MEDITERRANEAN ACTION PLAN
MED POL

UNITED NATIONS ENVIRONMENT PROGRAMME

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

FINAL REPORTS ON RESEARCH PROJECTS DEALING WITH BIOACCUMULATION AND TOXICITY OF CHEMICAL POLLUTANTS

RAPPORTS FINAUX SUR LES PROJETS DE RECHERCHE TRAITANT DE LA BIOACCUMULATION ET DE LA TOXICITE DES POLLUANTS CHIMIQUES

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This volume is the fifty-second issue of the Mediterranean Action Plan Technical Reports Series.

This series contains selected reports resulting from the various activities performed within the framework of the components of the Mediterranean Action Plan: Pollution Monitoring and Research Programme (MED POL), Blue Plan, Priority Actions Programme, Specially Protected Areas and Regional Marine Pollution Emergency Response Centre for the Mediterranean.

Ce volume constitue le cinquante-deuxième 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.

INTRODUCTION

The United Nations Environment Programme (UNEP) convened an Intergovernmental Meeting on the Protection of the Mediterranean (Barcelona, 28 January - 4 February 1975), which was attended by representatives of 16 States bordering the Mediterranean Sea. The meeting discussed the various measures necessary for the prevention and control of pollution of the Mediterranean Sea, and concluded by adopting an Action Plan consisting of three substantive components:

- Integrated planning of the development and management of the resources of the Mediterranean Basin (management component);

- Co-ordinated programme for research, monitoring, exchange of information, assessment of the state of pollution and protection measures (assessment component);

- Framework convention and related protocols with their technical annexes for the protection of the Mediterranean environment (legal component).

All components of the Action Plan are inter-dependent and provide a framework for comprehensive action to promote both the protection and the continued development of the Mediterranean ecoregion. No component is an end in itself. The Action Plan is intended to assist the Mediterranean Governments in formulating their national policies related to the continuous development and protection of the Mediterranean area and to improve their ability to identify various options for alternative patterns of development and to make choices for appropriate allocations of resources.

MED POL - Phase I (1976-1980)

The Co-ordinated Mediterranean Research and Monitoring Programme (MED POL) was approved as the assessment (scientific/technical) component of the Action Plan.

The general objectives of its pilot phase (MED POL - Phase I), which evolved through a series of expert and intergovernmental meetings, were:

- to formulate and carry out a co-ordinated pollution monitoring and research programme taking into account the goals of the Mediterranean Action Plan and the capabilities of the Mediterranean research centres to participate in it;

- to assist national research centres in developing their capabilities to participate in the programme;

- to analyse the sources, amounts, levels, pathways, trends and effects of pollutants relevant to the Mediterranean Sea;

- to provide the scientific/technical information needed by the Governments of the Mediterranean States and the EEC for the negotiation and implementation of the Convention for the Protection of the Mediterranean Sea against Pollution and its related protocols.

- to build up consistent time-series of data on the sources, pathways, levels and effects of pollutants in the Mediterranean Sea and thus to contribute to the scientific knowledge of the Mediterranean Sea. MED POL - Phase I initially consisted of seven pilot projects (MED POL I - VII), which were later expanded by additional six pilot projects (MED POL VIII - XIII), some of which remained in a conceptual stage only.

MED POL - Phase I was implemented in the period from 1975 to 1980. The large number of national research centres designated by their Governments to participate in MED POL (83 research centres from 15 Mediterranean States and the EEC), the diversity of the programme and its geographical coverage, the impressive number of Mediterranean scientists and technicians (about 200) and the number of co-operating agencies and supporting organizations involved in it, qualifies MED POL as certainly one of the largest and most complex co-operative scientific programmes with a specific and well-defined aim ever undertaken in the Mediterranean basin.

The overall co-ordination and guidance for MED POL - Phase I was provided by UNEP, acting as the secretariat of the Mediterranean Action Plan (MAP). Co-operating specialized United Nations Agencies (ECE, UNIDO, FAO, UNESCO, WHO, WMO, IAEA, IOC) were responsible for the technical implementation and day-to-day co-ordination of the work of national research centres participating in the pilot projects.

MED POL - Phase II (1981-1990)

The Intergovernmental Review Meeting of Mediterranean Coastal States and First Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution, and its related protocols (Geneva, 5-10 February 1979), having examined the status of MED POL - Phase I, recommended that during the 1979/80 biennium a Long-term pollution monitoring and research programme should be formulated.

Based on the recommendations made at various expert and intergovernmental meetings, a draft Long-term (1981-1990) Programme for Pollution Monitoring and Research in the Mediterranean (MED POL - Phase II) was formulated by the Secretariat of the Barcelona Convention (UNEP), in co-operation with the United Nations Agencies which were responsible for the technical implementation of MED POL - Phase I, and it was formally approved by the Second Meeting of the Contracting Parties of the Mediterranean Sea against pollution and its related protocols and Intergovernmental Review Meeting of Mediterranean Coastal States of the Action Plan held in Cannes, 2-7 March 1981.

- development of sampling and analytical techniques for monitoring the sources and levels of pollutants. Testing and harmonization of these methods at the Mediterranean scale and their formulation as reference methods. Priority will be given to the substance listed in the annexes of the Protocol for the prevention of pollution of the Mediterranean Sea by dumping from ship and aircraft and the Protocol for the protection of the Mediterranean Sea against pollution from land-based sources (activity A);

- development of reporting formats required according to the Dumping, Emergency and Land-Based Sources Protocols (activity B);

- formulation of the scientific rationale for the environmental quality criteria to be used in the development of emission standards, standards of use or guidelines for substaces listed in annexes I and II of the Land-Based Sources Protocol in accordance with Articles 5, 6 and 7 of that Protocol (activity C);

- epidemiological studies related to the confirmation (or eventual revision) of the proposed environmental quality criteria (standards of use) for bathing waters, shellfish-growing waters and edible marine organisms (activity D);

- development of proposals for guidelines and criteria governing the application of the Land-Based Sources Protocol, as requested in Article 7 of that Protocol (activity E);

- research on oceanographic processes, with particular emphasis on surface circulation and vertical transport. Needed for the understanding of the distribution of pollutants through the Mediterranean and for the development of contingency plans for cases of emergency (activity F);

- research on the toxicity, persistence, bioaccumulation, carcinogenicity and mutagenicity of selected substances listed in annexes of the Land-Based Sources Protocol and the Dumping Protocol (activity G);

- research on eutrophication and concomitant plankton blooms. Needed to assess the feasibility of alleviating the consequences and damage from such recurring blooms (activity H);

- study of ecosystem modifications in areas influenced by pollutants, and in areas where ecosystem modifications are caused by large-scale coastal or inland engineering activity (activity I);

- effects of thermal discharges on marine and coastal ecosystems, including the study of associated effects (activity J);

- biogeochemical cycle of specific pollutants, particularly those relevant to human health (mercury, lead, survival of pathogens in the Mediterranean Sea, etc.) (activity K);

The general long-term objectives of MED POL - Phase II were to further the goals of the Barcelona Convention by assisting the Parties to prevent, abate and combat pollution of the Mediterranean Sea Area and to protect and enhance the marine environment of the Area. The specific objectives were designed to provide, on a continuous basis, the Parties to the Barcelona Convention and its related protocols with:

- information required for the implementation of the Convention and the protocols;

- indicators and evaluation of the effectiveness of the pollution prevention measures taken under the Convention and the protocols;

- scientific information which may lead to eventual revisions and amendments of the relevant provisions of the Convention and the protocols and for the formulation of additional protocols;

- information which could be used in formulating environmentally sound national, bilateral and multilateral management decisions essential for the continuous socio-economic development of the Mediterranean region on a sustainable basis;

- periodic assessment of the state of pollution of the Mediterranean Sea.

The monitoring of, and research on, pollutants affecting the Mediterranean marine environment reflects primarily the immediate and long-term requirements of the Barcelona Convention and its protocols, but also takes into account factors needed for the understanding of the relationship between the socio-economic development of the region and the pollution of the Mediterranean Sea.

For this purpose, monitoring was organized on several levels:

- monitoring of sources of pollution providing information on the type and amount of pollutants released directly into the environment;

- monitoring of nearshore areas, including estuaries, under the direct influence of pollutants from identifiable primary (outfalls, discharge and coastal dumping points) or secondary (rivers) sources;

- monitoring of offshore areas (reference areas) providing information on the general trends in the level of pollution in the Mediterranean;

- monitoring of the transport of pollutants to the Mediterranean through the atmosphere, providing additional information on the pollution load reaching the Mediterranean Sea.

Research and study topics included initially in the MED POL - Phase II were:

- study of pollutant-transfer processes (i) at river/sea and air/sea interface, (ii) by sedimentation and (iii) through the straits linking the Mediterranean with other seas (activity L);

The Contracting Parties at their 6th Ordinary Meeting (Athens, October 1989) agreed to:

(a) Re-orient the research activities within MED POL in order to generate information which will also be useful for the technical implementation of the LBS protocol in addition to supporting monitoring activities;

(b) replace as from 1990 research activities A-L by the following five new research areas:

Research area I - Characterization and measurement

This area will include projects which cover the characterization (identification of chemical or microbiological components) and measurement development and testing of methodologies of specified contaminants;

Research area II - Transport and dispersion

This area will include projects which aim at improving the understanding of the physical, chemical and biological mechanisms that transport potential pollutants from their sources to their ultimate repositories. Typical topics will be atmospheric transport and deposition, water movements and mixing, transport of contaminants by sedimentation and their incorporation in biogeochemical cycles. Priority will be given to the provision of quantitative information ultimately useful for modelling the system and contributing to regional assessments;

Research area III - Effects

This area will include projects relevant to the effects of selected contaminants, listed in Annexes I and II of the LBS and Dumping protocols, to marine organisms, communities and ecosystems or man and human populations. Priority will be given to effects and techniques providing information useful for establishing environmental quality criteria;

Research area IV - Fates/Environmental transformation

This area will include projects studying the fate of contaminants (including microorganisms) in the marine environment such as persistence or survival, degradation, transformation, bioaccumulation etc. but excluding transport and dispersion which is dealt in area II;

Research area V - Prevention and control

This area will include projects dealing with the determination of the factors affecting the efficiency of waste treatment and disposal methods under specific local conditions as well as the development of environmental quality criteria and common measures for pollution abatement;

(c) define target contaminants or toher variables at periodic intervals depending on the progress of implementation of the LBS protocol;

(d) select project proposals on the basis of their intrinsic scientific validity, their Mediterranean specificity, and encourage whenever possible bilateral and multilateral projects among Mediterranean countries from the north and the south of the basin.

As in MED POL - Phase I, the overall co-ordination and guidance for MED POL - Phase II is provided by UNEP as the secretariat of the Mediterranean Action Plan (MAP). Co-operating specialized United Nations Agencies (FAO, UNESCO, WHO, WMO, IAEA, IOC) are responsible for the technical implementation and day-to-day co-ordination of the work of national centres participating in monitoring and research.

The present volume includes final reports on research projects implemented under Research Activities G and K dealing with the bioaccumulation and toxicity of chemical pollutants. Final editing and compilation of this volume was done by Mr. G.P. Gabrielides, FAO Senior Fishery Officer (Marine Pollution) while Ms V. Papapanagiotou, FAO Secretary, was responsible for the typing.

INTRODUCTION

Le Programme des Nations Unies pour l'environnement (PNUE) a convoqué une réunion intergouvernementale sur la protection de la Méditerranée (Barcelone, 28 janvier - 4 février 1975) à laquelle ont pris part des représentants de 16 Etats riverains de la mer Méditerranée. La réunion a examiné les diverses mesures nécessaires à la prévention et à la lutte antipollution en mer Méditerranée, et elle s'est conclue sur l'adoption d'un Plan d'action comportant trois éléments fondamentaux:

- Planification intégrée du développement et de la gestion des ressources du bassin méditerranéen (élément "gestion");

- Programme coordonné de surveillance continue, de recherche, d'échange de renseignements et d'évaluation de l'état de la pollution et des mesures de protection (élément "évaluation");

- Convention cadre et protocoles relatifs avec leurs annexes techniques pour la protection du milieu méditerranéen (élément juridique).

Tous les éléments du Plan d'action étaient interdépendants et fournissaient le cadre d'une action d'ensemble en vue de promouvoir, tant la protection que le développement continue de l'écorégion méditerranéenne. Aucun élément ne constituait une fin à lui seul. Le Plan d'action était destiné à aider les gouvernements méditerranéens à formuler leurs politiques nationales en matière de développement continu et de protection de zone de la Méditerranée et à accroître leur faculté d'identifier les diverses options s'offrant pour les schémas de développement, d'arrêter leurs choix et d'y affecter les ressources appropriées.

MED POL - Phase I (1976 - 1980)

Le programme coordonné de surveillance continue et de recherche en matière de pollution de la Méditerranée (MED POL) a été approuvé au titre de l'élément "évaluation" (scientifique/technique) du Plan d'action.

Sa phase pilote (MED POL - Phase I) avait les objectifs généraux ci-dessous, élaborés au cours d'une série de réunions d'experts et de réunions intergouvernementales:

- formuler et exécuter un programme coordonné de surveillance continue et de recherche en matière de pollution en tenant compte des buts du Plan d'action pour la Méditerranée et de l'aptitude des centres de recherche méditerranéens à y participer;

- aider les centres de recherche nationaux à se rendre plus aptes à cette participation;

- étudier les sources, l'étendue, le degré, les parcours, les tendances et les effets des polluants affectant la mer Méditerranée;

- fournir l'information scientifique et technique nécessaire aux gouvernements des pays méditerranéens et à la Communauté économique européenne pour négocier et mettre en oeuvre la Convention pour la protection de la mer Méditerranée contre la pollution et les protocoles y relatifs;

- constituer des séries chronologiques cohérentes de données sur les sources, les cheminements, les degrés et les effets des polluants de la mer Méditerranée et contribuer par là à la connaissance scientifique de cette mer.

La Phase I du MED POL comportait à l'origine sept projets pilotes (MED POL I - VII) auxquels sont venus ultérieurement s'ajouter six autres (MED POL VIII - XIII) dont certains n'en sont restés qu'au stade de la conception.

La Phase I du MED POL a été mise en oeuvre au cours de la période 1975 - 1980. Le grand nombre de centres de recherche nationaux désignés par leurs gouvernements pour participer au MED POL (83 centres de recherche de 15 Etats méditerranéens et de la CEE), la diversité du programme et sa couverture géographique, l'effectif impressionnant de scientifiques et techniciens méditerranéens (environ 200) ainsi que la quantité d'organismes coopérants et d'organisations d'appui qui y étaient engagés permettent sans conteste de caractériser le MED POL comme l'un des programmes de coopération scientifique les plus vastes et les plus complexes, comportant un objectif spécifique et bien défini, qui ai jamais été entrepris dans le bassin mediterranéen.

La coordination et la direction générales de MED POL - Phase I ont été assurées par le PNUE, faisant fonction de secrétariat du Plan d'action pour la Méditerranée (PAM). Les organismes spécialisés coopérants des Nations Unies (CEE - Commission économique pour l'Europe, ONUDI, FAO, UNESCO, OMS, OMM, AIEA, COI) étaient chargés de l'exécution technique et de la coordination quotidienne des travaux des centres de recherche nationaux participant aux projets pilotes.

MED POL - Phase II (1981 - 1990)

La réunion intergouvernementale des Etats riverains de la Méditerranée chargés d'évaluer l'état d'avancement du Plan d'action et première réunion des Parties contractantes à la Convention pour la protection de la mer Méditerranée contre la pollution et aux protocoles y relatifs (Genève, 5-10 février 1979), ayant examiné la situation de la Phase I du MED POL, a recommandé que, durant la période biennale 1979 - 80, soit formulé un programme à long terme de surveillance continue et de recherche en matière de pollution.

Sur la base des recommandations énoncées lors des diverses réunions d'experts et réunions intergouvernementales, un projet de programme à long terme (1981 - 1990) de surveillance continue et de recherche en matière de pollution (MED POL - Phase II) a été formulé par le secrétariat de la Convention de Barcelone (PNUE), en coopération avec les organismes des Nations Unies chargés de l'exécution technique de MED POL - Phase I, et il a été officiellement approuvé lors de la deuxième réunion des Parties contractantes à la Convention pour la protection de la mer Méditerranée contre la pollution et aux Protocoles y relatifs et réunion intergouvernementale des Etats riverains de la mer Méditerranée chargée d'évaluer l'état d'avancement du Plan d'action, qui s'est tenue à Cannes du 2 au 7 mars 1981.

L'objectif général à long terme de la Phase II du MED POL était de concourir à la réalisation des objectifs de la Convention de Barcelone en aidant les Parties contractantes à prévenir, réduire et combattre la pollution dans la zone de la mer Méditerranée ainsi qu'à protéger et améliorer le milieu marin dans cette zone. Les objectifs particuliers étaient de fournir constamment aux Parties contractantes à la Convention de Barcelone et aux Protocoles y relatifs:

- les renseignements dont elles avaient besoin pour appliquer la Convention et les protocoles;

- des indications et une évaluation de l'efficacité des mesures prises pour prévenir la pollution en application de la Convention et des protocoles;

- des renseignements scientifiques qui pourraient servir à réviser et modifier les dispositions pertinentes de la Convention et des protocoles et à rédiger des protocoles additionnels;

- des informations qui pourraient servir à formuler sur les plans national, bilatéral et multilatéral, les décisions de gestion, respectueuses de l'evnironnement, qui seraient indispensables à la poursuite du développement socio-économique de la région méditerranéenne;

- une évaluation périodique de l'état de pollution de la mer Méditerranée.

La surveillance continue des polluants affectant le milieu marin de la Méditerranée ainsi que la recherche menée à leur sujet répondent en premier lieu aux prescriptions immédiates et à long terme de la Convention de Barcelona et des protocoles y relatifs, mais elles tiennent également compte des facteurs requis pour la compréhension des relations existant entre le développement socio-économique de la région et la pollution de la mer Méditerranée.

A cette fin, la surveillance continue était organisée à plusieurs niveaux:

- surveillance continue des sources de pollution fournissant des renseignements sur la nature et la quantité des polluants directement libérés dans l'environnement;

- surveillance continue des zones situées à proximite du littoral, y compris les estuaires, et qui sont sous l'influence directe de polluants émis par des sources identifiables primaires (émissaires, rejets et sites côtiers d'immersion) ou secondaires (cours d'eau);

- surveillance continue des zones du large (zones de référence) fournissant des renseignements sur les tendances générales du niveau de pollution en Méditerranée;

- surveillance continue du transfert des polluants à la Méditerranée par voie atmosphérique, fournissant des renseignements supplémentaires sur la charge polluante qui atteint la Méditerranée.

Les sujets de recherche et d'étude inclus initialement dans MED POL - Phase II étaient les suivants:

- mise au point de techniques d'échantillonnage et d'analyse pour la surveillance des sources et des niveaux de pollution. Essai et harmonisation de ces méthodes à l'échelle méditerranéenne, et formulation de méthodes de référence. Substances figurant sur les listes de priorité des protocoles sur les opérations d'immersion et sur la pollution d'origine tellurique (activité A);

- mise au point de la présentation type des rapports à soumettre en application des protocoles relatifs à l'immersion, à la pollution résultant de situations critiques et à la pollution d'origine tellurique, (activité B);

- élaboration des fondements scientifiques des critères de qualité de l'environnement qui serviront à définir des normes d'émission, des normes d'usage ou des directives concernant les substances énumérées dans les annexes I et II du protocole relatif à la pollution d'origine tellurique, conformément aux articles 5, 6 et 7 de ce protocole (activité C);

- études épidémiologiques relatives à la confirmation (ou révision éventuelle) des critères de la qualité de l'environnement (normes d'usage) proposés pour les eaux servant à la baignade, à la culture de coquillages et à l'élevage d'autres organismes marins comestibles (activité D);

- mise au point de projets de directives et de critères régissant l'application du protocole relatif à la pollution d'origine tellurique, conformément à l'article 7 de ce protocole (activité E);

- recherches sur les processus océaniques, et particulièrement sur la circulation en surface et les déplacements verticaux. Cette information est nécessaire à la connaissance de la répartition des polluants en Méditerranée et à la mise au point de plans pour parer aux situations critiques (activité F);

- recherches sur la toxicité, la persistance, la bioaccumulation et le caractère cancérigène et mutagène de certaines substances énumérées dans les annexes du protocole relatif à la pollution d'origine tellurique et du protocole relatif aux opérations d'immersion (activité G);

- recherches sur l'eutrophisation et les floraisons de plancton qui l'accompagnent. Cette information est nécessaire pour évaluer la possibilité de prévenir les effets et les dégâts causés par ces floraisons périodiques (activité H);

- étude des modifications de l'écosystème dans les zones soumises à l'influence des polluants et dans celles où ces modifications sont dues à d'importantes activités industrielles sur la côte ou à l'intérieur des terres (activité I);

- effets des pollutions thermiques sur les écosystèmes marins et côtiers, y compris l'étude des effets connexes (activité J);

- cycle biogéochimique de certains polluants intéressant particulièrement la santé (mercure, plomb, survie des organismes pathogènes dans la mer Méditerranée, etc.) (activité K);

- étude des processus de transfert des polluants (i) aux points de contact entre les cours d'eau et la mer et entre l'air et la mer, (ii) par sédimentation et (iii) à travers les détroits qui relient la Méditerranée aux mers voisines (activité L).

Les Parties contractantes au cours de leur sixième réunion ordinaire (Athènes, octobre 1989) ont convenu de:

(a) réorienter les activités de recherche menées dans le cadre du MED POL en sorte qu'elles engendrent des informations qui soient également utiles pour l'application technique du Protocole tellurique, en plus de l'appui apporté aux activités de surveillance continue;

(b) à compter de 1990, remplacer les activités A à L par les cinq nouveaux domaines de recherche ci-après:

Domaine de recherche I - Caractérisation et dosage

Ce domaine englobera des projets de recherche en matière de caractérisation (identification de constituants chimiques ou microbiologiques) et de dosage (mise au point et éssai de méthodes) de contaminants donnés;

Domaine de recherche II - Transfert et dispersion

Ce domaine englobera des projets visant à approfondir notre connaissance des mécanismes physiques, chimiques et biologiques qui véhiculent les polluants potentiels de leurs sources à leurs dépôts ultimes. Les sujets étudiés porteront notamment sur le transfert et le dépôt atmosphériques, les mouvements et le brassage des eaux, le transfert des contaminants par sédimentation et leur incorporation dans les cycles biogéochimiques. Priorité sera accordée à l'obtention de données quantitatives servant, en dernier ressort, à la modélisation des systèmes et à l'établissement des évaluations régionales;

Domaine de recherche III - Effets

Ce domaine englobera des projets relatifs aux effets de certains contaminants énumérés aux annexes I et II du Protocole tellurique et du Protocole relatif aux situations critiques: effets sur les organismes, les communautés et les écosystèmes marins, effets chez l'homme et parmi les populations humaines. Priorité sera accordée aux effets et techniques fournissant des données utiles pour établir les critères de qualité du milieu;

Domaine de recherche IV - Destinées/transformation dans l'environnement

Ce domaine englobera des projets portant sur l'étude de la destinée des polluants (micro-organismes y compris), dans le milieu marin, et notamment sur la persistance et la survie, la dégradation, la transformation et la bio-accumulation, etc., mais non sur le transfert et la dispersion qui sont traités dans le domaine II;

Domaine de recherche V - Prévention et lutte antipollution

Ce domaine englobera des projets traitant de la détermination des facteurs conditionnant l'efficacité des méthodes d'épuration et d'élimination des déchets sous des conditions locales spécifiques ainsi que de l'établissement de critères de qualité du milieu et de mesures communes de réduction de la pollution;

(c) définir des contaminants cibles ou d'autres variables à des intervalles périodiques en fonction de l'état de l'avancement de l'application du Protocole tellurique;

(d) choisir les propositions de projet sur la base de leur valeur scientifique intrinsèque, leur spécificité méditerranéenne et, chaque fois que possible, encourager les projets bilatéraux et multilatéraux entre les pays méditerranéens du nord et du sud du bassin.

Comme lors de la Phase I du MED POL, la coordination et la direction générales de la Phase II étaient assurées par le PNUE, par l'intermédiaire du secrétariat du Plan d'action pour la Méditerranée (PAM). Les organismes spécialisés coopérants des Nations Unies (FAO, UNESCO, OMS, OMM, AIEA, COI) étaient chargés de l'exécution technique et de la coordination quotidienne des travaux des centres de recherche nationaux participant au programme de surveillance continue et de recherche.

Le présent volume comprend les rapports finaux sur les projets de recherche exécutés au titre des activités G et K traitant de la bioaccumulation et la toxicité des polluants chimiques. La préparation, l'édition et la compilation de ce volume ont été assurées par M. G.P. Gabrielides, FAO Fonctionnaire Principal des Pêches (Pollution Marine), et Mme V. Papapanagiotou, Secrétaire FAO était chargée de la dactylographie.

CONTENTS/TABLE DES MATIERES

DIFFERENT RESPONSES TO ENVIRONMENTAL MERCURY IN EDIBLE SPECIES OF THE NORTHERN TYRRHENIAN SEA

by

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A B S T R A C T

The subject of this paper is a three year investigation on the different Hg environmental impact and related responses in important edible species of the northern Tyrrhenian sea. Results are reported on: a) the Hg assessment in the studied species; b) its relation with organism length and marine sediment contamination; c) the possible causes of the different metal contents among the species, and in particular between two similar species taken as an example; d) detoxification mechanisms based on mercury binding proteins and on Se protective effects.

1. INTRODUCTION

It has long been known that the northern Tyrrhenian sea is affected by the geological anomaly of Mt. Amiata. The above results in the presence of Hg in the surface 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, in which the metal content was found to be very high (Barghigiani et al., 1986a, Barghigiani et al., 1986b).

The subject of attention in this study is a screening of the Hg contents in different-sized organisms of important edible species from the northern Tyrrhenian sea, the investigation of the possible causes of the high Hg concentration differences between different species of flatfish, and research on the most probable detoxication mechanisms: Hg binding proteins and Hg-Se relation.

The preliminary results on flatfish seemed to indicate a much lower content of mercury in S. vulgaris than in other species. Research was thus performed on sole and L. boscii, another commercial flat fish, to understand the causes of this difference. The feeding behavior and some important biological aspects were considered.

Generally, high tissue levels of heavy metals are associated with specific sequestering proteins the function of which is still the subject of investigation. A low molecular weight protein "metallothionein", rich in cysteine and capable of binding heavy metals

such as Cd, Zn, Cu and Hg, has been isolated from fish (Marafante, 1972; Olafson and Thompson, 1974; Noel-Lambot et al., 1978; Overnell and Coombs, 1979). Therefore, considering the potential toxic effects of Hg on marine biota and consequently on human health, C. linguatula and L. boscii were chosen in order to investigate the subcellular distribution and the possible presence of low molecular weight Hg binding proteins such as metallothionein.

Furthermore, within the investigation on the detoxication mechanisms the Hg-Se relation was studied in the most important edible species. Indeed, it must be pointed out that selenium, in addition to its role as an essential micronutrient for normal growth and reproduction, exerts an antagonistic effect in mercury poisoning (Spallholz et al., 1981) and divergent results are reported on the relation between the two elements in the literature. Among these results an almost perfectly linear Hg-Se correlation, with a 1:1 molar ratio, was observed in marine mammals and a linear correlation and a 1:16 ratio were found in marine fish (Koeman et al., 1975), while in swordfish a non-linear correlation was found (Freeman et al., 1978) and in freshwater fish no correlation was found (Froslie et al., 1985). Leonzio et al. (1982) also reported a correlation between mercury and selenium in fish but other authors (Cappon and Smith, 1981; Cappon and Smith, 1982) found no correlation between the two elements either in marine or freshwater fish.

2. MATERIALS AND METHODS

2.1 Collection and choice of species

The sampling was carried out off the Tuscany coast, from the promontory of Argentario to the gulf of Follonica (Fig. 1), in two annual trawl surveys for three consecutive years (spring and summer 1985, 1986 and 1987).

In each survey 30 hauls were made lasting one hour each and distributed in the area according to a randomized stratified design (Grosslein and Laurence, 1982).

The species chosen were the ones considered most important both commercially and from the standpoint of their abundance in the study area. The following species were selected: Merluccius merluccius, Eledone cirrhosa, Trisopterus minutus capelanus, Nephrops norvegicus.

Three species of flatfish were also chosen: Solea vulgaris, Citharus linguatula and Lepidorhombus boscii.

During the trawling, samples of sediments were also collected.

2.2 Hg and Se analyses

Samples were analyzed for Hg after digestion with concentrated HNO_3 in a pressurized decomposition system at 120°C for 6 hours. The measurements were performed by flameless atomic absorption spectrometry with a Coleman MAS 50 Perkin Elmer. The organic mercury was determined using the method of Capelli et al. (1979).

Selenium was determined by hydride generation atomic fluorescence spectrometry using hydrobromic acid-based aqueous matrices according to the method of D'Ulivo (1989). The dry weight was evaluated 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: 0.789±0.074 μg g^{-1} Hg; 1.62±0.12 μg g^{-1} Se) and DOLT-1 (dogfish liver: 0.225±0.03 μg g^{-1} Hg; 7.34±0.42 μg g^{-1} Se) of the National Research Council of Canada.

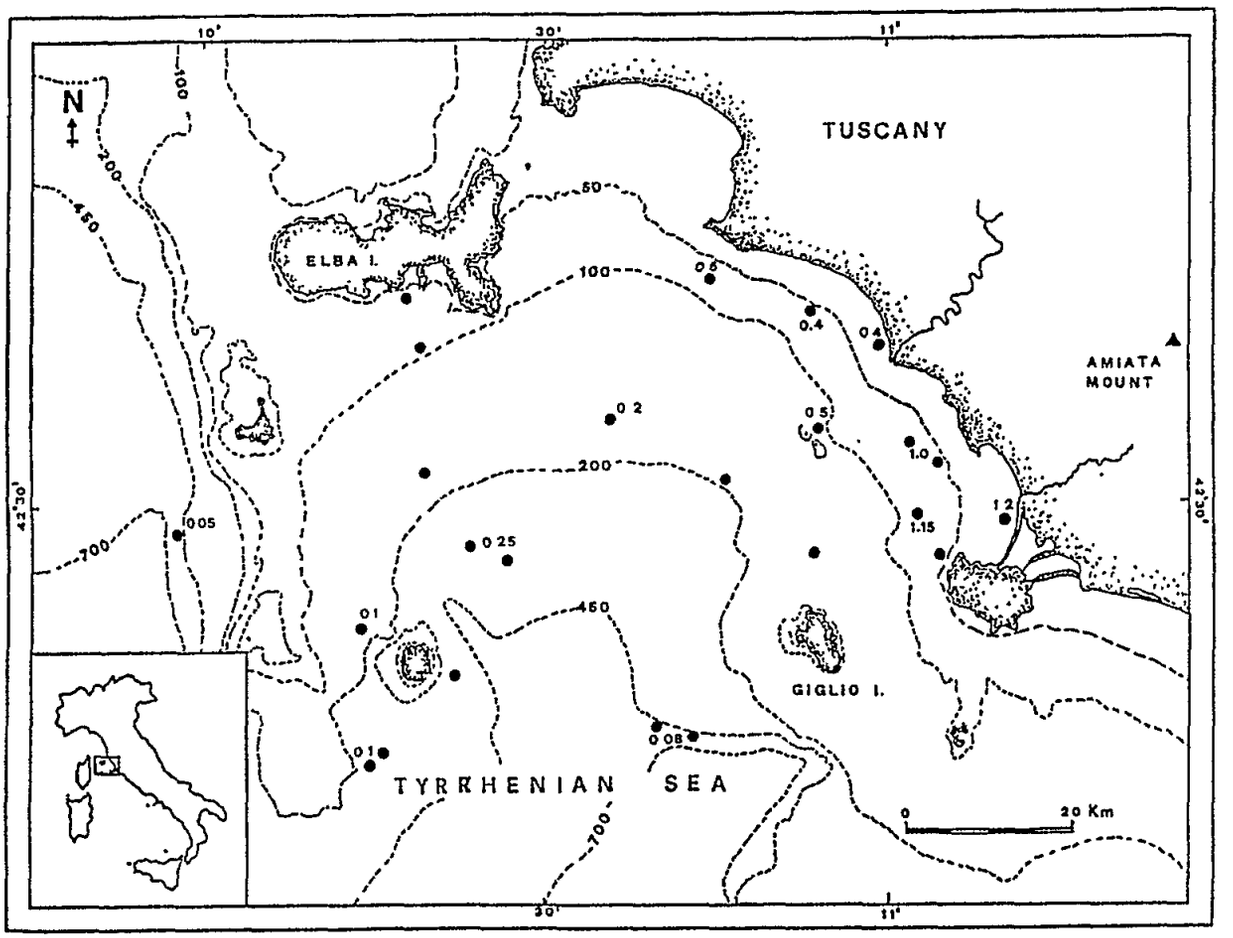

Fig. 1 Study area and Hg concentration in sediments (μg g^{-1} d.w.)

2.3 Determination of age and stomach content of S. vulgaris and L. boscii

The age of the fish was determined on the basis of the relation between length and age in S. vulgaris and L. boscii collected in Italian coastal areas, reported in the literature (Bello and Rizzi, 1987; Ramos, 1982).

Taxonomic and mercury determinations were performed on the stomach contents of all the studied specimens. The number of specimens

analyzed was 44 for L. boscii and 35 for S. vulgaris.

2.3.1 Subcellular fractionation and isolation of Hg-binding proteins in C. linguatula and L. boscii

Small amounts of tissue (2-3g) were homogenized with an Ultraturrax homogenizer in Tris-HCl buffer, 20 mM, pH 8.6, 1:1 (w/v). The homogenate was centrifuged at 40,000 x g for 20 min and the clear supernatant was recentrifuged for an additional 40 min spun again at the same gravitational force.

Separate 0.5 ml aliquots of liver and muscle supernatant were applied to Sephadex G-75 columns (1x60cm), and eluted with Tris-HCl buffer, 20 mM, pH 8.6 at a flow rate of 20 $ml.hr^{-1}$. The column had been previously calibrated with Cd-thionein (cd-Mt) (purified from goldfish liver (Carpené and Vasak, 1989). Fractions were collected and analyzed for their absorbance at 254 nm corresponding to the aromatic aminoacids. Hg, Zn, and Cu were also determined in order to test for the possible presence of Mt-like proteins. Aliquots of the pellet and supernatant were held for Hg analyses.

2.3.2 Heavy metal determinations in pellet, supernatant and eluate in C. linguatula and L. boscii

Mercury was measured in pellet, supernatant and Sephadex G-75 eluate. The analyses were performed by flameless Atomic Absorption Spectrophotometry (AAS) with a Perkin Elmer Coleman MAS 50 mercury analyzer on the samples previously digested as described before.

Zn and Cu were determined in the gel filtration eluate by flame AAS, aspirating the samples directly, with an Instrumentation Laboratory spectrophotometer Mod. 11.

3. RESULTS AND DISCUSSION

From Fig. 2 it can be seen that in all the studied organisms, with the exception of E. cirrhosa, the muscle Hg concentrations increase with the increase in length.

What was observed for E. cirrhosa is not attributable to the fact that samples were collected in different hauls, but is probably due to this organism's life cycle of less than two years, and hence the difference of the exposure times between the specimens of small size and those of larger size is limited. It should also be emphasized, with regard to E. cirrhosa, that even the smaller specimens display mercury concentrations that are above the value of 0.7 $\mu g\ g^{-1}$ fresh weight, the limit indicated by EC standards as the maximum for edible parts of comestible marine organisms (EEC, 1984).

The highest total Hg contents were observed in M. merluccius. However, in all the analyzed species the mercury contents of larger-sized specimens exceed the limit, except for sole . In this organism the metal content increases with length as in the two other species of

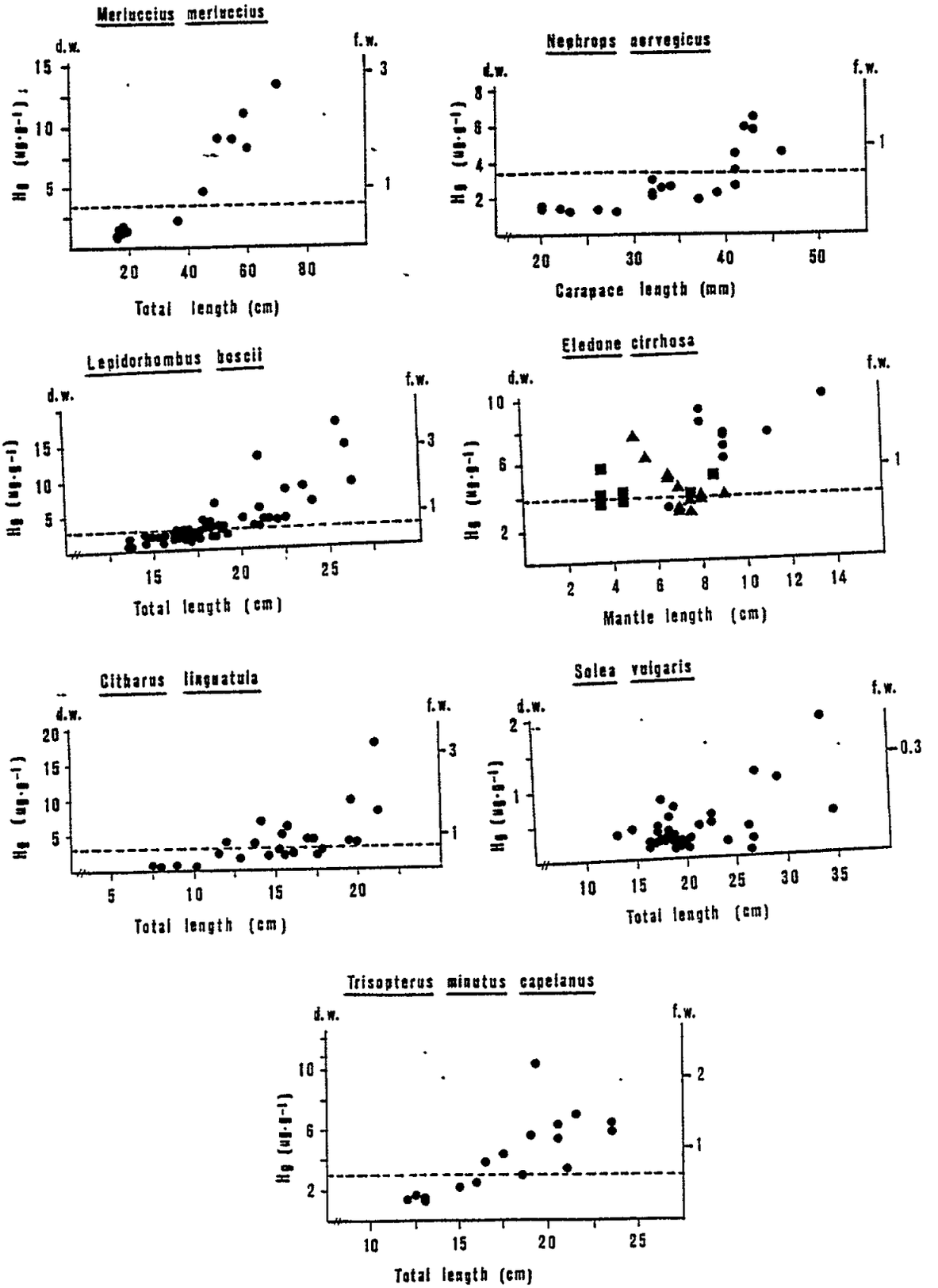

Fig 2 Hg concentrations versus organism length. The horizontal broken line shows the limit of 0.7 µg g^{-1} f.w. indicated for edible parts of fish by the EC standards. For E. cirrhosa (●) from station at the center of the study area, (▲) from station near Monte Cristo, (■) from station near the coast.

flatfish considered here, but while in C. linguatula and in L. boscii the Hg levels are high, especially in the specimens of larger size, in sole they do not exceed 2 μg g^{-1} d.w . It must be pointed out that in this area S. vulgaris lives in shallower waters (0-80 m) than C. linguatula (0-260 m) and L. boscii (60-540 m), and the total Hg concentration of sediments decreases with the distance from the shore, that is, it decreases with increasing depth (Baldi and Bargagli, 1982).

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

3.1 Stomach contents and Hg concentration in L. boscii and S. vulgaris

It has been shown that the Hg increase with length is significantly higher in L. boscii than in S. vulgaris. Furthermore, the differences in concentration between organisms of the same age but of different species were still evident and thus could not be attributed to the higher growth rate of sole with respect to megrim.

The data on the stomach contents of L. boscii and S. vulgaris (Table I) are in agreement with other literature data on the feeding behavior of the two considered species (Whitehead et al., 1986; De Groot, 1971; Braber and De Groot, 1973).

In L. boscii crustaceans represent the highest percentage of ingested organisms, but the highest amount of mercury is taken up from fish. The mercury intake from cephalopods is low because they are present in small amounts in L. boscii's diet and sepiolides are less contaminated by the metal. Indeed, their life cycle, i.e. exposure to the environment, is about 6 to 9 months (Boletzky, 1975).

In S. vulgaris polychaetes and crustaceans are present almost in the same amount, but mercury uptake is mainly due to the former organisms. Amount and mercury content of the molluscs are not reported in the table because only shells were found in the stomach.

The average mercury concentration of stomach contents was higher in L. boscii (2.2 μg g^{-1} d.w.) than in S. vulgaris (1.0 μg g^{-1} d.w. in the stomach plus 0.617 μg g^{-1} d.w. in the remaining part of the alimentary tract). The average mercury content found in the full alimentary tract (stomach plus intestine content) was 0.191 μg for megrim (n=40) and 0.106 for sole (n=21). If the average mercury is considered for all the collected organisms, this was 0.174 μg for megrim (44 specimens) and 0.063 μg for sole (35 specimens).

From these data it can be concluded that the feeding behaviour seems to be a determining cause of the different mercury concentration found in muscle tissue of the two species, even if other factors such as a different physiology could also play an important role.

Table I
Stomach content and total ingested Hg.

	Class	Group	No. of Specimens	Total d.w. (g)	Hg ($\mu g\ g^{-1}$ d.w.)	Hg µg
L. boscii	Polychaeta					
	Cephalopoda	Sepiolidae	2	0.450	0.880	0.396
	Crustacea	Euphasiacea	235	1.256	1.024	0.510
		Isopoda	26	0.330	2.029	0.770
		Decapoda (a)	3	0.224	2.680	0.610
		Mix (b)	7	0.160	6.503	1.050
			tot.217	1.970		
	Osteichthyes	Gadidae & Cupleiformes	7	1.030	3.602	3.710
S. vulgaris	Polychaeta	Mix	26	0.417	1.485	0.617
	Bivalvia & Gasteropoda	Mix	7	-	-	-
	Crustacea	Amphipoda	108	0.376	0.465	0.175
		Mix	20	-	-	-
	Osteichthyes	-	-	-	-	-

3.3 Hg subcellular distribution and binding proteins in C. linguatula and L. boscii

As regards the concentrations and distribution of Hg between pellets and supernatant in muscle of C. linguatula and L. boscii, more than 90% of the metal was found in pellets, whereas in liver a high Hg concentration was also found in the supernatant.

Figure 3 shows the Sephadex G-75 chromatogram pattern of muscle supernatant obtained from C. linguatula and L. boscii; most of the Hg is bound to high molecular weight ligands (HWL). At the elution volumes corresponding to metallothionein (Mt) only traces of Hg were found; Zn and Cu contents fell below the detection limits of the instrument (Zn: 1.2 ng ml^{-1}).

Unlike with muscle, the gel filtration of liver samples of L. boscii revealed the presence of a consistent amount of Hg bound to low molecular weight ligands (LWL) having the same retention time as goldfish liver Cd-Mt; with Hg, different amount of Cu and Zn were found (Fig. 4).

The higher percentage of Hg in the pellets than in the soluble fraction seems to be a general fate independent of the animal species and the operating conditions (natural or laboratory) (Koeman et al., 1973; Wageman and Hobden, 1986). This is not surprising because Hg is mostly present as methyl-Hg, which is lipophilic and has a low affinity for Mt as well (Chen et al., 1973). By contrast, the inorganic Hg has one of the stronger affinities, among heavy metals for thionein (Kagi and Koijma, 1987) and can induce Hg-Mt in fish (Bouquegneau, 1979;

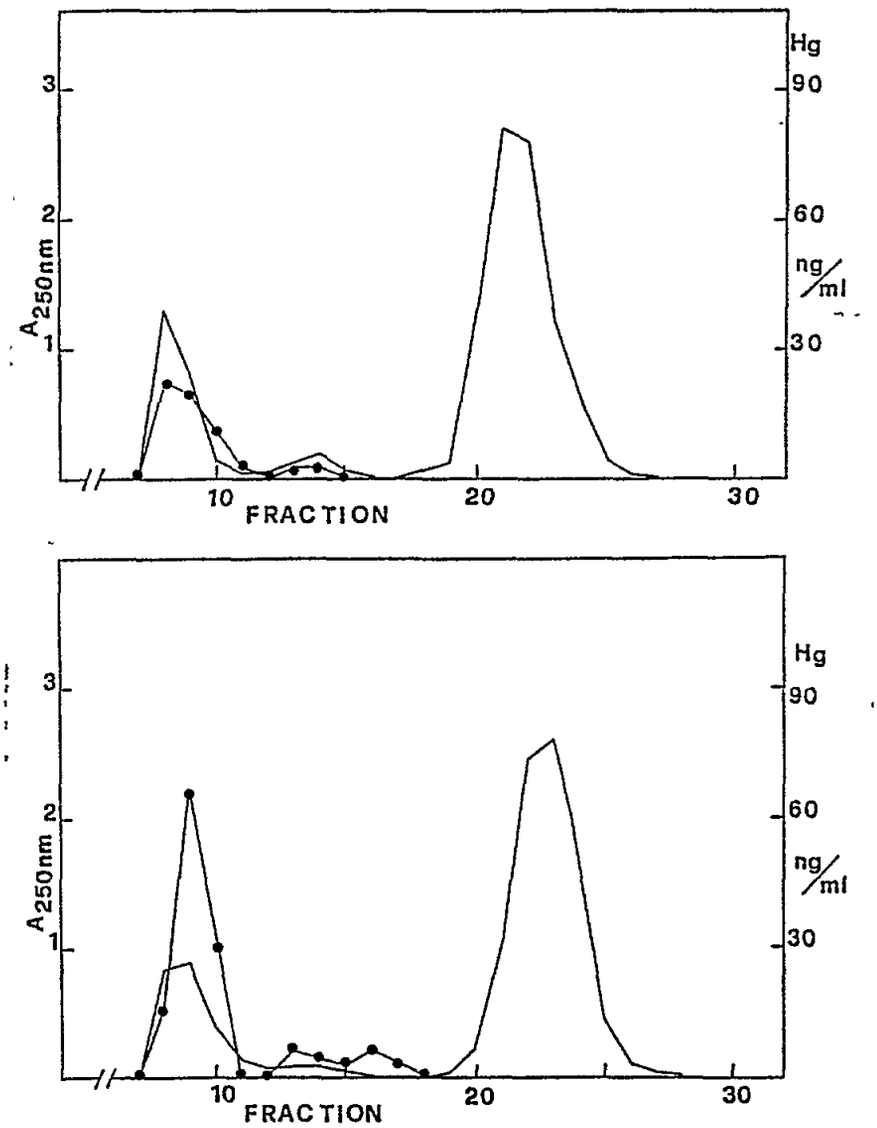

Fig. 3 Sephadex G.75 chromatography of muscle cytosol from C. linguatula (A) and L. boscii (B). A_{254} ---- ; Hg •----•

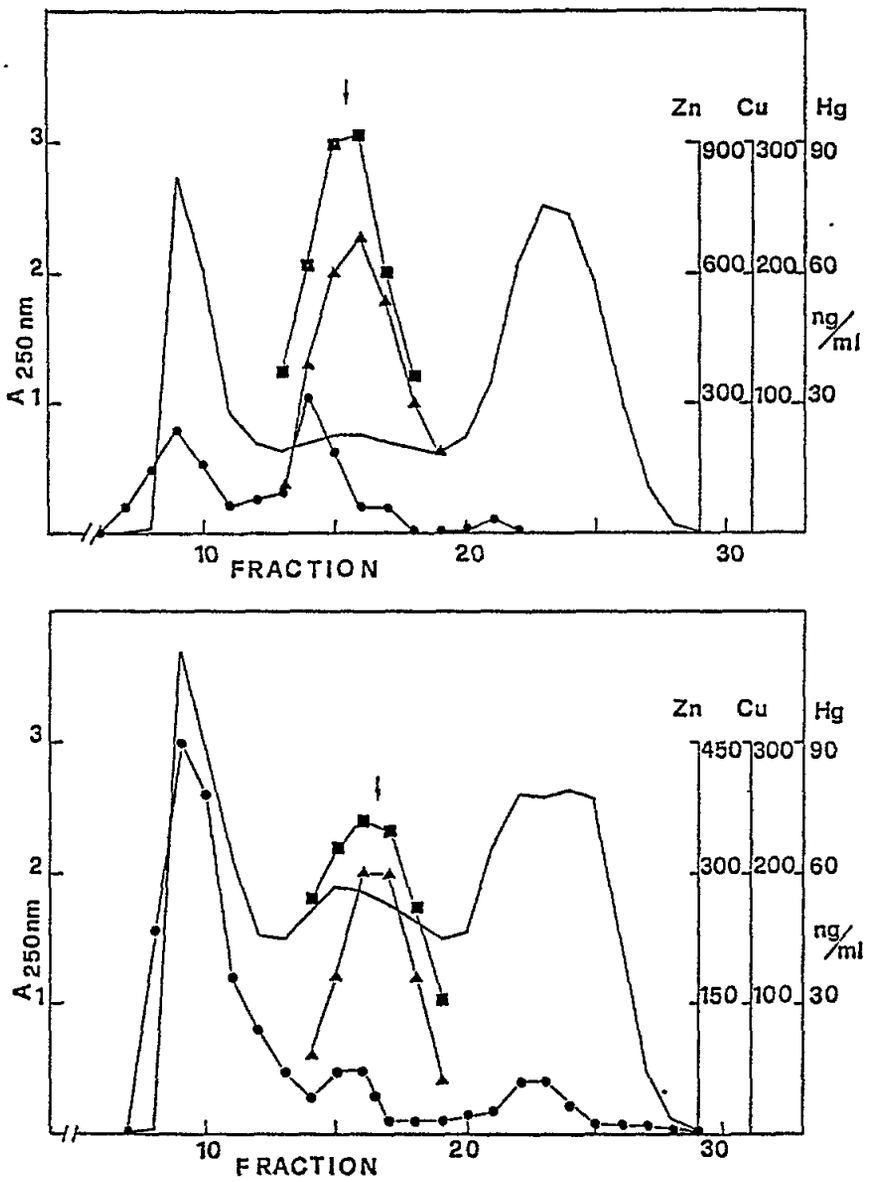

Fig. 4 Sephadex G.75 chromatography of liver cytosol from two specimens of L. boscii. A_{254} --- ; Hg ●----● ; Zn ■----■ ; Cu ▲----▲

Marafante, 1976) but it is inferior to Cd as a Mt inducer (Piotrowski et al., 1974).

However, binding of Hg^{2+} to LWL in the liver of L. boscii seems of little significance as a protective mechanism since only a small amount (5%) of the hepatic metal is bound to the LWL. In muscle of both species Hg is almost exclusively bound to the HWL; it could be bound in the methylated form, as it was found in sea lion (Lee et al., 1977).

3.3 Hg-Se relationship

As regards Se, from the data reported in Figure 5 no evident relation appears between this element and Hg in any of the studied species, independently of their feeding behavior, ecological features and phylogenetic level. This is in contrast with some previous literature data on Se contents in marine organisms, which however are in contrast with other literature data on this element, as was reported in the introduction. Stress must be laid on the fact that the hydride generation method allows accurate determination of Se at the nanogram level but may suffer form several interference problems (D'Ulivo, 1989) that might explain the different results reported in the literature and which have been removed by the method used here.

The only evident difference between the data reported in Figure 5 seems to be represented by the Se concentration range, which in Eledone cirrhosa is 2 to 11, and 1.2 to 5 ppm in the other species. Furthermore, in this cephalopod two different trends of SE with respect to Hg levels can be observed, depending on the different stations and probably due to geochemical and environmental factors and to different life cycle phases as well.

However, these data are only preliminary and need to be supported by more information.

4. ACKNOWLEDGMENTS

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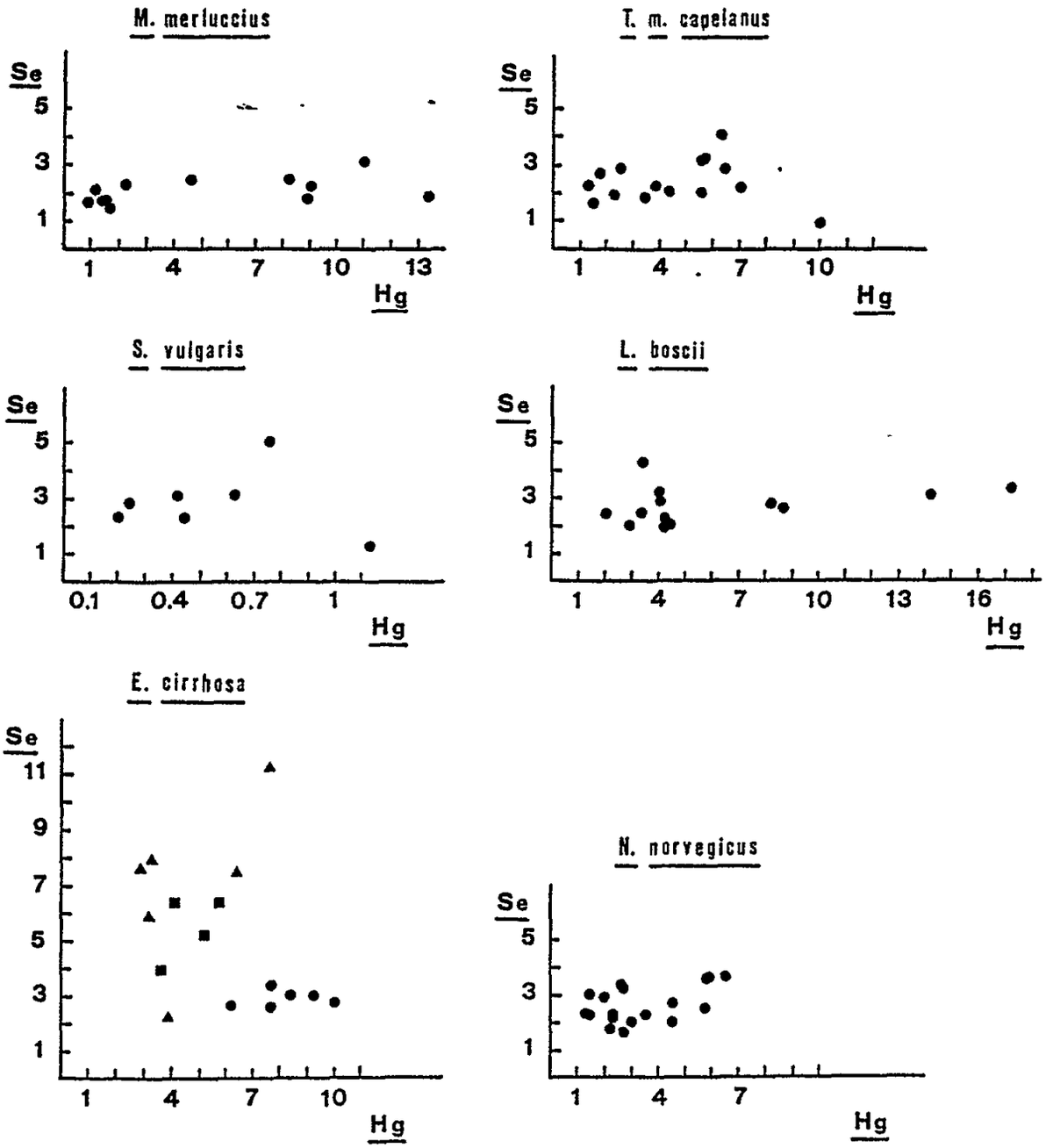

Fig. 5 Relation between Hg and Se concentrations expressed in µg g^{-1} d.w. For E. cirrhosa: (•) station at the center of the area, (▲) near Monte Cristo, (■) near the coast

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CADMIUM IN WATER, SEDIMENTS AND BENTHIC ORGANISMS FROM A STRETCH OF COAST FACING THE THERMOELECTRIC POWER PLANT AT TORVALDALIGA (CIVITAVECCHIA, ROME).

by

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A B S T R A C T

With a view to demonstrating the eventual synergic effects between temperature and bioaccumulation of cadmium, a series of analyses on organism gathered along a stretch of coast in front of the thermoelectric power plant at Torvaldaliga (Civitavecchia, Rome) was carried out at depths of 0.30m and 4m between October 21, 1987 and January 13, 1990. The organisms chosen were Ulva rigida C. Agardh. Corallina elongata, Ellis and Solander, Posidonia oceanica (L) Delile; Patella caerulea (L) and Paracentrotus lividus (LmK). In addition, samples of water and sediment from the six chosen sites were analysed.

Atomic absorption spectrophotometrical analysis showed that the area examined did not register particular episodes of cadmium pollution although the values revealed in the organisms indicate the existence of differentiated influence caused by the thermal increase in the bioaccumulation of cadmium by part of the organism examined, with effects which were, on the average, positive in U. rigida and negative in P. caerulea.

There is no evidence of significant influence in the other species, although a SE/NW gradient becomes evident and increases from the southern stations towards those of the north. It is similarly evident in this phenomenon the importance of other factors, such as age, dimension of the organisms and, above all, the local hydrodynamic conditions.

1. INTRODUCTION

Different from the effects of mercury, the effects of a rise in temperature in the bioaccumulation phenomena and in the release of cadmium in the marine environment have not yet been entirely clarified.

The bioaccumulation of heavy metals on the part of marine organisms can occur by different physiological processes; among these the most significant generally are the ingestion of food and the direct absorption by contact of the area with the external liquid medium, as with the respiratory surfaces (Boyden and Romeril, 1974; Phillips, 1977).

The scope of the present research has been to verify whether thermal variations, which generally exert a notable influence on many physiological processes, are capable of modifying the bioaccumulation of cadmium in some coastal benthic organisms. The occasion for this study was offered by the activation on the Latium coast, just north of Civitavecchia, in the Torvaldaliga area where a new thermoelectric power plant (TVN) is operating.

The Torvaldaliga area has been the object of several research studies, since 1965, advanced by Prof. Taramelli and colleagues of the Department of Animal and Human Biology of the University of Rome. (Taramelli and Herzel, 1969; Taramelli et al., 1981; Chimenz et al., 1985); these studies aimed at the characterization of the coastal benthic community sited in the marine depth close to the thermoelectric power plant. It was thus interesting to evaluate the effects revealed at various levels of the marine environment by the current situation.

The action exerted by the thermal increase on the bioaccumulation of cadmium is only partially known (Cairns et al., 1975). As in the case of mercury, there appears an unfavorable effect on the bioaccumulation in the organisms, caused by the higher temperatures (Vernberg and O'Hara 1972). From the sparse data available on the subject - obtained from laboratory experiments - appears that there is a greater accumulation of cadmium as a result of higher temperatures. From this point of view it differs from the behaviour of other heavy metals in which these effects cannot be attributed solely to an acceleration of the metabolism of the organisms, or to their particular physiological processes - such as the rhythm of the branchial ventilation or the intake of food due to increased thermal levels - but may be linked to the characteristics of the specific means of intake of the cell's element.

Phillips (1976), recognized this peculiar behaviour of cadmium in experiments on Mytilus edulis at various temperatures and levels or salinity, and Jackim et al. in 1977 reached the same conclusions while studying the rhythm of the bioaccumulation of metal at 10°C and 20°C on the part of Mytilus edulis, Mya arenaria and Mulinia lateralis. Eisler (1971), encountered a not dissimilar presence of the phenomenon in the fish Fundulus heteroclitus.

Our research, because of the thermal waste-water discharged at Torvaldaliga, encouraged us to verify the existence of this synergism in natural conditions.

2. MATERIALS AND METHODS

2.1 Locality

The location chosen for our samplings comprises the Latium coast NW of Civitavecchia, precisely at 42°05'23''N 11°47'11''E (Fig. 1). This area, named Torvaldaliga, is characterized today by the presence of two large thermoelectric power plants, of which the more recent came into operation in 1986. The power plant known as Torvaldaliga south is formed by four groups capable of a total output of 1174MW, while the

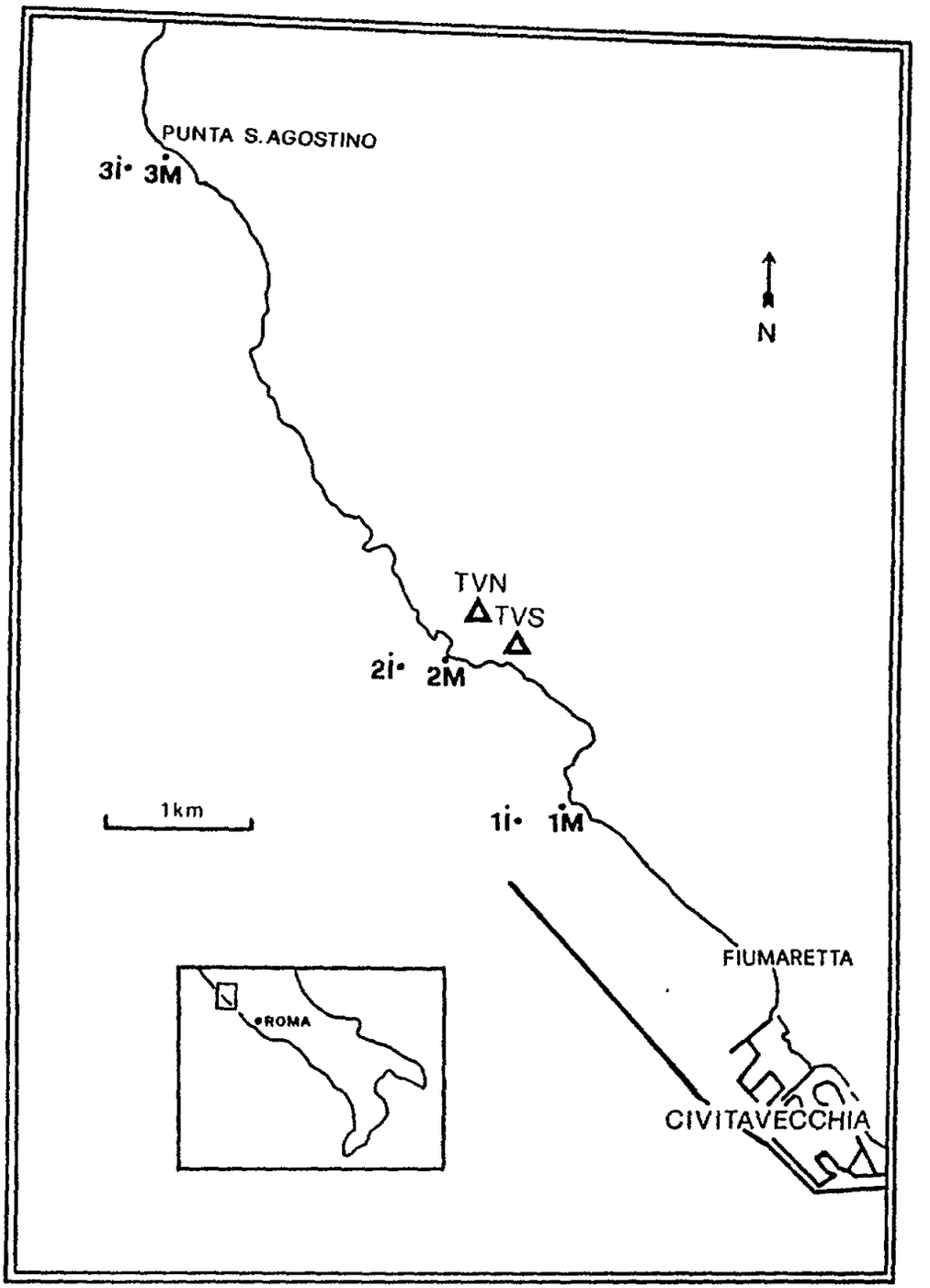

Fig. 1 Locality

power plant at Torvaldaliga north consists of four groups, each with a capability of 660MW.

Both power plants use sea water for cooling the condensers, this water being supplied by appropriate intake suction pipes. The water, after circulating is discharged into the sea by way of two thermal waste-water outflows. The outflow at Torvaldaliga north is about 320,000 m^3 of hot water per hour when at maximum capacity, which, according to data furnished by ENEL, should amount to 500,000 m^3 daily. This water, which enters the sea at 1.5 - 2m/s at all seasons presents a δT of about 7°C than that of the marine waters of the zone.

The dominant substratum along this stretch of coast is rocky but has a sub-horizontal trend. For this reason the coast is never excessively steep. The current that lapse the coast has a tendency from SW towards NW and is inclined to deviate northwards the flow of water arriving in the sea from the thermal discharges.

Six stations were chosen along this coast for sampling. Of these, the first three, indicated by the letter M, were situated at a depth of 0.3m at the extremity of the mesolitoral; the other stations, indicated by the letter I, were installed in the infralitoral along the depth contour of 4m, in correspondence with the three first mentioned.

Stations 1M and 1I were located near Punta Mattonara, stations 2M an 2I in front of Torvaldaliga north and Torvaldaliga south, while 3M and 3I were located on the coastal segment facing Punta S. Agostino. These stations placed along a 10 Km stretch of coast, present varied environmental conditions an in particular 1 and 3 (M and I), further distant from the power plants, functioned as control stations whereas stations 2 (M and I) were those most directly concerned with the effect of the thermal wastewater discharges.

2.2 Methodology

The species chosen for our research were the algae Ulva rigida, C.Agardh and Corallina elongata, Ellis and Solander, the phanerogam Posidonia oceanica (L) Delile, the mollusc Patella caerulea (LmK) and the echinoderm Paracentrotus lividus (LmK). All species belong to the benthic population and fairly plantyful in the area examined. The algae and the animal organisms, together with the samples of sediment and water, were collocated by hand at the six chosen stations in October and November 1987 and in February and June 1989 (Table I and Ibis); P. oceanica was collected in March, June and October 1989 and in January 1990 at stations 1I, 2I and 3I.

For an examination of the eventual special variability of the cadmium content, five samples of P. oceanica, distant 1-3m from one another and more or less connected at the centre and at the extremities of a rectangle 1-2m per side, were collocated at each station. At the lower coastline, the samples were collocated using scuba diving equipment (ARA). During each investigation water and air temperatures were registered, together with meteo- marine conditions and morphological aspects of the substratum and the vegetable growth. The

Table I

Sampling in stations 1M, 2M, and 3M.

	1st sampling 21/10/1987			2nd sampling 2/2/1989			3rd sampling 15/6/1989		
	St. 1M	St. 2M	St. 3M	St. 1M	St. 2M	St. 3M	St. 1M	St. 2M	St. 3M
water	+	+	+	+	+	+	+	+	+
sediments	+	+	+	+	+	+	+	+	+
ORGANISMS:									
Ulva rigida	abs.	abs.	+	+	+	+	+	+	+
Patella caerulea	+	+	+	+	+	+	+	+	+
Paracentrotus lividus	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.

Table I-bis

Sampling in stations 1I, 2I, and 3I.

DATE: 7/11/1987	St. 1I	St. 2I	St. 3I
water	+	+	+
sediments	+	+	+
ORGANISMS			
Corallina elongata	abs.	+	+
Paracentrotus lividus	+	+	+

samples were placed in plastic containers of 1 litre each and were preserved by freezing at -25°C. The preparation was performed in compliance with instruction from F.A.O. (Bernhard, 1976).

The selected metal was cadmium; the cadmium value in the organisms on the soft parts only whereas the analyses of sediment involved five phases to determine:

a) the easily converted cadmium;
b) carbonate linked cadmium;
c) cadmium linked to Fe and Mn oxides;

d) cadmium linked to the organic substance;
e) total cadmium; (Table II)

Before mineralization, the algae and invertebrates underwent different treatments. The algae were treated with a solution of CH_3COONa, 1M at pH5 to remove deposits of $CaCO_3$, particularly conspicuous in Corallina elongata. As regards Patella and Paracentrotus it was sufficient, after thawing, to separate the soft parts of the animals from the shells and dermoskeletons. Only the gonads of Paracentrotus lividus were analysed; this was in order to avoid contamination of the samples by the intestinal contents. The soft parts of Patella caerulea were homogenized before being weighed and mineralized.

The real mineralization followed a similar process. After determination of the dry weight (DW) about 4 or 5gr of the homogenized samples were placed on a beute on a thermostat plate and heated to a temperature of 90-100°C with the addition of 20ml of redistilled water. The mineralization then proceeded with the addiction of a nitric-perchloric-sulfuric mixture (25+25+1). Subsequently HNO_3 was added until a perfectly clear solution was obtained. The saline residue was then reduced with 10ml of redistilled H_2O and 1ml of HCl at 100°C.

The determination of the cadmium was effected on samples of solution raised to a volume of 50ml using an AAS Perkin Elmer 5100 Zeeman supplied by a graphite furnace and equipped with an A60 autosampler. With a view to reducing the possibility of error and thus obtaining greater accuracy,we prepared some standards by the addition method.

Regarding P. oceanica, the following procedure was effected. The leafed material was sorted in laboratory and eventually scraped with a plastic blade to remove the epibionts, discarding the less coloured basal parts and those parts with massive presence of epibionts. The samples were then stove dried at 105°C to a constant weight and then ground in an electric agate mortar.

Digestion was achieved as follows: 0.5 of dry material with 10ml of HNO_3 was placed in a teflon container and heated to 100°C in three hours; digestion was completed in the same teflon containers which subsequently opened were treated with 3ml of H_2O_2 at 35% on a hot thermoplate at 85°C for an hour. Finally the solution was transferred into flasks of 50ml, brought up to volume with deionized water (Brix and Lyngby, 1983; Brix et al., 1983a; Brix et al., 1983b; Krishnamurty et al., 1976).

The dosage of metal was calculated by standard addition method with AAS furnished by graphite furnace (Perkin Elmer 5000 Zeeman HGA500 with automatic sampler).

The analytic data of the 60 samples analysed (5 for each of the three stations, repeated 4 times in the annual cycle), are reported in Table II. The cadmium content is expressed in µg dry weight (DW). The animal and vegetable organisms were collected by the Zoological Institute of Rome University; the analysis of the cadmium content of

Table II
Cd content in sediments.

SEDIMENTS ($\mu g\ g^{-1}$ dw)	Station 1M 21/10/1987	Station 1I 7/11/1987	Station 2M 21/10/1987	Station 2I 7/11/1987	Station 3M 21/10/1987	Station 3I 7/11/1987
FRACTION 1 Cd exchangable	0.0010 ± 1	0.006 ± 0.5	0.015 ± 1	0.004 ± 0.5	0.011 ± 1.5	0.090 ± 5.7
FRACTION 2 Cd bounded to carbonates	0.033 ± 5.6	< D.L.	0.007 ± 0.0011	< D.L.	< D.L.	< D.L.
FRACTION 3 Cd bounded to Fe & Mn oxides	0.052 ± 0.006	0.015 ± 0.004	0.042 ± 0.004	0.025 ± 0.0011	0.038 ± 0.0017	0.021 ± 0.0045
FRACTION 4 Cd bounded to organic matter	0.014 ± 0.0032	0.005 ± 0.0012	0.008 ± 0.002	0.005 ± 0.0015	0.013 ± 0.0011	0.007 ± 0.0010
TOTAL CADMIUM	0.230 ± 0.014	0.072 ± 0.006	0.150 ± 0.012	0.146 ± 0.011	0.154 ± 0.011	0.166 ± 0.024
WATER	< D.L.	< D.L.	< D.L.	< D.L.	< D.L.	< D.L.

the samples of algae, animals, H_2O and sediment was effected by the section "small quantities of elements", laboratory of applied toxicology of the I.S.S. and the analysis of P. oceanica was carried out in the central laboratory of hydrobiology, Rome.

3. RESULTS

The results obtained are summarized in Tables II to VIII.

Ulva rigida: the results evidence a series of accumulation values indicative of an entity somewhat varied temporally (Tables III, IV, V; Fig. 2).

The sampling taken in February 1989 revealed a very high cadmium content at station 2M (273±23 $\mu g\ g^{-1}$) fresh weight (FW), reduced at station 1M (35±2 $\mu g\ g^{-1}$) and of intermediate level at station 3M (122±11 $\mu g\ g^{-1}$). Alternatively, the data obtained in June 1989 revealed a very different trend, with average values of 6.2 $\mu g\ g^{-1}$ FW for the station at Punta Mattonara (1M), 7.6 $\mu g\ g^{-1}$ FW at the Torvaldaliga station (2M) and 21.7 $\mu g\ g^{-1}$ at the Punta S. Agostino station.

Corallina elongata: this seaweed was collected in October at the surface stations and in November at stations 2I and 3L of the infralitoral. The average cadmium values are fairly homogenous in the October samplings, with a maximum of 51±3 $\mu g\ g^{-1}$ FW at the punta S. Agostino station; on the contrary, the maximum for November is registered at the infralitoral station 2I with a value of 75±7 $\mu g\ g^{-1}$ FW (Tables III, IV; Fig. 3).

Posidonia oceanica: a preliminary examination of the average cadmium content, reveals constantly inferior values at station I, facing Punta Mattonara (Table VI). The analysis of variants between stations (Table VII) indicates significant differences ($P <= 1\%$) in June 1989 and January 1990. In the first instance the cadmium content was greater in the Posidonia samples collected at station 2 (3.47 DW) with respect to those of stations 1 and 3 (2.22 and 3.31 DW respectively).

In the second case it were the samples of station 3 that revealed the maximum cadmium content, 3.87 DW with respect to stations 1 and 2, where cadmium was present in value of 3.07 and 3.34 respectively. The average seasonal contents varied between 2.19 $\mu g\ g^{-1}$ DW (October 1989) and 3.43 $\mu g\ g^{-1}$ DW (January 1990). The average of the 60 data on which the present research is based was 2.81 DW (Table VI; Fig. 4).

4. DISCUSSION

Examining the results obtained, it may be concluded that there is no significant environmental pollution by cadmium in the interested zone since in the majority of cases the values registered did not differ greatly from those registered in Mediterranean areas not subject to pollution, as regards water and sediments. Therefore, the proximity

Table III

Cd contents in organisms.

ORGANISMS	PERIOD	STATIONS	LENGTH (mm)	Cd µg g^{-1} Mean ± SD	No of samp-lings	t°C Water
U. rigida	Oct. '87	St. 3M	30- 70	27 ± 2		20 °C
	Feb. '89	St. 1M	30- 70	25 ± 3		14 °C
	Feb. '89	St. 2M	30- 40	273 ± 23		21 °C
	Feb. '89	St. 3M	30-120	121 ± 11		13 °C
	June '89	St. 1M	30-120	6 ± 1		25 °C
	June '89	St. 2M	30- 60	8 ± 1		27 °C
	June '89	St. 3M	30-150	22 ± 2		27,5°C
C.elongata	Oct. '87	St. 1M	20-30	35 ± 3		20 °C
	Oct. '87	St. 2M	20-30	38 ± 3		22 °C
	Oct. '87	St. 3M	15-30	51 ± 4		20 °C
	Nov. '87	St. 2I	30-45	75 ± 7		21 °C
	Nov. '87	St. 3I	30-45	35 ± 3		20 °C
P.caerulea	Oct. '87	St. 1M	25.6	909 ± 40	40	20 °C
	Oct. '87	St. 2M	24.6	419 ± 40	40	22 °C
	Oct. '87	St. 3M	34.4	1140 ± 47	40	20 °C
	Feb. '89	St. 1M	24.3	481 ± 26	40	14 °C
	Feb. '89	St. 2M	24.7	417 ± 23	40	21 °C
	Feb. '89	St. 3M	24.95	833 ± 74	40	13 °C
P.lividus	Nov. '87	St. 1I	38.73	52 ± 4	15	21 °C
	Nov. '87	St. 1I	40.46	74 ± 6	15	21 °C
	Nov. '87	St. 3I	38.28	121 ± 10	15	20 °C

Patella caerulea: this gastropod, communal to all intermediate littoral stations revealed somewhat elevated levels of cadmium content, with a maximum average of 1140±46 µg g^{-1} FW in October at 3M; in February the minimum value was 419 µg FW (Tables III, IV; Fig. 5).

Paracentrotus lividus: present in the infralitoral stations, the gonads of P. lividus always revealed a modest average cadmium content, with a minimum of 52±4 µg Kg^{-1} FW at station 1I and a maximum of 121±11 µg kg^{-1} FW at station 3I (Tables III, IV; Fig. 6).

of Torvaldaliga to the port of Civitavecchia does not appear to influence, in a marked manner, the environmental presence of the metal. However, in the majority of the samples there appears to be a gradient in the element's presence, characterized by increased values proceeding from SE (stations 1M L) towards NW with maximum values at station 3 near Punta S. Agostino. We believe that the levels of cadmium observed were influenced, notably at times, by the local hydrodynamic values,

Table IV

Mean concentrations of cadmium in water, sediments (mg g^{-1} DW) and marine organisms (µg g^{-1} FW) from Torvaldaliga area.

	St.1M	St.2M	St.3M	St.1I	St.2I	St.3I
water	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.
sediments (µg g^{-1} DW)	0.230	0.150	0.154	0.072	0.146	0.166
ORGANISMS: (µg g^{-1} FW)						
Ulva rigida	35	273	122	-	-	-
Corallina elongata	35	38	51	-	-	-
Patella caerulea	909	419	1140	-	-	-
Paracentrotus lividus	-	-	-	52	74	121

Table V

Cadmium concentrations (µg g^{-1} FW) attained by Ulva rigida from Torvaldaliga area.

	1st sampling 21/10/1987		2nd sampling 2/2/1989		3rd sampling 15/6/1989	
Stations:	WATER t°C	Cd µg g^{-1} FW	WATER t°C	Cd µg g^{-1} FW	WATER t°C	Cd µg g^{-1} FW
St. 1M	20°C	-	14°C	35 ± 2.9	25°C	6.2
St. 2M	22°C	-	21°C	273 ± 23	27°C	7.6
St. 3M	20°C	27 ± 2.3	13°C	122 ± 11	27.5°C	21.7

depending on the morphological characteristics of the coastline at the different stations. In fact, station 3, close to Punta S. Agostino, is characterized by a reduced hydrodynamism with respect to the other stations; this is due to the presence of low rock formations and pebbly barriers towards the open sea; these favour the slackness of water.

In the sampled species, the bioaccumulation of cadmium displays evident and conspicuous temporal fluctuations in Ulva rigida, even at

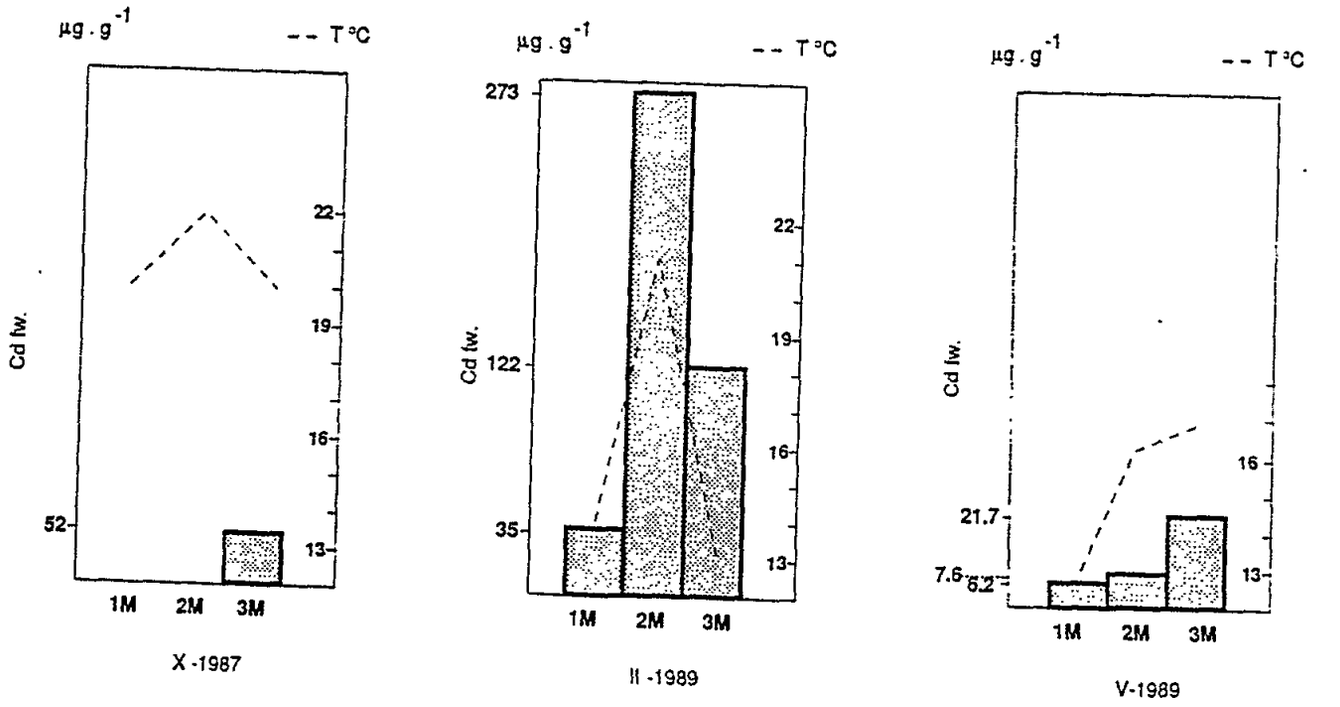

Fig. 2 Concentrations of Cd in Ulva rigida

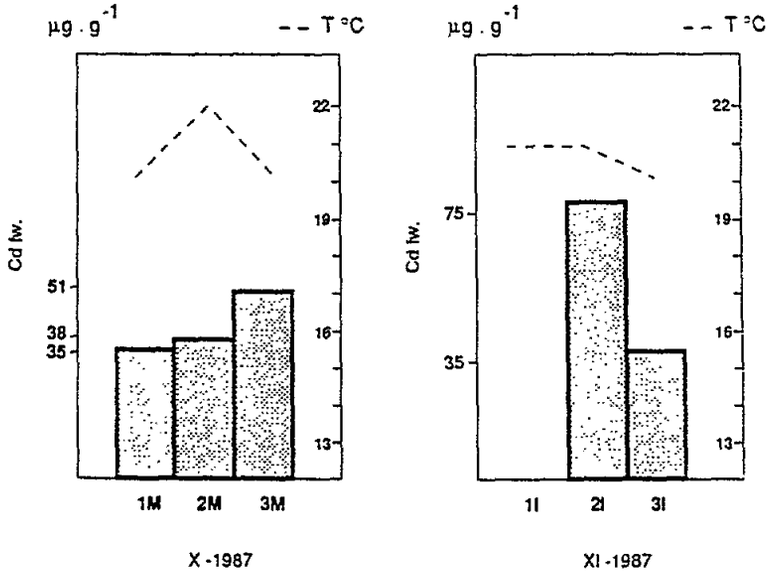

Fig. 3 Concentrations of Cd in Corallina elongata

Table VI

Cadmium contents (µg g^{-1} DW) in 60 samples of *Posidonia oceanica*.

Station No	Sample No	Measurements	DATES 08/03/89	07/06/89	04/10/89	13/01/90
1		WATER t°C	13°C	19°C	19°C	16.5°C
1	1	Cd CONTENTS µg g^{-1} DW	2.85	2.13	2.45	3.41
	2		2.48	2.33	2.36	3.13
	3		2.38	2.21	1.83	2.85
	4		2.30	2.08	1.99	2.97
	5		2.32	2.35	1.48	2.97
2		WATER t°C	17°C	23.8°C	21°C	16.5°C
2	1	Cd CONTENTS µg g^{-1} DW	2.59	3.98	2.16	3.22
	2		2.28	3.02	2.63	2.99
	3		2.23	3.26	2.30	3.14
	4		3.21	2.91	2.22	3.68
	5		2.97	4.20	2.86	3.65
3		WATER t°C	14°C	20°C	18.5°C	8.5°C
3	1	Cd CONTENTS µg g^{-1} DW	3.34	2.35	2.05	3.88
	2		3.25	4.02	2.32	3.78
	3		2.14	2.60	2.09	3.82
	4		2.34	3.46	2.33	4.03
	5		2.90	4.11	1.71	3.86

the same location. An examination of the complete data regarding the three samplings of *Ulva rigida* does not permit an univocal conclusion regarding the correlation existing between the accumulation of metal on the part of the algae, and the water temperature. For instance there exists a negative correlation between the bioaccumulation of the element and the summer increase in the water temperature. In contrasst to this the February samples reveal the presence of a marked maximum of concentration of the metal in the samples taken at stations 2M where the waters are consistently warmer as a result of the waste water discharges. At this station the increased water temperature may have effectively influenced results to the extent of varying the rhythm of absorption and excretion of the element and to influencing the duration of the annual cycle of *U. rigida*.

However, the high values of the element (Cd) measured at station 2M in February, could also represent a greater environmental presence of the metal, more or less localized and due to natural or anthropogenic factors; it could also be partly due to the meteorological and marine conditions, which in January and February 1989 were characterized by an exceptionally calm sea and a consequent reduction in hydrodynamism and circulation for a protracted period.

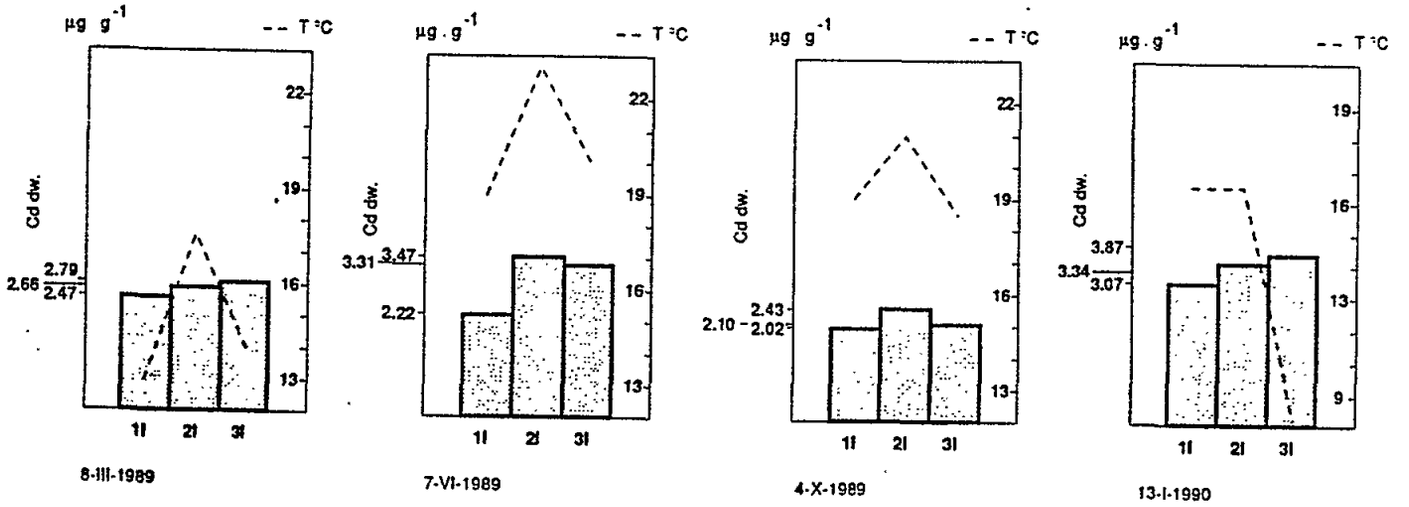

Fig. 4 Concentrations of Cd in Posidonia oceanica

Moreover one may hypothesize a correlation between the entity of the bioaccumulation of the metal and the dimensions of Ulva rigida sprouts, since we found at station 3M algal sprouts of greater dimension than those at other stations.

Regarding C. elongata, a slow growing perennial rhodophicean, consistently registered cadmium levels inferior to those of Ulva rigida, with a slight tendency towards greater accumulation at depths of 4m. This species, which although like P. lividus does not appear to be influenced in its bioaccumulation rhythm by the thermal variations caused by the waste-water discharge, reproposes the existence of a south-north in the presence of the metal.

P. oceanica, a phanerogam widely diffused along the Tyrrenian coast, revealed the lowest bioaccumulation at station I facing Punta Mattonara; this was apparently due to factors linked to the hydrodynamics of the area controlled and to considerable seasonal variations. Very little emerges regarding the effects of the cooling water from the thermoelectric power plant on the bioaccumulation od cadmium in P. oceanica, considering that the plants collected at the station in front of the thermoelectric station at Torvaldaliga evidence bioaccumulation values not unlike those revealed at station 3 facing Punta S. Agostino, and only in June significantly superior by 0.15 µg g^{-1} DW with respect to station 3 facing Punta S. Agostino and by 1.25 µg with respect to station 1 (Punta Mattonara).

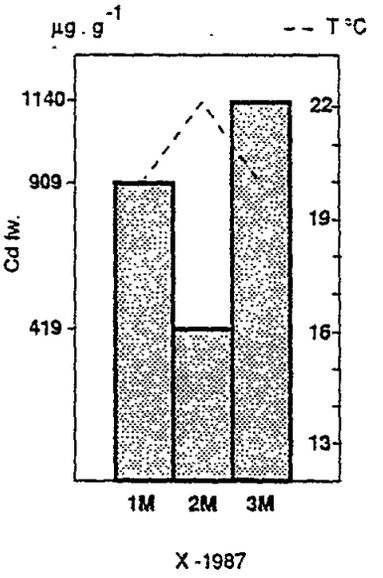

Fig. 5 Concentrations of Cd in Patella caerulea

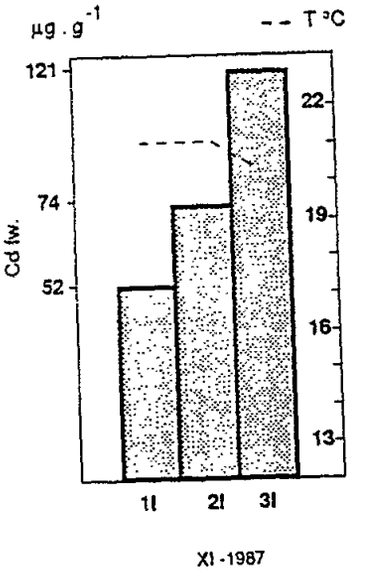

Fig. 6 Concentrations of Cd in Paracentrotus lividus

Table VII

Number of samples (n), means (M), standard deviations (S.D.) and analysis of variance for each station of cadmium contents ($\mu g\ g^{-1}$) in leaves of Posidonia oceanica.

	STATION 1			STATION 2			STATION 3			VARIANCE ANALYSIS	
DATE	n	M	S.D.	n	M	S.D.	n	M	S.D.	F	P%
08/03/89	5	2.47	0.23	5	2.66	0.43	5	2.79	0.54	0.78	-
07/06/89	5	2.22	0.12	5	3.47	0.58	5	3.31	0.80	6.96	1%
04/10/89	5	2.02	0.40	5	2.43	0.30	5	2.10	0.25	2.31	-
13/01/90	5	3.07	0.22	5	3.34	0.31	5	0.10	0.10	16.58	1%

Table VIII

Number of samples (n), Means (M), standard deviations (S.D.) and intervals of variability of seasonals cadmium contents ($\mu g\ g^{-1}$ DW) in leaves of Posidonia oceanica.

DATE	n	M	S.D.	VARIABILITY
08/03/89	15	2.64	0.41	2.23 - 3.34
07/06/89	15	3.00	0.79	2.08 - 4.20
04/10/89	15	2.19	0.35	1.48 - 2.86
13/01/90	15	3.43	0.41	2.85 - 4.03
ALL SAMPLES	60	2.81	0.68	1.48 - 4.20

P. caerulea, a gastropod mollusc and a primary consumer, for a notable capacity of cadmium bioaccumulation has been revealed, at station 2M, (affected by the waste-water discharge), showed the lowest level of cadmium content. It appears to be negatively influenced in the accumulation process by the higher environmental temperatures and being a surface species is certainly sensibly affected by the warm and less dense waste-water discharged into the sea.

Thus, the synergism studied could react in a harmonious manner in Ulva rigida and in Patella caerulea; however, the interference due to the effect of the changing local hydrodynamic conditions recommends further investigation to obtain, by a monthly or bi-monthly campaign, articulated research in the Torvaldaliga area, with a view to noting the trend of cadmium bioaccumulation in closer relation to the variations of environmental parameters.

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IMPORTANCE DU MACROPLANCTON GELATINEUX DANS LE STOCKAGE ET LE TRANSFERT DES METAUX POLLUANTS (CADMIUM, CUIVRE, PLOMB ET ZINC) EN MEDITERRANEE NORD-OCCIDENTALE

par

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R E S U M E

Nous avons recherché les concentrations en cadmium, cuivre, plomb et zinc dans certaines espèces gélatineuses. Les concentrations en métaux traces chez les Siphonophores sont en général faibles (domaine de variation pour le cadmium: 0,1-1,8 µm g^{-1}, le cuivre: 1,6-34,7 µg g^{-1}, le plomb: de la limite de détection à 10,6 µg g^{-1} et le zinc: 19-177 µg g^{-1}). Parmi les Siphonophores, les Vélelles prélevées à 5 km des côtes, dans le courant Ligure, ont une concentration moyenne en cadmium légèrement supérieure à celles prélevées dans la Baie de Villefranche ou même plus au large à environ 40 km du rivage alors que pour le plomb les concentrations sont plus élevées à la côte. Parmi les espèces étudiées, les Mollusques étudiés (Gymnosomes et Thécosomes) ont les concentrations les plus élevées en métaux. Pour les Salpes (Tuniciers), le domaine de variation est pour le cadmium: 0,1-4,3 µg g^{-1}, le cuivre:0,1-26,0 µg g^{-1}, le plomb: de la limite de détection à 6,4 µg g^{-1}, et pour le zinc: 12-135 µg $^{-1}$. Il est à noter la très forte concentration en métaux trouvée dans les pelotes fécales de Pegea confoederata et de Salpa maxima. Le rapport de la concentration en métal dans les féces à la concentration en métal dnas les Salpes (Salpa maxima et Pegea confoederata) est compris entre 2, 6 et 7, ce qui est en accord avec les données de la littérature. Les résultats montrent l'importance du macroplancton gélatineux dans le cycle biogéochimique des métaux traces.

1. INTRODUCTION

Les organismes gélatineux constituent une part importante de la biomasse dans les eaux océaniques et côtières pendant diverses périodes de l'année. Ces organismes peuvent modifier le transport et le cycle des métaux traces dans les eaux. Le contenu en métaux traces dans la macroplancton gélatineux est encore peu connu. Le but de cette étude a été de rechercher les concentrations en cadmium, cuivre, plomb et zinc dans certaines espèces gélatineuses ainsi que les variations spatio-temporelles éventuelles de ces concentrations. Parmi les métaux considérés, deux d'entre eux le cuivre et le zinc sont des éléments indispensables à la vie mais deviennent toxiques quand ils sont présents dans le milieu en grande quantité, tandis que le cadmium et le plomb n'ont aucun rôle biologique ou physiologique connu et sont responsables de nombreux effets toxiques. Seuls quelques travaux,

auxquels nous nous réfèrerons dans la discussion, existent à l'heure actuelle, sur le contenu en métaux traces du macroplancton gélatineux (Fowler et al. 1985; Krishnaswami et al., 1985 et Roméo et al. 1987).

2. MATERIELS ET METHODES

Les échantillons ont été pris, soit à la côte, soit au large, à une profondeur comprise entre 10 et 25 m, pour éviter la contamination de surface et tout dommage à ces organismes très fragiles et pour obtenir le plus grand nombre possible d'échantillons. La pêche a été réalisée à l'aide d'un filet à plancton de maille 680 µm pour les échantillons côtiers tandis que pour ceux du large un filet Omori a été utilisé.

Après la pêche, les animaux ont été laissés dans de l'eau de mer filtrée à température ambiante pour qu'ils éliminent leur contenu stomacal. Puis les échantillons ont été identifiés, rincés rapidement avec de l'eau bidistillée et lyophilisés. Dans certains cas (Salpes: espèce Pegea confoederata et Salpa maxima), les pelotes fécales ont pu être récupérées et traitées comme les échantillons.

En vue de l'analyse, les échantillons ont été pesés sous hotte à flux laminaire, puis minéralisés à l'aide d'acide nitrique "suprapur". Les autres métaux ont été analysés par spectrophotométrie d'absorption atomique à flamme (appareil Philips, Pye Unicam SP9) pour le cuivre et le zinc et sans flamme (four graphite avec programmateur vidéo PU 9095); la limite de détection pour le plomb et le cadmium est de 100 ng l^{-1}. Les procédés analytiques ont été contrôlés régulièrement en analysant des matériaux standard de référence (hépato-pancréas de homard "Tort-1" fourni par le Conseil National de la Recherche du Canada).

2.1 Espèces prélevées

Les échantillons ont été prélevés soit à 500 m de la côte, à la station hydrologique "B" (43°41'10''N, 7°19'E) à l'entrée de la baie de Villefranche-sur-mer en différentes périodes de l'année entre 1987 et 1989: soit en deux points au large du Cap Ferrat sur la radiale Nice-Calvi: l'un à 3 milles soit environ 5 km (station L_1: 43°38'8''N, 7°23'4''E, mai 1989), localisé dans le courant Ligure, l'autre à 28 milles (station L_2: 43°24'8'N, 7°52'E, mars 1988) dans le front hydrologique du bassin Liguro-Provençal, mais en dehors du courant Ligure. Au cours d'une croisière océanographique, des organismes ont été collectés en septembre 1989 sur la radiale Nice-Calvi à 23 Milles de la côte (station L'_2).

La taxonomie des animaux récoltés est présenté ci-dessous:

*Embranchement des CNIDAIRES: *Classe des SIPHONOPHORES
Ordre: Physonectes
Famille: Velellidae: Velella velella

n=20 (10/5/89) côte
n=22 (16/5/89) large (station L_1)
n= 8 (15/9/89) large (station L'_2)

Ordre: Cystonectes
Famille: Agalmidae: Nanomia bijuga
n= 2 (18/5/89) côte
Agalma elegans
n= 4 (19/6/87) côte
Hippopodius hippopus
n= 3 (19/6/87) côte

Famille: Diphyidae: Chelophyes appendiculate
n=10 (19/6/87) côte
n= 1 (25/3/88) large (station L_2)
n= 1 (14/4/89) côte
n= 1 (16/5/89) large (station L_1)

Famille: Abylidae: Abylopsis tetragona
n= 2 (19/6/87) côte
n= 1 (25/3/88) large (station L_2)
n= 1 (14/4/89) côte
n= 1 (23/5/89) côte

*Classe des MEDUSES ACALEPHES

Ordre: Rhizostomes
Cotylorhiza tuberculata
n= 6 (15/9/88) côte

Rhizostoma pulmo
n= 3 (1/6/89) côte

*Embranchement des CNIDAIRES
Ordre des Tentaculés: Pleurobrachia ctenophora
n= 1 (16/5/89) côte

Ordre des Nus: Beroe ovata
n=12 (24/3/88) côte

*Embranchement des MOLLUSQUES: *Classe des GASTEROPODES
Ordre des Ptéropodes Thécosomes
Cavolinia inflexa n= 2 (25/3/88) large (L_2)
n= 2 (14/4/89) côte
n= 3 (16/5/89) large (L_1)
n= 2 (5/12/89) côte
Clio pyramidata n= 3 (25/3/88) large (station L_2)

Ordre des Ptéropodes Gymnosomes
Pneumodermopsis canephora
n= 1 (25/3/88) large (station L_2)
n= 2 (14/4/89) côte
n= 4 (16/5/89) large (station L_1)
n= 1 (5/12/89) côte

*Embranchement des TUNICIERS: *Classe des THALIACES
Sous-Classe des Pyrosomides:
Pyrosoma atlanticumn= 1 (18/5/89) côte

Sous-Classe des Salpides: Salpes
Pegea confoederata n= 5 (16/5/89) large (L_1)
+n= 3 pelotes fécales
Thalia democratica n= 2 (14/4/89) côte
n= 2 (18/5/89) côte
Salpa maxima n= 3 (25/3/88) large (station L_2)
+n= 1 pelotes fécales
Salpa fusiformis n= 1 (25/3/88) large (station L_2)
Ihlea punctata n= 4 (25/3/88) large (station L_2)

3. RESULTATS

Les tableaux suivants donnent les résultats (exprimés en µg de métal par g de poids sec) des analyses de métaux traces effectuées sur les différents organismes étudiés.

Le tableau I montre que les concentrations en métaux traces chez les Siphonophores sont en général faibles, seule l'espèce Nanomia bijuga présente des concentrations élevées pour les quatre métaux etudiés (malheureusement, seulement deux échantillons de cette espèce ont pu être prélevés). Le domaine de variation des concentrations en métaux traces chez les Siphonophores est indiqué en bas du tableau I (l'espèce N. bijuga n'est pas comprise dans ce domaine).

Un grand nombre de Vélelles ont été prises en 89 tant à la côte qu'au large (50 échantillons en tout). Parmi l'ensemble des Siphonophores l'espèce Velella velella semble concentrer faiblement les métaux traces. En effet, nous pouvons remarquer que les concentrations en zinc sont plus élevées chez les espèces Hippopodius hippopus et Abylopsis tetragona et les concentrations en cuivre sont plus élevées chez Chelophyes appendiculata que chez les autres Siphonophores.

Chez les Scyphoméduses (tableau II) Cotylorhiza tuberculata, organismes de taille importante, nous pouvons noter que les concentrations en zinc sont significativement plus faibles dans l'ombrelle (36 µg g^{-1}) que dans les tentacules et organes des animaux (103 et 131 µg g^{-1}, respectivement).

En ce qui concerne les Cténophores (tableau III), l'espèce Pleurobrachia ctenophora (un seul échantillon) semble concentrer particulièrement les métaux par rapport à l'espèce Beroe ovata (12 échantillons) où le zinc est en très faible concentration (29 µg g^{-1}).

Les Mollusques gélatineux (tableau IV) ont des concentrations relativement élevées en zinc par rapport aux Cnidaires, aux Cténophores et aux Tuniciers.

Tableau I

Concentrations en métaux traces (exprimées en $\mu g\ g^{-1}$) des Cnidaires: Siphonophores.

ESPECES ETUDIEES	Cadmium	Cuivre	Plomb	Zinc
Velella velella				
(10/5/89-côte)	0,3 ± 0,1	2,4 ± 0,8	0,3 ± 0,3	35 ± 9
(n = 20)				
(16/5/89-large L_1)	0,8 ± 0,3	2,9 ± 1,3	0,1 ± 0,1	30 ± 6
(n = 22)				
(15/9/89-large L'_2)	---	2,1 ± 0,2	---	43 ± 11
(n = 8)				
Moyenne générale	0,6 ± 0,3	2,6 ± 1,0	0,2 ± 0,2	34 ± 9
(n = 50)				
Domaine de variation Velella velella	**0,2 - 1,6**	**1,6 - 7,6**	**L.D. - 1,6**	**19 - 70**
Nanomia bijuga				
(18/5/89-côte)	2,7	57,1	24,0	196
(n = 2)	12,7	98,0	33,9	183
Agalma elegans				
(19/6/89-côte)	---	5,4 ± 1,5	---	33 ± 8
(n = 4)				
Hippopodius hippopus				
(19/6/87-côte)	0,5 ± 0,3	3,2 ± 1,3	---	131 ± 8
(n = 3)				
Chelophyes appendiculata				
(19/6/87-côte)	1,1 ± 0,3	10,8 ± 6,5	5,3 ± 1,9	66 ± 26
(n = 10)				
(25/3/88-large L_2)	1,7	34,7	---	58
(n = 1)				
(14/4/89-côte)	1,6	17,1	3,4	176
(n = 1)				
(16/5/89-large L_1)	1,3	14,7	---	107
(n = 1)				
Abylopsis tetragona				
(19/6/87-côte)	0,7	10,5	---	112
(n = 2)	0,9	5,0	---	112
(25/3/88-large L_2)	0,8	6,5	---	177
(n = 1)				
(14/4/89-côte)	0,7	7,6	3,0	99
(n = 1)				
(23/5/89-côte)	---	4,9	---	166
(n = 1)				
Domaine de variation Siphonophores	**0,1-1,8**	**1,6-34,7**	**L.D.-10,6**	**19-177**

Tableau II

Concentrations en métaux traces (exprimées en $\mu g\ g^{-1}$) des Cnidaires: Scyphoméduses.

ESPECES ETUDIEES	Cadmium	Cuivre	Plomb	Zinc
Cotylorhiza tuberculata (15/9/88-côte) (n = 6)				
Ombrelle	1,4 ± 0,4	1,3 ± 0,1	---	36 ± 10
Tentacules	1,7 ± 0,1	1,7 ± 0,2	---	106 ± 3
Organes	2,2 ± 0,7	2,0 ± 0,2	---	131 ± 17
Domaine de variation Cotylorhiza tuberculata	0,8 - 2,7	1,2 - 2,3	---	22 - 149
Rhizostoma pulmo (1/6/89-côte) (n = 3)	---	3,5 ± 0,5	---	110 ± 41
Domaine de variation Rhizostoma pulmo		3,1 - 4,0	---	72 - 154

Tableau III

Concentrations en métaux traces (exprimées en $\mu g\ g^{-1}$) des Cténophores.

ESPECES ETUDIEES	Cadmium	Cuivre	Plomb	Zinc
Pleurobrachia ctenophora (16/5/89-côte) (n = 1)	2,5	25,6	3,2	177
Beroe ovata (25/3/88-côte) (n = 12)	1,4 ± 0,4	2,8 ± 0,8	---	29 ± 9
Domaine de variation Beroe ovata	0,8 - 2,0	1,5 - 4,4	---	18 - 48

Tableau IV

Concentrations en métaux traces (exprimées en $\mu g\ g^{-1}$) des Mollusques.

ESPECES ETUDIEES	Cadmium	Cuivre	Plomb	Zinc
Cavolinia inflexa				
(25/3/88-large L_2)	2,4	3,6	---	184
(n = 2)	2,4	5,1	---	205
(14/4/89-côte)	0,4	1,4	3,0	81
(n = 2)	1,1	1,3	3,0	52
(16/5/89-large L_1)	1,4 ± 0,6	1,8 ± 0,2	3,0 ± 0,1	96 ± 17
(n = 3)				
Clio pyramidata				
(25/3/88-large L_2)	3,1 ± 1,0	3,1 ± 0,2	---	242 ± 22
(n = 1)				
Pneumodermopsis canephora				
(25/3/88-large L_2)	3,9	8,7	---	325
(n = 1)				
(14/4/89-côte)	1,2	4,6	3,0	106
(n = 2)	6,9	9,4	3,0	118
(16/5/89-large L_1)	1,5 ± 0,2	3,3 ± 1,0	3,0 ± 0,1	109 ± 61
(n = 4)				
Domaine de variation Mollusques	**0,4 - 6,9**	**1,3 - 9.4**	**2,8 - 3,2**	**52 - 325**

Les concentrations en métaux chez les Tuniciers (Salpes) semblent extrêmement variables (tableau V). A l'exception de l'espèce Pyrosoma atlanticum, les concentrations en zinc chez les Salpes paraissent faibles. Les pelotes fécales de Pegea confoederata et de Salpa maxima ont des concentrations élevées en métaux.

4. DISCUSSION

Parmi les 137 échantillons analysés en 1987-88-89, il ne semble pas y avoir de variations spatio-temporelles significatives dans les concentrations en métaux traces sauf celles notées ci-dessous, ni de changements notables par rapport aux échantillons prélevés à la côte en 1984 (Roméo et al., 1987). La plus grande source de variations pourrait provenir des différences entre espèces et, peut-être, à l'intérieur d'une même espèce (ou d'un même ordre) entre les tailles. De ce fait, nous avons entrepris d'analyser nos résultats suivant une approche allométrique, en nous référant aux travaux de Boyden (1974, 1977) qui a établi des relations générales entre le contenu en éléments traces et le poids d'organismes du type:

Tableau V

Concentrations en métaux traces (exprimées en $\mu g\ g^{-1}$ poids sec) des Tuniciers: Salpes.

ESPECES ETUDIEES	Cadmium	Cuivre	Plomb	Zinc
Pyrosoma atlanticum (18/5/89-côte) (n= 1)	4,3	12,4	3,9	135
Pegea confoederata (16/5/89-large L_1) (n = 5)	0,3 ± 0,0	11,9 ± 1,3	4,5 ± 2,5	51 ± 4
Pelotes fécales de P. confoederata (16/5/89-large L_1) (n = 3)	2,1 ± 0,0	59,3 ± 18,1	---	352 ± 25
Thalia democratica (14/4/89-côte) (n = 2)	0,1 0,3	6,4 26,0	2,0 0,5	32 48
(18/5/89-côte) (n = 1)	0,1	8,6	0,6	24
Salpa maxima (25/3/88-large L_2) (n = 3)	1,7 ± 1,5	2,5 ± 1,0	---	20 ± 8
Pelotes fécales de Salpa maxima (25/3/88-large L_2) (n = 1)	4,3	15,0	---	118
Salpa fusiformis (25/3/88-large L_2) (n = 1)	0,3	8,5	---	10
Ihlea punctata (25/3/88-large L_2) (n = 4)	0,7 ± 0,2	2,3 ± 0,3	---	18 ± 3
Domaine de variation Salpes	0,1 - 4,3	0,1 - 26,0	L.D. - 6,4	12 - 135

$Y = a\ W^b$, a et b étant des constantes, Y, le contenu en éléments traces et W, le poids de l'organisme.

Si b = 1, la concentration en métaux traces est indépendante de la taille (Y/W = a) et la liaison des métaux traces à des constituants chimiques particuliers peut expliquer cette indépendance.

Si b < 1, il y a une relation entre l'accumulation des métaux et la surface des organismes, la prise de l'élément trace se faisant par adsorption.

Si b > 1, la concentration en métaux augmente avec la taille des organismes.

Des relations allométriques ont pu être ainsi établies entre le contenu total en métaux traces présent dans l'organisme (ng totaux) et le poids (W exprimé en milligramme).

Chez les Siphonophores, nous avons pris, pour établir ces relations, l'exemple des Vélelles dont nous possédions un grand nombre d'échantillons de taille différente (n = 50)

$Cu = 9,0\ W^{0,797}$ (erreur sur b = 0,058; r = 0,891 p<0,001)
$Zn = 73\ W^{0,875}$ (erreur sur b = 0,054; r = 0,922 p<0,001)
$Cd = 1,3\ W^{0,846}$ (erreur sur b = 0,109; r = 0,744 p<0,001)

Le tableau I indiquait que les Vélelles prélevées le 16 mai 1989 à 5 km des côtes, dans le courant Ligure (station L_1) avait une concentration moyenne en cadmium légèrement supérieure ($0,8 \pm 0,3\ \mu g^{-1}$) à celles trouvées dans les Vélelles de la baie de Villefranche du 10 mai ($0,3 \pm 0,1\ \mu g\ g^{-1}$) ou même très au large à environ 40 km, à la station L'_2 pour le prélèvement du 15 Septembre 1989 ($0,4 \pm 0,2\ \mu g\ g^{-1}$). Nous avons donc cherché à voir si cette tendance se retrouvait dans les relations allométriques:

- pour les 22 échantillons prélevés dans le courant Ligure (station L_1), nous trouvons:

  $Cd = 2,0\ W^{0,854}$ (erreur sur b = 0,129; r = 0,829 p<0,001);

- pour les 28 échantillons restant (côte et L'_2 confondus):

  $Cd = 1,3\ W^{0,795}$ (erreur sur b = 0,079; r = 0,893 p<0,001)

Dans ce dernier cas, les coefficients a et b étant plus faibles, les concentrations sont plus faibles.

En ce qui concerne le plomb, certains échantillons de Vélelles avaient une concentration inférieure à la limite de détection, pour les autres Vélelles, le calcul des relations allométriques donne des valeurs de b proches ou supérieurs à 1, mais les coefficients de corrélation r ne sont pas très significatifs, il semble donc difficile de conclure. Toutefois on peut remarquer que ce sont les échantillons de petite taille qui ont les concentrations les plus faibles en plomb. La moyenne des concentrations en plomb chez les Vélelles prélevées à la côte le 10/5/89 est de $0,3\ \mu g\ g^{-1}$, alors qu'elle est de $0,1\ \mu g\ g^{-1}$ plus au large mais dans le courant Ligure à 5 km de la côte, le 16/5/89. Nous n'avons pu effectuer les analyses de plomb des Vélelles prises très au large (station L'_2).

Chez les Cténophores Beroe ovata, prélevés à la côte le 25/3/88 (n = 12), les relations allométriques trouvées sont les suivantes:

$Cu = 2,4\ W^{1,006}$ (erreur sur b = 0,145; r = 0,925 p<0,001)
$Zn = 85\ W^{0,840}$ (erreur sur b = 0,107; r = 0,945 p<0,001)

Dans le cas du cadmium l'erreur sur b est trop importante pour que l'on puisse conclure à une quelconque relation. D'autre part, les déterminations des concentrations en plomb chez Beroe ovata n'ont pu être effectuées.

Chez les Tuniciers (Salpes), comme nous n'avions pas un assez grand nombre d'échantillons de chaque espèce, nous n'avons pas essayé d'établir des relations allométriques.

5. CONCLUSIONS

Ce travail, effectué dans le cadre d'un contrat FAO-MEDPOL Phase II, est l'une des rares études concernant les métaux traces polluants et les organismes du macroplancton gélatineux en Méditerranée. A notre connaissance, seuls Krishnaswami et al., 1985 et Fowler et al., 1985 ont abordé ce sujet. Nos résultats sont en bon accord avec les leurs. L'originalité de notre travail est d'avoir pu prélever un grand nombre d'échantillons (plus de 130) appartenant à des espèces variées. Nos résultats diffèrent quelque peu de ceux présentés dans le rapport précédent, du fait que nous avons pu obtenir au cours de l'année 1989 plusieurs échantillons d'une même espèce gélatineuse.

L'approche allométrique de nos résultats peut suggérer que le cuivre, le zinc et le cadmium se lieraient par adsorption ($b<1$) chez les Siphonophores ainsi que le zinc chez les Cténophores, par contre chez ces derniers organismes, les concentrations en cuivre seraient indépendantes de la taille des animaux (b très proche de 1).

Nous n'observons dans aucun cas d'augmentation de la concentration en métaux avec la taille des organismes ($b>1$) que nous avons étudiés.

Deux résultats semblent importants sur les espèces dont nous avions le plus grand nombre d'échantillons, à savoir les Siphonophores Velella velella:

- les concentrations plus élevées en cadmium dans les Vélelles prélevées à 5 km du rivage mais dans le courant Ligure que celles trouvées chez les Vélelles prélevées à la côte ou très au large. Ce courant venant d'Italie, transporte les pollutions industrielles de la région de Gènes, ce qui peut donc influencer la teneur en polluants dans les organismes marins.

- les concentrations plus fortes en plomb des Vélelles prises à la côte le 10 mai 1989, par rapport aux concentrations de celles du large (courant Ligure); les eaux côtières étant nettement plus riches en plomb soluble que les eaux du large, du fait des retombées atmosphériques riches en plomb tétraéthyle provenant des gaz d'échappement des automobiles.

Pour les Salpes, il est à noter la très forte concentration en métaux trouvée dans les pelotes fécales de Pegea confoederata et de Salpa maxima. Les Salpes sont des herbivores et leurs pelotes fécales

sont constituées en majeure partie par du phytoplancton digéré; les algues phytoplanctoniques concentrant en général les métaux à partir de l'eau de mer.

Le rapport concentration en métal dans les fèces/concentration en métal dans les Salpes (Salpa maxima et Pegea confoederata) est compris entre 2,6 et 7. Les travaux de Krishnaswami et al. (1985) donnent des rapports de 2 pour le zinc et le cuivre et de 22 pour ^{210}Pb.

Si nous comparons les résultats des concentrations en métaux dans les fèces de Pegea confoederata (n = 3) à celles que nous avons publiées (Roméo et al. 1988) dans le matériel particulaire recueilli dans une trappe à sédiments (placée à une profondeur de 200 m, en zone côtière de la Mer Ligure, programme Dyfamed) qui sont de 0,7 µg Cd g^{-1}, 46 µg Cu g^{-1} et 150 µg Zn g^{-1}, nous constatons que les concentrations en métal dans les fèces sont en général supérieures à celles des particules provenant de la trappe. En descendant dans la colonne d'eau, les fèces se décomposent en particules dont une partie est recyclée, les métaux associés à ces particules repassent alors dans la chaîne alimentaire, l'autre partie sédimente. Les pelotes fécales contribuent donc, d'une manière ou d'une autre, au transport des métaux dans la colonne d'eau.

Des résultats (Fowler et al. 1987) sur des analyses effectuées sur des trappes à sédiment et sur des échantillons de zooplancton de Méditerranée Nord-Occidentale ont montré que les radionuclides ^{141}Ce et ^{144}Ce envoyés dans l'atmosphère lors de l'accident de Tchernobyl, se retrouvaient à 200 m de profondeur en mer en quelques jours. Ceci ne pouvait pas être expliqué uniquement par les lois physiques (loi de Stokes) qui évalueraient ce transport à quelques années. L'hypothèse la plus probable suggérée par Fowler et al. (1987), était le transport vertical de ces radionuclides par les pelotes fécales produites par le zooplancton après ingestion de phytoplancton et de particules contaminées des couches de surface.

6. REFERENCES

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ENZYMIC ASPECTS OF THE XENOBIOTIC METABOLIZING SYSTEM IN *Mytilus galloprovincialis* Lam.

by

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A B S T R A C T

The results presented show that in the digestive gland of mussels injected with 0.1, 0.5 and 2.5 mg/animal of Arochlor 1254 (a commercial mixture of PCBs) and then cleaned in sea water for 2, 4 and 8 days there is a significant uptake of the organic xenobiotic compounds (from 7 to 218 $\mu g\ g^{-1}$ w.w.).

The components of Arochlor 1254 are present in the tissue essentially in the same proportion as in the original Arochlor 1254 mixture, indicating a low metabolic rate of these compounds. Moreover, both NADPH and NADH cytocrome c reductase activities present in the digestive gland microsomes are similar in control and treated mussels (0.1 - 0.5 mg Aroclor 1254/animal) being lower in the mussels injected with the highest dosage. Interestingly, the Benzo(a)pyrene hydroxylase activity is decreased in the animals treated with high concentration of Arochlor 1254, but slightly and significantly enhanced in the microsomal preparation of the animals treated with 0.1 mg/animal of Arochlor 1254 and maintained in clean sea water for 4-8 days.

1. INTRODUCTION

It is well known that in the endoplasmic reticulum of vertebrate cells a multienzymatic system is present, named MFO (Mixed Function Oxygenase), involved in the formation of oxidized metabolites of endogenous compounds, such as steroid hormones and also organic xenobiotics compounds such as pesticides, aromatic hydrocarbons, etc. which are taken up by the cells. The more polar oxidized derivatives are usually conjugated (Scoppa, 1968) and successfully excreted, although it has been demonstrated that active metabolites deriving from organic xenobiotics may react with cellular components and this often results in DNA damage (Stegeman, 1981). It is important to note that the MFO system is inducible in vertebrates by treatment with organic xenobiotic substances (Bothelo *et al.*, 1979; Stegeman, 1981). In relation to these findings, it has also been recently proposed to evaluate the possibility of the induction of MFO activity in marine organisms (Payne, 1976). This, in fact, may help to quantify the biological effects exerted by the accumulation of environmental pollutants in the tissues of the marine animals.

For this experimental work the mussel has been chosen because it is a sedentary, filter-feeding animal, able to accumulate within its

tissues many of the pollutants present in sea water (Sprague, 1971) and for these reasons it has been often utilized as a biological indicator in monitoring programs. It has also been considered a test organism to evaluate the biological effects that the accumulation of pollutants may exert on the physiology of the animal, with the aim of quantifying the biological impact of pollutants.

In addition, since it has been demonstated that there are differences in MFO activity in the digestive gland of mussels sampled at different periods of the year (Livingstone and Farrar, 1984; Stegeman, 1985), the cytocrome P-450 content and related Benzo(a)pyrene hydroxylase activity were evaluated in November- December (animals with immature gonads) and in June-July (mussels with mature gonads) to evaluate the role of the physiological status of the animal in response to the accumulation of xenobiotics for a possible future field application of these results.

2. MATERIALS AND METHODS

2.1 Animals

Specimens of Mytilus galloprovincialis Lam., 4-6 cm long, were collected from Palmaria (Italy); mussels were maintained at 15°C in synthetic seawater prepared according to La Roche et al. (1970) with maximum 35 salinity. Animals were acclimatized to laboratory conditions for at least one week before testing.

Mussels were treated with different concentrations of Arochlor 1254, a mixture of PCBs, and detoxified for 1, 4 or 8 days. The digestive gland of the animals was then analyzed to evaluate the total concentration of Arochlor 1254 and the components of the complex mixture of organic xenobiotics selectively accumulated in the tissues. At the same time, the effects of the organic xenobiotics on MFO were evaluated by testing the activity of NADH cytochrome c reductase, NADPH cytochrome c reductase, Benzo(a)pyrene hydroxylase and the content of cytochrome P-450.

2.2 Microsomal preparation

Microsomes from digestive gland were prepared as described previously (Viarengo et al., 1986). All steps in the microsome isolation procedure were performed at 0-4°C.

The tissues were homogenized in isotonic 900 mM sucrose (20% glycerol) buffered with 20 mM HEPES pH 7.6, containing 1 mM EDTA, 5 mM DTT, 0.66 mM phenylmethylsulphonyl-fluoride (PMSF); trypsin inhibitor (120 mg/100 ml) and leupeptin (3 µg ml^{-1}) were added to the homogenization medium to act as antiproteolytics (Viarengo et al., 1986).

The homogenate was filtered through gauze and then centrifuged at 30.000 x g for 20 minutes to remove cell debris, nuclei, mitochondria and lysosomes. The resulting supernatant was centrifuged at 150.000 x g for one hour to obtain the microsomal pellet.

Microsomal fractions were either used immediately after preparation or frozen at -80°C. Before use, the pellets were carefully resuspended in the corresponding homogenization buffer (1 ml g^{-1} starting material).

2.3 Biochemical assays

Protein content was evaluated according to Hartree's (1972) modification of the method developed by Lowry et al. (1951), using bovine serum albumin as a reference protein.

2.4 Assay of enzymic activities

All enzymic tests were performed at 25°C. Benzo(a)pyrene hydroxylase activity was assayed according to Nebert and Gelboin (1968), using quinine sulphate as a standard. A concentration of 0.03 nmol of 3-hydroxybenzo(a)pyrene per ml in 1 N NaOH gives a fluorescence intensity which is equal to that given by 0.3 µg quinine sulphate per ml in 0.1 N H_2SO4 at an excitation wavelength of 400 nm and an emission wavelength of 522 nm (Rickert and Fouts, 1970).

NADPH and NADH cytochrome-c-reductase activities were assayed spectrophotometrically according to Omura and Takesue (1970) and Sottocasa et al. (1975), respectively.

2.5 Procedure for PCB analysis

The concentration of PCBs in the tissues of the mussels treated with Arochlor was measured essentially as described in the U.S. Dept. of Health, Education and Welfare, Food and Drug Administration, Pesticide Analytical Manual, Vols. I and II, Washington, D.C., 1972.

The quantitative gas chromatographic analysis of the total PCBs content of the examined tissues was performed with a DANI 3800 HR PTV and a glass column b (lenght 1.8 mm, diameter 3 mm) containing 3% OV-101 on Chromosorb W-HP (80-100 mesh) as described previously (Contardi et al., 1985). Analytical separations of the different PCBs was obtained utilizing OV1-101 coated 25 m fused silica capillary column, 0.33 mm i.d., carrier gas hydrogen , 12 psi column head pressure. Temperature program: 4 min -40°C isothermal, 40°C ----> 140° C prgr rate 10°C/min, 3 min 140°C isothermal, 140°C ----> 200°C prgr rate 1.6°C/min and then 200°C 30 min isothermal.

Cytochrome P450 levels were determined according to Ray and Estabrook (1970), assuming an extinction coefficient of 91 nM-1 x cm^{-1}.

3. RESULTS AND DISCUSSION

The data presented in Table I show the concentration values of Arochlor 1254 present in the digestive gland of mussels injected with 2.5, 0.5, 0.1 mg Arochlor 1254/animal and then kept in clear sea water for different periods of time. The concentration of PCBs was evaluated after separation of the Arochlor components by gas chromatography on a silica column. As shown in Table I the Arochlor concentrations range from about 7.1 µg g^{-1} wet weight in the animals treated with 0.1 mg of

xenobiotic to 218 μg g^{-1} wet weight found in the digestive gland of mussels treated with 2.5 mg of Arochlor.

During the recovery period after the treatment with the organic xenobiotic, the concentration of PCBs increases from the second to the fourth day by about 20-40% and in the following 4 days it remains

Table I

Arochlor 1254 levels in the digestive gland of mussels treated with different concentrations of the xenobiotics (PCBs) and then kept in clean sea water for different periods of time. Each value represents the mean ± SE of at least 4 experiments, each involving 14-16 animals.

Treatment mg/animal	Time (days)	Arochlor 1254* μg g^{-1} wet weight
control	4	2.37 ± 0.3
0.1	2	7.10 ± 2.1
0.1	4	8.20 ± 2.2
0.5	2	36.00 ± 8.5
0.5	4	59.00 ± 6.2
0.5	8	61.21 ± 7.8
2.5	2	154.12 ± 17.1
2.5	4	218.60 ± 21.7

* The concentration of PCBs was evaluated by gas chromatography after clean-up of the extracts on a silica-gel column.

stable. This trend of Arochlor accumulation was quite similar regardless the different concentrations of Arochlor utilized.

Interestingly enough, the concentration of PCBs found in the digestive gland of treated mussels appears to be of the same order of magnitude as that which in the rat liver can stimulate MFO activity and, in particular, the synthesis of citochrome P450 and Benzo(a)pyrene hydroxylase activity. Figure 1 shows the chromatograms of the Arochlor 1254 components separated by gas chromatography using a capillary column as described under Materials and Methods (Panel A) and the components of the xenobiotic mixture extracted from the digestive gland of mussels treated for 4 days with 0.5 mg/animal of PCBs (Panel B). It should be noted that the chromatographic technique allows the separation of about 42 compounds and that all these Arochlor 1254 components are present in similar proportions in the extracts from mussel digestive glands to those from the original injected mixture. The same results were obtained by analyzing the tissue PCBs content from mussels treated with different Arochlor concentrations and maintained in clean sea water for different time periods (data not shown). These results furnish a first indication that the rate of metabolism of the Arochlor components in mussels digestive gland is extremely limited; no oxidized metabolite accumulation or presence is detected after 8- day recovery period.

The data presented in Tables II-IV show the values of the activity of the more important microsomal enzymes involved in the electron transport chain of the MFO. Furthermore, the data reported concern the microsomal preparations obtained from control animals as well as the results from mussels treated with different Arochlor concentrations and then kept in clean sea water for different periods. As shown in Tables II and III the values of NADPH and NADH cytochrome c reductase activities in the microsomal preparations made from the digestive gland, are essentially constant, or slightly lower when the animals are treated with 2.5 mg Arochlor/mussel. Only in the preparations obtained from mussels injected with 0.5 mg PCBs/mussel after a period of 4 days

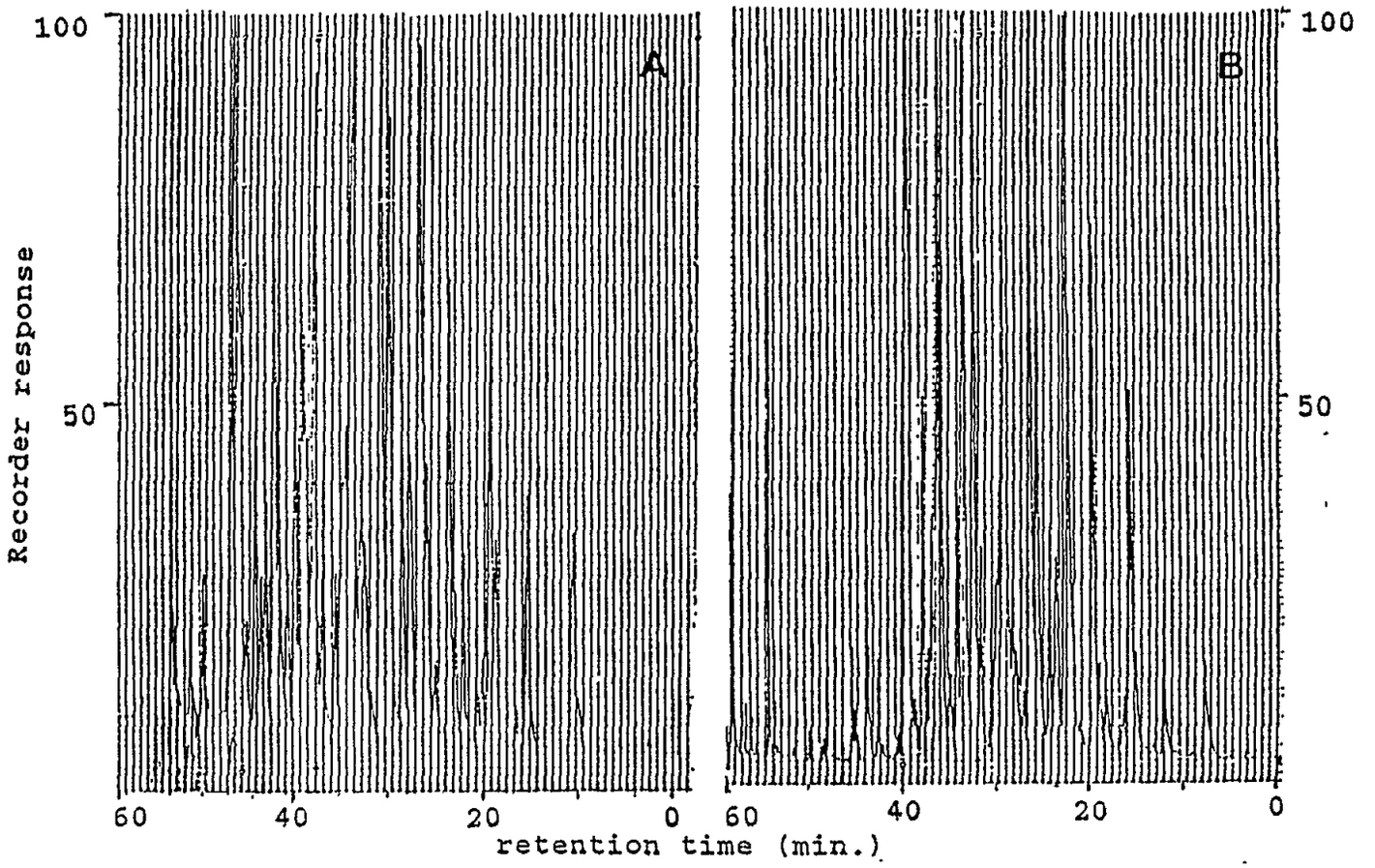

Fig. 1 Gas chromatographic separation by capillary column of Arochlor 1254 components from pure Arochlor 1254 (Panel A) and from digestive gland extracts of mussels treated with 5 mg animal^{-1} xenobiotic mixture and then kept in clean sea water for 4 days (Panel B).

Table II

In vivo effects of different concentrations of Arochlor 1254 on NADPH-cytochrome c reductase activity in the digestive gland of mussels treated with the xenobiotic and then kept in clean sea water for 4 and 8 days. Each value represents the mean ± SE of at least 4 experiments,each involving 14-16 animals.

Treatment	NADPH-cytochrome c reductase activity 4 days	8 days
Controls	10.1 ± 3.7	11.3 ± 3.5
Arochlor (0.5 mg/animal)	11.7 ± 3.4	9.1 ± 3.4
Arochlor (2.5 mg/animal)	8.4 ± 3.1	11.2 ± 2.4

* expressed in nm reduced cit. c min^{-1} mg^{-1} protein.

Table III

In vivo effects of different concentrations of Arochlor 1254 on NADH-cytochrome c reductase activity in the digestive gland of mussels treated with the xenobiotic and then kept in clean sea water for 4 and 8 days. Each value represents the mean ± SE of at least 4 experiments, each involving 14-16 animals.

Treatment	NADPH-cytochrome c 4 days	reductase activity* 8 days
Controls	161.1 ± 18.3	172.8 ± 24.1
Arochlor (0.5 mg/animal)	213.3 ± 34.6	173.4 ± 27.1
Arochlor (2.5 mg/animal)	130.8 ± 15.4	ND

* expressed in nm reduced cit. c min^{-1} mg^{-1} protein.
ND = not determined.

Table IV

In vivo effects of different concentrations of Arochlor 1254 on Benzo(a)pyrene-Hydroxylase activity in the digestive gland of mussels treated with the xenobiotic and then kept in clean sea water for 2, 4 and 8 days. Each value represents the mean ± SE of at least 4 experiments, each involving 14-16 animals.

Treatment	Benzo(a)pyrene hydrolase activity* 2 days	4 days	8 days
Controls	0.0022 ± 0.0020	0.023 ± 0.0025	0.021 ±
Arochlor (0.1 mg/animal)	ND	0.036 ± 0.003	0.028 ± 0.002
Arochlor (0.5 mg/animal)	0.020 ± 0.0023	0.026 ±	ND
Arochlor (2.5 mg/animal)	0.014 ± 0.0026	0.019 ± 0.0021	0.016

* expressed in nm 8-OH.BP min^{-1} mg^{-1} protein.
ND = not determined.

was there an increase of NADH activity which, due to the variability of the results, appears to be significant at 95% probability level.

The data presented in Table IV show the values of Benzo(a)pyrene hydroxylase activity in the microsomal preparations obtained from the digestive gland of mussels treated with different Arochlor 1254 concentrations and then maintained in clean sea water for 2, 4 and 8 days. In the animals treated with the highest concentration of PCBs, a decrease of enzyme activity was observed; on the contrary, the activity of Benzo(a)pyrene hydroxylase was found slightly but significantly enhanced in the microsomal preparations obtained from mussels treated with a low Arochlor concentration (0.1 mg/animal) and maintained in clean sea water for 8 days.

In Table V the data concerning the concentrations of cytochrome P-450 found in the microsomal preparations of Arochlor treated mussels and controls sampled in the May-July period are presented. As mentioned previously, the values of cytochrome P-450 show only a minimal decrease in the animals treated with the highest concentrations of PCBs; the data concerning cytochrome P-450 show that in samples from a set of experiments performed in the November-December period, the concentration of such compounds in the preparation is under the limit of sensitivity of the method employed.

To ascertain whether cytochrome P-450 is always present in the endoplasmic reticulum throughout the year, although in minimal undetectable amounts, a polyacrylamide gel electrophoresis analysis of the microsomal preparation was performed. This technique allows us to detect the presence of minimal amounts of proteins belonging to the cytochrome P-450 family. The results of such analysis, when compared

Table V

In vivo effects of different concentrations of Arochlor 1254 on cytochrome P-450 content of mussels treated with the xenobiotic and then kept in clean sea water for 2 and 4 days. Each value represents the mean ± SE of at least 4 experiments, 14-16 animals.

Treatment	Cytochrome P-450 content*	
	2 days	4 days
Controls	0.042 ± 0.006	0.040 ± 0.007
Arochlor (0.1 mg/animal)	ND	0.043 ± 0.007
Arochlor (0.5 mg/animal)	0.045 ± 0.008	0.041 ± 0.005
Arochlor (2.5 mg/animal)	0.040 ± 0.008	0.037 ± 0.009

* expressed in nm/mg protein.
ND = not determined.

with similar data obtained from rat liver microsomes, clearly indicate that minimal amounts of cytochrome P-450 are always present in mussel digestive gland preparations even if the compound is not detectable by UV technique (data not shown). These data indicate that regardless the high variability, which is probably related to seasonal physiological variations, the metabolism of important biological lipophilic substances such as steroid hormones is always active although at an extremely low rate.

In conclusion, the results presented indicate that at the moment the utilization of the values of MFO activity (and in particular of Benzo(a)pyrene hydroxylase activity) as a specific stress index can be recommended only as part of the set of data including the evaluation of a general stress index (such as lysosomal stability or rate of protein catabolism). In fact, negative results may depend on the general stress syndrome caused by xenobiotic accumulation which, as reported by Viarengo and Moore (1981), is often associated to a net increase of the rate of protein catabolism, frequently involving a decrease of the endoplasmic reticulum content and/or a decrease of some associated enzymic activity (Penning and Scoppa, 1977).

Moreover, these facts and the data concerning seasonal variations of MFO activity in the digestive gland of mussels give a strong indication that particular care must be taken in the interpretation of field results and that a correct comparison of such data must also involve the analysis of the gametogenic status of the mussels.

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TRANSPORT AND TOXICITY OF METAL POLLUTANTS TO MARINE ORGANISMS

by

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A B S T R A C T

Sublethal and lethal toxicity tests of heavy metals on two species of marine invertebrates were performed. In addition, the transport of Cs, Cd and Hg across the isolated gill epithelia, used as a modelling system in the metal transport, was studied. Under the continuous flowing test conditions, lethal toxicity concentration of the methyl mercury ($96hLC_{50}$=0.031 mg l^{-1}) is more than one order of magnitude lower than that for the mercuric chloride ($96hLC_{50}$=1.04 mg l^{-1}). Influxes of the radioactive labeled mercurial compounds and caesium were studied across perfused isolated gill preparations of the shore crabs Carcinus mediterraneus. The results show an increased transfer of methyl mercury over inorganic form across perfused epithelial cells. The ^{137}Cs net transport gill epithelia was from the haemolymph side crossing basolateral and apical surfaces into the bathing medium. The effect of methyl mercury (5 µg Hg l^{-1}) on transbranchial potentials (TBP) and chloride fluxes in isolated perfused Carcinus gill filaments was investigated. Methyl mercury compound had reduced TBP values from -3.6±0.6 mV in the control groups to values close to zero. Chloride fluxes were reduced by inorganic and organic mercurial forms by 57 and 64% of the control group, respectively. After 1-8 days of exposure of the sea urchins Sphaerechinus granularis in the 10 µg Cd l^{-1} sea water continuous flowing system, gametes were isolated and fertilized. Most of them (3-8 days exposure) exhibited delay in developmental sequences, irregular fertilization membrane formation, and malformations in the early prism stage and swimming blastula.

1. INTRODUCTION

In recent years considerable efforts have been directed into the improvement of toxicological methods and our understanding of mechanisms of the interactions of toxic substances with living matter, which is poor in terrestrial and aquatic toxicology. Therefore, our work was centered on studies of transport and effects of metals and their interaction with marine organisms. Furthermore, various sequences of developmental stages of marine organisms have been suggested for testing of a wide range of pollutants in the marine environment.

Effect of the mercuric chloride and methyl mercury chloride on lethal toxicity in the shrimps Palaemon elegans was tested. In addition, the effects of mercuric compounds on chloride fluxes and TBP

(transbranchial potentials) of the isolated perfused gills of the crustacean Carcinus mediterraneus was studied. Transport of caesium in the isolated gill epithelia was investigated. Moreover, a study was undertaken to investigate the effects of cadmium on delay in the fertilization membrane formation, first cleavage time, swimming blastula, early prism stage and late pluteus of the sea urchins Sphaerechinus granularis.

2. MATERIALS AND METHODS

Experiments were performed on isolated gills from the brackish water acclimated crabs Carcinus mediterraneus, collected for experiments from the middle Adriatic region. Sea urchins Sphaerechinus granularis and shrimps Palaemon elegans were collected on the Istrian coast of the Adriatic (Rovinj region).

Tests for the acute lethal toxicity of the mercurial compounds to the shrimps P. elegans were performed according to UNEP (1987) suggestions. For the acute toxicity test shrimps were acclimated for 2 weeks at 20°C and 38 salinity. During the experimental procedure animals were not fed.

The posterior gills of the shore crabs C. mediterraneus were cut off at their base and prepared for perfusion studies. The gills were perfused with diluted sea water (SDW 460 mOsmol l^{-1}; 239 mM Cl^-) in a solution identical to the external bathing solution.

TBP values were measured by a Keithley Instruments 601 electrometer with Ag-AgCl Ingold electrodes. Chloride and caesium fluxes were traced by radioactive isotopes Cl-36 and Cs-137, respectively. Detailed methodological description was addressed by Lucu and Siebers (1986a, 1987). Methyl mercury was dissolved in acetone and mercury chloride in distilled water. In previous experiments acetone was added to the control solution (2 µl/50 ml) and no effect of TBP and chloride fluxes was observed. Under the control condition the TBP values were stable for several hours in the range from -3 to -4 mV (negative polarity referring to the basolateral side).

Sea urchins were kept in running sea water aquaria. Sperms and eggs were collected by the equatorial opening of the shell. Concentrations of gametes in the suspension were from 1 to $5\text{-}10^3$ eggs/ml and from 1 to $3\text{-}10^7$ sperm/ml. The time from the beginning of fertilization to a fully formed fertilization membrane was measured. Sperm suspensions were treated with various cadmium concentrations for 5 minutes; eggs were inseminated and further development after fertilization membrane appearance was blocked by sodium lauril sulfate.

Cadmium concentrations in selected sea urchins developmental phases were determined by a Varian-Techtron AA6 Atomic Absorption Spectrophotometer. The sediment in the vial containing a suspension of eggs was dried and 0.1-0.3 g fresh weight was dissolved in concentrated nitric acid (Merck p.a.) and diluted by redistilled water for atomic absorption.

3. RESULTS AND DISCUSSION

3.1 Acute toxicity tests

Under the static test conditions (renewal once daily) acute toxicity of Hg ($HgCl_2$ form t=20°C) in the shrimps Palaemon elegans was 0.116 mg l^{-1} ($96hLC_{50}$=0.101 to 0.134 mg l^{-1}; lower and upper confidence limits). We have also checked lethal toxicity of the two mercurial compounds in the shrimps at 10°C under continuous flowing test conditions. ($96hLC_{50}$ for $HgCl_2$ was 1.04 mg l^{-1} and for CH_3HgCl was 0.031 mg l^{-1}). According to these results methyl mercury is at least for more than one order of magnitude more toxic compound than inorganic $HgCl_2$.

3.2 Fluxes of mercury and caesium across perfused Carcinus gill preparations; Effects of mercury on TBP and Cl^- fluxes

Influxes of radioactive labeled $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ from the external medium through the apical and basolateral sides of the isolated perfused gills were studied (Table I). Preliminary results have shown an increased transfer of methyl mercury through the membrane barriers of the perfused epithelial gill cells. Hydrophobic methyl mercury forms are expected to be more bioavailable than hydrophilic ($HgCl_2$) ones (Gutknecht, 1981).

Table I

Influx of two labeled mercurial compounds from bathing sea water (DSW) through perfused gills of shore crab Carcinus mediterraneus. The concentration of the added stable isotope Hg in the bathing and perfusion identical solutions was 10 µg Hg^{2+} l^{-1}. The external medium consisted of $^{203}HgCl_2$ or $CH_3{}^{203}HgCl$ of activities 51200 ± 2200 cpm/ml or 53100 ± 2500 cpm/ml, respectively. Each of the three perfusion periods of 30 min. consisted the means ±S.E. of 5 observations.

	1st period	2nd period (cpm/g tissue/h)	3rd period
$^{203}HgCl_2$	5100 ± 450	5123 ± 812	4375 ± 602
$CH^{3203}HgCl$	8200 ± 700	7944 ± 410	6700 ± 350

The effects of two mercurial compounds, $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ on chloride fluxes of the isolated perfused gills of the crustacean C. mediterraneus were studied by Lucu and Siebers (1986a, 1986b).

After addition of 10 µg Hg^{2+} ($CH_3{}^{203}HgCl$ form) on the basolateral membrane side, TBP values were increased from -3.5 mV to a value close to zero. TBP has been described as an active potential generated by an unequal distribution of Na^+ and Cl^+ as a consequence of the active transport processes (Siebers et al., 1985). The effect of Cu on

positively charged potential (polarity in reference to the perfusion side) of the similar magnitude and reversed polarity compared with our results, has been described in the gills of sea water acclimated flounders (Stagg and Shuttleworth, 1982).

Both mercurial compounds inhibited chloride influxes and the values were 57 to 64% of the control (Table II).

Table II

Effect of merucry perfused from the basolateral side of the isolated Carcinus mediterraneus gill preparation on chloride fluxes, $J_{a-->b}$ = flux from the apical (a) to the basolateral (b) side, and transbranchial potentials. The perfusion solution was diluted sea water (360 mmol Cl l^{-1}) identical to the external bathing solution. The values are given as means of 5 observations.

Treatment	Chloride influxes ($Jcl_{a--->b}$; $\mu M\ g^{-1}\ h^{-1}$)	TBP (mV)
Control	245 ± 64	-3.6 ± 0.6
$HgCl_2$ added; 100 µg Hg l^{-1}	139 ± 43	-
CH_3HgCl added; 5 µg Hg^{2+} l^{-1}	158 ± 48	0.3 ± 0.9

Effects of diuretic amiloride (Merck, Sharp Dohme, Munich) on caesium fluxes from the apical (bathing side) to the basolateral-haemolymph side (influxes) and in the opposite direction (effluxes) were studied (Table III). Cs-137 effluxes are greater than influxes, showing that the basolaterally oriented gill side is more permeable to Cs-137 than the apically oriented one. Therefore, the Cs-137 transport factors are larger from the haemolymph side to the bathing side (effluxes) than the fluxes in the opposite direction (Table III). In addition, influxes and effluxes of Cs-137 was inhibited by 0.1 mM amiloride added during the experiment to the bathing solution (Lucu and Jelisavčić, 1988). This relatively high concentration of amiloride showed a similar effect on Na inhibition from the apical side of the gill preparation as reported by Lucu and Siebers (1986a, 1986b). Since Cs have similar physico-chemical behavior to K (Bryan, 1961; Bryan and Ward, 1962; Beauge and Sjodin, 1968) we suggest that Cs competes with amiloride sensitive K effect.

3.3 Cd toxicity tests on the sea urchin embryonic stages

The embryonic development of the fertilized gametes of the sea urchin Sphaerechinus granularis Lam. treated during a 1 to 8 days in 10 µg Cd l^{-1} in the constant flowing toxicological system were studied. Fertilized gametes of the sea urchins previously exposed 3-8 days in 10 µg Cd l^{-1} exhibited a high percentage of malformations irregular fertilized membranes and cleavage formations, abnormal and less movable

Table III

Caesium fluxes through the isolated perfused Carcinus gill epithelia. Mean values of 6 observations ±S.E. TBP = transbranchial potential (mV).

	Transport factors		TBP
	Influxes	Effluxes	
CONTROL	1.65 ± 0.20	6.49 ± 1.04	-2.1
AMILORIDE	0.93 ± 0.11	4.70 ± 0.46	-3.9

blastula stages. In addition, the effect of Cd on the delay in the fertilization membrane formation and further sequences of the early developmental stages (first cleavage and late blastula) was examined. Delay in fertilization membrane formation of the Cd exposed gametes (comparing to the non-exposed Cd gametes) was observed. According to these results, low cadmium chronic exposure of the mature individuals is more harmful to the embryonal development than short-term exposure of the fertilized eggs. Most the effects described here call for further studies during a sea urchin's life cycle. These results were explained in more detail by Lucu and Obersnel (1987).

Sea urchin spermatozoa move in vigorous circles until they take contact with an egg. It is well known that the boring movement of the spermatozoa is a prerequisite for fertilizing of the sea urchin eggs. The movement of the sea urchin and mammalian spermatozoa is influenced by the interaction between the sperm cell components and environmental factors. Therefore, at the beginning, acrosomal tubules of spermatozoa were affected by cadmium in contact with the egg surface. The differences in the swimming activity of the sperms incubated at various concentrations may be the consequence of heavy metal interaction with unspecified enzymatic systems which regulates spermatozoan motility. The inhibition of the choline acetyl transferase (ChAT) system by cadmium could change spermatozoan activity and impair their functioning. According to Dwivedi (1985) cadmium at lower concentration levels can decrease spermatozoan ChAT. The fertilized eggs and their developmental stages were less permeable to cadmium up to the formation of skeletal structures 15 hours after fertilization. Increased cadmium concentrations in the early developmental stages, 15-40 hours after fertilization, were most probably due to cadmium accumulation (or adsorption) into the newly formed skeletal structures.

4. CONCLUSIONS

In the perfused gill preparations of marine crab Carcinus mediterraneus, transport of the serious potential pollutants Cd, Hg and Cs was studied. Furthermore, lethal toxicity tests ($96hLC_{50}$) of the mercury on adult shrimps and low level Cd effects on the sea urchins embryonic stages were investigated.

Table IV

Effects of cadmium on the embryonal development phases of the sea urchins Sphaerechinus granularis Lam. the sea urchins were exposed to 10 µg Cd^{2+} l^{-1}. In various time intervals (1-8 days) the gametes were isolated and fertilized. Percentages of hte normal developed stages ±S.E. proportions in 3 experiments were presented. The time after fertilization is presented in brackets.

Time of exposure to 20 µg Cd l^{-1} (days)	Formed fertilization membrane (3 min.)	First cleavage (1.5 h)	Swimming blastula (10 h)	Early prism stage (24 h)	Late pluteus (48 h)
Control (not exposed)	98.5±0.012	98.3±0.013	98.8±0.015	96.0±0.019	95.5±0.02
1 day	98.6±0.012	98.7±0.011	97.5±0.016	96.4±0.019	93.5±0.25
3 days	67.5±0.047*	91.4±0.028**	92.7±0.026	84.3±0.036**	67.4±0.047*
5 days	42.5±0.049*	79.6±0.04*	81.3±0.039*	51.6±0.05*	38.5±0.048*
8 days		54.3±0.05*	63.6±0.048*	34.4±0.047*	18.7±0.039*

*, ** significantly different from control group at the 1% and 5% levels, respectively.

The fluxes of two radioactive labeled mercurial compounds $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ were studied across the perfused gill preparations of shore crab Carcinus mediterraneus. The results have shown an increased transfer of methyl mercury over the inorganic $HgCl_2$ form across the perfused epithelial cells.

For the shrimp Palaemon elegans under continuous flowing test conditions the methyl mercury is at least for one order of magnitude more toxic ($96hLC_{50}$=0.031 mg l^{-1}) than inorganic $HgCl_2$ form ($96hLC_{50}$=1.04 mg l^{-1}).

Sea urchins Sphaerechinus granularis exposed for 3 to 8 days in 10 ug Cd l^{-1} sea water showed, after fertilization, irregularity in the fertilization membrane formation and delay and malformations in the early developmental stages.

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PERIODICITY AND CAUSES OF IRREGULAR MACRO- AND MICRO-PLANKTON BLOOMS APPEARING IN EUTROPHIED AREAS IN NORTHERN ADRIATIC AND THE GULF OF TRIESTE

by

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A B S T R A C T

The monitoring of macroplankton shows that in the Adriatic sea 9 species of Scyphomedusae and one cubomedusa are present, even if not permanent.

The interaction between jellyfish and fisheries shows that the evolution in the fishing yield (monthly distribution) appears to be influenced by the increase of the jellyfish caught only in some periods. This, however, does not imply that an analysis of daily data might show damage due to the behaviour of the net in the case of Rhizostoma pulmo, whereas damage due to Pelagia noctiluca are mainly of a toxicological-health care nature to man and of a hygiene-veterinary nature as far as the catch is concerned.

The sequence of vitellogenesis as related to the size increase of the oocytes of Pelagia noctiluca (Forskål) (Scyphozoa,Semaeostomeae) was examined to assess the influence of climatic factors on its reproductive period in the central and northern Adriatic sea. Oocytes of all stages were always present in the ovaries of adults down to bell diameter 3.5 cm. Thus, reproduction occurs throughout the year in this area.

1. INTRODUCTION

This project has been set up due to the fact that large blooms of micro- and macroplankton have been observed in some coastal areas of the Adriatic sea. Since 1977, coastal and offshore swarmings of Pelagia noctiluca have been recorded in the Gulf of Trieste, which were more frequent in 1977-1980 and ended in 1986. During July-September 1988 and 1989 microplankton blooms were observed, supported by several species of Radiolaria and Phytoflagellata.

In the initial stage of the research project, the monitoring of macroplankton with the collection of hydroclimatic data in the Gulf of Trieste was carried out. The second step of the research was the analysis of damage caused by macroplankton blooms and the study of the influence of environmental conditions on reproduction using the gonad maturity.

2. MATERIALS AND METHODS

The data on jellyfish occurrence and distribution in the Gulf of Trieste were obtained by:

- monitoring on 4 daily hauls since 1983 from a 14 ton motor trawler with a 200 Hp diesel engine, equipped with a 35 m long, 12.5 m large net, with 40 mm mesh size, owned by the Fishermens' Co-operative Society S. Vito of Marano Lagunare.

- monthly monitoring since 1978 on the coast from Trieste to Grado by researchers of the Department of Biology, University of Trieste, and by experts working under contract for C.I.M.A.M. (qualitative data).

The data on P. noctiluca distribution in the northern and central Adriatic sea were recorded during seasonal cruises of the Laboratory of Marine Biology and Fishery of Fano, as part of the fish stock monitoring programme granted by the Ministry of Merchant Marine. Double oblique hauls were made with a 300-500 µm mesh size FAO net. Specimens with 3.5-5 cm diameter between opposite rhopalia were selected from those collected to avoid excessive variations in the number of oocytes and to avoid comparison with specimens containing immature gonads (with diameter ≥ 3.5 cm) or at the end of their life (with diameter of 6-8 cm). A total number of 60 specimens over a five year period (1981-1985) (1 specimen/month/year) were examined. One ovary was excised from each specimen and cut into two parts. One piece was fixed in 4% neutralized formaldehyde in filtered sea water for an in toto examination with a Zeiss Videoplan videoanalyser. The examination consisted of counting and measuring all the oocytes in a 500 µm wide section of the gonadial ribbon. Diameter sizes were arranged in 10 µm classes from 10 µm to 320 µm.

The second part of each ovary was fixed for 2 h at 5°C in 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.25, made isotonic with sea water (1150 mOsm) by adding 0.35 M NaCl and 2 mM $CaCl_2$, washed in the same buffer solution (0.4 M NaCl) and post-fixed in 1% OsO_4 for 1 h at 5°C. After washing in the buffer, and through rising concentrations of ethanol, the specimens were washed in propylene oxide and embedded in EPON-Araldite. Semi-thin sections were stained with toluidine blue, PAS-green malachite, and methylene-azur blue II-basic fuchsin; ultrathin sections were stained with lead citrate and uranyl acetate.

Hydrological data were taken from the Unità Sanitaria Locale (USL) of Trieste and processed in the Department of Biology.

3. RESULTS AND DISCUSSION

The monitoring of macroplankton show the following results:

The large aggregations of P. noctiluca, present in the Adriatic sea from 1976, are strongly reduced from May 1986. In 1987 and 1988 only few specimens were detected (Avian and Rottini Sandrini, 1988; Rottini Sandrini et al., 1990). Other species, such as Rhizostoma pulmo, coastal scyphomedusa with a scyphystoma stage, is normally present in the Gulf of Trieste (Table I) with a peak in April-June, and a second in August-October. There is often a third peak in January-February. The annual distribution of this species exhibits some fluctuations, perhaps in relation with meteoclimatic factors (Fig. 1) (Del Negro, 1988). In May-June 1989 blooms of Chrysaora hysoscella, a jellyfish uncommon in the Adriatic sea (Del Negro et al., 1990) and Aurelia aurita were recorded in the Gulf of Trieste (Fig. 1 and 2).

In the Middle Adriatic, near the coast, South the Po delta and in the Venice Lagoon another species of jelly-fish was observed in 1987 and 1988, during the warm season: Carybdea marsupialis (Table I) which cause envenomations to the bathers. Its biological cycle is still unknown (Rottini Sandrini and Avian, 1990a).

The collection of hydroclimatic data in the Gulf of Trieste shows a mid-term cycle of the seawater temperature which seems to be related with the bloom period of P. noctiluca (Rottini Sandrini and Avian, 1990b). This research is in progress.

The analysis of damage caused to fishing by macroplankton blooms shows that the presence of large quantities of jellyfish, with their corresponding weight, affects the proper functioning of fishing nets, as already stated (Rottini Sandrini et al., 1984), both blocking and altering the shape of the mouth, as well as increasing the strain on the engine. These observations have been obtained from daily information concerning the quantity of the catch and the monitoring of R. pulmo. In the case of high concentration of these jellyfish in the fishing area, the damage recorded is directly on the working of the net and its efficiency; damage which can mean the loss of a single catch and even the breaking of the net. Such occurrences are, however, infrequent and may be avoided by moving the fishing boat and avoiding the next catches in an area free from jellyfish. The reduction of the catch in a casting, or over the day, does not directly imply a drastic reduction in the average monthly catch. Nevertheless, the occasional loss and/or reduction in the fish yield in a casting, along with the need to redirect the fishing boat in order to find areas free from jellyfish, mounts up to financial losses which are difficult to quantify. In addition, the presence of the large specimens of jellyfish, very different in size to the content of the catch, can cause damage by crushing the commercial fish when the net is hawled in.

In the Gulf of Trieste, contrary to observations by Piccinetti-Manfrin and Piccinetti (1984) in the mid-Adriatic, no direct damage has been recorded owing to the inefficient work of the nets, traceable to the presence of large quantities of P. noctiluca. According to the present study, it is more of a health/poisoning problem for workers during the catch sorting stage, and on the catch itself, with possible consequential changes being recorded after veterinary checks. The fish may eat whole, or part of P. noctiluca or to be stung by them, with a possible resulting modification in their organoleptic property.

Table I

Historical data on the presence in the Adriatic sea of Scyphozoa (from Rottini Sandrini and Avian, 1990b).

A: Carybdea marsupialis; B: Nausithoe punctata; C: Paraphyllina intermedia; D: Chrysaora hysoscella; E: Pelagia noctiluca; F: Drymonema dalmatinum; G: Aurelia aurita; H: Discomedusa lobata; I: Cotylorhiza tuberculata; L: Rhizostoma pulmo.

year	A	B	C	D	E	F	G	H	I	L
1790	-	-	-	-	?	-	-	-	-	-
1830	-	-	-	-	+++?	-	-	-	-	-
1837	-	-	-	-	-	-	+	-	-	-
1838	-	-	-	-	-	-	+	-	-	-
1843	-	-	-	-	+?	-	-	-	-	-
1874	-	-	-	+	-	-	+	-	-	-
1875	-	-	-	+	-	-	+	-	+	+
1877	-	-	-	-	-	-	-	+	-	-
1879	-	-	-	-	+	-	-	-	-	-
1880	-	-	-	-	-	-	+	-	-	-
1881	-	+	-	-	-	-	-	-	-	-
1884	-	-	-	+	-	+	+	-	+	+
1895	-	-	-	-	+	+	-	-	-	-
1896	-	-	-	-	-	-	+	-	-	-
1899	-	+	-	+	-	-	+	-	+	+
1900	-	+	-	+	-	-	+	+	+	+
1901	-	+	-	-	-	-	+	+	-	+
1902	-	+	-	+	-	-	+	+	+	+
1903	-	-	-	-	-	-	-	-	-	+
1904	-	-	-	-	-	-	-	+	-	-
1905	-	-	-	+	-	-	-	-	-	-
1908	-	-	-	+	-	+	+	+	+	+
1909	-	-	-	+	-	-	+	+	-	+++
1910	-	-	-	+	+	+	+	+	-	+
1911	-	-	-	+	+	-	+	+	+	+
1912	-	-	-	-	-	-	+	-	-	-
1913	-	+	-	-	+	-	-	-	-	-
1914	-	-	-	-	+	-	-	-	-	-
1915	-	-	+	-	+	-	+	-	+	+
1921	-	-	-	+	-	-	-	-	+	+
1922	-	+	+	-	+	+	-	-	-	+
1931	-	-	-	-	-	+	-	-	-	-
1940	-	-	-	-	-	+	-	-	-	-
1945	-	-	-	-	-	+	-	-	-	-
1952	-	-	-	-	-	-	+	-	-	-
1953	-	-	-	-	-	-	+	-	-	-
1962	-	-	-	-	-	-	+++(2)	-	-	-
1976	-	-	-	-	+	-	-	-	-	+*
1977	-	-	-	-	++	-	-	-	-	+*
1978	-	-	-	-	+++	-	-	-	-	+*
1979	-	-	-	-	+++	-	+*	-	-	+*
1980	-	-	-	-	+++	-	+*	-	+*	+*
1981	-	-	-	+*	+++	-	+*	-	+*	+*
1982	-	-	-	++*	+++	-	+*	+*	+*	++*
1983	-	-	-	-	+++	-	+*	+*	+*	+++*
1984	-	-	-	-	+++	-	+*	+*	++*	++*
1985	-	-	-	+*	+++	-	+*	+*	+*	+*
1986	+	-	-	-	++	-	+*	-	+*	+*
1987	+(1,2)	-	-	+*	+*	-	+*	-	+*	+*
1988	+(1)	-	-	+*	+*	-	+*	-	+*	+*
1989	-	-	-	++*	+*	-	+++*	-	-	+*

+: few or single specimens; ++: several specimens; +++: very high number of specimens; *: Author's personal observations; ?, doubtful calssification. (1): Piccinetti, 1987, 1988, unpublished observations. (2): Vio, 1962, 1987, unpublished observations.

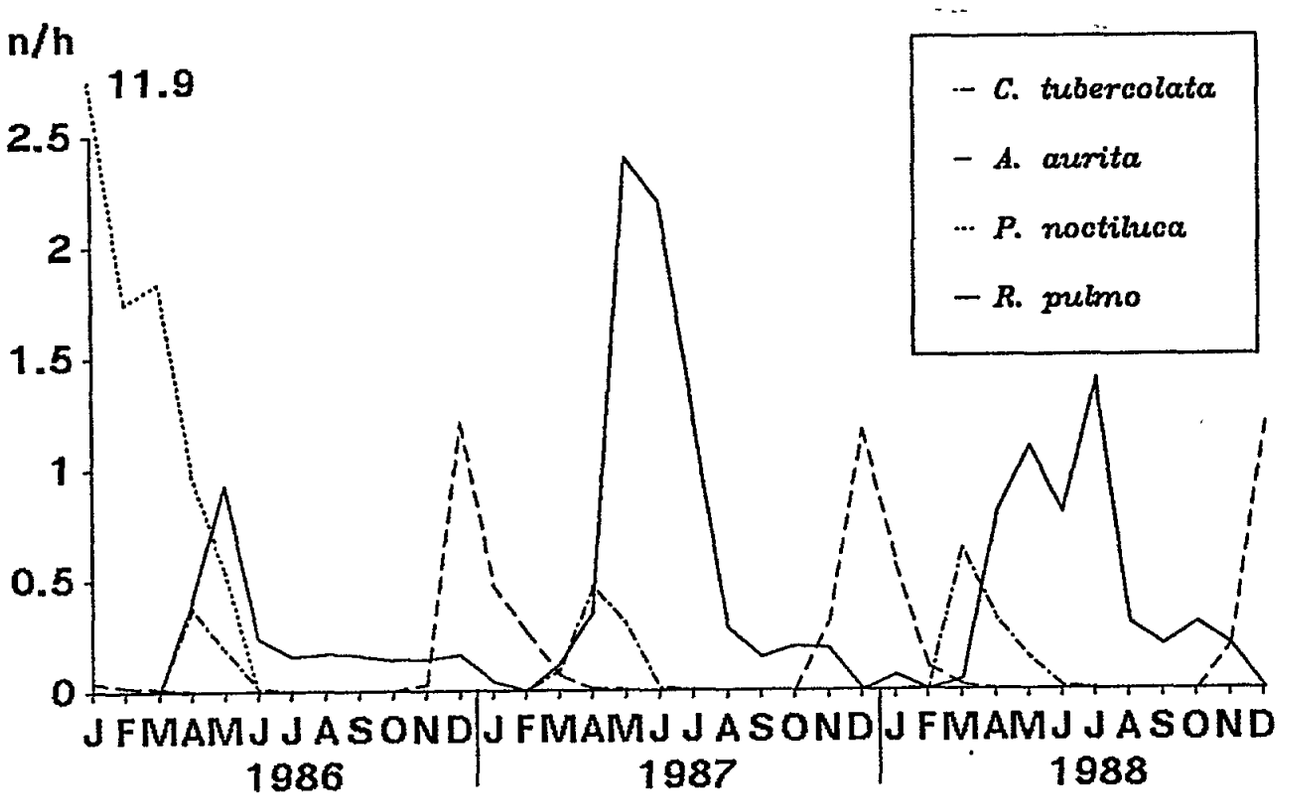

Fig. 1 Monthly distribution of R. pulmo, C. tubercolata, P. noctiluca and A. aurita (from Del Negro, 1988)

In both cases, therefore, fishermen may be faced with financial losses: the need to recast, change fishing area, as well as the inability to sell all their fish if affected by jellyfish, with the consequent need to sell them for other uses (eg. fish meal), causes more or less significant loss (Giorgi et al., 1985; Rottini Sandrini et al., 1986).

The histological analysis of the ovaries of P. noctiluca, carried out in order to see the seasonal influence on the reproduction shows that there are always ovocytes in all the stages of development. Accordingly, P. noctiluca in the Adriatic sea reproduces throughout the year. The seasonal abundance of the ovocytes in a gonad is, on the contrary, variable, with a minimum in summer and two peaks during spring and autumn (Fig. 3). This quantitative distribution is related to the seawater temperature, to the metabolic rate of P. noctiluca and to the food availability in the examined area (Avian et al., 1991; Giorgi et al. 1991; Rottini Sandrini and Avian, 1990a).

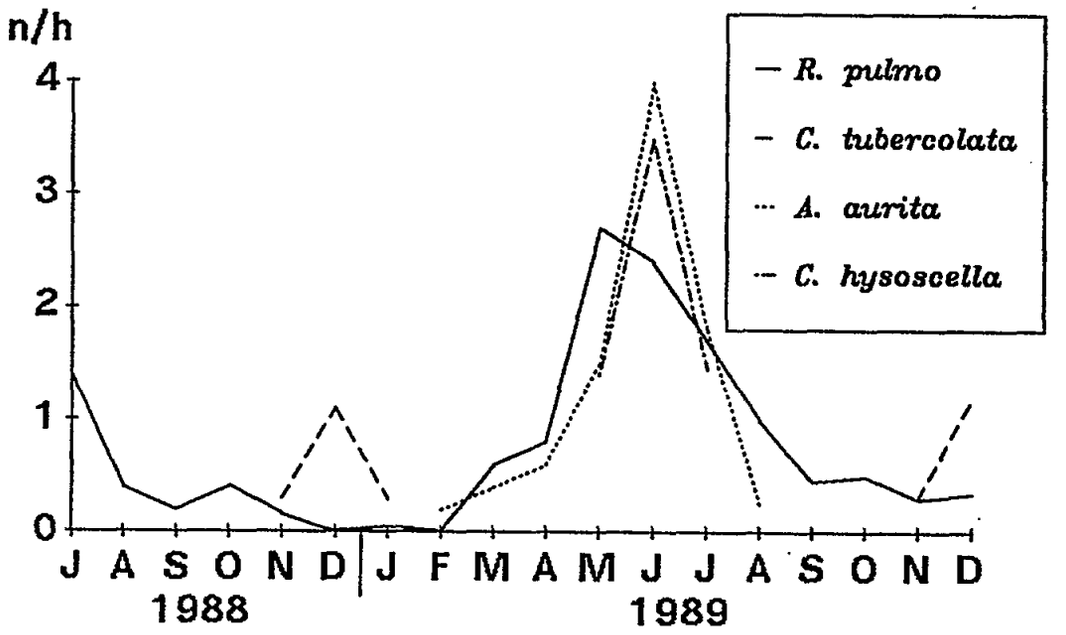

Fig. 2 Monthly distribution of R. pulmo, C. tubercolata, P. noctiluca and A. aurita (from Del Negro et al., 1990)

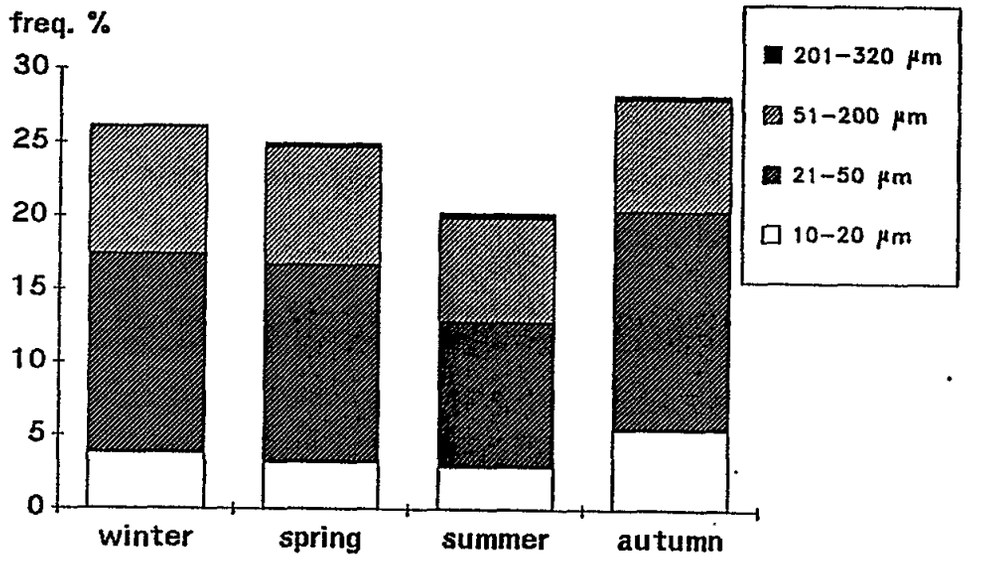

Fig. 3 Seasonal size distribution of the oocytes of P. noctiluca. Four diameter classes. Each column represents the total (100 %) frequency for one season. The sum of the four columns represents the total (100 %) frequency of the oocytes counted and measured (from Rottini Sandrini and Avian, 1990a)

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TESTING THE REFERENCE METHOD FOR THE ANALYSIS OF DDTs AND PCBs IN MARINE ORGANISMS

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A B S T R A C T

A testing of the UNEP/FAO/IOC/IAEA Reference Method No. 14 "Determination of DDTs and PCBs in selected marine organisms by packed column gas chromatography" was carried out following the procedure as closely as possible with the aim of evaluating the recovery, precision and accuracy of the method and to identify the major sources of variability. From the results it appears that a good level of recovery and precision may be obtained when the concentrations of organochlorine residues in marine biota are relatively high, but the precision falls for lower concentrations. The lack of marine samples of certified organochlorine residue concentrations accounts for the general difficulty in evaluating the accuracy of the analytical method in our own laboratory. An intercomparison exercise between laboratories which use the Reference Method is suggested.

1. INTRODUCTION

In June 1984, an expert consultation meeting was held at FAO headquarters to: (i) discuss the results of a testing exercise carried out in the years 1983 and 1984 by selected laboratories of the Mediterranean area; (ii) to review and revise the Reference Methods in the light of these results and of new developments in methodologies and analytical instrumentation.

Since considerable modifications of Reference Method No. 14 (UNEP/FAO/IAEA, 1982) had been suggested, a new draft issue was prepared by UNEP in co-operation with FAO, IOC and IAEA (1985). The revised version of Reference Method No. 14 was tested in our laboratory and the results are discussed below.

2. MATERIALS AND METHODS

All reagents listed in the Reference Method No. 14, Rev. 1 (UNEP/FAO/IOC/IAEA, 1985) were available at the Institute of Marine Biology, Venice, or were easily purchased from local suppliers, with the notable exception of internal standards 1,1-dichloro 2,2-diphenylene, 2,5,2′,6′-tetrachlorobiphenyl, Aroclor 1254 and Aroclor 1260 of certified composition. Of the suggested internal standards, only 2,5,2′,6′-tetrachlorobiphrnyl was found and bought from Foxboro/Analabs, USA. A solution of 2,4,5-trichlorobiphenyl, obtained from the International Laboratory of Marine Radioactivity (ILMR), Principality of Monaco, was also used as internal standard.

Standard stock solutions were prepared by dissolving 100 mg of each compound (HCB, alpha HCH, gamma HCH, aldrin, dieldrin, pp'DDE, pp'DDD, pp'DDT) and of Aroclor 1254 and Aroclor 1260 in 100 cm^3 iso-octane; by appropriate dilution of the stock solutions, working solutions were prepared on a bimonthly basis.

Composite samples of mussel, anchovy and sardine tissues, collected from the Gulf of Venice and prepared according to guidelines given in UNEP/FAO/IAEA (1984), were Soxhlet extracted for eight hours with n-hexane. Subsamples of 20 g fresh tissues, blended with 60 g anhydrous sodium sulphate, or 5 g freeze-dried tissues were used so that no more than 1 g fat was extracted.

The extracts were evaporated under vacuum to 5 cm^3. Extractable organic matter (EOM) was determined by weighing the residue after evaporating 1 cm^3 extract to constant weight. Extracts were then shaken with concentrated sulphuric acid to remove interfering lipids, reduced to 0.5 cm^3 and applied to a silica gel micro-column, which was eluted with 7 cm^3 n-hexane (fraction 1) and then with 10 cm^3 25% diethyl ether in n-hexane (fraction 2). Fraction 1 contained HCB, pp'DDE and PCBs. Fraction 2 contained HCH isomers, pp'DDD and pp'DDT. The eluates were then concentrated to 1 cm^3 for gas chromatographic analysis.

Analyses were carried out using a Carlo Erba FV 2351 gas chromatograph equipped with a Ni-63 electron capture detector. A GC glass column, 200 x 0.4 cm i.d., packed with 5% OV-101 on Chromosorb W AW-DMCS, was prepared according to the recommended procedure. Gas chromatographic conditions were: injection port temperature 225°C, oven temperature 200°C, detector temperature 250°C. Nitrogen was used as the carrier gas at a flow rate of 60 cm^3 min^{-1}. The retention time of pp'DDT was between 14 and 15 min. The number of theoretical plates, calculated for pp'DDT, was between 2,800 and 3,300 for every set of analyses.

Identity of organochlorines was assumed from their appearance in the appropriate silica gel column fraction and from their retention time. The presence of pp'DDT and pp'DDD was also confirmed by dehydrochlorination to pp'DDE and pp'DDMU with alcoholic potassium hydroxide in selected samples.

Blanks were run for the entire procedure to control the background contamination introduced by reagents and equipment. To keep blank values as low as possible, i.e. below 0.01 ng μl^{-1} for HCB, HCH and DDTs and 0.1 ng μl^{-1} for PCBs, all glassware was treated with hot detergent solution, left to stand overnight, thoroughly rinsed with tap water, distilled water and acetone and dried at 220°C for several hours.

3. RESULTS AND DISCUSSION

The method was detailed enough and written in such a clear form that we did not find any problems in following the instructions step by step.

Preliminary to the analysis of organism tissues, it was necessary to control the GC column performance, the detection limit and the linear range for the ECD of our gas chromatograph. Then the instrument precision was estimated from repeated determinations of standard solutions. The peak pattern of eight organochlorine pesticides for the OV-101 column (theoretical plates number 3,050) is presented in Figure 1 to show the chromatographic resolution for the pesticides of interest, while detection limits and linear range for ECD and instrument precision are presented in Table I.

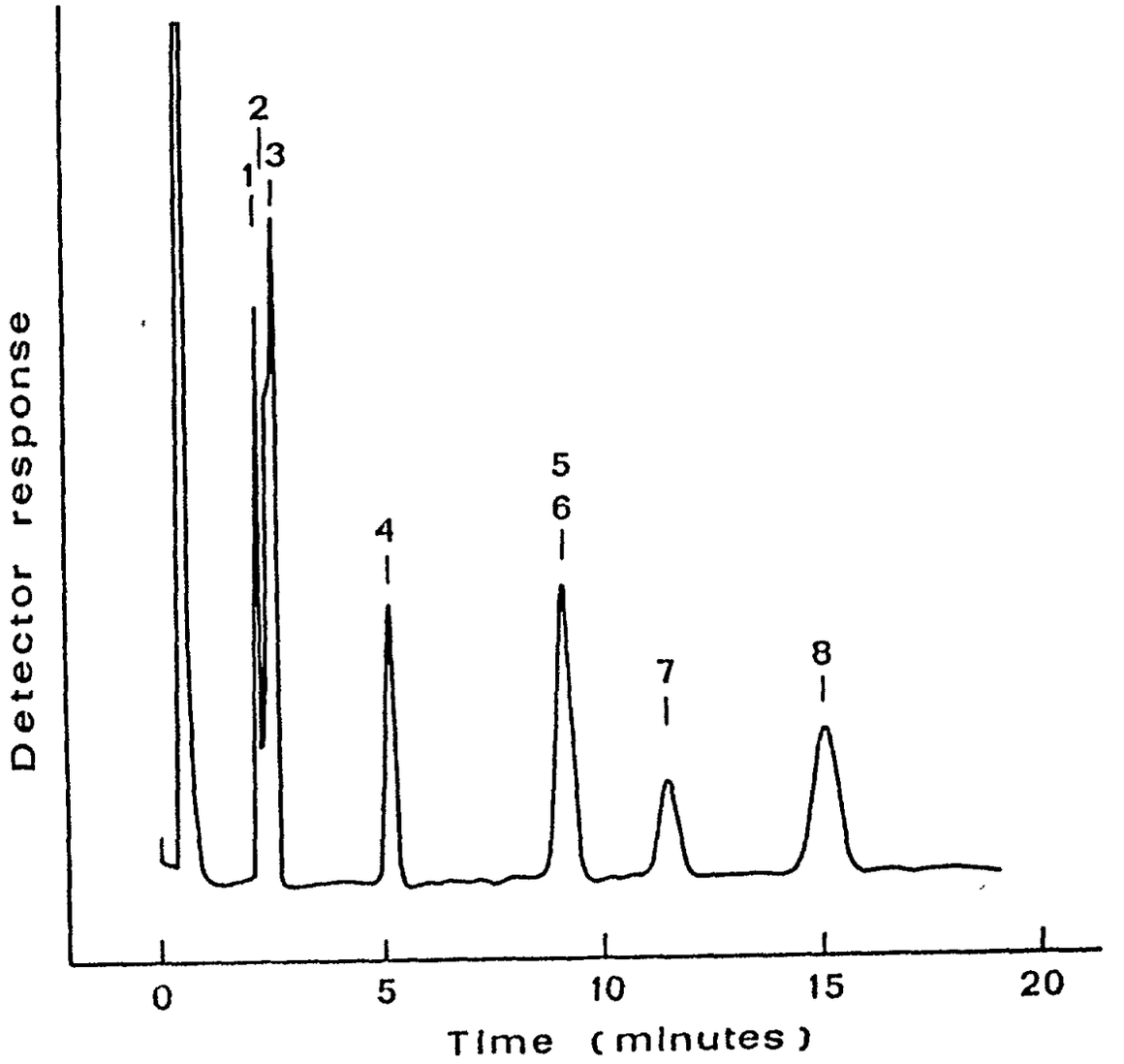

Fig. 1 Isothermal separation at 200°C of eight chlorinated pesticides on OV-101: 1. α-HCH, 2. HCB, 3. γ-HCH, 4. Aldrin, 5. Dieldrin, 6. pp'DDE, 7. pp'DDD, 8. pp'DDT

Table I

Testing of the gas chromatograph performance.
SD: standard deviation of five replicate injections.

Organochlorine compound	Detection limit (ng)	Linear Range (ng)	Instrument level (ng)	Precision % SD
HCB	0.005	0.005 - 0.200	0.100	1.5
α-HCH	0.005	0.005 - 0.200	0.100	1.9
γ-HCH	0.005	0.005 - 0.200	0.100	1.7
pp'DDE	0.010	0.010 - 0.600	0.100	2.0
pp'DDD	0.015	0.015 - 0.900	0.200	1.8
pp'DDT	0.020	0.020 - 1.00	0.200	2.1
PCB (Ar.1254)	0.200	0.200 - 10.0	5.00	1.8
PCB (Ar.1260)	0.200	0.200 - 10.0	5.00	2.8

The precision of the entire analytical procedure was estimated by the usual statistical methods (Snedecor and Cochran, 1967), extracting five subsamples from the same well homogenized sample of freeze-dried mussel tissues as well as carrying out in duplicate the analyses of composite samples of mussels, anchovies and sardines. The results are reported in Table II. If a relative standard deviation of ± 20% of the mean is considered to be an acceptable range of reproducibility for repetitive analyses of organochlorine residues in environmental samples, it appears that this level of precision may be easily obtained when the concentrations are relatively high, but the precision falls for lower concentrations.

Table II

Precision of the entire analytical procedure.
Concentrations expressed in ng g^{-1} wet weight.
SD: standard deviation of five repetitive subsamples.

Organochlorine compound	Mussels		Sardines		Anchovies	
	conc.	SD	conc.	SD	conc.	SD
HCB	0.15	0.16	1.47	0.82	0.86	0.37
α-HCH	0.11	0.20	0.49	0.31	0.32	0.23
γ-HCH	0.23	0.21	0.64	0.23	0.48	0.36
pp'DDE	1.21	0.44	18.30	1.90	12.70	1.8
pp'DDD	0.82	0.27	5.46	1.10	3.37	0.78
pp'DDT	0.52	0.25	3.42	0.58	3.10	0.80
PCB (Ar.1254)	17.60	1.56	-	-	-	-
PCB (Ar.1260)	-	-	209	11.0	182	8.5

As soon as 2,4,5-trichlorobiphenyl and 2,5,2',6'-tetrachlorobiphenyl became available, a recovery test was carried out for each organism analysed. To this end, two composite samples were made up using 80 mussels and 50 anchovies, respectively. Fresh tissues (soft parts of mussels and fillets of anchovies without skin) were blended and carefully homogenized. An aliquot of 150 g of fresh homogenate was freeze-dried. For the two matrices, five subsamples of fresh homogenate and five subsamples of freeze-dried homogenate were Soxhlet extracted after adding 1.0 ng of 2,4,5-trichlorobiphenyl and 2,5,2',6'-tetrachlorobiphenyl and analysed through the entire procedure. The results, shown in Table III, are quite satisfactory either as recovery or reproducibility for the PCB fraction; however, the recovery test did not include the compounds eluting in the DDT fraction, due to the lack of an appropriate internal standard.

Table III

Results of the recovery tests. Five subsamples analysed for each set of analysis.

Biota	Matrix	2,4,5-trichloro-biphenyl		2,5,2',6'-tetra-chlorobiphenyl	
		% recovery	SD	% recovery	SD
Mussels	fresh homogenate	91.6	3.6	92.0	4.2
	freeze-dried homogenate	94.8	4.5	93.8	4.0
Anchovies	fresh homogenate	90.4	3.5	90.9	4.7
	freeze-dried homogenate	92.1	4.3	94.5	3.8

Since marine samples of certified organochlorine residue concentration were not available, the accuracy of the analytical method was checked and compared with the accuracy obtained by other laboratories participating in an intercalibration exercise organized by ILMR, Principality of Monaco. A preliminary report on the intercalibration exercise of organochlorine compound measurements on shrimp homogenate and fish homogenate was distributed by IAEA (1987). A comparison of our results with the means and standard deviations given in that report, after rejecting outlying results by Chauvenet's and Dixon's tests, is shown in Tables IV and V. Our results generally fit in one standard deviation range, with the exception of pp'DDE which is within two standard deviation range; this means that our analytical performance does not differ significantly from that of the world-wide participating laboratories; however, the small number of reported results and their rather large dispersion reduce the possibility of estimating the "true values", and consequently the accuracy of our determinations.

The method of quantification of PCBs, adopted in this work, is based on peak height comparison between the Aroclor standard and the residue. Total height of peaks eluting later than pp'DDE has been used in an attempt to obtain the best average ECD response per weight for both the sample residue and the reference standard. Based on a paper of Sawyer (1978), the Reference Method No. 14, Rev. 1, recommends a quantification procedure based on individual ECD peak comparison

Table IV

Comparison of our results with means (X) and standard deviations (SD) of an intercalibration exercise organized by IAEA (1987). Organochlorine compound concentrations in shrimp homogenate (MA-A-3/OC) are expressed in ng g^{-1} dry weight).

Organochlorine compound	Our results	after Chauvenet's test		after Dixon's test	
	X	X	SD	X	SD
HCB	0.4	0.58	0.4	0.3	0.14
γ-HCH	0.2	2.8	4	2.8	4
pp'DDE	9.7	5.6	3.1	5.6	3.1
pp'DDD	0.2	0.67	0.82	0.67	0.82
pp'DDT	0.35	1.8	2.5	1.8	2.5
PCB (Ar.1254)	4.9	12	7	26	24
PCB (Ar.1260)	4.55	12	15	5.1	3

Table V

Comparison of our results with means (X) and standard deviations (SD) of an intercalibration exercise organized by IAEA (1987). Organochlorine compound concentrations in fish homogenate (MA-B-3/OC) are expressed in ng g^{-1} dry weight).

Organochlorine compound	Our results	after Chauvenet's test		after Dixon's test	
	X	X	SD	X	SD
HCB	1.95	1.4	0.72	1.4	0.72
γ-HCH	0.6	3.4	4.6	3.4	4.6
pp'DDE	75.5	180	94	180	94
pp'DDD	72.5	46	25	46	25
pp'DDT	70.0	66	42	66	42
PCB (Ar.1254)	848	500	210	760	580
PCB (Ar.1260)	695	480	190	480	190

between the residue peaks and peaks of identical retention times of an Aroclor reference standard, in which each individual Aroclor peak is a known weight percentage of the total. Thus, the need for an Aroclor standard of known composition in terms of weight percent of individual peaks is apparent, as is the need for uncommon internal standards listed in the Reference Method.

4. CONCLUSIONS

From the testing it appears that the instrument variability represents only a minor fraction of the variability of the entire analytical procedure.

An acceptable reproducibility (standard deviation of ± 20%) may be easily obtained by an experienced laboratory for replicate analyses of relatively high concentrations of organochlorine residues in marine biota, but the precision falls when low concentrations of organochlorine residues have to be analysed.

The lack of marine samples of certified organochlorine residue concentrations accounts for the general difficulty of evaluating the accuracy of the analytical method in own laboratory. In this respect, an intercomparison run between laboratories which use the Reference Method would be useful before participating in an intercalibration exercise between laboratories using different analytical procedures.

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