>-</td>
</tr>
<tr>
<td>Saanich Inlet</td>
<td>-</td>
<td>0.6</td>
<td>nd</td>
<td>2.6</td>
<td>na</td>
<td>0.2</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

nd: not detected; na: not analyzed

is transported in the Sn(II) form to the sea bottom, where it is easily utilized in the abiotic/biotic methylation reaction taking place in sediment to yield MMT and TMT (Donard and Weber, 1988). Guard et al. (1981) observed that, in anaerobic estuarine sediments, TMT can yield only volatile tetramethyltin by both biotic and abiotic pathways. No other hydride reducible (water soluble) methylnit tin was detected by Guard and co-workers. They suggested that the DMT released from the proposed reaction mechanism may be removed from the ecosystem as the very insoluble (CH₃)₂SnO but DMT more likely accumulates in the form of complexes with sulfur-containing ligands found in sediments. The findings of these two model studies and the pattern of MT distribution in the Saanich Inlet sediment clearly indicate that the methylation reaction of tin follows a different pathway in the Sn(II)-modified anoxic environments.

Craig and Rapsomanikis (1985) observed that methylating agents can transfer methyl groups to Sn(0) and Sn(II) by oxidative abiotic reaction to yield MMT, DMT and, to a lesser extent, TMT, but no detectable tetramethyltin. Rapsomanikis and Weber (1985) have shown that, in the presence of MaI only, MMT was the major product of abiotic methylation under reducing conditions. Halls et al. (1982) detected the formation of DMT, TMT and, to a lesser extent, MMT by bio-mediated
reactions in estuary sediment. The lower concentration of MMT detected by the group relative to DMT and TMT was due presumably to the inefficient extraction of MMT from the sediments as indicated by Tugrul et al. (1983a). Nevertheless, the MT findings of model experiments were similar to the MT distributions in sediments from relatively less polluted environments (see Table 3).

The studies carried out by Rapsomanikis and Weber (1985), and by Craig and Rapsomanikis (1985), have demonstrated that the abiotic methylation of inorganic tin under reducing conditions is a sequential type of reaction with diminishing yield at each step, while the products of biomediated chemical reactions of Sn(IV) and Sn(II), obtained by other groups from laboratory-simulated experiments, predict the formation of both volatile and non-volatile methylin compounds in different aquatic environments. As yet no model study is available in the literature to demonstrate the biochemical methylation reaction of tin to result predominantly in TMT formation in aerobic water and sediment environments.

Aboul Dahab (1988) has determined very high concentrations of water extractable methyl- and butylinins in the sediments from the Alexandria coastal belt (Table 4). MMT and TBT were found to be the predominant species, TMT being generally undetectable. This result is in good agreement with those of Tugrul et al. (1983a) in tin-polluted sediments though Aboul Dahab's (1988) total MT concentrations in sediments are much greater than the MT levels given in Table 3.

Fig. 4 Average values of 6 N HCl extractable inorganic tin (■) and total water extractable methylin (□) in sediments from different environments (after Tugrul et al., 1983a)
Table 4
Spatial concentrations of tin species (ng g\(^{-1}\) as Sn) in the Alexandria coastal belt (after Aboul Dahab, 1988).

<table>
<thead>
<tr>
<th></th>
<th>I Ref. St.</th>
<th>II Agamy</th>
<th>III Mex Bay</th>
<th>IV Western Harbour</th>
<th>V Off Eastern Harbour</th>
<th>VI Off Eastern Harbour</th>
<th>VII Eastern Coast</th>
<th>VIII Eastern Coast</th>
<th>IX Abu Kir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Station No.</td>
<td>4</td>
<td>1-3</td>
<td>5-11</td>
<td>12-14</td>
<td>15</td>
<td>16</td>
<td>17-18</td>
<td>19-26</td>
<td>27-35</td>
</tr>
<tr>
<td>No. of samples</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Inorg. Sn</td>
<td>310</td>
<td>488</td>
<td>2147</td>
<td>4720</td>
<td>2430</td>
<td>1080</td>
<td>3645</td>
<td>1075</td>
<td>2252</td>
</tr>
<tr>
<td>MMT</td>
<td>0</td>
<td>20</td>
<td>69</td>
<td>1000</td>
<td>85</td>
<td>0</td>
<td>500</td>
<td>41</td>
<td>127</td>
</tr>
<tr>
<td>DMT</td>
<td>15</td>
<td>12</td>
<td>35</td>
<td>77</td>
<td>70</td>
<td>45</td>
<td>23</td>
<td>23</td>
<td>43</td>
</tr>
<tr>
<td>TMT</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total MT</td>
<td>15</td>
<td>32</td>
<td>104</td>
<td>1136</td>
<td>155</td>
<td>45</td>
<td>545</td>
<td>64</td>
<td>173</td>
</tr>
<tr>
<td>% MMT/Total MT</td>
<td>0</td>
<td>63</td>
<td>68</td>
<td>88</td>
<td>0</td>
<td>0</td>
<td>91</td>
<td>68</td>
<td>70</td>
</tr>
<tr>
<td>% DMT/Total MT</td>
<td>100</td>
<td>37</td>
<td>32</td>
<td>7</td>
<td>45</td>
<td>100</td>
<td>4</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>% TMT/Total MT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MBT</td>
<td>0</td>
<td>41</td>
<td>57</td>
<td>330</td>
<td>45</td>
<td>55</td>
<td>138</td>
<td>60</td>
<td>83</td>
</tr>
<tr>
<td>DBT</td>
<td>10</td>
<td>17</td>
<td>49</td>
<td>305</td>
<td>65</td>
<td>60</td>
<td>120</td>
<td>63</td>
<td>67</td>
</tr>
<tr>
<td>TBT</td>
<td>35</td>
<td>40</td>
<td>187</td>
<td>975</td>
<td>185</td>
<td>160</td>
<td>260</td>
<td>63</td>
<td>252</td>
</tr>
<tr>
<td>Total BT</td>
<td>45</td>
<td>97</td>
<td>294</td>
<td>1610</td>
<td>295</td>
<td>275</td>
<td>518</td>
<td>186</td>
<td>401</td>
</tr>
<tr>
<td>% MBT/Total BT</td>
<td>0</td>
<td>38</td>
<td>19</td>
<td>22</td>
<td>15</td>
<td>20</td>
<td>27</td>
<td>32</td>
<td>20</td>
</tr>
<tr>
<td>% DBT/Total BT</td>
<td>22</td>
<td>15</td>
<td>16</td>
<td>19</td>
<td>22</td>
<td>22</td>
<td>23</td>
<td>34</td>
<td>15</td>
</tr>
<tr>
<td>% TBT/Total BT</td>
<td>78</td>
<td>47</td>
<td>65</td>
<td>59</td>
<td>63</td>
<td>58</td>
<td>50</td>
<td>34</td>
<td>65</td>
</tr>
</tbody>
</table>

Only our recent MT results in deltaic sediments are at levels comparable with those from the Alexandria coasts. The distribution of organotins in the sediments from Alexandria also indicate a close relationship between the total methyl- and butyltin concentrations. Total BT/total MT ratios were found to range between 1-3 whereas the sample from the Eastern Alexandria coasts which contained DMT only, had a BT/MT ratio of 6. Moreover, Aboul Dahab (1990) has obtained similar methyl- and butyltin distributions in the sediments collected from the same locations in 1989.

3.7 Biota

The methyltinics determined by Tugrul et al. (1983a) in various marine organisms are displayed in Table 5. Briefly, fish and limpet accumulate in their edible tissues methyltinics synthesized by microorganisms in the ambient waters and/or by the organism itself. As seen in Fig. 5 and Table 5, the total-tin and methyltin concentrations in the edible tissues of limpets of the same size changed monthly with the greatest concentrations occurring in the spring and early summer months, followed by sharp decreases during the summer months, indicating a short half-life and fast accumulation of MT species. Since limpets are filter feeders and inhabit the tidal zone, it is difficult to assess the degree of methylation of inorganic tin by the organisms itself. Nevertheless, the limpets collected in 1980 and 1982-1983 had a similar methyltin distribution in their edible tissues.
As previously pointed out by Tugrul et al. (1983a), the TMT species predominated in both sets of limpet samples collected from outside the Lamas Harbour, thus clearly indicating the influence of fresh water of the Lamas Stream on the MT bioaccumulation and subsequent degradation in limpet tissue. Furthermore, in limpets from outside the harbour, both inorganic and methylin concentrations were found to be consistently higher than in "inside" samples. The methylin content of the limpet shell demonstrates the significance of accumulation of bacterial-origin methyltns in the shell (Table 5).

Table 5

Average organotin, total tin concentrations (ng g⁻¹, dry weight) and methylin ratios in marine organisms from the northeastern Mediterranean coast, Turkey (after Tugrul et al., 1983a).

<table>
<thead>
<tr>
<th>Species</th>
<th>T-Sn</th>
<th>Me-Sn</th>
<th>Me₂-Sn</th>
<th>Me₃-Sn</th>
<th>Tin % in T-MeSn</th>
<th>Me/Me₃</th>
<th>Me₂/Me₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U. Moluccensis</td>
<td>260</td>
<td>27</td>
<td>2.6</td>
<td>1.2</td>
<td>6</td>
<td>23</td>
<td>2.2</td>
</tr>
<tr>
<td>Mullus barbatus</td>
<td>88</td>
<td>0.8</td>
<td>2.9</td>
<td>1.3</td>
<td>3</td>
<td>0.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Limpet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patella caerulea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.H. in. (Oct.'80)</td>
<td>13</td>
<td>2.8</td>
<td>1.2</td>
<td>0.7</td>
<td>16</td>
<td>4</td>
<td>1.7</td>
</tr>
<tr>
<td>L.H. out. (Oct.'80)</td>
<td>n.a.</td>
<td>0.4</td>
<td>0.2</td>
<td>2.8</td>
<td>-</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Soft tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.H. in. (June'80)</td>
<td>75</td>
<td>1.3</td>
<td>12</td>
<td>34</td>
<td>36</td>
<td>0.04</td>
<td>0.4</td>
</tr>
<tr>
<td>L.H. out. (June'80)</td>
<td>64</td>
<td>2.1</td>
<td>18</td>
<td>63</td>
<td>76</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>L.H. in. (Oct.'80)</td>
<td>50</td>
<td>4.8</td>
<td>4.2</td>
<td>35</td>
<td>52</td>
<td>0.14</td>
<td>0.1</td>
</tr>
<tr>
<td>L.H. out. (Oct.'80)</td>
<td>n.a.</td>
<td>2.4</td>
<td>7.8</td>
<td>0.8</td>
<td>-</td>
<td>3</td>
<td>9.8</td>
</tr>
<tr>
<td>Brown macro-algae*</td>
<td>9</td>
<td>n.d.</td>
<td>0.5</td>
<td>5.8</td>
<td>41</td>
<td>(a)</td>
<td>0.09</td>
</tr>
<tr>
<td>Chorophyta</td>
<td>37</td>
<td>n.d.</td>
<td>12</td>
<td>n.d.</td>
<td>18</td>
<td>(a)</td>
<td>(b)</td>
</tr>
<tr>
<td>(L.H., Apr.'80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seaweed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(L.H., Dec.'80)</td>
<td>250</td>
<td>16.8</td>
<td>37</td>
<td>0.9</td>
<td>11</td>
<td>19</td>
<td>41</td>
</tr>
</tbody>
</table>

(a): very small; (b): very large; L.H. in., Lamas Harbour inside; L.H. out., Lamas Harbour outside; n.d. not detected; n.a. not analyzed; * 40m depth near L.H.

Chlorophyta collected from the Lamas Harbour accumulated only DMT whereas seaweed from the same location contained MMT and DMT almost at the same level together with trace amounts of TMT, indicating different methylation pathways. Seidel et al. (1980) also observed only DMT and tetramethylin in some macroalgae samples from different sites of the US coastal waters. The methylin distribution in brown macro-algae and sediments from the locations off Lamas estuary is similar, which predicts that macroalgae accumulate MT species by methylating inorganic tin itself or directly from the sediments.
Fig. 5 Monthly variation of tin species in the edible tissue of limpet from Lamas Harbour.
4. CONCLUSIONS

The differences in the sampling, treatment and measurement methods followed by various groups make it difficult to assess the spatial distributions of alkyltins from the published data. This statement is confirmed by comparison of the methylytin concentrations measured by the various groups in the waters and sediments from different locations. For example, the methylytin concentrations found in the Canadian waters are consistently much higher than those measured in similar environments by other groups; this leads us to point out the possibility of incomplete recovery of methylytin species in water by the hydride generation-AA method and that of significant methyl and butyltin inputs from as yet unidentified sources to the Canadian lakes and rivers. Therefore, comprehensive intercalibration studies are needed to improve the reliability of alkyltin measurements in the environmental samples.

The detection of TMT and MBT compounds in the water column of the Marmara sea basin indicates the significance of the vertical transport of organotin compounds by fast sinking particulate matter. It also demonstrates the biochemical production of TMT in the intermediate water layers.

In relatively less contaminated and less productive sea waters and in unpolluted oxic sediments, the predominant form of methylytin species produced by biochemical reactions is trimethylytin. On the other hand, monomethyl- and dimethylytin species predominate in tin-polluted sea and most lake and river waters, and in tin-polluted harbour and bay sediments. This is all very similar to the relative distribution of methylytins in abiotic model studies, and indicative of a sequential type of reaction.

The results of a model study by Donard and Weber (1988) demonstrate that biomediated methylation reactions of tin under reducing conditions follow a pathway which yields water soluble MMT and TMT together with volatile tetramethylytin, stannane and methyl stannanes but only trace amounts of water soluble DMT. The distribution of water soluble products in this model experiment are consistent with the unexpected methylytin distribution in the unpolluted-anaerobic sediments of the Saanich Inlet, which did not contain measurable concentrations of water extractable dimethylytin. This interesting aspect of methylytin distribution in unpolluted-reducing environment should be confirmed by tin speciation studies to be conducted in the redox gradient zone and anoxic sediments of the Black sea.

The findings of abiotic methylation experiments are consistent only with the distribution pattern of methylytin species in the tin-modified waters and sediments in which MMT and DMT predominate. No model study yet exists to support and describe the predominant formation of TMT in unpolluted-oxic seawaters and sediments. Trimethylytin and, to a lesser extent, dimethylytin produced in the marine environment are of great importance in the large scale transport of tin in the oceans due to their low adsorption rates by fast sinking particulate matter.
5. REFERENCES


FATE AND DISTRIBUTION OF CHROMIUM IN WATERS, SEDIMENTS AND MUSSELS OF THE KASTEKA BAY

by

I. VUKADIN and N. ODZAK

Institute of Oceanography and Fisheries, 58000 Split, Yugoslavia

ABSTRACT

This paper is an attempt to determine Cr (III), Cr (VI) and total chromium in sea water, particulate matter, sediment and marine organisms.

Chromium (III) is a crucial element for some living organisms at low concentrations, while chromium (VI) is considered to be a moderate to severe toxic agent. Our results show that the chromium concentrations range from 0.1 - 0.5 µg dm⁻³ Cr(III) and 0.1 - 0.84 µg dm⁻³ in the sea water, from 10-20 mg kg⁻¹ DW in particulate matter and from 50-100 mg kg⁻¹ DW in sediment. Levels of this metal in marine organisms vary between below detection limit and 0.7 mg kg⁻¹ FW, with maximum values of 50 mg kg⁻¹ FW in same organisms.

1. INTRODUCTION

Chromium may exist in natural waters in many chemical forms. It is usually encountered in the oxidation states Cr (III) and Cr (VI). It is well known that each of these oxidized states has very different biological and toxicological properties. Chromium (III) is an essential element for some organisms whereas chromium (VI) is considered to be a moderate to severe toxic agent.

The study of the chemical speciation of Cr in the sea water has been a topic of increasing research interest for the past few years. Studies have been particularly concerned with the distribution of Cr between Cr (III) and Cr (VI). The chemical speciation of Cr in sea water has been examined by chemical analyses and theoretical calculation. The analytical results concerning the relative abundance of Cr (III) and Cr (VI) species have not always been constant, although thermodynamic calculations suggest that chromate should be the predominant form in the natural oxygenated environment (Nakayama et al., 1981).

The sources of chromium in the environment are waste chromates from electroplating corrosion inhibitors, waste waters from leather industry and leaching from sanitary land fills (Aboul-Dahab, 1989).

The major difficulties that arise in studies of speciation of Cr in natural waters, sediments and marine organisms include the provision of sufficient precision and sensitivity to detect very low concentrations, contamination during sampling and species alteration during sampling, handling and analyses.
2. EXPERIMENTAL

2.1 Study area

The work described here was carried out in the coastal area of the town of Split (Kastela Bay, middle Adriatic coast) where many industrial effluents are discharged into the sea (Fig. 1). The Kastela Bay, which receives considerable amounts of wastes, industrial and municipal effluents (still untreated) is one of the most threatened areas along the eastern Adriatic coast. An open sea station (Station 9) close to the Vis Island was used as a reference station.

2.2 Material and methods

Sea water samples were collected from two characteristic depths (surface and bottom layer) at five stations in the Kastela Bay (Fig. 1) in spring 1989.

Samples of marine organisms and sediments were collected from the same stations and at the same time as the sea water. Sediments were sampled with a gravity corer of 35 mm internal diameter in perspex tubes. Surface sediment (0-5 cm) and subsurface sediment (10-15 cm) were sampled and stored frozen until analysis. Sample collection, filtration and analyses were carried out as described by Kremling (1983). Acid precleaned Nucleopore filters of 0.45 μm were used. The filtrate from the latter represents the dissolved phase which includes dissolved Cr (III) and Cr (VI) species. Dissolved Cr was speciated as described by Cranston and Murray (1988). Cr (III) is scavenged from 250 ml sample by adding 2 ml of 0.01 M Fe (III)-hydroxide at pH 8 to the sample and shaking for 1 hour.

Both Cr (III) and Cr (VI) are quantitively co-precipitated by adjusting the Fe (II) ammonium sulfate to pH 8 and immediately adding this green precipitate to a sample.

During shaking, the iron (II) hydroxide is oxidized and chromium (VI) is reduced to chromium (III).

A double beam PE AAS was used applying a flameless technique.

Sediment samples were air dried, ground in agate mortar and sieved to pass 32 < dp < 75 μm (dp = pore diameter of sieves). Unsieved sediment was also analyzed for chromium content. Samples were dissolved totally with HF, HCl and HNO₃ acids and the dried residue taken up in 0.1 M HCl acid (UNEP/FAO/IAEA/IOC, 1984). Graphite furnace AAS technique was used for measuring Cr concentrations.

Samples of marine organisms (homogenates of ten mussels M. galloprovincialis), were prepared and digested with HNO₃ according to the method recommended by UNEP/FAO/IAEA/IOC, 1984. The results are expressed on a fresh weight basis (FW).
3. RESULTS AND DISCUSSION

3.1 Water and particulate matter

This preliminary study of chromium levels in sea water and particulate matter has shown Cr (III) to be the principal chromium form (Table 1). It was also confirmed that chromium is mainly bound to suspended matter in the sea.

Since there is a fair amount of organic material in natural sea water which is capable of binding Cr (III), such as organic acids, amino acids and humic acid (Nakayama et al., 1981), the main part of this metal is bound to the particulate part. Also other authors have obtained similar results (Jurasic and Prohic, 1986; Fajgelj and Kosta, 1987).

In the study area, Cr showed no distribution pattern. Rather high levels of this metal were recorded also from the open sea stations (Table 1) both in sea water and in suspended matter. This, on the one hand, could be accounted for by the geochemical cycles of this element (Paul and Meischner, 1976) and on the other hand, by the fact that this study was carried out in spring (June 1989), when biological activity is very high and a considerable quantity of biological material is produced in the sea through the photosynthetic processes or during the season with intensified organic productivity.
Increased chromium levels in the vicinity of some industrial plants (Stations 1c and 1b, Fig. 1) and municipal and industrial effluents (Vranjic basin) in the Kastela Bay are very likely to occur due to these effects.

Table 1

Chromium concentrations (µg dm⁻³) in sea water and suspended matter (mg kg⁻¹ DW) in the study area.

<table>
<thead>
<tr>
<th>Station</th>
<th>Depth</th>
<th>Cr(III)</th>
<th>Cr(VI)</th>
<th>Particulate Cr</th>
<th>Suspended matter (g dm⁻³ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.29</td>
<td>0.03</td>
<td>6.62</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.50</td>
<td>0.01</td>
<td>6.68</td>
<td>0.009</td>
</tr>
<tr>
<td>1b</td>
<td>0</td>
<td>0.26</td>
<td>0.03</td>
<td>14.19</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.88</td>
<td>0.06</td>
<td>19.65</td>
<td>0.008</td>
</tr>
<tr>
<td>1b₁</td>
<td>0</td>
<td>0.14</td>
<td>0.84</td>
<td>12.13</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.35</td>
<td>0.14</td>
<td>10.90</td>
<td>0.009</td>
</tr>
<tr>
<td>1c</td>
<td>0</td>
<td>0.48</td>
<td>0.04</td>
<td>13.36</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.38</td>
<td>0.13</td>
<td>14.84</td>
<td>0.008</td>
</tr>
<tr>
<td>1d</td>
<td>0</td>
<td>0.18</td>
<td>0.35</td>
<td>19.28</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.22</td>
<td>0.01</td>
<td>14.60</td>
<td>0.010</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0.06</td>
<td>0.46</td>
<td>13.54</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.21</td>
<td>0.24</td>
<td>24.62</td>
<td>0.010</td>
</tr>
<tr>
<td>BLANK</td>
<td></td>
<td>0.03</td>
<td>0.04</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Sediments and marine organisms

A preliminary study of the chromium content in marine sediment from both the coastal area (Kastela Bay) and the open sea (Stoncica cape near the Vis Island) showed almost negligible differences. The values recorded from the open sea station were even higher than those for the Kastela Bay. This may very likely be due to natural sources, that is to the high content of this element in volcanic and carbonate rocks (Paul and Meischner, 1976).

Spatial distribution of chromium in the Kastela bay sediment showed that the eastern part (Vranjic basin) had far higher levels of Cr. This is most probably due to the municipal effluents carrying considerable quantities of some heavy metals (Table 2).

Chromium content was also analysed in different sediment fractions (32 < dp < 75 µm). Low variability of chromium concentrations between
different sediment fractions is indicative of a rather even
distribution of this metal in sediment.

In some sediment samples (in fraction > 32 μm) Cr concentrations
were higher than in the fine fraction of the sediment. This could be
attributed to the fact that fraction > 32 μm represents about 70% of
the sediment.

The chromium content in marine organisms (mussels - *Mytilus
galloprovincialis*) showed maximum values in samples from the vicinity
of industrial and municipal effluent outfalls (Table 3, 1c).

**Table 2**

Content of Cr in the Kastela Bay sediments,
and at an open sea station (9) I. Vis (mg kg⁻¹ DW).

<table>
<thead>
<tr>
<th>Stations</th>
<th>Depth of sediments (cm)</th>
<th>Unsieved sediments</th>
<th>32&lt;dp&lt;75 (μm)</th>
<th>dp&lt;32 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 - 5</td>
<td>39.11</td>
<td>43.74</td>
<td>39.47</td>
</tr>
<tr>
<td></td>
<td>10 - 15</td>
<td>29.18</td>
<td>52.62</td>
<td>38.30</td>
</tr>
<tr>
<td>1b</td>
<td>0 - 5</td>
<td>39.86</td>
<td>56.37</td>
<td>55.27</td>
</tr>
<tr>
<td></td>
<td>10 - 15</td>
<td>60.60</td>
<td>59.81</td>
<td>61.54</td>
</tr>
<tr>
<td>1b₁</td>
<td>0 - 5</td>
<td>38.73</td>
<td>57.34</td>
<td>69.01</td>
</tr>
<tr>
<td></td>
<td>10 - 15</td>
<td>19.43</td>
<td>49.46</td>
<td>53.54</td>
</tr>
<tr>
<td>1c</td>
<td>0 - 5</td>
<td>50.80</td>
<td>59.75</td>
<td>36.55</td>
</tr>
<tr>
<td></td>
<td>10 - 15</td>
<td>43.65</td>
<td>35.59</td>
<td>32.62</td>
</tr>
<tr>
<td>9</td>
<td>0 - 5</td>
<td>40.74</td>
<td>96.00</td>
<td>50.02</td>
</tr>
<tr>
<td></td>
<td>10 - 15</td>
<td>32.22</td>
<td>102.05</td>
<td>39.11</td>
</tr>
</tbody>
</table>

**Table 3**

Chromium content in mussels (*Mytilus galloprovincialis*)
from the Kastela bay (mg kg⁻¹ FW).

<table>
<thead>
<tr>
<th>Station</th>
<th>Chromium content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vranjic (1c)</td>
<td>0.75</td>
</tr>
<tr>
<td>Jugovinil (1b₁)</td>
<td>0.70</td>
</tr>
<tr>
<td>Sv. Kaja (1c)</td>
<td>0.59</td>
</tr>
<tr>
<td>Kastel Stari (1b)</td>
<td>0.57</td>
</tr>
<tr>
<td>Institut (1d)</td>
<td>0.317</td>
</tr>
<tr>
<td>Ciovo</td>
<td>0.34</td>
</tr>
</tbody>
</table>
On the basis of this study, we tried to explain the behaviour of chromium in the marine environment. Cr (III) can be oxidized to Cr (VI) by catalytic action of oxides either by adsorption during sedimentation or in the marine sediments. Direct oxidation of Cr (III) with only dissolved oxygen, i.e. the theoretical redox equilibrium, cannot always be attained in practice (Nakayama et al. 1981).

Meanwhile Cr (VI) can be reduced in marine organisms or by some reducible materials. Organic complexes of Cr (III) are not oxidized and the trivalent state is stabilized. Thus chromium can be present in sea water in any form of inorganic Cr (III), Cr (VI) and organic species (Cranston and Murray, 1978).

This preliminary study points to some peculiarities in the distribution of this metal which calls for further and more intensive research.

4. CONCLUSION

The study of Cr levels in different parts of the Kastela Bay aim at identifying the major sources of chromium in the bay, their fate and distribution. Cr levels found in the bay, however, are of the same order of magnitude as those recorded from the open sea station. Therefore, the concentrations of this metal in this environment are not related to the pollution processes but rather attributed to natural sedimentological processes (Paul and Meischner, 1976).

5. REFERENCES


THE CYCLING OF MERCURY THROUGH THE MARINE ENVIRONMENT OF KASTELA BAY

by

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Institute of Oceanography and Fisheries
Split, Yugoslavia

ABSTRACT

Long-term uncontrolled discharge of mercury in the Kastela Bay (started in 1949 when the chloralkali plant of the PVC factory was set in operation) resulted in an excess accumulation of this metal in the majority of vital compartments of this aquatic area. Even though accurate data on the total quantity of deposited mercury are not available, judging from the assessments of different authors, at least 150000 kg of this toxic element have entered Kastela Bay. This assumption is based on the knowledge of technological processes (where mercury losses are inevitable with a minimum of 150-250 g Hg per ton of produced chlorine) as well as on the results of a number of measurements of Hg levels in sea water, sediments and characteristic marine organisms. The results obtained (mercury in seawater, sediments, phytoplankton, zooplankton, mussels and some commercial fish species) showed the transfer and bioaccumulation of mercury at different trophic levels in the marine environment (especially in the area near the chloralkali plant), indicating a biomagnification effect of mercury between the different levels of the food chain.

1. INTRODUCTION

Long-term studies of heavy metals (Zn, Cd, Pb, Cu, As, Hg) in seawater, sediments and some marine organisms in the coastal area of middle and southern Adriatic have shown that the areas in the vicinity of industrial centres are the most exposed since industry is the principal source of pollution by these microelements (Stegnar et al., 1980; Vukadin et al., 1982).

Mercury is one of the heavy metals with the widest distribution in nature. Due to natural mechanisms of its transformation to toxic methylmercury (Wood et al., 1968; Jensen and Jernelov, 1969; Kersten, 1988), uncontrolled disposal in aquatic areas may result in its bioaccumulation in biota. Since mercury (due to the chloralkali plant on the coast of the Kastela bay) is one of the most mentioned pollutants in this area, in 1985 (in the framework of UNEP MED POL Phase II programme) we directed our scientific interest to the studies of its ecological cycle. The principal goal of this research project was to establish:

a) mercury concentrations in sea water, sediments, phyto- and zooplankton, mussels and some commercial species of fish,

b) distribution of Hg among water, sediments and marine biota, and
3) the uptake of mercury by marine organisms at different trophic levels.

2. EXPERIMENTAL

2.1 Study area

2.1.1 Natural characteristics of the Kastela Bay

The Kastela Bay is the largest bay in the coastal region of central Dalmatia. It is an elongated basin near Split (Fig. 1) 14.8 km long and 6.6 km wide. It communicates with the adjacent Split channel through a 1.8 km wide inlet which is actually the deepest part of the bay (average depth of the inlet is 40 m with a maximum of 56 m). All the other characteristics of the bay are summarized in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kastela Bay, mid-Adriatic area.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Geographical position</th>
<th>43°31'N; 16°23'E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area</td>
<td>61 x 10^6 m^2</td>
</tr>
<tr>
<td>Volume</td>
<td>1400 x 10^6 m^3</td>
</tr>
<tr>
<td>Greatest depth</td>
<td>56 m</td>
</tr>
<tr>
<td>Mean depth</td>
<td>23 m</td>
</tr>
<tr>
<td>Mean time of water exchange</td>
<td>30 days</td>
</tr>
</tbody>
</table>

The sediments of the bay are composed of silt and fine sand. The Jadro River is the main source of fresh water in the bay with annual mean drainage of about 315 x 10^6 m^3 (about 10 m^3 s^-1 flow).

Kastela Bay is an area of high primary production in the Mediterranean Basin due to the weathering of nutrients from fields near Kastela Bay and from woodlands of the Split peninsula.

Along its northeastern coast there are: a new port, several cement plants, a chemical plant (PVC-factory), a shipyard and other factories. The consequences of urban and industrial wastes into the bay during the last 20 years are increasingly apparent. This is especially evident in shallow waters of the eastern part which is highly polluted by industrial and domestic wastes.

Kastela Bay is an area on the eastern Adriatic coast polluted for 40 years (1950-1990) by mercury released from the chloralkali plant of the PVC factory.
Fig. 1  Study area with sampling stations
Mercury inputs to the bay from this source were not measured prior to 1985 and the assessments in past years were based on the quantity of chlorine production and varied within a wide range (from several hundreds of kilograms to several tens of metric tons). In 1985, the factory undertook some technical and technological measures to reduce mercury losses. Chlorine production ceased in May 1990.

2.2 Sampling

Our research included five principal sediment sampling stations in the Kastela Bay (No 1, 1-a, 1-b, 1-c, 1-d) (Fig. 1). However, for the study of mercury distribution between sea water, sediments and biota, only two characteristic stations were selected: station No 1-b in the close vicinity of the chloralkali plant and station No 1 located in the middle of the Kastela Bay, ca. 4 km away from the direct pollution source.

Additionally, in two occasions in 1986, total mercury was measured in non-filtered surface water samples from sites located at 10, 250, and 500 m from the factory effluent outfall, from the central part of the bay (No 1) and from the inlet to the bay (No 1-d).

Sediments were collected from all stations at different periods from 1985 to 1988 (May and September 1985, July and December 1986, March and November 1987 and April 1988). Sediment samples were taken by a gravity corer equipped with perspex tubes during cruises with the research vessel "BIOS". The surface sediment layer (0-5 cm) of each sample was frozen and stored in polythene bags until the analysis.

Surface sea water samples were collected manually with dark Pyrex bottles from a plastic boat (seasonally, 4 times a year). The cleaning procedure of the bottles and the pretreatment of the samples before analysis were performed in accordance with the procedure described earlier (Horvat et al., 1987).

Phytoplankton for mercury analysis was collected only from stations No 1-b and No 1 and was sampled 4 times a year by repeated vertical hauls of phytoplankton net (mesh size 50 μm), concentrated by filtration, frozen and stored in polythene vials before analysis.

The composition of the phytoplankton community at both stations was quite similar, but relative contribution of different species varied to a large extent. While Dinoflagellates were the best represented group at station No 1-b, making up even 95% of the total phytoplankton community, at station No 1 diatoms prevailed with about 70% followed by coccolithophorids with "Microflagellates" with 25%, and dinoflagellates with 6%. Procorcentrum micans, P. minimum, P. triestinum, Gonvalaulax polyedra, Ceratium karstenii and Oxytoxum longicaps were the best represented species of dinoflagellates at station No 1-b, while Skeletonema costatum, Leptocylindrus adriaticus, Rhiposolenia alata f. gracilima, Chaetoceros curvisetus, Thalassionema nitzschoides and some other species of diatoms were dominant at station No 1.

Zooplankton samples were collected by vertical hauls from bottom to surface using the "HANSEN" net (73/100, silk No.3). (To our regret, due to technical reasons zooplankton was sampled by phytoplankton net
in March and June 1986 and apparently affected the results of those samplings). One twentieth of the sample was counted and the whole catch examined for rare species. Diversity index \( d \) as quantitative indicator of copepod group structure was estimated from the formula developed by Margaleff (1951): \( d = S - 1/N \ln N \), where \( S \) is the number of species, \( N \) the number of individuals per cubic metre and \( \ln N \) the natural logarithm of the number of individuals.

The zooplankton at the studied stations consisted predominantly of Copepoda – quantitatively the best represented zooplankton group. The percentage of Copepoda was about 70% at station No 1-b and about 65% at station No 1. At station No 1-b Cladocera followed Copepoda with 20% while Medusae, Siphonophora, Mollusca larvae, Ostroccida, Decapoda larvae, Chaetognatha, Cephalopoda, Tunicata and other groups were present amounting together to about 15%. At station No 1, the composition was similar. Cladocera followed Copepoda with about 19% and the above mentioned groups of zooplankton organisms were present with about 16%. As the Copepoda were the best represented zooplankton group of organisms, their composition was studied at the investigated stations, too. So, at station No 1-b among copepods Acartia clausi was dominant species (with about 65%), and Paracalanus parvus, Centropages typicus, Temora stylifera and Temora longicornis occurred in larger numbers among 25 copepod species. At station No 1 Centropages typicus, Acartia clausi, Temora stylifera and Ctenocalanus vanus were dominant among 28 copepod species.

Shellfish were collected seasonally (four times a year) from the station in the vicinity of the chloralkali factory (1-b) and from the coast of Ciclo Island (1-d) as a reference station. Shellfish samples were collected from the littoral tidal zone. All the samples were prepared "on board" or in our laboratory – following recommended procedures (UNEP/FAO/IOC/IAEA, 1984) – stored into polythene vials and frozen until analyses, which were performed at the Department of Nuclear Chemistry at the "J. Stefan" Institute in Ljubljana.

The following fish species were caught (by trawl) from the Kastela Bay in 1985/88: red mullet (Mullus barbatus), common sole (Solea vulgaris), common pandora (Pagellus erythrinus) and European hake (Merluccius merluccius).

2.3 Mercury analysis

The unfrozen sediment was wet sieved through a nylon net of 0.1mm mesh size and a fraction was taken for analysis of mercury and sealed in precleaned quartz ampoules. The soft part of mussels and fillet from fish were used for Hg analysis. The determination of Hg in sediments, phyto- and zooplankton, fish and mussels was carried out by neutron activation analysis (NAA) in the TRIGA Mark II reactor of the Institute "J. Stefan". Samples were irradiated together with standards in the rotating rack of the reactor at a flux of \( 1.8 \times 10^{12} \text{ n cm}^{-2} \text{sec} \) for about 20 hours. The sample was pyrolysed in a stream of air with oxygen-assisted catalytic combustion. Mercury was absorbed on a selenium impregnated paper. The gamma activity of the isolated radionuclide \(^{199}\text{Hg} \) was measured in a "3x3" "well-type" NaI(Tl) detector connected to a 400 channel analyser (Kosta and Byrne, 1969; Byrne and Kosta, 1974).
Determination of total mercury in sea water samples was carried out by cold vapour atomic absorption spectrophotometry (CV AAS) (a closed aeration technique coupled with a double amalgamation stage on a gold absorber) (Horvat et al., 1987). Determination of methyl mercury in fish and mussels was performed by gas chromatography (Horvat et al., 1988; Horvat et al., 1990).

The accuracy of the results obtained and standard errors of analyses were checked using SRMs from NBS (Washington), standard addition experiments and interlaboratory comparisons (with KFK Julich-West Germany, and IAEA laboratory in Monaco as co-operating laboratories) (Horvat et al., 1980; 1990; Horvat, in press).

3. RESULTS AND DISCUSSION

3.1 Mercury in sediments

The spatial and temporal distribution of total mercury in surface sediments from the study area during the four-year period in question are reported in Table 2 and in Figure 2.

Table 2

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Station</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-d</td>
</tr>
<tr>
<td>16/05/85</td>
<td>0.90</td>
</tr>
<tr>
<td>11/09/85</td>
<td>0.65</td>
</tr>
<tr>
<td>04/06/86</td>
<td>1.16</td>
</tr>
<tr>
<td>08/12/86</td>
<td>0.80</td>
</tr>
<tr>
<td>24/03/87</td>
<td>0.53</td>
</tr>
<tr>
<td>07/11/87</td>
<td>0.82</td>
</tr>
<tr>
<td>20/04/88</td>
<td>0.72</td>
</tr>
<tr>
<td>Average (X)</td>
<td>0.80</td>
</tr>
<tr>
<td>St.dev. (SD)</td>
<td>0.20</td>
</tr>
<tr>
<td>CV (%)</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Sediments from station No 1-b were the most polluted (5-22 mg kg\(^{-1}\) dw). Other zones contaminated by Hg from waste waters were restricted and localized along the coast of the Bay near anthropogenic installations (such as station No 1-c in front of a shipyard). During this period no trend of increase or decrease in mass mercury concentrations in surficial sediments was observed at any of the stations, because 5 cm of sediments probably correspond to more than 3 years of sedimentation.
Fig. 2 Histograms of mean concentrations of Hg\textsubscript{total} in surface sediments from Kastela bay during 1985/88. (arithmetical mean (X) and standard deviations (SD) for entire period)

The analysis of mean values (Fig. 2) clearly shows that the chloralkali factory is the principal Hg pollution source in the Kastela Bay, which means that the areas in the closest vicinity of stations No 1-b and No 1-c were most polluted. In station No 1-b Hg concentrations in sea water, phyto- and zooplankton and shellfish showed the strong anthropogenic influence on this marine ecosystem.

3.2 Mercury in sea water

Average concentrations for total Hg in unfiltered surface sea water at two stations (No 1 and 1-b) during the period of investigation are reported in Figure 3 and Table 4. The highest concentration of Hg was detected at station 1-b, indicating a strong influence of effluents from the chloralkali plant.
Fig. 3 Histograms of mean concentrations of Hg_{total} in unfiltered surface sea water at two stations during 1985/88

An additional comparison between these two stations (Tudor et al., 1990) shows that the concentration of mercury at station No 1 is directly proportional to that in front of the PFC factory \([(Hg_{SW})_{1} = 0.36 (Hg_{SW})_{1-b}] with a coefficient of 0.4. This points to the fact that both the increase and decrease in sea water coincide in time over the larger part of the bay (if not throughout the bay), and that underlying sediments are probably a source from which mercury might be released and introduced into the water column and plankton (Tudor et al., 1990).

Obtained results of additional measurements from 1986 are given in Table 3.

Mercury concentrations in sea water 10 m off the outfall were from 15 to 18 times lower than those in the effluent itself (6 \(\mu g\) dm\(^{-3}\)). During the first sampling, mercury concentrations decreased at a high rate with the distance of sampling site from the coast. In December of the same year mercury concentrations were, on the average, only two times lower at a station 500 m off the coast than near the coast.
Table 3
Mean total mercury levels in surface sea water samples collected in 1986 (ranges in brackets).

<table>
<thead>
<tr>
<th>Station</th>
<th>June Concentration (ng Hg dm$^{-3}$)</th>
<th>December Concentration (ng Hg dm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>the effluent of chlor-alkali plant</td>
<td>6000</td>
<td></td>
</tr>
<tr>
<td>10 m off the outfall</td>
<td>330 (135-535)</td>
<td>378 (249-444)</td>
</tr>
<tr>
<td>250 m off the outfall</td>
<td>50 (44-55)</td>
<td>254 (113-439)</td>
</tr>
<tr>
<td>500 m off the outfall</td>
<td>43 (37-46)</td>
<td>117 (60-354)</td>
</tr>
<tr>
<td>Central part of the bay (1)</td>
<td>47</td>
<td>97</td>
</tr>
<tr>
<td>Inlet to the bay (1-d)</td>
<td>25</td>
<td>58</td>
</tr>
</tbody>
</table>

Table 4
Total mercury content in sea water (ng Hg dm$^{-3}$), phytoplankton and zooplankton (μg Hg kg$^{-1}$ WW) at stations 1 and 1-b, and bioaccumulation factors between plankton and sea water.

<table>
<thead>
<tr>
<th>month/year</th>
<th>Sea water</th>
<th>Phytoplankton</th>
<th>Zooplankton</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 1-b</td>
<td>1 1-b</td>
<td>1 1-b</td>
</tr>
<tr>
<td>9/85</td>
<td>60 190</td>
<td>125 644</td>
<td>41 195</td>
</tr>
<tr>
<td>11/85</td>
<td>21 32</td>
<td>110 906</td>
<td>67 406</td>
</tr>
<tr>
<td>3/86</td>
<td>48 367</td>
<td>13 51</td>
<td>40 36</td>
</tr>
<tr>
<td>6/86</td>
<td>30 72</td>
<td>96 577</td>
<td>172 854</td>
</tr>
<tr>
<td>9/86</td>
<td>97 217</td>
<td>640 1030</td>
<td>130 220</td>
</tr>
<tr>
<td>12/86</td>
<td>58 117</td>
<td>90 380</td>
<td>49 122</td>
</tr>
<tr>
<td>3/87</td>
<td>12 58</td>
<td>128 182</td>
<td>46 61</td>
</tr>
<tr>
<td>6/87</td>
<td>4 23</td>
<td>16 90</td>
<td>30 99</td>
</tr>
<tr>
<td>12/87</td>
<td>7 17</td>
<td>38 220</td>
<td>47 47</td>
</tr>
<tr>
<td>4/88</td>
<td>34 110</td>
<td>69 894</td>
<td>68 236</td>
</tr>
</tbody>
</table>

Average 34 110 132 497 68 236
Std. dev. 29 109 183 364 46 249
CV (%) 84 99 138 73 67 106

Average ratio No 1 No 1-b
phyto : water 3850 4500
zoopl : water 1983 2140
3.3 Mercury levels in biota

Mean concentrations for total Hg in sea water, phyto- and zooplankton samples, collected from the central part of the bay (station 1) and close to the chloralkali plant (station 1-b) between 1985 and 1988, together with the concentration factors between plankton and sea water, are given in Table 4.

Even though the results showed no apparent pattern (due to the inadequate samplings in March and June 1986) it seems that Hg accumulated by phytoplankton was almost three times higher than in zooplankton.

Comparing Hg levels in sea water to those in plankton, the bioaccumulation factor between plankton and sea water varies from 1983 to 4500.

Even though the bioaccumulation factor between plankton and individual fish species decreased from 1 to 13 times depending on fish species (Zvonaric et al., 1987), Hg concentrations of total Hg in fish and shellfish of the Kastela Bay (Table 5 and Fig. 4) indicate that some species accumulated Hg at concentrations above the edible limits (Soles v. vulgaris, Pagellus erythrinus).

This becomes more serious if we take into account that more than 80% of total mercury accumulated by these marine organisms is in the methyl form.

The results obtained for some commercial fish species from this region are presented in Table 5.

Table 5

Mean mercury (methyl mercury) concentrations in some fish (muscle) species from the Kastela Bay and Split Channel (an area off the direct influence of mercury pollution).

<table>
<thead>
<tr>
<th>Date of Sampling</th>
<th>Mullus barbatus</th>
<th>Pagellus erythrinus</th>
<th>Merluccius merluccius</th>
<th>Solea vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>KASTELA BAY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 1985</td>
<td>405</td>
<td>526</td>
<td>193</td>
<td>810</td>
</tr>
<tr>
<td>Sept. 1986</td>
<td>370 (330)</td>
<td>730 (680)</td>
<td>170 (140)</td>
<td>-</td>
</tr>
<tr>
<td>July 1987</td>
<td>420 (380)</td>
<td>680 (680)</td>
<td>180 (170)</td>
<td>1370 (1030)</td>
</tr>
<tr>
<td>August 1987</td>
<td>470 (300)</td>
<td>260 (240)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 1988</td>
<td>620 (510)</td>
<td>1740 (1610)</td>
<td>490 (480)</td>
<td></td>
</tr>
<tr>
<td>840 (790)</td>
<td>1540 (1340)</td>
<td>430 (340)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPLIT CHANNEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 1988</td>
<td>120 (120)</td>
<td>480 (330)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Average total mercury concentrations in the 1985-1988 period varied within the following ranges:

0.37 - 0.84 ppm in red mullet, 0.26 - 1.74 ppm in common pandora, 0.17 - 0.49 ppm in hake and 0.81 - 1.37 in common sole.

Seasonal fluctuations of the total mercury concentrations in shellfish are given in Fig. 4. The results show the high mercury concentrations (mean value 1610 μg Hg kg⁻¹ ww) in shellfish collected near the chloralkali plant rendering them unsuitable for human consumption.

![Graph showing mercury concentrations over time](image)

**Fig. 4** Histograms of concentrations of Hg_{total} in shellfish (*Mytilus galloprovincialis*) from Kastela Bay during 1985/88, with arithmetical mean (X̄) for entire period

4. CONCLUSIONS AND RECOMMENDATIONS

Mercury content in the surface sediment layer (0-5 cm) has remained almost unchanged for the last five years. The highest concentrations have been reported for the vicinity of the chloralkali plant (about 10 mg kg⁻¹ of dry sediment) and the lowest for the bay inlet (0.8 mg kg⁻¹).
Mercury levels in the samples of unfiltered sea water are highest in the coastal belt close to the factory (100 - 500 μg Hg dm⁻³), radially decreasing going offshore. The proportionality between Hg levels in the sea water of the central bay part and that close to the chloralkali plant was established with a coefficient of 0.4.

Mercury levels in plankton and sea water are correlated. Established factors of biomagnification (through phyto- and zooplankton) are indicative of intensive accumulation of mercury by individual levels of the food chain.

Mussels from the area closest to the factory outlet are the most conspicuous example. From the data on mercury concentration in the sea water, sediment and biota as well as on the present anthropogenic input, it seems that significant quantity of mercury in the water originates from the bottom of the bay (Tudor et al., 1990).

On the basis of the results of this study we would like to recommend the following for future investigations:

- to establish the rate of mercury removal from the sediment (by dilution and remobilization processes) to the water column;

- to establish the proportion of dissolved and particulate mercury in sea water as well as the importance of suspended matter (particularly that of phytoplankton) on the partition coefficient;

- to assess the average mercury residence time in all compartments which can help in the calculation of the auto-purification rate of the Bay;

- to continue the monitoring of mercury and methyl mercury in living organisms, but limited to mussels - Mytilus galloprovincialis (as filter feeders) and to representatives of non-migratory fish species (common sole - Solea vulgaris and-or annular sea bream - Diplodus annularis).

5. REFERENCES


Horvat, M., in press. Determination of methylmercury in biological standard reference materials. (submitted to Water Air and Soil Pollution)


Kosta, L. and A.R. Byrne, 1969. Activation analysis for mercury in biological samples at nanogram level. Talanta, 16:1297-1303


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35. UNEP: Bibliography on marine pollution by organotin compounds. MAP Technical Reports Series No. 35. UNEP, Athens, 1989 (92 pages) (English only).


37. UNEP/FAO: Final reports on research projects dealing with eutrophication and plankton blooms (Activity H). MAP Technical Reports Series No. 37. UNEP, Athens, 1990 (74 pages) (parts in English or French only).


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51. UNEP/FAO: Final reports on research projects dealing with mercury, toxicity and analytical techniques. MAP Technical Reports Series No. 51. UNEP, Athens, 1991 (166 pages) (parts in English or French only).

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52. PNUE/FAO: Rapports finaux sur les projets de recherche traitant de la bioaccumulation et de la toxicité des polluants chimiques. MAP Technical Reports Series No. 52. UNEP, Athens, 1991 (66 pages) (parties en anglais ou français seulement).


