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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

FINAL REPORTS ON RESEARCH PROJECTS DEALING WITH EUTROPHICATION AND HEAVY METAL ACCUMULATION

RAPPORTS FINAUX SUR LES PROJETS DE RECHERCHE RELATIFS A L'EUTROPHISATION ET A L'ACCUMULATION DES METAUX LOURDS

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This volume is the one-hundred and fourth 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 cent-quatrième numéro de la série des Rapports techniques du Plan d'action pour la Méditerranée.

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

PREFACE

The 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 and 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 and appropriate allocations of resources.

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.

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.

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.

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

- 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);

- 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 contains the final reports on research projects dealing with eutrophication and heavy metal accumulation. 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.

PREFACE

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.

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.

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.

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 relatifs à l'eutrophisation et à l'accumulation des métaux lourds. 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.

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EUTROPHICATION PROCESSES AND ALGAL BLOOMS (RED-TIDES) IN IZMIR BAY

by

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ABSTRACT

Phytoplankton bloomings as well as physical and chemical characteristics of the water column were investigated spatially and temporally over a three-year period (1990-1992) in the eutrophic-hypertrophic "bloom sensitive" zones of the bay of Izmir.

The blooming species and their successions as well as the environmental parameters and their potential role on colour-tides were studied. Multiple regression models, principal component analysis (PCA) and multidimensional scaling (MDS) were used to calculate which factors were more effective during blooming seasons. It was observed that multiple interacting of soluble inorganic phosphate and nitrogen with physical factors such as light and temperature, in proper combination, led to the development of dinoflagellate or diatom dominated blooms. When phosphate levels in the water column were adequate, A. minutum, P. micans, and S. trochoidea dominated the dinoflagellate red-tides observed. On the contrary, diatoms such as T. allenii, T. anguste-lineata, the euglenoid E. gymnastica and the prasinophyte P. propulsum formed primarily light and temperature dependent bloomings in nitrogen rich waters. These results support that rapid changes of phosphate and nitrogen concentrations in eutrophic or hypertrophic shallow waters due to domestic sewage effluents may cause abnormal phytoplanktonic succession and fluctuations in primary production. The relationship between eutrophication processes and unusual frequent algal blooms is probably a common feature of shallow bays and needs to be incorporated into concepts of water quality management and physiological-ecological characteristics of nuisance bloom taxa.

1. INTRODUCTION

Red-tides and other noxious algal blooms dominated by toxigenic phytoplanktonic species have attracted increasing attention worldwide especially since the 1980's. In addition to neurotoxic, paralytic and diarrhoeic shellfish poisoning, sometimes only anoxia has caused mass mortalities of many marine consumers during these toxic and/or non-toxic blooms (Steidinger, 1983; Wyatt, 1990). These blooms have constituted a risk factor both as a threat to public health and aquaculture in sub-tropical regions such as Izmir Bay on the eastern coast of Aegean Sea (Jacques and Sournia, 1979; Montresor et al., 1990). Although the impact of red-tides

on some fish species has been documented for the region since the 1950's (Nümann, 1955; Acara and Nalbanto–lu, 1960; Büyükìºìk and Koray, 1984; Koray and Büyükìºìk, 1988; Koray, 1984, 1987, 1988, 1992a, 1992b; Koray et al., 1992a, 1992b), little is known about which species are responsible and how the primary and secondary ecological factors influence "colour-tide" communities.

The objectives of the present study were to identify the blooming species and their successions, to determine environmental factors affecting the dimensions of bloomings and, based on their concentrations, to estimate their potential roles in the red-tide communities. A multiple regression model was constructed to simulate the variation of phytoplankton concentration as chlorophyll a resulting from the combined effects of eutrophication.

2. MATERIALS AND METHODS

This study was conducted in the inner part of Izmir Bay (Lat. 38E20´- 38E42´N, Long. 29E25´ - 27E10´E) in which red-tides were observed (Fig. 1). This site was chosen because frequent bloomings occur in this region. The tidal amplitude is 0.2 m over this part, and the current velocities are 0.02-0.5 m sec^{-1}. The mean depth changes substantially towards the south but the observed maximum was 13 m. Sampling took place during the blooming period, between March and July. Bi-weekly or three-weekly visits to the four stations were carried out to obtain a detailed time series. Sampling, during red-tides, was not restricted only to this part of the bay but the whole expansion area was detected.

Water samples were collected from 0.5, 2.5, 5.0 and 10.0 m depth between 0900 and 1200 hours with a Hydro-Bios universal series water sampler (1.5 liter) from all four stations. To obtain the detailed vertical variability structure of the environmental factors and biological activity, station 3 was also sampled vertically with 0.5 m intervals down to 7.0 m with a portable Cole-Parmer Masterflex peristaltic sampling pump equipped with 15.0 m teflon tubing. The samples were fixed by neutral formaldehyde (2-4% final concentration) for biological investigations. For chemical analyses, 1 liter of seawater from each sampling depth was stored in polyethylene bottles in the dark and cool carrying boxes. Temperature and light were determined in situ. Inorganic nitrogenous nutrients, silica, phosphate, pH, salinity, Chlorophyll a and phaeopigment determinations were done in the laboratory according to the procedures of Strickland and Parsons (1972). Chlorophyll b and Chlorophyll c, total carotenoids and particulate organic carbon concentrations were also determinated during the 1991 sampling period.

For the determination of Fe, Zn and Cu content of phytoplankton cells, water samples were filtered through GF/C glass filters. The samples on the filter were dried at 110EC for a night period and were demineralized using wet ashing with $HClO_4$ - HNO_3 (1:5) and then analyzed by flame Atomic Absorption Spectrophotometry (Varian 1250). The same procedure was used for GF/C filtered, APDC-Chloroform extracted sea water.

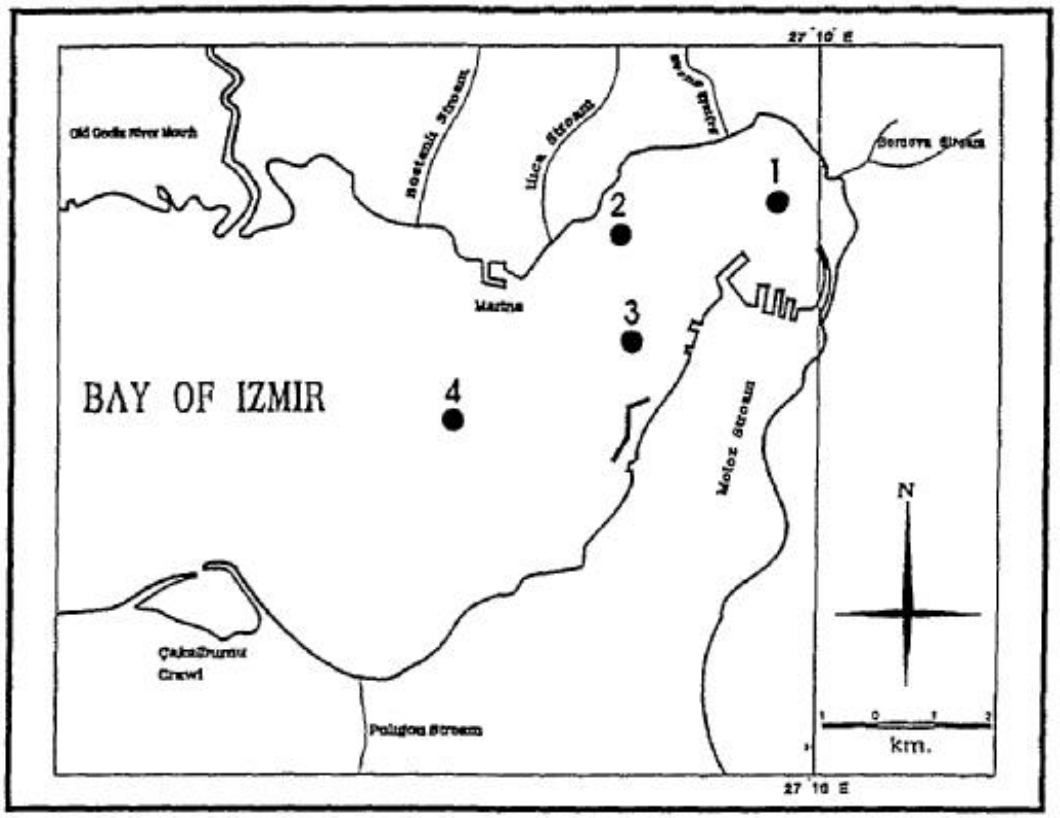

Fig. 1 Sampling locations in Izmir Bay

The general procedure for the measurement of primary productivity using light and dark bottles was as follows. A water sample from each depth of 0.5 m, 2.5 m and 5.0 m was collected between 09:00 and 09:30 hours, oxygen concentrations were determined, samples were placed into three light and dark BOD bottles, incubated in situ attached to a free floating drifter for 60 minutes. Incubations were stopped at the end of this period and final dissolved oxygen concentrations were determined. Primary productivity was calculated according to Strickland and Parsons (1972).

Cell abundances were estimated with Naubauer and Sedgwick-Rafter counting chambers after one week sedimentation of 500 cc. biological samples. The supernatant was eliminated by reverse siphon unit and samples were concentrated to 1.0-5.0 ml final volume gradually, in order to obtain convenient volume for counting procedures. Preferably, 1.00 ml final volume were used for counting for each chamber.

Species determinations were done at the Stazione Zoologica Anton-Dohrn during the Advanced Phytoplankton Course, 1990, UNESCO (Hasle and Syversten, 1990; Steidinger and Tangen, 1990; Heimdal, 1990; Throndsen, 1990).

Statistical analyses were performed using the PRIMER software package (FAO/IOC/UNEP, 1988).

To determine which element (N or Si) was the single nutrient limiting, F_n values were derived from the Monod (1942) equation;

$$F_d=P_m(N/K_s+N)$$

where P_m was the daily maximal production at optimal light from the light saturation curve, K_s (µmol l^{-1}, in this case µg-at l^{-1} =µmol l^{-1}) was the half-saturation constant. For nutrient limited growth, limiting factors were calculated as;

$$F_n=N/(K_s+N)$$

where F_n is the unitless fraction reflecting the degree of limitation of a particular nutrient, N is the nutrient concentration (µg-at l^{1}) and K_s as above. The nitrogen half-saturation constant (K_s) was accepted as 0.8 µmol l^{1} and temperature- dependent silicate half-saturation constant was

K_s=0.33 for T˜20.0 and K_s=2.5 for T>20.0 for general interpretations at community level (Keller, 1989).

In this study, an empirical multiple regression model was also used to develop a predictive equation between dependent variable Chl a, and the other variables including temperature, salinity, daily light, dissolved nitrogenous nutrients, orthophosphate and silica that were thought to be independent but potentially important to phytoplankton production.

The conventional equation is,

$$\hat{Y} = a + b_{Y1} . X_1 + b_{Y2} . X_2 + ... + b_{Yk} . X_k$$

Standardization of the dependent and independent variables were required to eliminate the effect of differences in measurement scale, to show the relative standardized strengths of the effects of independent variables on the Chl a and stabilize the variance (Sokal and Rohlf, 1981). For this purpose the variables were transformed to standard deviates,

$$y' = \frac{Y-\bar{Y}}{s_y} \qquad \text{and} \qquad x_j' = \frac{x_j-\bar{x}_j}{s_{xj}}$$

by subtracting the means and dividing by the standard deviation.
The standardized multiple regression was given as,

$$\hat{Y}' = b'_{Y1} . x_1' + b'_{Y2} . x_2' + ... + b'_{Yk} . x'_k$$

Here the coefficient b'_{Yj} were ß weights.

For the prediction equation, $\log_{10}$ transformation was preferred to simplify conversion to original measurement units and to normalize the data. The stepwise selection procedure was used to obtain an estimate of the regression coefficients and significant relationships. The standardized data were also used for PCA.

3. RESULTS AND DISCUSSION

During the 1990-1992 blooming season, fifty species of marine phytoplankton, representing seven different classes were identified (Table 1). A. minutum, G. simplex, N. scintillans, P. micans, P. triestinum, P. steinii, S. trochoidea, E. huxlei, T. allenii, T. anguste-lineata, E. gymnastica and P. propulsum were the species causing blooms most frequently. The most pronounced and widespread blooms were caused by P. micans and N. scintillans although A. minutum, G. simplex and S. trochoidea also occurred in the community. The huge pink-orange patches of N. scintillans were observed at the inshore and offshore localities between March and June and spread out the entire bay by wind. Coccolithophorides, diatoms and euglenoids were also associated with different types of "colour-tides" in Izmir Bay. On several occasions, E. huxlei caused "milky sea" appearance especially in inshore localities. During the monospecific and mixed blooms of N. closterium, P. pungens, T. allenii, T. anguste-lineata, E. gymnastica and P. propulsum seawater were strikingly discoloured to "bright-green".

Consistent monthly differences between some blooming species were observed; dinoflagellates generally reach their maxima in the middle of spring (March-April), whereas blooms of E. huxlei, T. allenii, E. gymnastica and P. propulsum occurred in late spring (May) or early summer (June) in warmer periods.

In Figs. 2-17, spatial and temporal patterns of the physico-chemical parameters are summarized for the 3-year period.

The temperature in the Izmir Inner Bay ranged from 14.5-25.6EC during the whole red-tide season of 1990. For time scales of two or three weeks, there was marked variations in temperature stratification (Fig. 2). The most pronounced discrepancies among the sampling depths were observed on 27 March, 16 May and 7 June with a maximum of 3.7EC (7 June, at station 2). Similarly, a slight temperature stratification was also discernable during the 1991 blooming season. During this period, a difference of 4.7EC was detected between surface and deep waters (4 June) but in the spring of 1992 the bay was almost completely mixed (max. range 1.2EC). However, the temperature stratification was not as distinct as during the 1983 toxic bloom (A. minutum) when a difference of 6.7EC was observed (Koray, 1984).

In 1990, the lowest salinity was observed during the second week of May and the highest just one week before and that was due to heavy rain. The gradient from 0.5 m to 10.0 m was small and deep water was a little denser (max. range 1.12‰). Similar vertical patterns were observed for temperature suggesting that the water column was relatively well mixed during the blooming period of 1990 (Fig. 3).

Table 1

Blooming species of phytoplankton in Izmir bay (Aegean Sea) during 1990-92. (without asterisk ≤100, * 10^2-10^3, ** 10^3-10^5, *** >10^5 cells l^{-1})

		1990								1991							1992		
SPECIES	Days	23	4	12	19	2	9	16	7	2	9	25	1	9	4	13	21	5	2
	Months	3	4	4	4	5	5	5	6	4	4	4	5	5	6	6	4	5	6
CYANOPHYCEAE																			
*** Oscillatoria sp.		-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-
DINOPHYCEAE																			
*** Alexandrium minutum		-	+	-	-	+	+	+	-	-	+	-	-	-	-	-	+	+	-
* Ceratium furca		-	+	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
* C. fusus		+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	+	-
* C. kofoidii		-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
C.tripos var.atlanticum		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
Ebria tripartita		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
*** Gymnodinium simplex		+	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-
* Gyrodinium spirale		-	-	+	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-
** Noctiluca scintillans		+	+	+	+	+	+	+	-	-	-	+	+	-	-	+	+	+	+
*** Oxytoxum scolopax		-	+	-	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+
Oxytoxum sp.		-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Polykrikos kofoidi		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
* Peridiniopsis rotunda		-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
*** Prorocentrum micans		+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
*** P. minimum		+	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
P triestinum		-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-	-
Protoperidinium claudicans		-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
* P. divergens		-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
P. leonis		-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
** P. longipes		-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
*** P. steinii		-	-	-	+	+	+	-	-	+	+	+	-	-	-	-	-	+	-
Pyrophacus horologium		-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
*** Scrippsiella trochoidea		+	-	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-
PRYMNESIOPHYCEAE																			
*** Emiliania huxlei		-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+
CHRYSOPHYCEAE																			
*** Bicosoeaca mediterranea		-	-	+	-	-	-	+	-	-	-	-	+	+	+	-	-	+	-
Dictyocha fibula var. messanensis		-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+
BACILLARIOPHYCEAE																			
Bacteriastrum hyalinum		-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
Cerataulina pelagica		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Chaetoceros gracilis		-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Coscinodiscus concinnus		-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
* C. granii		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lauderia borealis		+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
*** Nitzschia closterium		-	+	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-
N. sigma		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
* Pleurosigma elongatum		-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-
*** Pseudonitzschia pungens		-	+	-	+	+	+	-	-	-	-	+	+	+	-	-	-	+	-
* Rhizosolenia setigera		+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	+
** Skeletonema costatum		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
*** Thalassiosira allenii		-	+	-	-	-	+	+	+	-	-	-	-	+	+	+	-	+	+
*** T. anguste-lineata		+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	-	-
** T. rotula		+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
T. tenera		+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. weissflogii		-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
EUGLENOPHYCEAE																			
** Eutreptiella gymnastica		-	-	+	-	+	+	-	+	-	-	-	-	-	+	+	-	-	+
* Eutreptia sp.		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PRASINOPHYCEAE																			
* Nefroselmis rotunda		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
* Pyramimonas grossii		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
** P propulsum		-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-
* Tetraselmis chui		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
Incertae sedis																			
Thalassomonas cf. minima		-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-

Temporal variation in surface water salinity was higher and correlated with increasing precipitation during the 1991 blooming season (max. range 2.1‰). The same figure was also observed in 1992, however, the difference with depth was more evident with a range of 3.1‰.

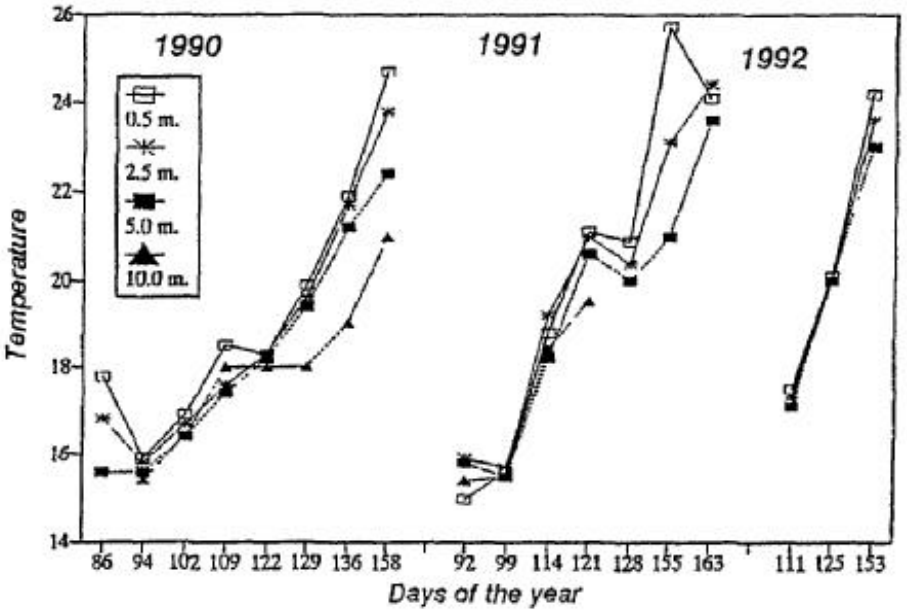

Fig. 2 Temperature variations at the sampling depths during the study period

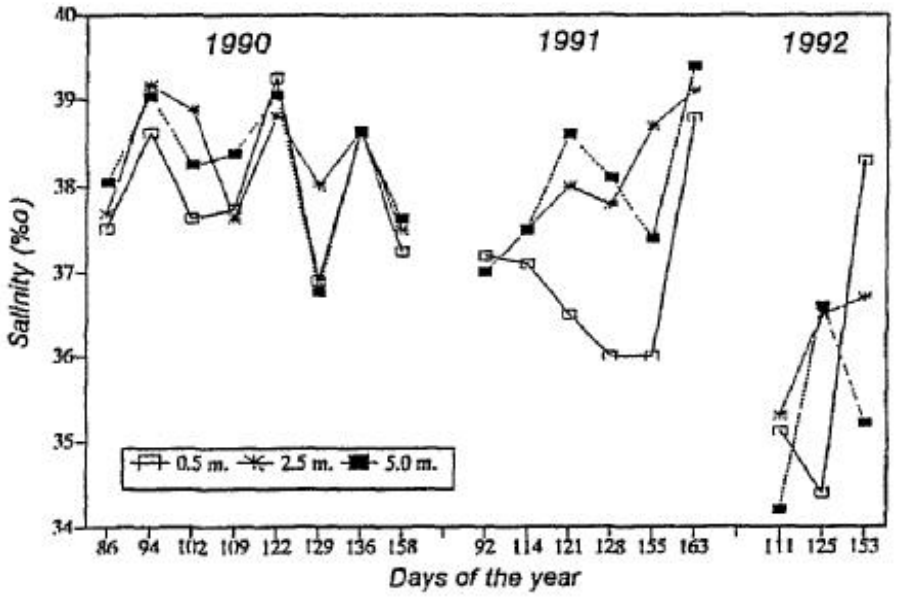

Fig. 3 Salinity variations at the sampling depths during the study period

The pH variability was considerable ranging from a minimum of 7.48 (4th April) to a maximum of 8.16 (27th March). pH values were typically higher on 2 May and 7 June (at almost all sampling depths) as compared to other sampling periods (Fig. 4). In general, variability increased with cell number when blooming species A. minutum, P. micans (2nd May) and E. gymnastica (7th June) were dominant in the phytoplankton community. The fact that these similar trends were also observed for oxygen, Chl a concentrations and light intensity (Figs 5, 6 and 7) strongly suggest that pH variability was controlling the phytoplankton productivity in surface waters. A sharp decrease in oxygen concentrations was observed at 2.5 m, 5.0 m and 10.0 m sampling depths with a minimum of 1.3 mg l^{-1} (on 7 June, at 5.0 m). Similarly, mean concentrations of Chl a were generally higher at the surface with a maximum observed on 7 June (0.5 m, 188.24 mg m^{-3}) and 16 May (2.5 m: 112.52 mg m^{-3}; 5.0 m: 58.25 mg m^{-3}; 10.0 m: 47.88 mg m^{-3}) during mixed blooms of T. allenii and E. gymnastica (7 June) and C. furca, E. huxlei and T. allenii (16 May). As shown clearly in Figs 5, 6 and 7 algal photosynthesis supported oxygen reserve of water (10 m) when sufficient light was available throughout the blooming period. However, oxygen deficiency that was due to the decay of sedimented material, was mainly consistent with increasing depth. This pattern suggests that mass mortality was caused by only oxygen deficiency during non-toxic blooms when toxic species disappeared. In 1991, the succession of algal biomass (Chl a) and pH followed a similar pattern due to high photosynthetic activity of blooming species such as A. minutum, P. triestinum, N. closterium, P. pungens and T. allenii. However, the relationship between photosynthetic activity and pH rapidly disappeared in early summer (4 June). There was a sharp zooplankton biomass peak during this unequilibrium period in May (Fig. 8). The peak was caused mainly by a rapid increase of heterotrophic ciliates (Helicostomella subulata) and copepods (copepodites of Acartia clausi and, nauplii and adults of Oithona nana) which feed on phytoplankton blooming species (phaeopigment: 20.77 mg m^{-3}). During late spring and summer, the zooplankton biomass was lower (phaeopigment: 0.53 mg m^{-3}) and in June, Chl a increased dramatically (130 mg m^{-3}, T. allenii bloomings). Increases in Chl a in the euphotic zone were associated with increasing light intensity in the same period (Figs 6 and 7). When a T. allenii bloom peaked, the pH started to increase and remained steady until 13 June (pH: 8.0). The variations in Chl a, phaeopigment, pH and light intensity were generally related to each other except oxygen in the 1992 blooming season.

Figs 9, 10 and 11 show the observed bi- or three weekly changes in inorganic nitrogenous nutrient concentrations. Nitrate and ammonium were the principal nitrogen species utilized and typically higher in surface than in deep layers due to a large supply of nutrients to the surface layer from urban sewage. In 1990, early-spring mean concentrations of nitrate N, ammonium N and nitrite N exceeded 21.87, 44.17 and 10.45 µg-at l^{-1} respectively. In the beginning of the following spring bloom of P. micans on 12 April, dissolved nutrient stocks disappeared rapidly, nitrate N, ammonium N and nitrite N surface concentrations decreased to 3.89, 6.27 and 3.23 µg-at l^{-1}. Decreases in nitrate N were consistent with decreases in ammonium N in the water column until 2 May; however, the increasing tendency of nitrite N with depth reflected nitrification processes near the bottom layer. Late spring mean concentrations of nitrogenous nutrients increased gradually but in early summer they were utilized during harmless blooms of T. allenii and E. gymnastica.

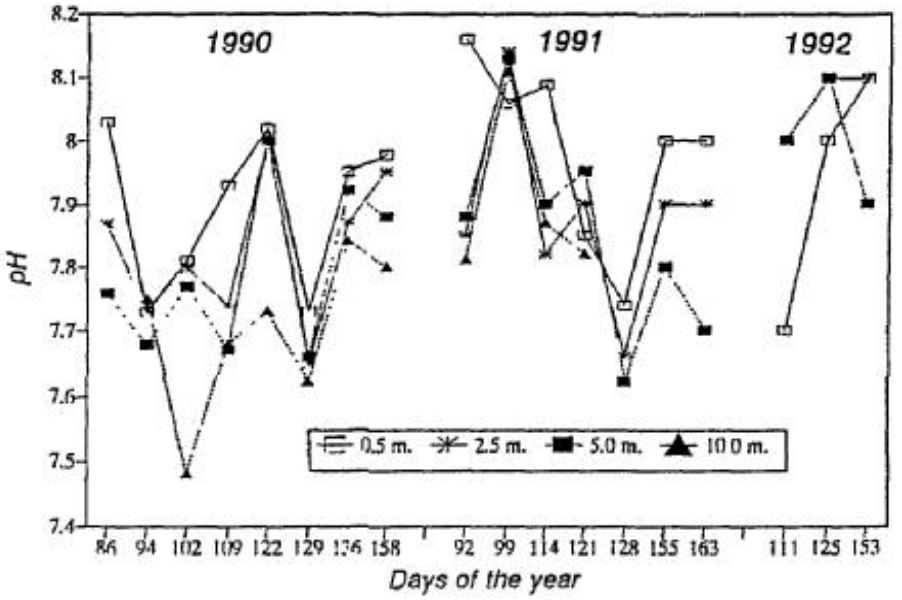

Fig. 4 pH variations at the sampling depths during the study period

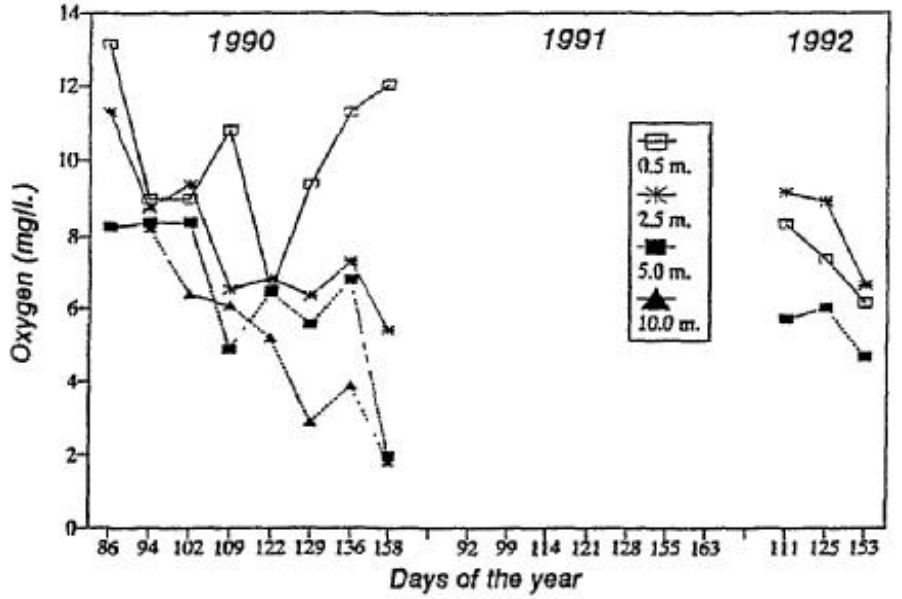

Fig. 5 Dissolved oxygen variations at the sampling depths during the study period

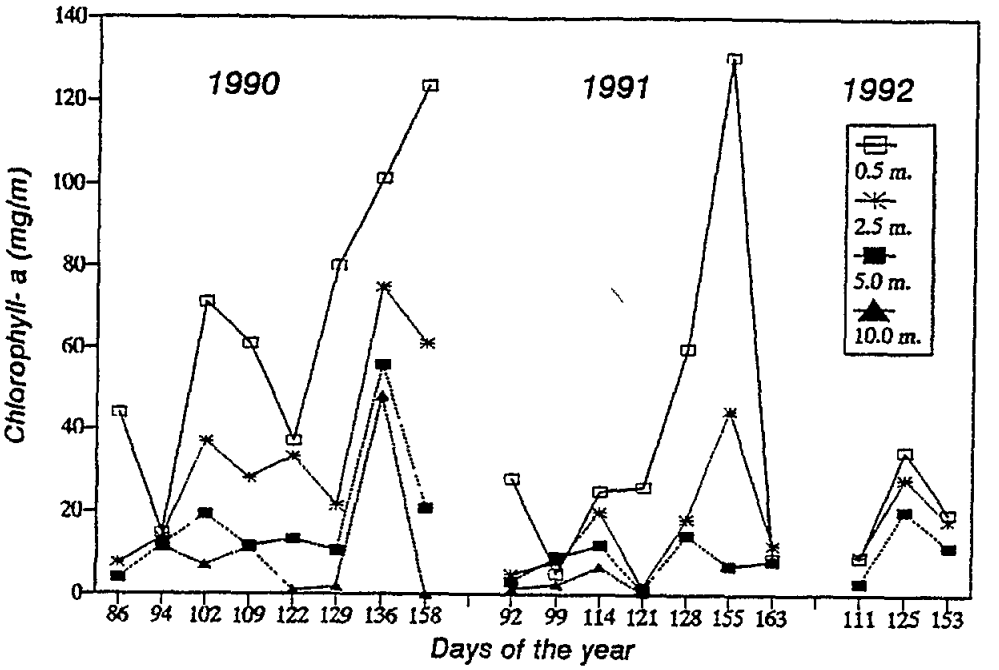

Fig. 6 Chlorophyll a variations at the sampling depths during the study period

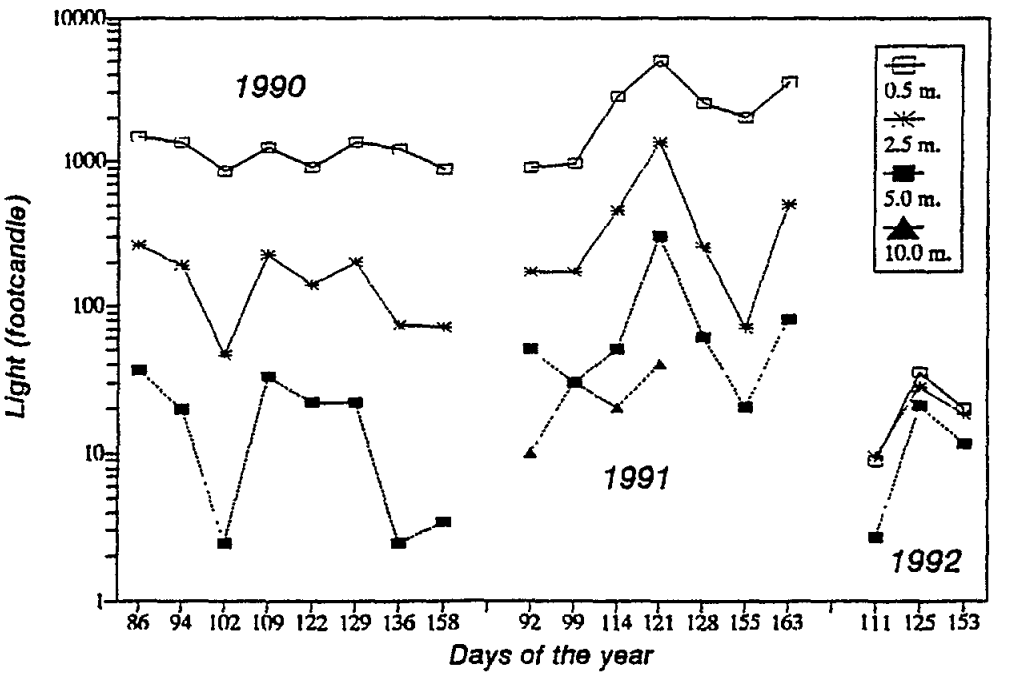

Fig. 7 Light variations at the sampling depths during the study period

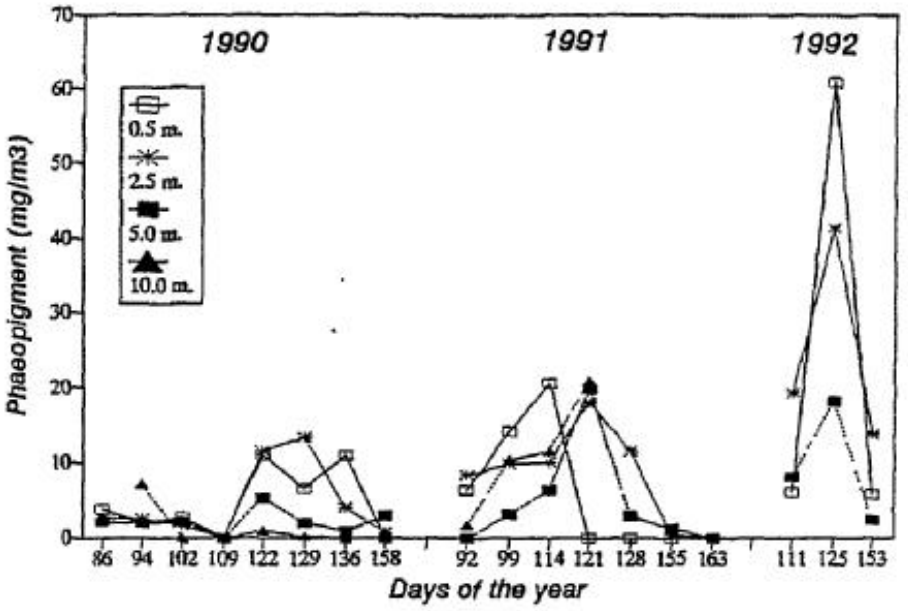

Fig. 8 Phaeopigment variations at the sampling depths during the study period

The same pattern was observed during the 1991 and 1992 blooming seasons, however, nitrate and nitrite concentrations were obviously lower. At the surface, salinity also declined due to a more rainy period during the red-tide season of 1991. Nitrate concentrations at the surface increased from almost zero to 4.25 µg-at l^{-1} between 2 April and 4 June, even though ammonia and nitrite showed fluctuations.

During 1992 all nitrogenous nutrient concentrations were high on 5 May and decreased on 2 June in the whole water column.

As indicated by vertical distributions in Fig. 12 and Fig. 13, orthophosphate P and silicate concentrations were generally higher in the surface water. During the 1990 blooming season, the mean concentrations of P were high on 12 April (4.3 µg-at l^{-1}) and rapidly decreased during the following weeks owing to its utilization by the blooming species.

In 1991, phosphate concentrations declined rapidly over the first three sampling days (2nd, 9th and 25th April) due to utilization of this nutrient by mixed blooms of A. minutum, P. micans, P. triestinum and T. anguste-lineata and rose again on 1st and 9 May (max.: 5.97 µg-at l^{1} at surface). However, P concentrations were gradually increasing below surface until late spring, except when T. anguste-lineata bloomings occurred. Such an increase with depth in P concentrations indicates a rapid

turnover rate of P and a low photosynthetic activity below surface due to low light penetration during blooms. Remineralization processes of P were evident especially at the 10.0 m depth with strong temporal variability; however, the fact that mean concentrations of P did not exhibit a significant trend on a spatial and temporal scale, strongly indicates that sewage inputs are more significant sources of P than remineralization.

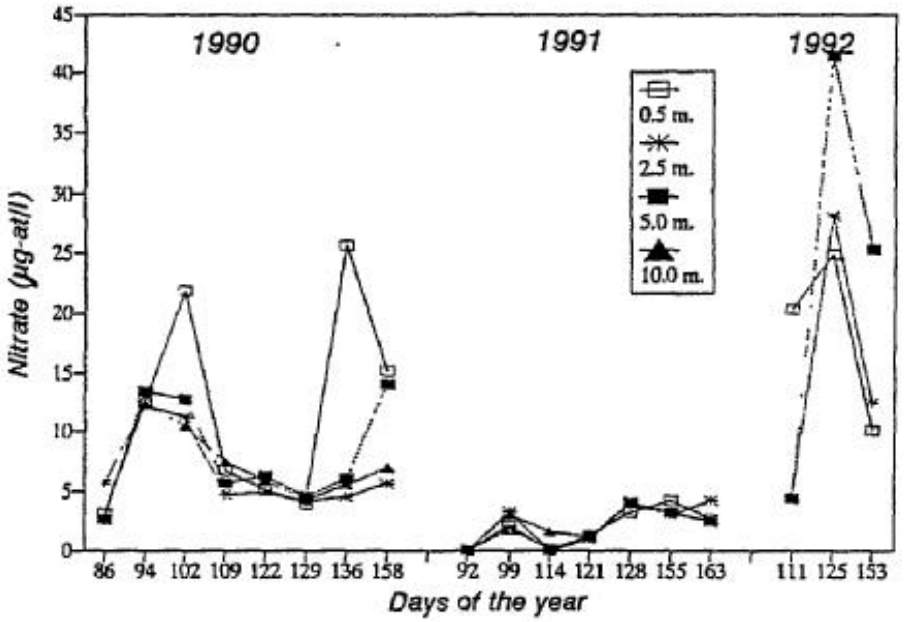

Fig. 9 Nitrate variations at the sampling depths during the study period

When diatoms dominated in early spring with T. anguste-lineata and in late spring with T. allenii, the decline in Si concentrations was due to the utilization of this nutrient. In mid-spring when dinoflagellates A. minutum, G. simplex, P. micans and S. trochoidea dominated the red-tides, Si accumulation was considerable. Depth profiles of silica showed a subsurface maximum. After mid-spring, Si concentrations tended to increase with depth due to the low assimilation and remineralization processes in the bottom water. This pattern strongly supported that Si was mainly supplied by the remineralization of diatom frustules after diatom blooming.

Profiles of the N:P and Si:P ratios are shown in Figs 14 and 15. There was a gradual decline in the Si:P ratio during the early-spring bloom of diatom T. anguste-lineata. When the T. anguste-lineata bloom was replaced by the dinoflagellate one of P. micans in mid-spring, a clear decrease in the N:P ratio was observed while the Si:P ratio increased gradually due to the Si remineralization from sedimented diatom frustules. This recycled Si was utilized in late spring by the minute diatom T. allenii. These changes in the Si:P and N:P ratios most likely indicate that N and Si control the blooming succession in Izmir Bay.

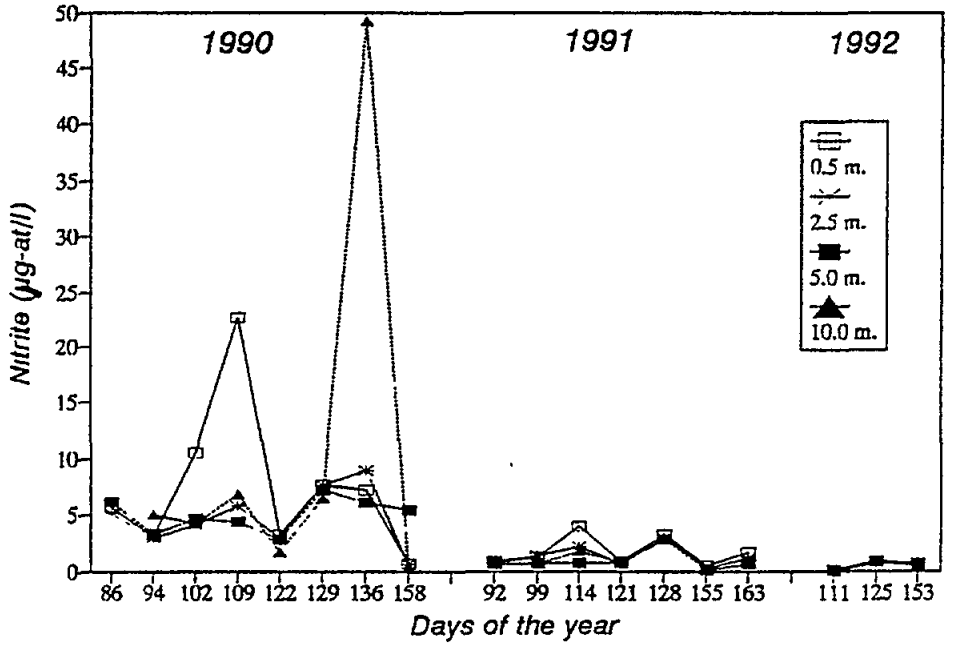

Fig 10 Nitrite variations at the sampling depths during the study period

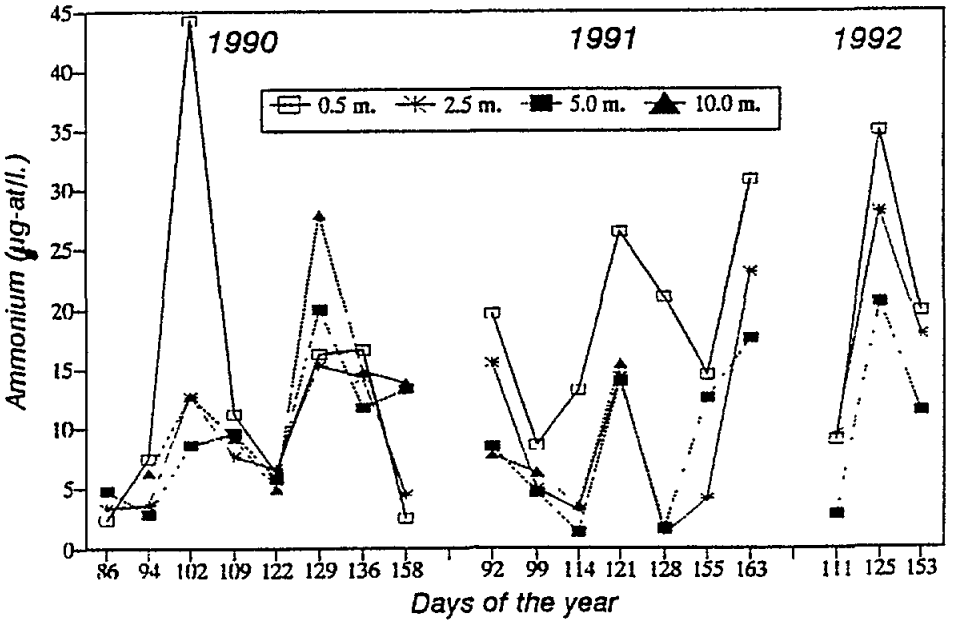

Fig. 11 Ammonia variations at the sampling depths during the study period

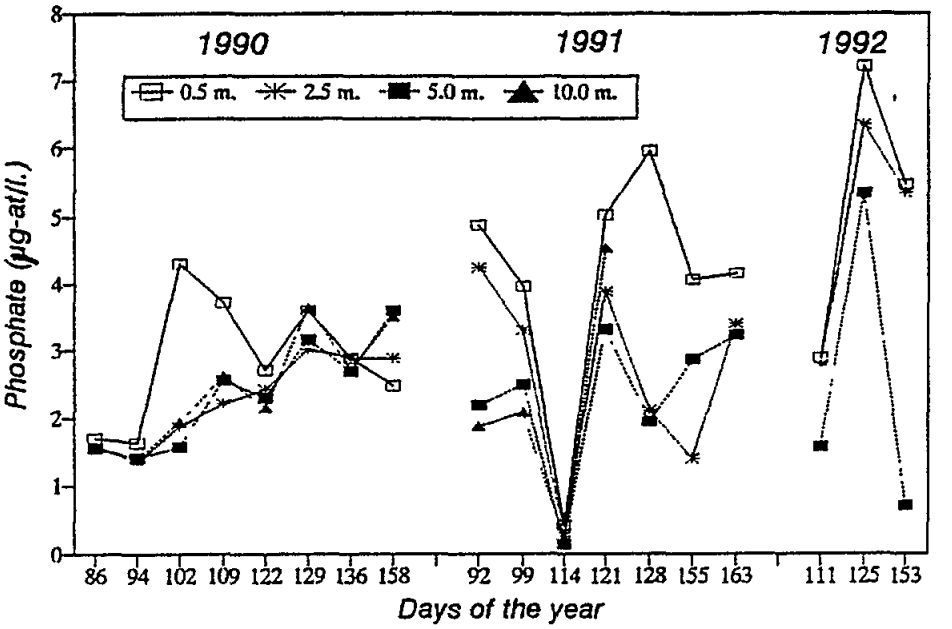

Fig. 12 Orthophosphate P variations at the sampling depths during the study period

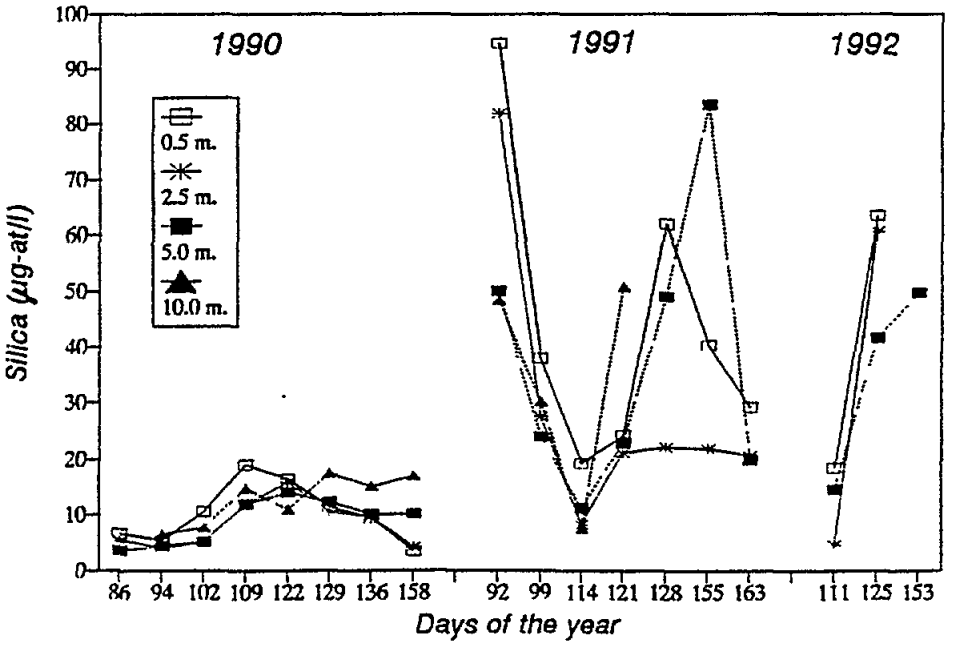

Fig. 13 Silica variations at the sampling depths during the study period

The successive potential growth patterns of diatoms and dinoflagellates were also clearly followed with F_n values of nitrate N and Si (Fig. 16). In the last week of March (27 March) and between the second week of April (19 April) and the second week in May (9 May), F_n values of nitrate N showed that the availability of this nutrient controlled the species competition and led to P. micans dominated dinoflagellate blooms. Although nitrate-N frequently reached limiting values, Si was never limiting during this period. The low levels in nitrate F_n also coincided with C. furca dominated blooms in the second week of May (16 May) at the sampling station 4. F_n values of Si were typically inversely related to nitrogen F_n s. Si F_n values reached their minima (0.54) during the blooms of the diatoms T. anguste-lineata (4 April) and T. allenii (16 May-7 June). There were also large increases and a stable plateau in Si F_n s between 19 April and 16 May during the dinoflagellate (P. micans) dominated bloom, due to the remineralization of sedimented frustules from the last diatom blooming (T. anguste-lineata). The fluctuations in nitrate F_ns were related to excessive growth of the euglenoid flagellate E. gymnastica in the sampling stations.

In 1992, primary productivity was measured in situ and the first experiment took place on 21 April when the algal spring bloom consisting mainly of S. costatum and P. triestinum reached its peak (Table 2). The sharp photosynthetic activity peak was observed on 5 May. The peak was caused mainly by a rapid increase of the prasinophyte T. chui (17.21 g C m^{-2} day^{-1}). During early summer, almost all of the primary productivity was due to the euglenoid flagellate E. gymnastica.

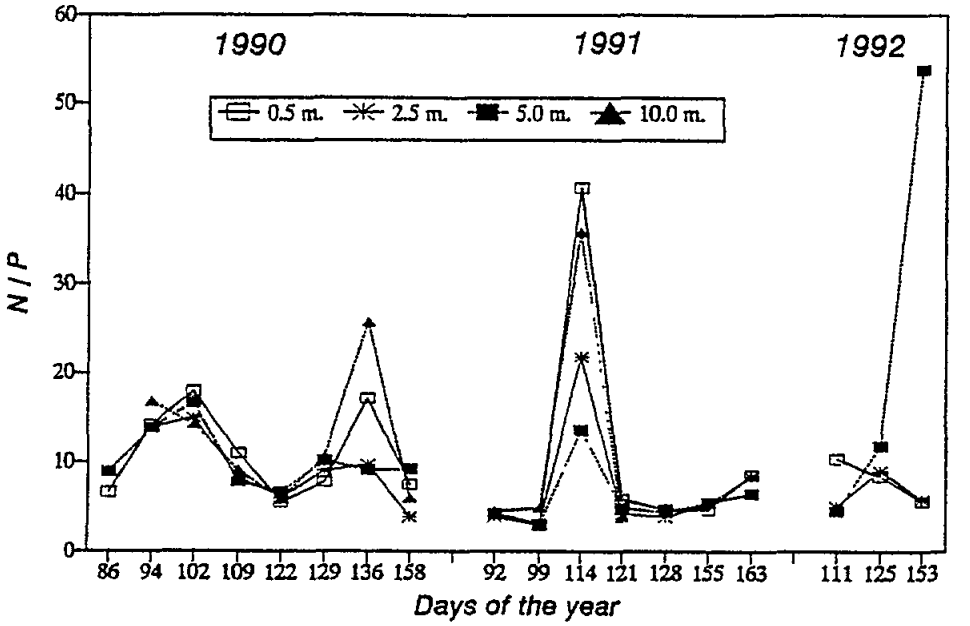

Fig. 14 Variations of the total N : P ratio at the sampling depths during the study period

Bi- or three weekly concentrations of Fe, Cu and Zn in the water column are summarized in Table 3 for the 1991 blooming season. The trace metal concentrations tend to decrease curvi-linearly from the surface to 6 m; however, the data show a small supply of Zn and Cu from the sediment to bottom waters. High surface concentrations can be explained by inputs from sewage, which flows directly into the upper water layers.

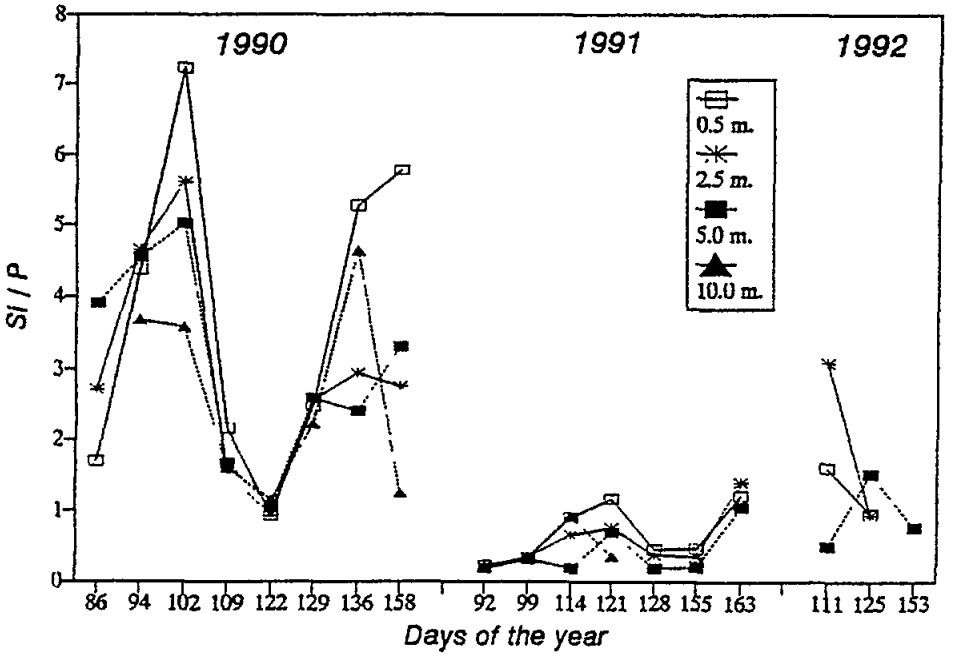

Fig. 15 Variations of the Si : P ratio at the sampling depths during the study period

Table 2

The results of in situ primary productivity measurements

Date	Integrated PP (gr C m^{-2} day^{-1})	Pmax (gr C m^{-3} h^{-1})
21.4.1992	7.80	0.769
5.5.1992	17.21	2.300
6.6.1992	10.52	0.968

The relationships between the trace metals and bloomings were not very clear. Based on the data, the variations of particulate organic carbon (POC) were not proportional with Fe, Cu and Zn suggesting other controlling factors (Fig. 17). However, trace metal stimulations on some species were prounced. For instance, the blooms of P. micans and N. scintillans obviously became frequent and spread when

Fe concentration were high in ambient water (1-5 May) whereas bloomings of these species were markedly inhibited at the high surface levels of Cu. On the other hand, cellular Fe, Cu and Zn accumulations were species dependent (Table 4).

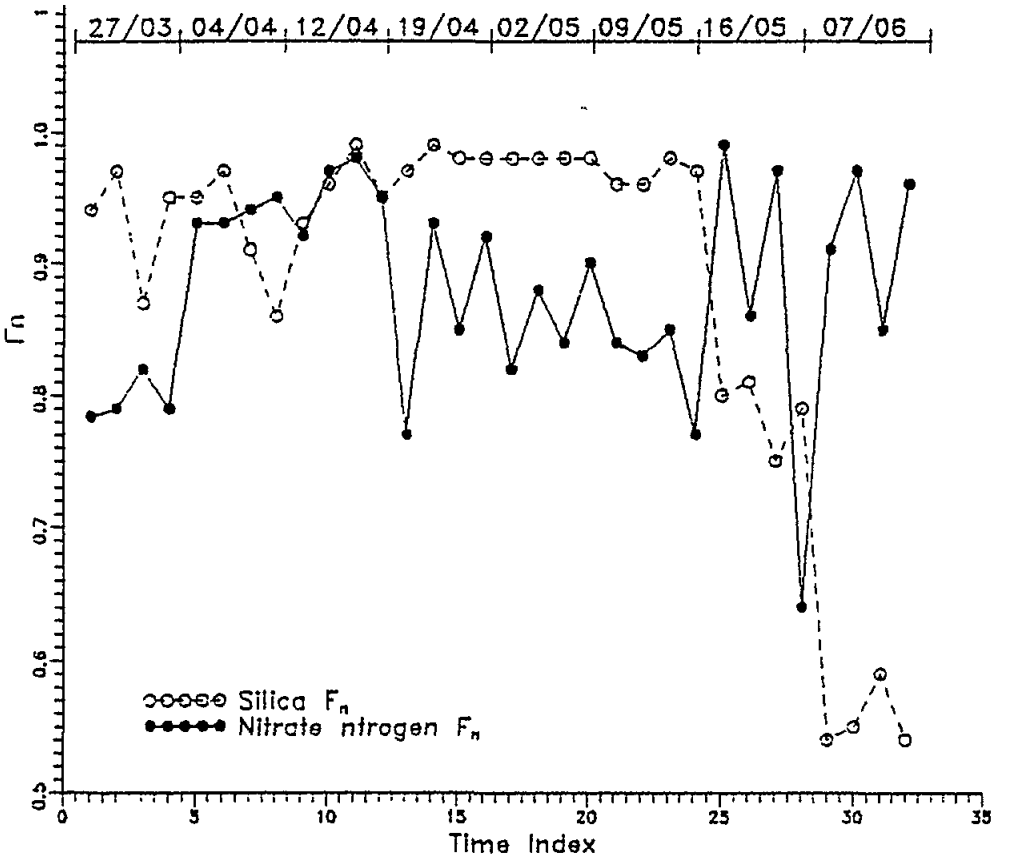

Fig. 16 Weekly variations in F_n values during the sampling period

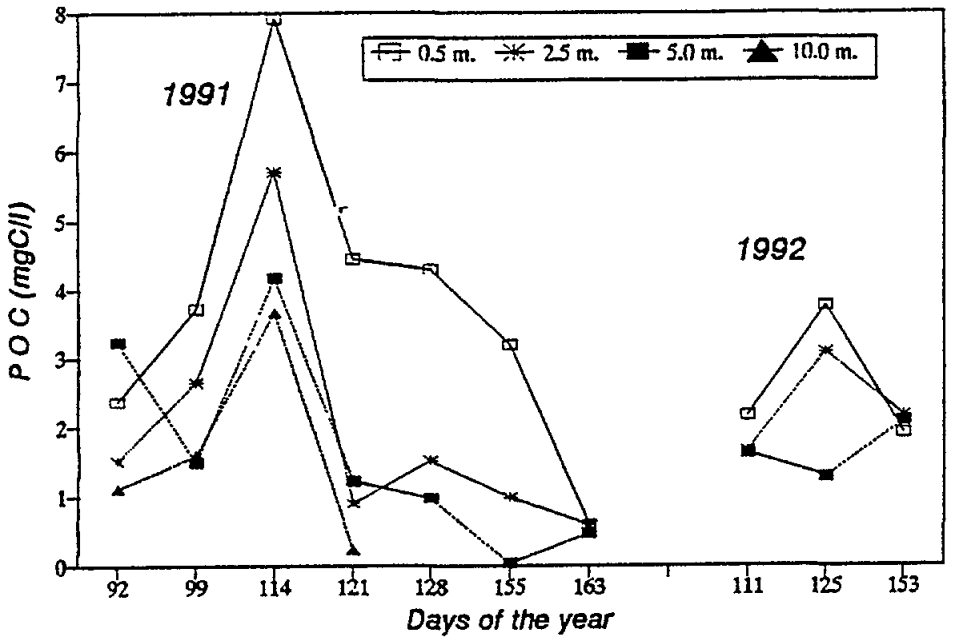

Fig 17 POC variations at the sampling depths during the study period

Table 3

Concentrations of Fe, Cu and Zn with depth during the blooming season of 1991 (mg l^{-1})

	DATE								
Depth(m)	2.4	9.4	25.4	30.4	1.5	9.5	4.6	13.6	5.7
0.5	5.320	0.056	0.045	0.062	0.089	0.073	0.028	0.025	0.120
1.0	0.166	0.087	0.022	0.134	0.078	0.781	0.129	0.028	nd.
1.5	0.062	0.028	nd.	0.058	0.019	1.139	0.117	0.011	0.198
2.0	0.058	0.086	nd.	0.019	nd.	0.148	0.039	0.064	0.034
2.5	0.224	0.025	0.028	0.006	0.028	0.031	0.034	0.045	0.213
Fe 3.0	0.193	0.019	nd	0.036	0.019	0.078	0.025	0.336	0.042
3.5	0.039	0.019	0.036	0.031	0.047	0.232	0.014	0.022	nd.
4.0	0.092	0.042	0.017	0.025	-	0.053	0.126	0.040	-
4.5	0.103	0.058	0.019	0.028	-	0.098	0.006	0.207	-
5.0	0.056	0.089	0.019	0.025	-	2.408	0.008	0.207	-
5.5	0.106	0.016	0.070	0.022	-	-	-	-	-
6.0	0.084	0.593	0.005	0.019	-	-	-	-	-
0.5	0.287	0.039	0.051	0.052		0.025	0.056	0.049	0.025
1.0	0.125	0.034	0.070	0.026		0.022	0.052	0.036	-
1.5	0.077	0.032	-	0.018		0.017	0.031	0.032	0.017
2.0	0.081	0.029	-	0.014		-	0.028	0.038	-
2.5	0.073	0.022	0.054	0.025		0.020	0.023	0.029	0.020
Zn 3.0	0.068	0.024	-	0.036		0.026	0.030	0.048	0.026
3.5	0.088	0.040	0.030	0.031		0.063	0.021	0.089	0.063
4.0	0.063	0.028	0.049	0.035		-	0.021	0.042	-
4.5	0.097	0.034	0.044	0.040		-	0.021	0.022	-
5.0	0.046	0.022	0.035	0.019		-	0.030	0.035	-
5.5	0.072	0.018	0.038	0.031		-	-	-	-
6.0	0.201	0.024	0.066	0.029		-	-	-	-
0.5	0.055	nd.	nd.	nd.		nd.	0.004	nd.	nd.
1.0	nd.	nd.	nd.	0.004		nd.	0.004	nd.	nd.
1.5	nd.	nd.	nd.	nd.		0.004	nd.	nd.	nd.
2.0	0.004	nd.	nd.	nd.		nd.	nd.	0.004	nd.
2.5	0.008	nd.	nd.	nd.		nd.	nd.	0.004	nd.
Cu 3.0	0.008	nd.	nd.	0.004		nd.	0.013	0.004	nd.
3.5	0.008	nd.	nd.	nd.		nd.	0.004	nd.	nd.
4.0	nd.	nd.	nd.	nd.		nd.	0.004	nd.	nd.
4.5	0.008	0.008	nd.	nd.		nd.	0.004	nd.	nd.
5.0	nd.	0.004	nd.	nd.		nd.	-	0.102	-
5.5	0.017	0.004	nd.	nd.		-	-	-	-
6.0	0.018	nd.	nd.	nd.		-	-	-	-

Table 4

Fe, Cu and Zn accumulation in the cells of three important blooming species in the bay of Izmir (μg g^{-1} dry weight)

Species	Fe	Cu	Zn
P. micans	245.39	31.08	208.34
N. scintillans	1232.87	11.17	679.90
E. gymnastica	843.19	17.8	1033.53

For the data based on the 1990 blooming season, the multiple regression equation in standard format with Chl a, orthophosphate P, temperature, light, Si, ammonium N and nitrate N was highly significant ($F=17.551$, $p<0.05$) respectively and explained 52% of the total variance in the data (Table 5). The standard partial regression coefficients indicated that as P, temperature, light and nitrate N increased, Chl a increased. Si and ammonium N increases affected negatively the phytoplankton production. This pattern clearly established Si and N controlled bloom succession (Pingree et al., 1977). Although P was the most important parameter effecting the equation, never limiting because of rapid recycling and continuous inputs from sewage.

Table 5

Parameters of multiple regression analysis in standard format for dependent variable Chl a (standardized variables)

Variables	Reg. Coeff.	Fcal	Sig.
Orthophosphate P	0.499	20.474	0.000
Temperature	0.358	20.241	0.000
Light	0.288	15.656	0.000
Si	-0.217	5.369	0.022
Ammonium N	-0.214	5.306	0.023
Nitrate N	0.152	4.029	0.048

For predictive purposes, data were $\log_{10}(Y_i+1)$ transformed and conventional regression without intercept was obtained as a function of the same variables.

$$\log(\text{Chl a}+1) = 1.445\log(PO_4^{-3}+1)+0.676\log(T+1)+0.132\log(L+1)$$
$$-0.518\log(SiO_4+1)-0.128\log(NH_4^{+}+1)+0.199\log(NO_3^{-}+1)$$

The six environmental independent variables described . 92% of the total variance, however, effects of ammonium and nitrate were almost negligible in the final predictive regression ($p<0.46$, Table 6).

Table 6

Parameters of multiple regression equation in conventional format for dependent variable Chl a ($\log_{10}(Y_i+1)$) transformed variables)

Variables	Reg. Coeff.	Fcal	Sig.level
Orthophosphate P	1.445	7.425	0.008
Temperature	0.358	10.724	0.001
Light	0.288	10.466	0.002
Si	-0.217	4.604	0.034
Ammonium N	-0.214	0.535	0.466
Nitrate N	0.152	0.541	0.464

The principal component (PC) analysis performed on the standardized nutrients, primary ecological factors, light, primary (Chl a) and secondary (phaeopigments) production units described . 95% of the total variance within the nine PCs (Fig. 18, Table 7). The first seven PCs which were used to calculate multiple regression equations explained . 88% of the variation in production during the blooming season. The eigenvectors were included in Table 8. Practically, the first PC can be interpreted as a nutrient concentration and biomass component (P, ammonium N, Si and Chl a). PC 2 summarized biomass and photosynthetic activity (Chl a, DO, pH, nitrite N) while the other PCs were generally interpreted as nutrient and primary ecological factor components.

Both multiple regression coefficients and principal components indicated that dimensions of blooms increased with increasing P, nitrate N, light and temperature. Succession was mainly controlled with Si and ammonium N. This information also suggests that phosphorus controls the amount of phytoplankton biomass during the red-tide season; however, phosphorus loading in the system is adequate and never limiting.

The reason why blooms of diatoms and dinoflagellates dominates each other sequentially is of some interest. Direct competition for nutrients among species appears to be responsible because the diatom blooms collapse before the onset of a dinoflagellate red-tide. Si depletion often controls this succession and causes a collapse on diatom blooms. For instance, dinoflagellate P. micans blooms in early spring when Si limits diatom growth.

Blooming alternatives for the bay of Izmir were summarized with a multidimensional scaling analysis using integrated species counts (Fig. 19). According to the non-metric analysis, there have been four species groups which competed each other, these were A. minutum + P. triestinum, P. micans + C. furca, P. steini + O. scolopax, Thalassiosira spp. + N. closterium. Although, polymixed blooms may be caused by the last three groups together when adequate nutrients are available, the blooms formed by A. minutum and/or P. triestinum characteristically separated other clusters.

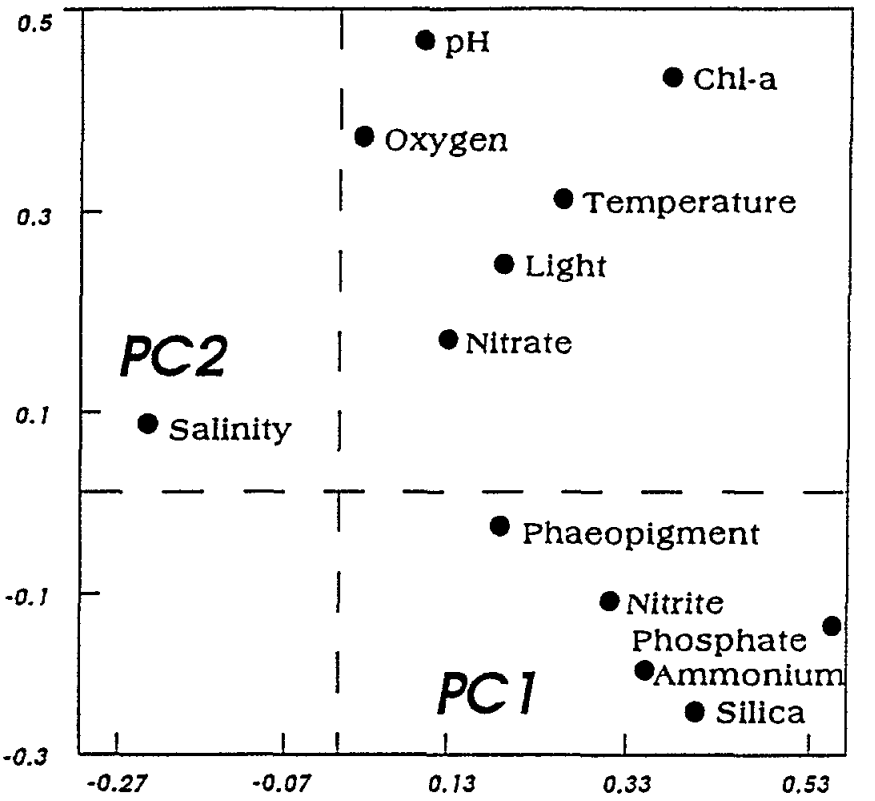

Fig. 18 Plot of the first two principle components

The increase of available P with a rapid turnover rate, enables the red-tide species to utilize the available nitrogen preferably in ammonium form during the dinoflagellate bloom term. Although remineralization of Si from the diatom frustules continues during this period, dinoflagellate blooms spread out until N is almost completely utilized. Within days, different types of competitions among dinoflagellate species may cause mixed community structure. For instance, toxic A. minutum bloom occurs when temperature stratification is pronounced with a minimum difference between the lowest and highest temperature levels in water column of 6°C. When this situation occurs, cell numbers of A. minutum reach millions in subsurface waters (2-5 m). Otherwise, P. micans can compete successfully with A. minutum especially at the surface and reach excessive cell numbers.

Table 7

The results of PCA

Component Number	Percent of Variance (Eigens)	Cumulative Percentage
1	24.573	24.573
2	17.753	42.327
3	12.983	55.310
4	11.057	66.367
5	8.666	75.034
6	6.946	81.979
7	4.810	86.789
8	4.090	90.880
9	3.824	94.704
10	2.121	96.825
11	1.870	98.695
12	1.305	100.000

In any case, some anoxic zones may be formed due to high photosynthetic rate and decaying of sedimented cells. As pointed out by Jones (1972) and Jones and Lee (1982), there exist clear relationships between total P, algal biomass and hypolimnia anoxia in freshwater ecosystems (Harris, 1986). The straightforward relationship between phytoplankton biomass and P in the present study evidently supports that oxygen depletion is a function of increased P loading in bottom waters of inshore localities during the warm red-tide season. In addition to anoxia, PSP toxicity may be another risk factor in Izmir Bay when the dominant species is A. minutum following high nutrient loads from rivers. However, there was no A. minutum dominated bloom in 1990-1992, and an examination of the ecological data suggests that the weak temperature stratification due to abnormal climatic changes during spring 1990 should have been the cause of this event. But, inverse effect of gradually increasing pollution on the life cycles of red-tide forming dinoflagellates must be another reason.

4. CONCLUSION

The generally increasing levels of Chl a, nutrients, increasing occurrence of toxic or non-toxic red-tides and other noxious algal blooms suggest a progressive eutrophication in Izmir Bay. In the next years, only deterioration processes in the sediment may sustain the blooms without further loading of nutrients from the rivers.

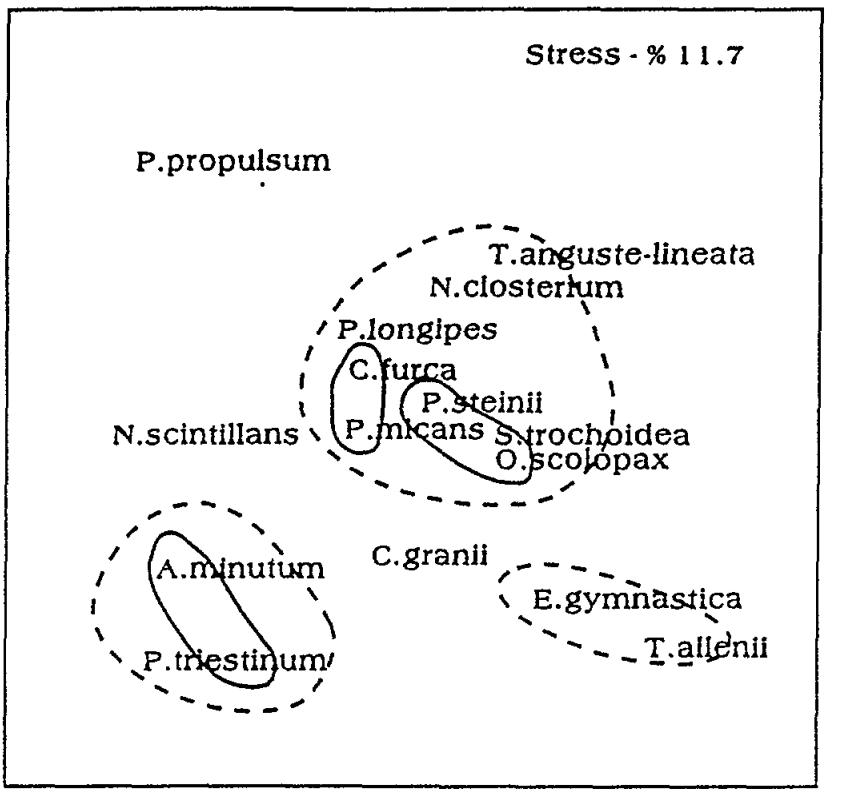

Fig. 19 Blooming alternatives in the bay of Izmir

Table 8

The component weights of the first seven PCs

Variables	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
P	0.516	-0.148	0.083	-0.092	-0.049	0.059	-0.016
Ammonium N	0.351	-0.260	-0.319	-0.022	-0.260	-0.210	-0.454
Nitrate N	0.127	0.139	-0.360	-0.038	-0.733	0.118	0.253
Light	0.232	0.279	-0.279	0.309	0.287	-0.397	-0.130
Temp.	0.258	0.289	0.320	-0.495	-0.080	-0.112	0.008
Si	0.384	-0.291	0.239	0.262	0.023	0.015	-0.309
Chl a	0.338	0.449	0.055	-0.105	-0.053	0.148	0.081
Oxygen	0.055	0.486	0.378	0.113	-0.000	0.126	-0.342
pH	0.007	0.434	-0.414	0.324	0.101	0.044	-0.030
Nitrite N	-0.262	0.037	0.251	0.420	-0.431	0.275	-0.030
Salinity	0.177	-0.041	0.368	0.486	-0.175	-0.409	0.556
Phaeo.pig.	0.331	-0.127	-0.092	0.200	0.272	0.697	0.262

The continuous inputs and sedimentation of the particulate insoluble material from the domestic sewage is also supported by remains of algal blooms. This sedimented thick organic mud covers almost the whole benthic surface on which the dinoflagellate benthic resting zygotic cysts and even temporary resting stages collect; it affects negatively the life cycles of the cyst and bloom forming dinoflagellates such as S. trochoidea and A. minutum. Consequently, in the near future, evident differentiation in the community structure of spring red-tides succession is awaiting in Izmir Bay. DSP causing P. micans and N. scintillans or PSP reason A. minutum dominated community structure is one of the probable alternatives. Another one may be anoxia reason flagellate (e.g. E. gymnastica) blooms. For this reason, progressive eutrophication processes and toxic PSP-DSP or non-toxic anoxia causing algal blooms including red-tides in Izmir Bay must be carefully followed to detect this undesirable point in the next years.

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QUANTITATIVE ASSESSMENT OF EUTROPHICATION: CRITERIA DEVELOPMENT FOR THE MANAGEMENT OF COASTAL WATERS

by

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SUMMARY

A number of methods on data analysis was evaluated for the quantitative assessment of eutrophication. Three standard data sets derived from oligotrophic, mesotrophic and eutrophic areas of the Aegean sea were used to establish criteria for characterizing trophic levels through nutrient and phytoplankton concentrations. The ranking of sampling sites using many variables was also studied, by applying multicriteria-choice methods. These multicriteria evaluation methods were shown to be an effective methodological tool in assessing eutrophication. In addition, this approach is compatible with multicriteria methods applied to policy-making and therefore the problem of eutrophication can be integrated with plan and project evaluation in environmental management.

Numerical classification by the group average clustering algorithm, based on phytoplankton community data seems to be an efficient method to assess water quality. For the improvement of the discrimination of the method, the following stepwise procedure is proposed: a) reduction of the original data matrix by removing the rare species, b) no scaling of the original data values, c) use of the Bray-Curtis coefficient of resemblance, d) identification of distinct groups of sites with objectivity by the non-parametric randomization test ANOSIM.

The quantification of eutrophication at a spatial scale was also investigated. A number of methods for surface interpolation were tested in order to select the most appropriate data analysis procedure. The development of a categorical map based on trend analysis and phytoplankton cell number concentration scaling showed a

clear spatial pattern of the system quality. The techniques proposed in this paper form an integrated methodological system for eutrophication evaluation of coastal marine areas.

1. INTRODUCTION

Marine eutrophication is the process of enrichment of the sea with plant nutrients, nitrogen and phosphorus, that stimulate aquatic primary production (Vollenweider et al., 1992). However, the characterization of the tropic state of coastal areas has already been a rather complicated problem due to a number of reasons: (a) The trophic classification of coastal waters cannot be easily performed since there is always uncertainty in the definition of oligotrophic and eutrophic conditions. Certain studies in the past have associated oligotrophy with the absence of measurable nutrient concentrations (Thomas, 1970; McCarthy and Goldman, 1979). On the other hand since eutrophication is defined by simply referring to organic nutrient enrichment from external sources (Bigar and Corey, 1971), the threshold of eutrophic conditions cannot be accurately set. It is therefore, a problem for marine scientists to define levels of nutrient and phytoplanktonic parameters characterizing the trophic state; (b) The study of functional interactions between independent variables (temperature, salinity, nutrient concentrations) and response variables (chlorophyll, phytoplankton cell number) may lead to an underestimate of the relative contribution made by each parameter within the system (Giovanardi and Tromellini, 1992) or to artifacts due to the violation of statistical assumptions (Vounatsou and Karydis, 1991); (c) The discrimination between polluted and control sites may be discussed if the data do not satisfy the methodological assumptions of the statistical methods (Clarke and Green, 1988; Karydis, 1992; Karydis, 1994) and (d) data processing in pollution studies is mainly based on methodology from biological indicators (Whilm and Dorris, 1968) and statistical evaluations (Warwick, 1988). All these techniques however, cannot be used with current research methods applied to policy analysis and coastal management (Hartog et al., 1989; Moriki and Karydis, 1994).

The present study is concerned with the quantification of eutrophication. The research has been partitioned into four objectives: (a) The development of classification scales for coastal waters based on nutrient and phytoplankton variables. This approach enables the establishment of criteria for coastal water quality and can be used as a tool for characterizing the level of eutrophication on a routine basis with a minimal amount of sampling; (b) The assessment of eutrophication using multicriteria choice methods. This type of analysis can be integrated into management approaches including social and economic values; (c) Identification and quantification of eutrophic trends based on phytoplankton community analysis. The measurement of changes in the structure of natural marine communities is widely used for the detection of man's impact in the marine environment. In this work the emphasis is given in the data preprocessing and the choice of suitable similarity indices and clustering algorithms so as to increase the discriminant power of the multivariate methods and (d) The quantification of eutrophication at a spatial

scale was studied by defining the horizontal dispersion of phytoplankton biomass. It was therefore, attempted to organize categorical maps illustrating eutrophic, mesotrophic and oligotrophic areas by combining the eutrophication scale developed with Geographic Information Systems.

2. CRITERIA FOR THE CLASSIFICATION OF COASTAL WATERS

2.1 The analysis of the system variability

The classification of coastal and inland waters into oligotrophic, mesotrophic and eutrophic has been based so far on mean values and standard deviations of the fundamental parameters characterising trophic levels (Heyman et al., 1984; Ignatiades et al., 1992). In order to set up a useful classification criterion and establish a probabilistic system for predicting and testing critical values of environmental variables (Georgopoulos and Seinfeld, 1982) various attempts were made in the past to fit field data of nutrients, chlorophyll and phytoplankton to known distribution functions (Heyman et al., 1984; Ignatiades et al., 1992; Giovanardi and Tromellini, 1992). Although various distributions seem to fit to the data such as beta and gamma distributions (Heyman et al., 1984), most research has been based on variable normalization (Cassie, 1962; Digby and Kempton, 1987; Jongman et al., 1987). This is probably because normality is the most important assumption of the parametric statistical methods (Zar, 1984). Among the univariate statistical methods, the analysis of variance and regression, both assuming normally distributed variables (Ott, 1988) are widely used; ANOVA for identifying pollution trends and regression for understanding the relationships of the variables within the system. In addition, numerous multivariate statistical methods also require normality (Morrison, 1988; Stevens, 1992). The most common technique to normalize data is the logarithmic transformation.

The logarithmic transformation has a predominant place in environmental variables: it has the effect of compressing the upper end of the measurement scale and thus reducing the importance of large values relative to smaller values in the data matrix. However, it is the large values that describe extreme environmental conditions being the critical values of the systems. In addition, big overlaps in nutrient and phytoplankton distributions occur between oligotrophic-mesotrophic and mesotrophic- eutrophic water masses (Ignatiades et al., 1992). This is probably due to seasonal variations of nutrients and phytoplankton (Pagou and Ignatiades, 1988) as well as to the difficulty to define the boundaries of the water masses used as sources of data.

In this section, inorganic nutrient concentrations and phytoplankton cell numbers have formed the basis of a classification system of coastal water quality; the proposed system is taking into account the following two points (a) the whole data processing is based on non-parametric methodology and (b) three standard data sets were used to define the central tendency of each variable for different trophic states. The three data sets come from areas known as eutrophic, mesotrophic and oligotrophic.

2.2 Source of data

The variables examined in this study include inorganic nutrient concentrations of phosphate, nitrate, nitrite and ammonia as well as chlorophyll concentrations and phytoplankton cell numbers. All these variables are of paramount importance in characterizing the trophic state of the marine environment and they are widely used for assessing eutrophication (Vollenweider et al., 1992). The basic idea was to depict statistical trends from water masses characterized by different trophic levels and therefore, different degree of oligotrophy and eutrophication. The data used originated from two areas of the Aegean Sea (a) Saronikos Gulf: inshore and offshore gulf water; sampling was carried out during 1997-1981 and (b) Island of Rhodes: offshore pelagic water; the water samples were collected during 1983-1984. The station locations are given in Figure 1. The sampling procedures and the laboratory methodology of the chemical and biological parameters have been given in previous studies (Ignatiades et al., 1981; Ignatiades et al., 1983; Karydis et al., 1983; Karydis et al., 1987).

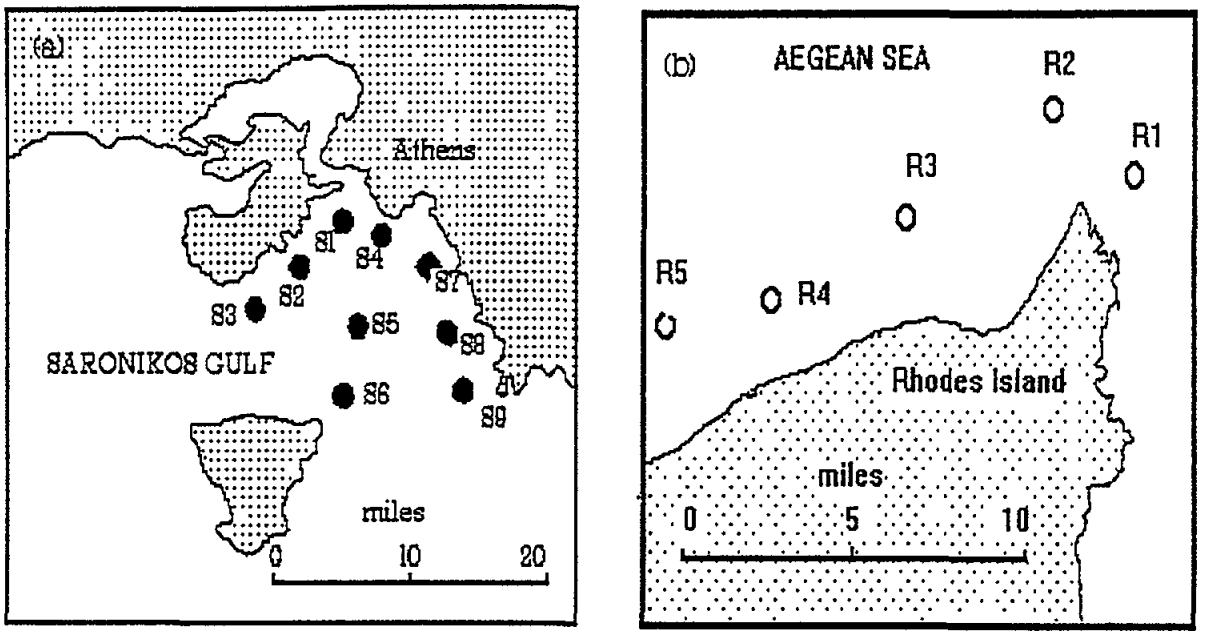

Fig. 1 Sampling locations (a) Saronikos Gulf (b) Rhodes Island

The nutrient, chlorophyll and phytoplankton variables were classified into three data sets representing three different water types (Ignatiades et al., 1992; Karydis, 1996).

Type A: Inshore Gulf Water; Eutrophic: Sampling (347 observations) was carried out monthly from two (2) stations (3 standard depths) located within the sewage dispersion field of Saronikos Gulf (stations S1, S2).

Type B: Offshore Gulf Water; Mesotrophic: The data (579 observations) were collected from seven (7) stations (3 standard depths) located in the remaining area of Saronikos Gulf (stations S3-S9).

Type C: Offshore Pelagic Water; Oligotrophic: Five cruises were carried out along the S.E. side offshore the Island of Rhodes. The data (197 observations) were collected from five (5) stations (6 standard depths) on a seasonal basis (stations R1 - R5).

2.3 Eutrophication criteria development

The nutrient frequency distribution profiles after the exclusion of the outliers (Karydis, 1994) showed a strong deviation from normality with a well expressed skewness to the right. Figure 2 illustrates the profiles of certain nutrients (phosphate, nitrate, ammonia) for the eutrophic water type (inshore gulf water) and oligotrophic water type (offshore pelagic water) as an example. Previous work (Ignatiades et al., 1992) has shown that these data sets could not be normalized by the logarithmic transformation and only the Box and Cox transformations have given satisfactory results. However, in some cases normality has not been obtained. In the present work it was found more appropriate to calculate some non-parametric summary statistics estimators. These estimators were the median value (M) of each variable, the lower quartile (LQ), the upper quartile (UQ), the minimum (Min) and the maximum (Max) values. The median was chosen as a measure of central tendency, the difference between the upper and lower quartiles as a dispersion measure and the difference between maximum and minimum values as the range of the values. The results are shown in Tables 1 and 2. It is observed that there is a gradual decrease in the median values of the nutrient variables. The interquartile range (IQR) that is the difference between UQ-LQ is much smaller compared to the range of each variable indicating the existence of outlying values. Similar tendencies were observed in the phytoplankton data.

However, there is no overlapping between the upper quartile values of Chl-á concentrations with the higher trophic state; the only overlapping observed in phytoplankton cell number was between the upper quartile of the mesotrophic data set and the lower quartile of the eutrophic data set.

Median values were used to develop a eutrophication scale. This scale is given in Table 3. The values in Table 3 resulted in a reasonable scaling of the nutrient / Chl-á concentrations and phytoplankton cell number to four trophic levels. When the concentrations of the variable examined are below the median value of the oligotrophic system then the waters are characterized as oligotrophic (O). If the measurements lie between the median values of the oligotrophic and mesotrophic systems, then the water mass is characterized as lower mesotrophic. The next category is upper mesotrophic and if the concentrations measured exceed the critical value which is the median concentration of the eutrophic system, they indicate eutrophic type of coastal environment.

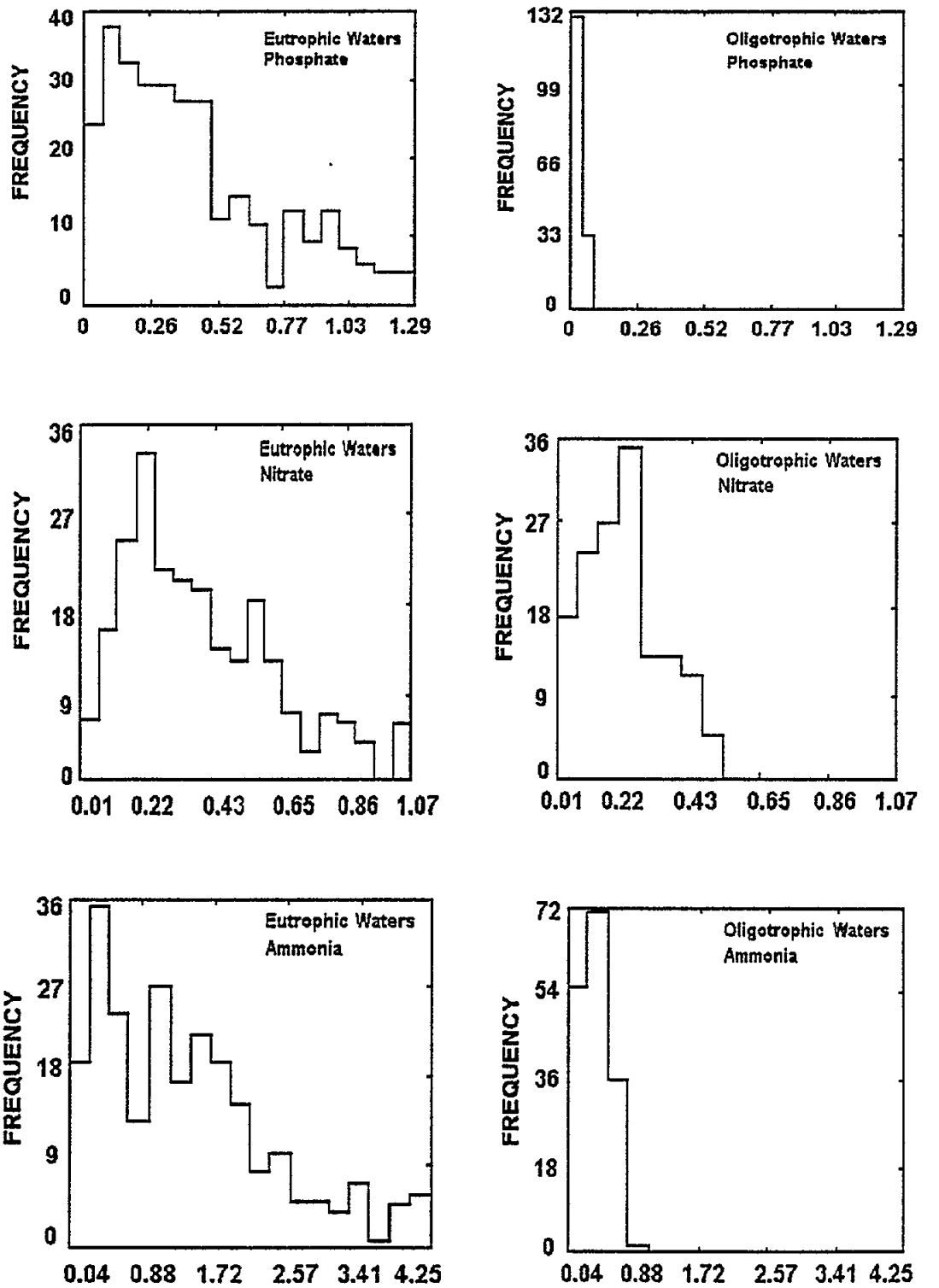

Fig. 2 Nutrient frequency distributions of the raw data after the exclusion of outliers. Upper part of the figure. Inshore Gulf Water from Saronikos Gulf. Lower part of the figure: Offshore pelagic water from Rhodes

Table 1

Statistical characteristics of the nutrient data sets.
(M = median; LQ = lower quartile; UQ = upper quartile; Min = minimum value;
Max = maximum value; N = number of observations.
Nutrient concentrations in µg-at l^{-1})

Water Type	Nutrient	M	LQ	UQ	Min.	Max.	N
TYPE A Eutrophic Waters	Phosphate	0.42	0.20	0.87	0.01	13.72	347
	Nitrate	0.57	0.27	1.45	0.01	24.11	347
	Nitrite	0.20	0.07	0.45	0.01	3.35	347
	Ammonia	1.47	0.62	2.54	0.06	17.4	275
TYPE B Mesotrophic Waters	Phosphate	0.10	0.06	0.16	0.01	3.50	595
	Nitrate	0.32	0.17	0.69	0.01	37.00	595
	Nitrite	0.09	0.04	0.21	0.01	1.86	595
	Ammonia	0.91	0.51	1.28	0.02	11.42	559
TYPE C Oligotrophic Waters	Phosphate	0.03	0.02	0.06	0.01	4.09	197
	Nitrate	0.26	0.16	0.56	0.02	5.06	195
	Nitrite	0.03	0.01	0.05	0.01	0.64	182
	Ammonia	0.41	0.25	0.60	0.04	12.27	189

Table 2

Statistical characteristics of phytoplankton variables.
(Statistical notation as indicated in Table 1
Phytopl. = Phytoplankton cell number (cells/l); chl-á = chlorophyll á in µg l^{-1})

Water Type	Phytopl.	M	LQ	UQ	Min.	Max.	N
TYPE A Eutrophic Waters	Phytopl.	172348	44688	691365	646677	$29x10^6$	290
	Chl-á	0.79	0.44	2.03	0.01	32.4	316
TYPE B Mesotrophic Waters	Phytopl.	36600	13600	160243	146643	$3x10^6$	511
	Chl-á	0.36	0.19	0.66	0.02	7.01	607
TYPE C Oligotrophic Waters	Phytopl.	4240	2240	7440	5200	152090	147
	Chl-á	0.08	0.06	0.11	0.01	0.84	118

2.4 Concluding remarks

In this section critical values were given that define the trophic levels in the Aegean Sea. These critical values can be considered as typical concentration limits and it is suggested to be used as a more reliable quantitative procedure in water quality studies and

coastal management. Furthermore, the framework of the method implies that such a system of water quality criteria can be set up in any particular type of aquatic environment and be used for classification of water masses when the trophic level is under question.

Table 3

Different water trophic categories depending on median nutrient and phytoplankton values from the three data sets (Type A, B and C).
(O = oligotrophic waters; LM = lower mesotrophic; UP = upper mesotrophic and E = eutrophic waters)

Nutrient	Ranges for each trophic category			
	O	LM	UM	E
Phosphate (µg-at P/l)	<0.03	0.03-0.10	0.10-0.42	>0.42
Nitrate (µg-at N/l)	<0.26	0.26-0.32	0.32-0.57	>0.57
Nitrite (µg-at L/l)	<0.03	0.03-0.09	0.09-0.20	>0.20
Ammonia (µg-at N/l)	<0.41	0.41-0.91	0.91-1.47	>1.47
Phytopl. (cells/l)	<4240	4240-36600	36600-172348	>172348
Chlorophyll á (µg/l)	<0.08	0.08-0.36	0.36-0.79	>0.79

There are three main points in the present work (a) a large volume of data was used to eliminate the possibility of artifacts (b) non-parametric statistics was used throughout and (c) the three data sets can be used as a type of "external standards" in water quality studies. Once the trophic level has been assessed, further data analysis can be carried out using multivariate methodologies such as multidimensional statistical analysis and multicriteria evaluation methods. These techniques will be presented in the next sections.

3. MULTICRITERIA EVALUATION METHODS

3.1 The nature of environmental problems

The complexity of environmental problems has increased since social and economic functions affect the environment in its totality and vice versa. The development of tourism along the Mediterranean coasts is having a positive effect on the economy of the countries and a negative effect on the environmental quality; but deteriorated marine environment might have a secondary negative effect on the income from tourism. It is therefore necessary for the managers and decision makers to reconcile between development and environmental quality. This complication has increased the number of people affected by environmental degradation and therefore the number of participants in environmental decision making. Due to the seriousness of environmental issues, environmental quality has to be taken into account in policy analysis and planning.

On the other hand it has been difficult to integrate information from different scientific disciplines: data from social, economic and environmental sciences seem to be difficult to integrate in the same analytical procedures. There is a number of reasons that account for the incompatibility of ecological with social and economic values. The quantification of environmental values is based on biological indicators (Whilm and Dorris, 1968; Karydis et al., 1983) and statistical evaluations (Ignatiades et al., 1985; Austen and Warwick, 1989) with low interpreting value for decision makers. Multivariate procedures have been proved adequate in facing the complicated nature of pollution induced ecological disturbances (Warwick, 1988; Karydis, 1992) and have been applied in the field of environmental quality assessment and management in order to aid the decision-making process (Karydis and Coccossis, 1990; Ignatiades et al., 1992). All these techniques cannot be used with current methods of analysis applied on complex planning problems and coastal management (Hartog et al., 1989). Integrated management approaches, including social and economic values, are often examined with multicriteria choice methods (Zeleny, 1982; Nijkamp et al., 1990). These methods have been developed to face the conflicting nature of problems in policy analysis and have been applied in the field of physical planning and environmental management (Hartog et al., 1989; Nijkamp, 1990).

The present section is concerned with the application of multicriteria analysis on environmental data, in order to examine the effectiveness of the methodology in eutrophication assessment. The case study is based on the coastal area of the city of Rhodes. This coastal zone is characterized by intense use by tourists and therefore, there is an obvious impact of tourism on the coastal marine environment (Karydis and Coccossis, 1990).

3.2 The coastal area of Rhodes: a case study

Experimental design

Data collection: Sampling was carried out at ten nearshore locations (Fig. 3) along the coastal area of the city of Rhodes, Greece, on a monthly basis for one year. Stations RH3, RH4 and RH5 located near the harbour had been characterised eutrophic whereas, stations RH7 and RH9 located one mile offshore were characterised oligotrophic (Karydis, 1992). The rest of the sampling sites were used for swimming and recreational purposes. Water samples were collected from the surface water (1m) and used for nutrient determinations and qualitative and quantitative analysis of the phytoplankton community. The methodological procedures are described by Karydis and Coccossis (1990).

Raw data matrix: The eutrophication assessment was carried out for the summer period (April-October). This is due to the fact that high temperatures and stratified waters during the summer, enhance the trophic state of the coastal system. In addition, it is during the summer period that the coastal area is used for recreational purposes. The mean values of phosphate, nitrate, ammonia and phytoplankton were calculated and the results are shown in Table 4. It is observed

that the highest values of nitrate and cell number were observed at stations RH3, RH4 and RH5. On the contrary, the lowest values were recorded at the two reference stations (RH7 and RH9) as well as at RH10.

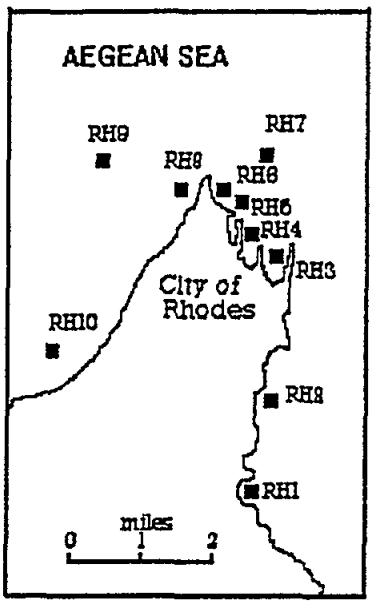

Fig. 3 Coastal area of the city of Rhodes: station locations

Table 4

Mean values of nutrients (μg-at l^{-1}) and phytoplankton cell number (cells l^{-1} x 10^4) along the coastal area of Rhodes, during the stratified period (May-October)

Variable	RH1	RH2	RH3	RH4	RH5	RH6	RH7	RH8	RH9	RH10
Phosphate	0.13	0.08	0.07	0.07	0.07	0.10	0.08	0.11	0.08	0.07
Nitrate	0.84	0.47	1.76	4.37	2.45	0.57	0.38	0.34	0.27	0.30
Ammonia	0.84	0.63	0.48	0.69	0.67	0.51	0.56	0.75	0.79	0.65
Phytopl.	2.06	1.86	5.39	33.92	8.38	1.79	0.93	2.47	0.80	0.72

Scale development: The Median (M), Lower Quartile (LQ), Upper Quartile (UQ), Minimum (Min) and Maximum (Max) values of each variable (PO_4, NO_3, NO_2, NH_3) shown in Table 1 formed the basis for the validation of eutrophic levels of the data which are presented in Table 4. An ordinal ranking was applied to the different levels of eutrophication. To values corresponding in the range "O" of the Table 3 the ordinal number (1) was assigned, reflecting the most favorable environmental quality

(oligotrophic conditions). Values ranging in the "LM" interval were characterized by the ordinal number (2), indicating a tendency of the system towards mesotrophic conditions. The ordinal number (3) was used to characterize values within the "UM" interval, indicating advanced mesotrophic conditions. Finally, the ordinal number (4) was used for values of the "E" interval of Table 3, indicating eutrophic conditions. This data ranking has been used extensively in environmental policy analysis (Nijkamp, 1980) and numerical classification (Boesch, 1977). Ordinal scaling being a semi quantitative variable can be used to express any type of data. It also has the advantage of smoothing out minor differences that would contribute noise into the system.

The summary scorecard: A summary scorecard displaying nutrient - phytoplankton loading of the ten coastal stations is presented in Table 5. The scores resulted from a combination of Tables 4 and 3. It is observed that during the summer most of the stations had a tendency towards upper mesotrophy. The great advantage of the scorecard is the fact that forms the summary of a large evaluation study. These criteria can be measured on any measurement scale since their final expression, through the ordinal scale, is dimensionless. These scores formed the basis for the application of the multicriteria choice methods.

Table 5

A scorecard of ten stations and four criteria for assessing eutrophic levels along the coastal area of the city of Rhodes

Variable	RH1	RH2	RH3	RH4	RH5	RH6	RH7	RH8	RH9	RH10
Phosphate	3	2	2	2	2	2	2	3	2	2
Nitrate	4	3	4	4	4	3	3	3	2	2
Ammonia	3	4	4	4	4	4	3	4	2	2
Phytopl.	2	2	3	4	3	2	2	2	2	2

Multicriteria choice-methods analysis: The principle of multiple criteria methods is the classification of alternative choice possibilities on the basis of various criteria (Nijkamp and Voogd, 1986). In the present study the sampling locations are the alternative choice possibilities while the nutrient variables and the phytoplankton cell number were the criteria. Multicriteria evaluation offers a variety of methods but all of them obey the same principle: the pairwise comparison of the scores for all the alternatives and for each criterion. These methods rank the alternatives (stations) according to their scores. Two multicriteria methods were used in the present study: the regime method and the concordance analysis. Methodological details about these two methods have been given elsewhere (Janssen, 1992; Moriki and Karydis, 1994). The results are shown in Table 6. Two groups of stations were formed: (a) one group including the eutrophic stations RH4, RH3 and RH5 as well as the stations RH1 and RH8 that seem to show eutrophic trends (b) the group of the oligotrophic stations RH7, RH9 and also the stations RH2, RH6 and RH10 which seem to portray oligotrophic characteristics.

Table 6

Multicriteria analysis of the data matrix of Table 5.
Two methods were applied, regime and concordance analysis.
Both indicated the same eutrophication trends for the ten stations

Regime Method	
Station	Score
RH4	1.000
RH3	0.833
RH5	0.833
RH1	0.667
RH8	0.556
RH2	0.389
RH6	0.389
RH7	0.222
RH9	0.056
RH10	0.056

Concordance Analysis	
Station	Score
RH4	17.000
RH3	14.000
RH5	14.000
RH1	7.000
RH8	7.000
RH2	- 3.000
RH6	- 3.000
RH7	-11.000
RH9	-21.000
RH10	-21.000

Methodological Evaluation and Conclusions: The complexity of natural ecosystems requires the use of variables for water quality management. Nutrients and phytoplankton cell number seem to provide adequate information for discriminating between eutrophic and unimpacted sites. In the present study a non-parametric procedure has been chosen. In addition, the results were expressed in a non-metric form through scaling. This way, the shortcomings resulting from the violation of the methodological assumptions of statistical procedures can be avoided and therefore, the resulting information is fully compatible to the methodologies used by planners and decision makers. This way it was possible for the data to be analysed by multicriteria choice-methods and a ranking of the sampling sites to be obtained. Further data analysis can be carried out by multivariate techniques such as cluster analysis and multidimensional scaling. The multicriteria analysis can satisfy the requirements of a multivariate procedure for the assessment of environmental impacts caused by human activities and can be added to the available methods used for coastal management studies. An application on multivariate statistics on the results of multicriteria evaluation methods (Moriki and Karydis, 1994) has shown very satisfactory discrimination among eutrophic, mesotrophic and oligotrophic coastal waters.

4. IDENTIFICATION OF EUTROPHIC TRENDS BASED ON PHYTOPLANKTON COMMUNITY ANALYSIS: A SPECIES APPROACH

4.1 Introduction

Although multivariate methods based on nutrient and chlorophyll concentrations have been widely used for eutrophication assessment (Karydis, 1992),

little effort has been made for the evaluation of water quality based on phytoplankton community analysis (Clarke, 1993). In 1982, Field et al. (1982) outlined a strategy for the analysis of data on community structure. The basic components of the above strategy were the following: a) The construction of an abundance array whose rows are the set of species present in a sample, while the columns represent different sampling units; b) The relationship between two samples is expressed with a coefficient measuring similarity or dissimilarity in species composition; c) The resulting triangular matrix of similarities between every pair of samples is used to classify the samples into groups, using hierarchical agglomerative clustering methods; d) The result of the classification can be presented with a dendrogram in the two-dimensional space.

The above strategy has been adopted in a number of published studies (Clarke, 1993). However, most of these studies were concerned with pollution assessment using the benthic community structure (Warwick, 1993; Agard et al., 1993). In the present study, an approach has been made for the assessment of eutrophication levels, based on the phytoplankton community structure. A number of scaling methods and resemblance measures were tested, in order to maximize the discrimination between a eutrophic and an oligotrophic system.

4.2 Field work: Sampling design

Water samples were collected from two stations located in the coastal area of the city of Mytilini. Sampling site M1 was placed near the sewage outfalls of Mytilini and M2 offshore. The station locations are given in Figure 4.

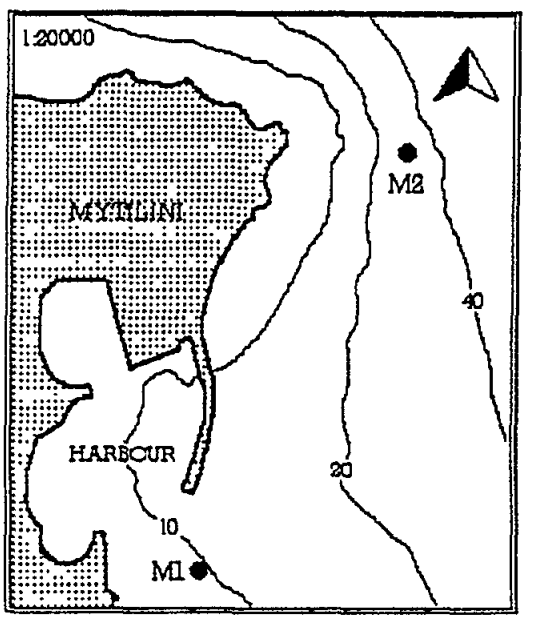

Fig. 4 The coastal area of Mytilini: station locations

M1 and M2 were previously characterized as eutrophic and oligotrophic respectively (Karadanelli et al., 1992). Fourteen samplings took place between February 1992 and May 1993.

Station M1 was sampled at 1, 5 and 10 m, while M2 was sampled at 1, 5, 10, 20 and 30 m. Water samples were collected with a VanDorn sampler and were fixed with lugol solution. The quantitative and qualitative analysis of the samples was carried out on an inverted microscope (Lund et al., 1958).

4.3 Data analysis

The original data-matrix consisted of 218 rows (phytoplankton species) and 111 columns (sampling units). Mean abundance was calculated for each species, during summer (May-October) and winter (November-April), dividing the original data-set into two subsets (summer and winter); the resulting data-matrices (one for each period) had 8 columns (the three sampling depths of station M1 and five of station M2). Numerical classification of the eight sampling units was performed by the group-average clustering algorithm, using different resemblance measures, species numbers and data scaling. The statistical significance of the clusters formed by the clustering procedure was tested using the non-parametric randomization test ANOSIM (Clarke and Green, 1988).

Selection of resemblance measures: The Bray-Curtis similarity measure, the euclidean distance and the absolute distance were used, since they have shown efficiency in discriminating polluted sites (Siokou-Frangou and Papathanassiou, 1991; Karydis, 1992).

Bray-Curtis similarity measure (BC): The BC similarity coefficient estimates the percent similarity (PS) between the j and k sampling units according to the formula (Bray and Curtis, 1957):

$$PS_{jk} = \left(\frac{2W}{A+B} \right) \times 100$$

where $W = \sum_{i=1}^{s} [\min(x_{ij}, x_{ik})]$, $A = \sum_{i=1}^{s} x_{ij}$, $B = \sum_{i=1}^{s} x_{ik}$

and x_{ij}, x_{ik} the abundance of the ith species in the j and k sampling unit, respectively and s the total number of species.

Euclidean Distance (ED): The ED distance in s-dimensional space between the jth and kth sampling unit, denoted by $d_{(j,k)}$, is given by the formula (Pielou, 1984):

$$d_{(j,k)} = \sqrt{\sum (x_{ij} - x_{ik})^2}$$

Absolute Distance (AD): The AD distance is the sum of the absolute differences taken over the s species:

$$d_{(j,k)} = \sum |x_{ij} - x_{ik}|$$

For expressing the distance between sampling units as percent similarity, the distance matrices were transformed to similarity matrices (relative scale 0-100) by the formula:

$$Z_{ij} = \frac{(X_{max} - X_i)}{X_{max}} \times 100$$

where x_{max} is the maximum value observed.

Species elimination: Elimination of the original data-matrices was performed, excluding species which occurred in the sampling units less than 10 times annually. The data-matrices resulting from the reduction had 56 rows, instead of the 218 of the original data matrix.

Data scaling: Metric Scaling. The metric scale is the natural scale used to measure the abundance of phytoplankton species.

Binary Scaling. The original and eliminated data-matrices were expressed in a binary form (Everitt, 1981). Values of species abundance exceeding the mean value of a sample were expressed by the state 1, otherwise state 0.

Ordinal Scaling. The scaling was based on the sample mean μ and the sample standard deviation ó, which determine the location and the shape of the frequency distribution of each variable (Sokal and Rohlf, 1981). To values lower than (μ-ó) the ordinal number (1) was assigned. Values ranging between (μ-ó) and (μ) were characterised by the ordinal number (2), while to values between (μ) and (μ+ó) the ordinal number (3) was assigned. Finally, the number (4) was used for values greater than (μ+ó).

Classification: The group-average clustering algorithm was chosen to produce the dendrogram from the similarity matrix, since it joins two groups of the samples together at the average level of similarity between all members of one group and all members of the other (Sneath and Sokal, 1973).

Statistical significance between groups of sampling units: The non-parametric randomization test ANOSIM was used (Clarke and Green, 1988) to determine the statistical significance of the results of the clustering procedure. The ANOSIM test relies on the rank order of similarities in the original matrix and determines whether sampling units which appear to be in the same clusters, form distinct, significantly different groups.

4.4 Results and discussion

Two groups of sampling units were formed by the clustering procedure, in every case. A eutrophic, consisting of the 3 sampling depths of station M1 and an oligotrophic, containing the 5 sampling depths of station M2. The formation of two distinct groups, regardless of the method used for the treatment of the original data-set, supports the view that the phytoplankton community data can be used as an efficient method for the assessment of eutrophication levels.

For the evaluation of the different techniques used to achieve higher resolution between the eutrophic and the oligotrophic group, the ANOSIM test significance level was considered (Table 7).

In general, the resolution between the eutrophic and oligotrophic group was higher in summer, perhaps due to the lack of water circulation, which favours the development of extreme conditions of eutrophication.

Among the three resemblance measures used, the best discrimination, both in summer and winter period, was achieved using the Bray-Curtis coefficient, especially when the natural scale (metric scale) of the species abundance was used. The Bray-Curtis coefficient is widely used in ecology and seems to be one of the most reliable coefficients of resemblance in many cases (Clarke, 1993). On the other hand, almost similar classification trends were shown by both euclidean and absolute distances, perhaps because no large differences were observed for the species abundance values between the sampling units. Euclidean distance emphasizes the larger differences of values, since each difference is squared and then summed, while absolute distance places less emphasis on large differences (Karydis, 1992).

The exclusion of the rare species of phytoplankton improved the resolution of the method, in every case. This fact supports the view that the rare species add noise to the signal carried by the phytoplanktonic community structure. On the contrary, no improvement of the resolution of the method was achieved using binary or ordinal scaling.

As a conclusion, the best discrimination, both in summer and winter, was achieved using the Bray-Curtis coefficient of resemblance, on the reduced data matrix with no scaling of the original values (Fig. 5).

Numerical classification by the group average clustering algorithm, based on phytoplankton community data seems to be an efficient method to assess water quality. As a conclusion, the following stepwise procedure is proposed: a) reduction of the original data matrix by removing the rare species, b) no scaling of the original data values, c) use of the Bray-Curtis coefficient of resemblance, d) identification of distinct groups of sites with objectivity by the non-parametric randomization test ANOSIM.

Table 7

ANOSIM test significance levels for differences between clusters

I. SUMMER PERIOD A. All species considered			
	Metric	Scales Binary	Ordinal
BC	0.797*	-	-
AD	0.673*	0.698*	0.544*
ED	0.705*	0.698*	0.568*
B. Rare species excluded			
	Metric	Scales Binary	Ordinal
BC	0.806*	-	-
AD	0.673*	0.906*	0.624*
ED	0.721*	0.906*	0.655*
II. WINTER PERIOD A. All species considered			
	Metric	Scales Binary	Ordinal
BC	0.894*	-	-
AD	0.667*	0.523*	0.420
ED	0.667*	0.523*	0.412
B. Rare species excluded			
	Metric	Scales Binary	Ordinal
BC	0.903*	-	-
AD	0.667*	0.670*	0.633*
ED	0.667*	0.670*	0.623*

* Statistically different clusters at the 0.05 probability level

5. SPATIAL QUANTIFICATION OF EUTROPHICATION USING CATEGORICAL MAPS

5.1 Introduction

It is important in coastal management studies to be able to quantify the system structure and system quality. The monitoring of the marine environment at a spatial scale allows the quantitative assessment of a pollution problem as well as the detection of spatial changes in time. This is particularly important in eutrophication studies due to phytoplankton seasonality and dynamics. However, there are very

limited applications in categorical maps for describing eutrophication. This may be due to the fact that spatial heterogeneity, a predominant feature in marine ecology, significantly contributes to the complexity of the spatial pattern. Spatial heterogeneity is a universal phenomenon, existing in ecological systems at all scales (Pielou, 1977; Whittaker and Levin 1977).

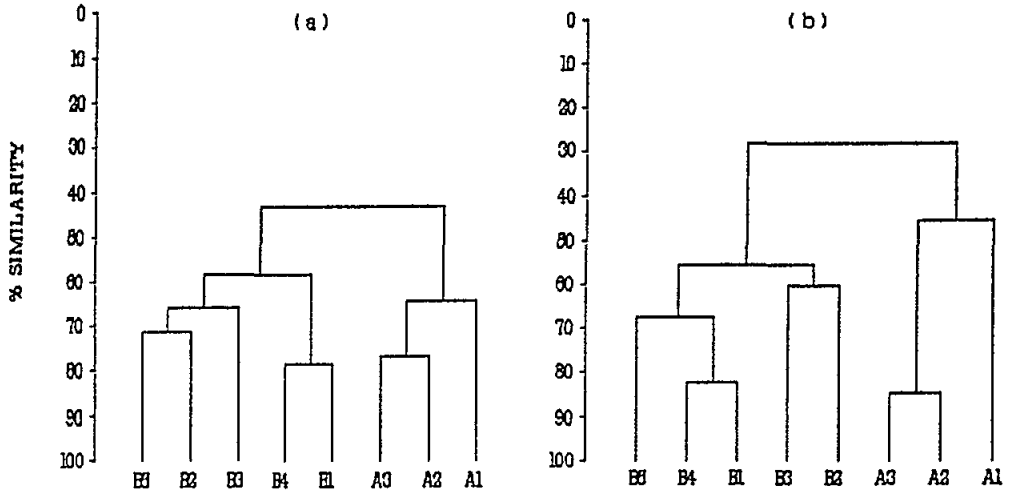

Fig. 5 Dendrograms for group-average clustering of the reduced data matrix with the Bray-Curtis coefficient in summer (a) and winter (b) period; (A1, A2, A3 and B1, B2, B3, B4, B5, represent different depths of the eutrophic and oligotrophic stations, respectively)

In the present section the possibility of forming a categorical map for describing the spatial distribution of a eutrophic field is attempted and a number of spatial interpolation methods were tested.

5.2 Case study: the Gulf of Alexandroupolis

Field work

Alexandroupolis Gulf is located in the northern part of the Aegean Sea. The bulk of runoff comes from the river Evros; in addition there are agricultural runoffs from the coastal zone used for agriculture. Samples were collected from twenty five stations (Fig. 6). Oceanographic casts were carried out by the Hydrographic Boat "Nautilos" in March 1982. Phytoplankton samples were analysed quantitatively and qualitatively using inverted microscope (Ignatiades et al., 1983). More methodological details have been given by Friligos and Karydis (1988).

Phytoplankton cell number (number of cells/l) varied between 4800 cells/l and 10,400,000 cells/l. The horizontal distribution of phytoplankton in the Alexandroupolis Gulf is given in Figure 7.

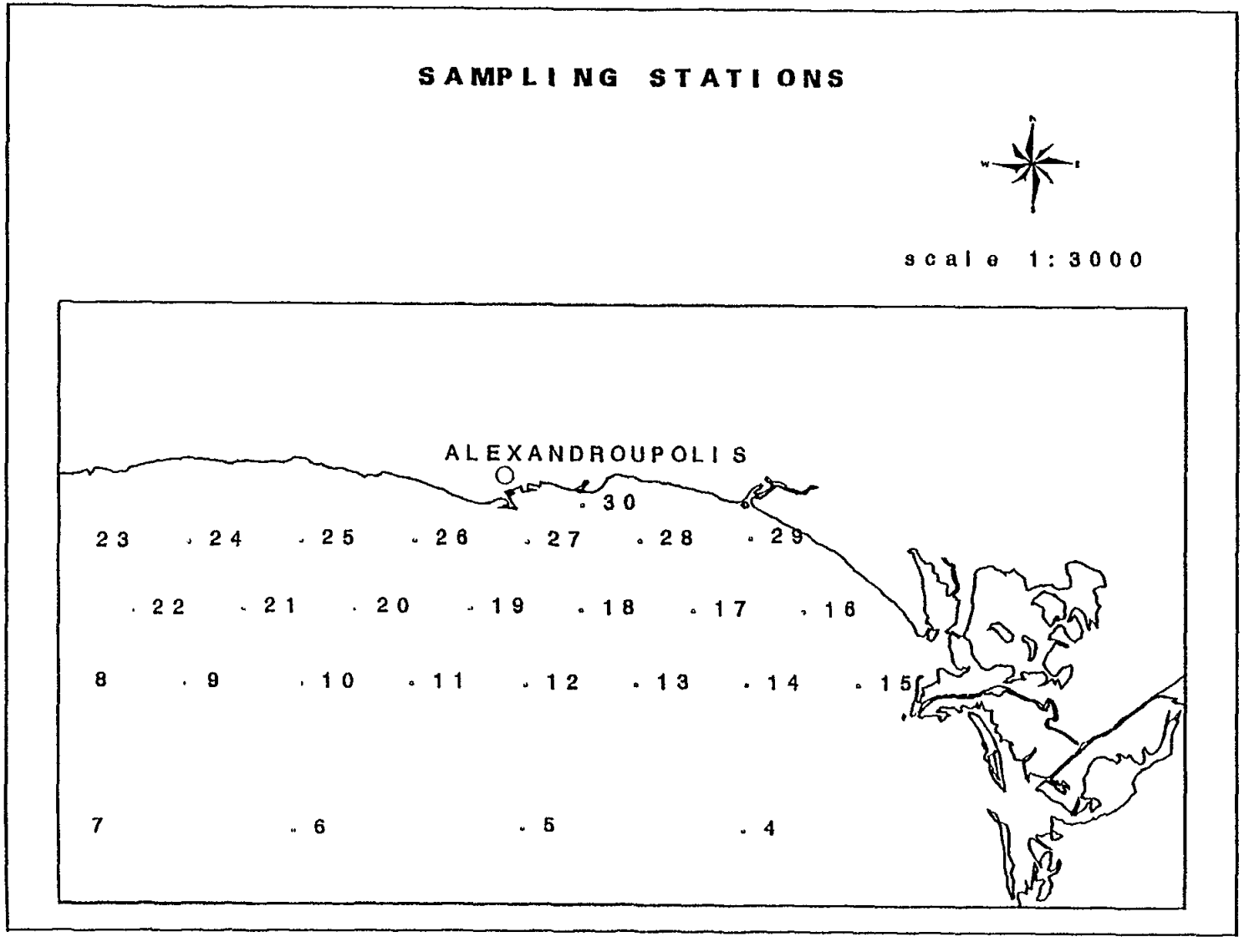

Fig. 6 Coastal area of Alexandroupolis: station locations

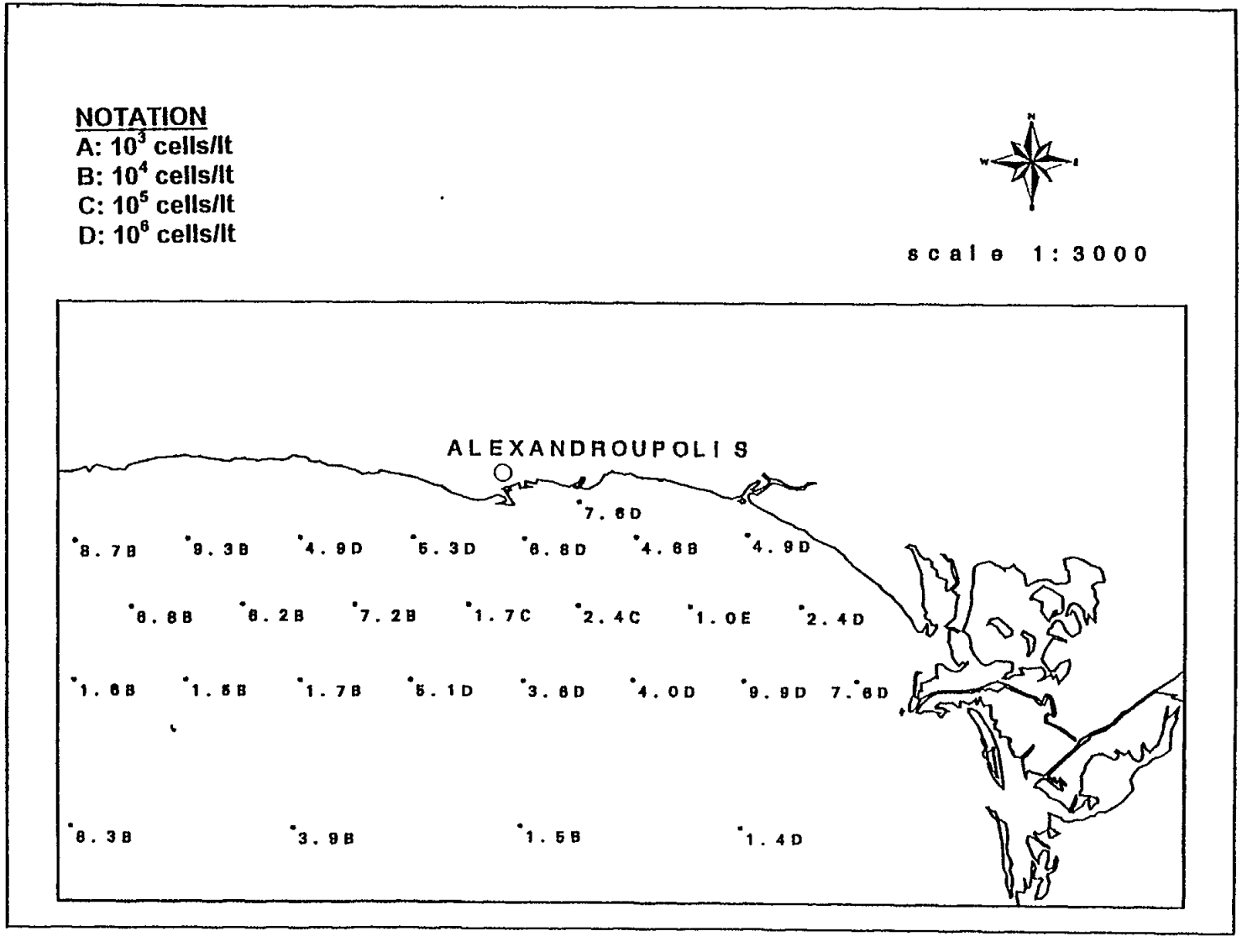

Fig. 7 Numerical map of horizontal distribution of phytoplankton

The second part of the case study is concerned with the development of a contour and a categorical map illustrating the trophic status of the Gulf. A number of spatial interpolation methods were tested and the goodness-of- fit of the derived surface to the data was evaluated. The contour map developed with Kriging (Cressie, 1991) is given in Figure 8.

This analysis did not give a good fitting of the data as the trend analysis which was finally applied.

Trend analysis: The trend surface interpolator uses a polynomial regression to fit a least-squares surface to the input points. It is a smoothing technique and the idea is to fit the data by least-squares using a function of the form

$$f((x,y)) = \sum_{r+s<p} a_{rs} x^r y^s$$

of which the first few functions are,

a	flat
a+bx+cy	linear
$a+bx+cy+dx^2+exy+fy^2$	quadratic

The above formulas cover two dimensions. However, there may be extension to three or more dimensions. The integer p is the order of the trend surface.

If we label 1, x, y, x^2 xy, y^2, ..., as $f_1(x)$,.....,$f_p(x)$ and the coefficients as b_1,.. .., b_p, the problem is multiple regression of Z(xi) on (f_1(xi)),.....,f_p(xi)). There are P=(p+1)(p+2)/2 coefficients which are normally chosen to minimize the

$$\sum_{1}^{N} [Z(xi) - f(xi)]^2$$

where Z(xi) are the observations of the surface Z we wish to map.

The process above is of statistical nature and is known as polynomial regression.

In the analysis of trends, the data are seen as being subject to error,but there is some reason for seeing the ordinates Z(xi) as the sum of two components. These components is assumed to be the result of a different physical process, probably on different scales (of the independent variable x in this case). The component varying more slowly with the independent variable is described as the trend and the other as the residual or the deviation from the trend.

The following quantity is frequently used empirically as a measure of the degree to which the trend fits the data.

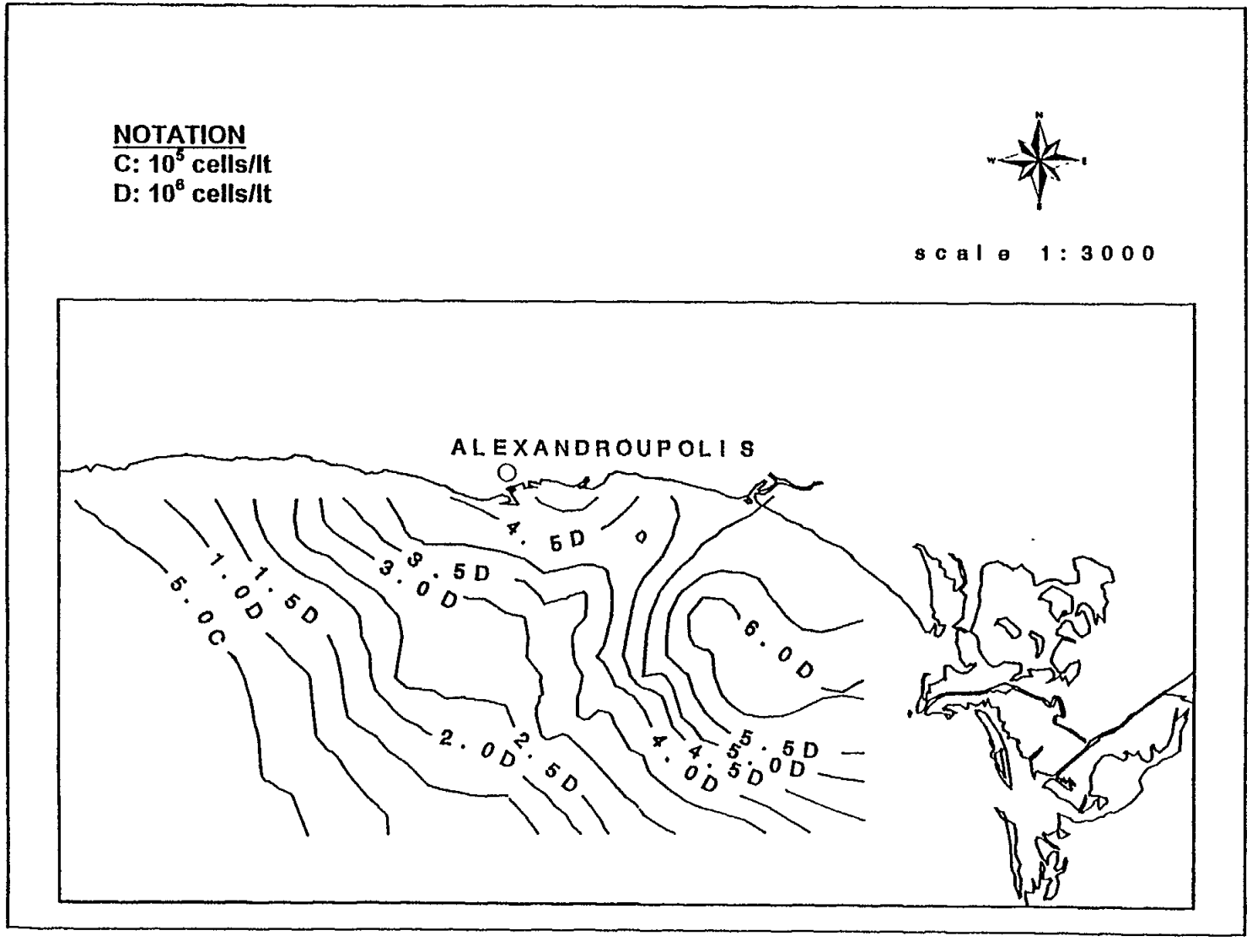

Fig. 8 Contour map illustrating horizontal phytoplankton distribution

$$R^2 = 1 - \frac{G\{Z(xi) - Z(xi)\}^2}{G\{Z(xi) - Z(xi)\}^2} = \frac{G\{Z(xi) - Z(xi)\}^2}{G\{Z(xi) - Z(xi)\}^2}$$

where Z(xi) is the estimated value of Z(xi) and

Z(xi) = GZ(xi) / Í the arithmetic mean of the N data values.

The expression in the denominator of these fractions is then just the variance of all the observations about this mean.

It is $0 < R^2 < 1$ and when R=1 then Z(xi) = Z(xi), so all the data lies on the regression curve and consequently this curve interpolates the data (there is no residual or deviation). On the other hand, when R=0, Z(xi) = Z(xi) (constant) for each i, and the analysis fails to show any trend.

Consequently, values of R^2 between 0 and 0.2 suggest that the trend is not well established and values of R^2 between 0.8 and 1 are often thought to show a significant trend in the data.

Finally, it is important to notice that the surface generated would seldom pass through the original data points since it performs a best fit for the entire surface. Moreover, as the order of the polynomial is increased, the surface being fitted becomes progressively more complex. A higher order polynomial will not always generate the most accurate surface; it is dependent upon the data. The lower the rms error, the more closely the interpolated surface represents the input points. An undesirable feature of trend surfaces is a tendency to wave edges to fit points in the center. The contours derived from trend analysis are illustrated in Figure 9.

An environmental gradient was observed, the highest concentrations being recorded near the coastal zone. It is observed that there is an area of high phytoplankton density in the Eastern part of the Alexandroupolis Gulf whereas, a eutrophic field also seems to be located near the urban zone of Alexandroupolis. However, the distribution pattern is lacking clarity and the information provided by the numerical map does not contribute to a water quality classification pattern.

Categorical mapping. Phytoplankton scaling: Phytoplankton cell numbers were transformed into an ordinal scale described in the second part of the present report. The ordinal number (1) was used to express oligotrophy (the most favourable environmental condition) whereas, the ordinal number (4) was used to express eutrophic conditions. Numbers (2) and (3) were used to express lower mesotrophic and higher mesotrophic conditions respectively. The categorization of phytoplankton cell number in the sampling sites is presented in Figure 10.

The categorical map is given in Figure 11. A clear horizontal pattern is presented illustrating the four trophic states of Alexandroupolis Gulf. It is also observed that minor heterogeneities have been smoothed out and the boundaries of the eutrophic areas can be easily defined. In conclusion, this method can be applied

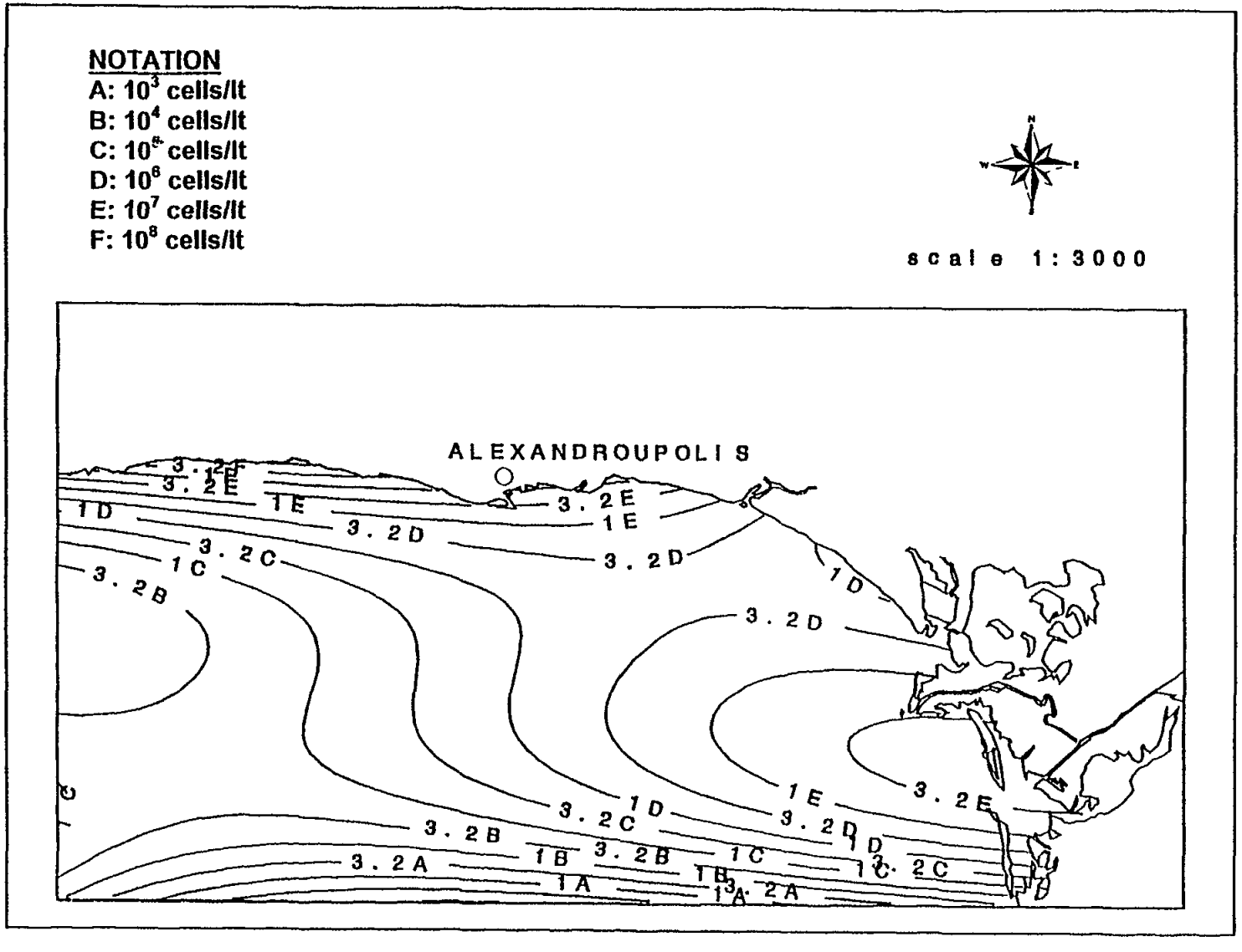

Fig. 9 Horizontal phytoplankton distribution based on trend analysis

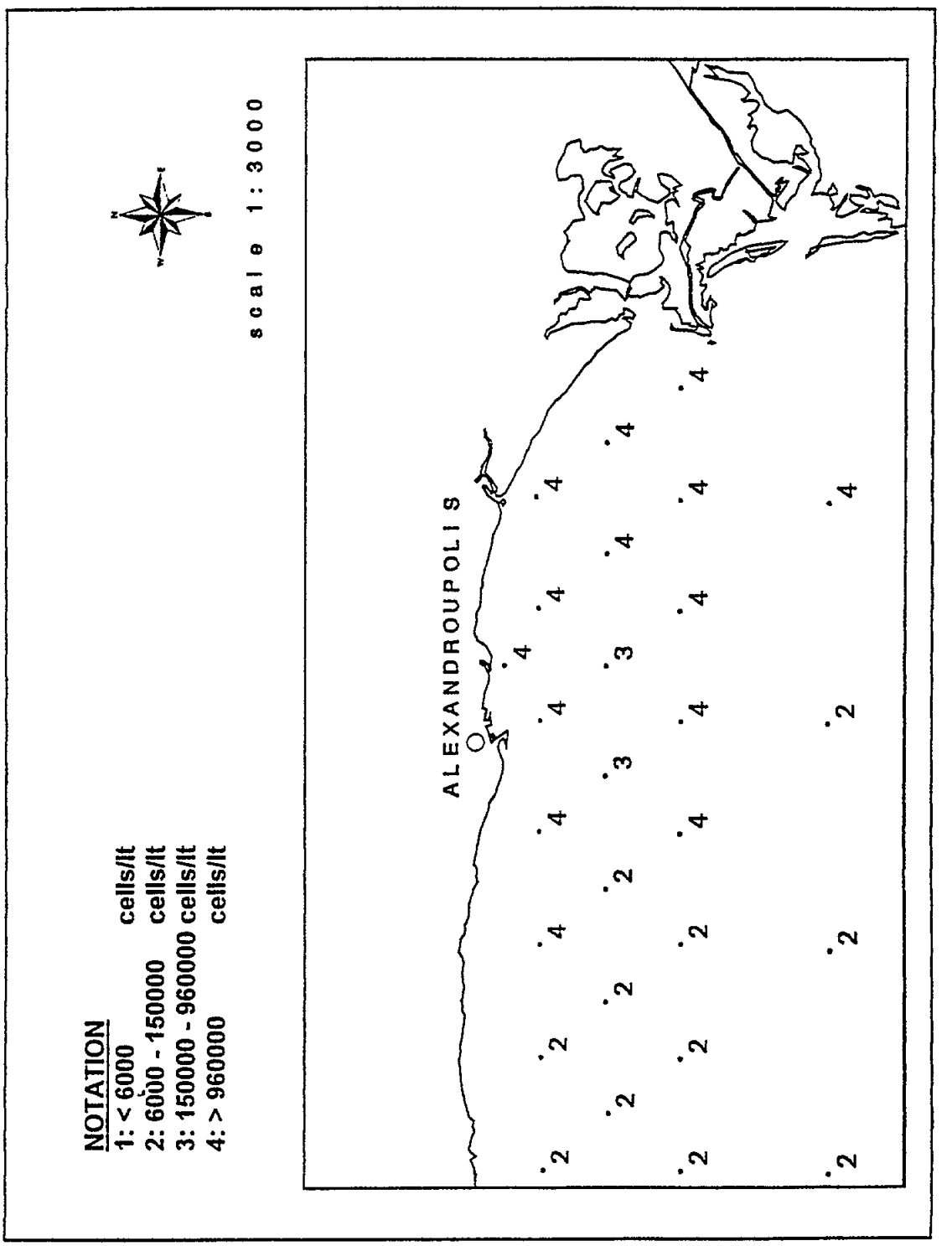

Fig. 10 Categorization of phytoplankton cell numbers in the sampling sites

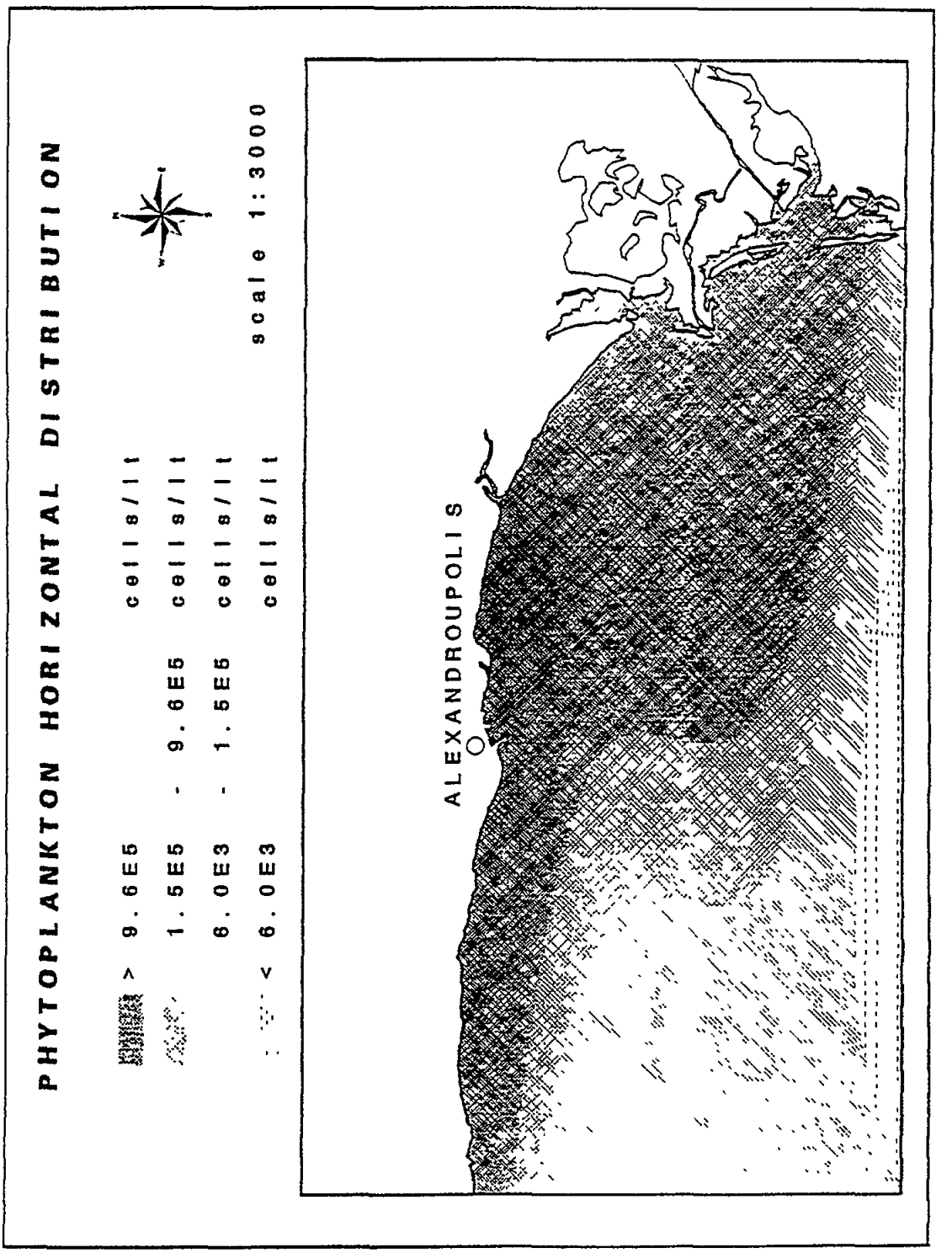

Fig. 11 Categorical map showing the four different trophic levels from oligotrophy to eutrophic conditions

as a tool for spatial quantitative assessment of eutrophication on a routine basis. Efforts to develop this type of categorical maps should concentrate around two points:

(a) The use of a suitable surface interpolation method. In this particular work the trend analysis has given the best fitting of the data. However, other methods should also be tried out to quantify spatial patterns of eutrophication,

and

(b) The scaling of the variables using ordinal scaling.

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CONTRIBUTION A L'ÉTUDE DES FLUX DE MATIÈRE DANS LES EAUX CÔTIÈRES SYRIENNES (EN FACE DE LATTAQUIÉ). RÔLE DU PLANCTON DANS LE TRANSFERT DE QUELQUES MÉTAUX LOURDS

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R E S U M E

Une première étude hydrochimique et zooplanctonique dans les eaux côtières Syriennes a eu lieu au cours des périodes printanière et estivale des années 1991 et 1992. Les principaux résultats obtenus indiquent les points suivants:

- des variations spatio-temporelles importantes pour les différents paramètres hydrochimiques et zooplanctoniques;
- une richesse relative des eaux étudiées en espèces zooplanctoniques (jusqu'à 112 espèces, dont les copépodes sont les plus importants);
- une certaine sensibilité du zooplancton aux effets de la pollution;
- des teneurs des eaux en métaux lourds étudiés (Cd, Cu, Pb et Zn) comparables à celles indiquées pour les zones modérément polluées en Méditerranée;
- des concentrations de ces métaux dans le zooplancton comparables à celles relevées dans la littérature méditerranéenne, et parfois plus élevées.

1. INTRODUCTION

Les travaux relatifs à l'étude de l'écosystème pélagique du bassin Levantin sont peu nombreux par rapport à ceux effectués en Méditerranée occidentale; ils traitent surtour de la composition du plancton et des caractéristiques physico-chimiques de cet écosystème. Parmi les travaux déjà réalisés en Méditerranée orientale, nous citerons les travaux de: Dowidar et El Maghraby (1970), Lakkis (1971 et 1973), Kimor et Wood (1975), Moraitou-Apostolopoulou (1985), Lakkis et Zeidane (1987), Pancucci-Papadopoulou et Anagnostaki (1989) et Pancucci-Papadopoulou et al. (1992).

Au cours de ces dernières années, les chercheurs ont commencé à s'intéresser à l'étude des effets de la pollution sur l'écosystème pélagique du bassin Levantin. Parmi les travaux qui ont été effectués sur les métaux lourds en Méditerranée orientale, nous pouvons mentionner les travaux de: Papadopoulou et al. (1976), Uysal et al. (1986), Uysal (1992), Nacopoulou et Catsiki (1992), Voutsinou-Taliadouri et al. (1992). Tous ces travaux portent sur la concentration des métaux lourds dans l'eau ou dans quelques organismes marins, souvent benthiques, ou des poissons. L'importance du stockage des métaux lourds dans le zooplancton n'a cependant pas suscité d'études élargies au niveau de la Méditerranée, si l'on excepte les travaux de Roméo et al. (1985 et 1987) et de Damoglu et Uysal (1992).

Vu l'importance des données relatives à l'écosystème pélagique des eaux syriennes, nous avons entrepris des recherches sur l'évolution spatio-temporelle de quelques paramètres hydrochimiques (salinité, température, sels nutritifs, chlorophylle a) et biologiques (abondance et biomasse du zooplancton ainsi que certains flux de matière au sein de l'écosystème zooplanctonique). Les concentrations du cadmium, du cuivre, du plomb et du zinc ont été déterminées dans l'eau de mer et dans certains groupes zooplanctoniques. Parmi ces métaux étudiés, deux d'entre eux (cuivre et zinc) sont des éléments indispensables à la vie et deviennent toxiques quand ils sont présents 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 (Singer, 1973).

2. MATÉRIELS ET MÉTHODES

2.1 Dates et stations de prélèvements

Les prélèvements hydrologiques et zooplanctoniques ont été effectués lors de 12 sorties réalisées en deux ans: entre mars et octobre 1991 et entre avril et octobre 1992, soit à raison d'une sortie par mois en moyenne.

Trois stations de prélèvements ont été retenues; elles sont situées en face de la ville de Lattaquié, à 500 mètres environ de la côte et à 5-10 mètres de profondeur (fig. 1). Les caractéristiques de ces stations sont les suivantes:

Station 1: située en face de l'Institut de Recherches Marines, loin de l'influence directe de toute sorte de pollution.

Station 2: située à l'entrée du port de Lattaquié et considérée comme une station polluée par les hydrocarbures. En plus, une partie des rejets urbains de la ville de Lattaquié débouche au sein du port.

Station 3: située en face de l'estuaire de la rivière "Al-Kabire Al-Chimali". Ce cours d'eau est soumis à l'influence de quelques rejets urbains et industriels de quelques usines au cours de son parcours.

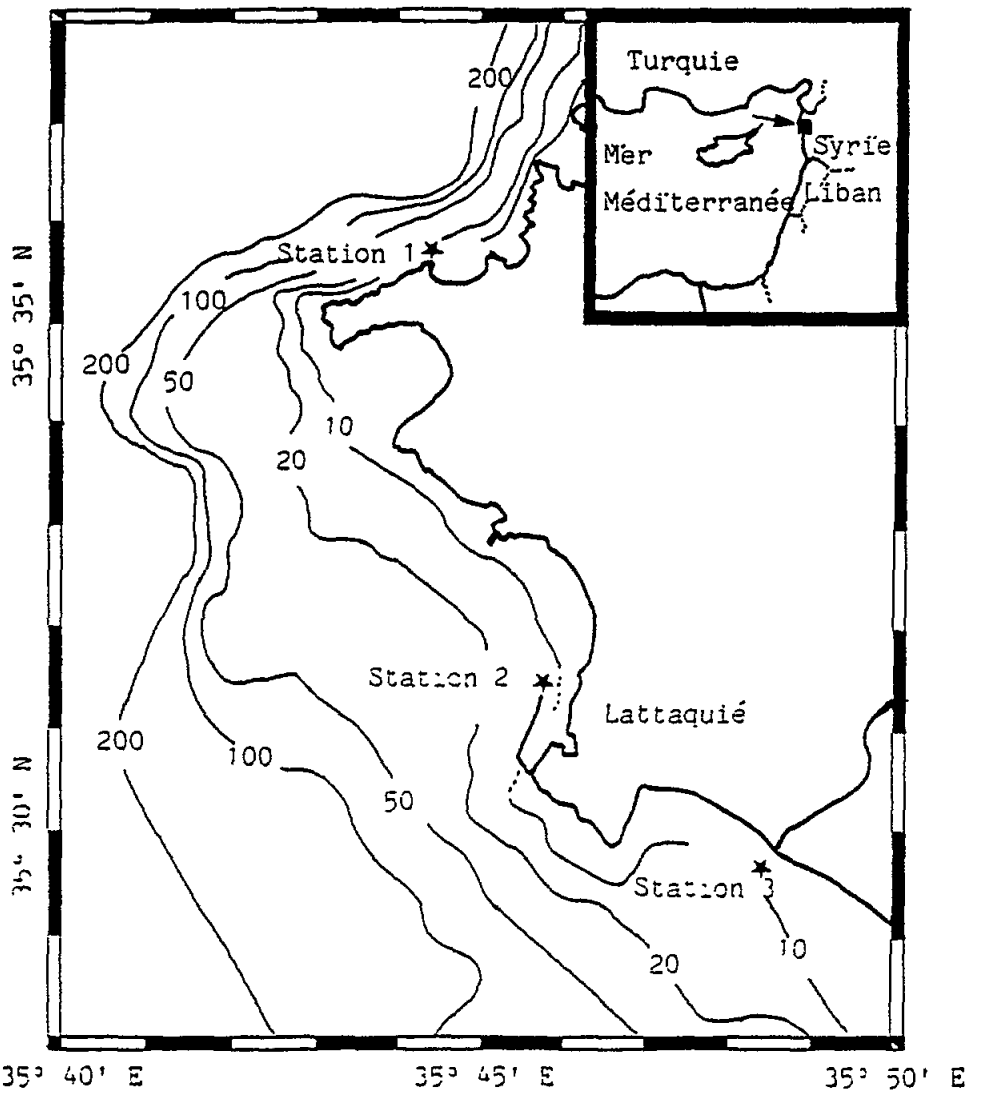

Fig. 1 Localisation géographique des stations de prélèvements

Les échantillons d'eau ont été prélevés à 0,5 mètre sous la surface de l'eau, à l'aide d'une bouteille de prélèvement Wildco (modèle 1520 C 20) d'une capacité de 2 litres.

Les prélèvements zooplanctoniques ont été effectués avec un filet à maillage de 150 μm, de 22 cm de diamètre d'ouverture et de 1,5 m de longueur. Les échantillons destinés à l'étude de la biomasse ont été prélevés à deux niveaux (0 et 5 mètres). Ceux destinés au dosage des métaux lourds dans le zooplancton ont été réalisés en pêche verticale entre -5 et 0 mètre en 1991, et uniquement à -0,5 mètre en 1992. Les échantillons de la biomasse ont été fixés au formol neutralisé à 4%.

2.2 Méthodes analytiques

Des mesures hydrologiques telles que la température et la salinité ont été effectuées sur place à l'aide d'un S.C.T. mètre YSI (modèle 33); de plus, un dosage ultérieur de salinité a été effectué selon la méthode volumétrique de Knudsen. Le pH a été mesuré à l'aide d'un pH-mètre de laboratoire. Les orthophosphates ont été analysés selon la méthode de Murphy et Riley (1962). Le dosage de l'azote ammoniacal est effectué par la méthode de Koroleff (1969). Le dosage de l'azote nitreux est réalisé selon la méthode de Benschneider et Robinson (1952); l'azote nitrique est mesuré après réduction sur colonne de cadmium traitée au cuivre selon la technique de Wood et al. (1967); les nitrates transformés en nitrites sont dosés par la suite selon la méthode de Benschneider et Robinson (1952). Les mesures spectrophotométriques ont été réalisées à l'aide d'un spectrophotomètre Philips (PU 8680).

Le dosage des métaux lourds (Cd, Cu, Pb et Zn) dans l'eau de mer a été effectué à l'aide d'un spectrophotomètre d'absorption atomique avec flamme (Perkin Elmer 2380) après une procédure de chélation-extraction à l'aide de l'ammonium 1-pyrrolidine dithiocarbamate (Filippelli, 1987). Les mêmes métaux lourds ont été mesurés dans des extraits zooplanctoniques après minéralisation de ces extraits à l'aide de l'acide nitrique (suprapure; la technique de l'absorption atomique avec flamme a également été appliquée).

La détermination du zooplancton est faite jusqu'à l'espèce et le comptage des individus de chaque espèce déterminée est réalisé dans des sous-échantillons fractionnés en boite de Motoda. L'abondance est calculée pour chaque espèce, d'abord par pêche ensuite par m^3 (à partir de la vitesse du bateau, du trajet parcouru et de l'ouverture du filet). L'abondance par m^2 est calculée par intégration sur la couche étudiée 0-5 m (méthode du trapèze).

La biomasse en poids sec par m^2 d'une espèce est calculée en multipliant l'abondance par m^2 par le poids sec moyen d'un individu; les valeurs du poids sec moyen ont été tirés des travaux de Nival et al. (1974) et de Baker (1990). La biomasse en azote a été calculée en utilisant le rapport N/PS emprunté à la littérature pour chaque espèce (Beers, 1966; Bougis, 1974; Gorsky et al., 1987).

Nous avons utilisé les données recueillis de six sorties effectuées en 1991 pour l'étude de la biomasse et de quelques flux de matière. L'abondance totale du zooplancton en surface a fait l'objet d'une comparaison entre 1991 et 1992.

Le flux de la production secondaire (Pr II) a été calculé, pour chaque groupe, à partir de l'équation suivante:

$$Pr\ II = Ke\ .\ B$$

où B est la biomasse et Ke est le taux de croissance exponentielle de l'organisme calculé à partir des équations de Sheldon et al. (1972) et de Baker (1990).

Le flux d'excrétion (E) est calculé à partir de l'équation:

$$E = C_{exc} . B$$

avec:

C_{exc} = taux d'excrétion spécifique ($\mu atg.N/\mu atg.N.J^{-1}$)

déduit de certains travaux spécialisés (cf. Baker, 1990).

Le flux de la mortalité (cadavre) a été calculé à partir de l'équation suivante:

$$Cadavre = M . B$$

où M est le coefficient de la mortalité. Les valeurs de M sont tirées de la littérature spécialisée (cf. Baker, 1990), pour les différents groupes zooplanctoniques.

3. RÉSULTATS

3.1 Caractéristiques hydrologiques des eaux étudiées

L'évolution spatiale (entre stations) de la température des eaux indique que ce paramètre se comporte de la même manière dans toutes les stations étudiées et tout au long de la période d'étude. La température des eaux a varié pendant cette période entre 15 et 32EC; les valeurs maximales ont été relevées en période estivale (au mois d'août 1991 et de juillet 1992). Une différence de la température des eaux (jusqu'à 7EC) a été enregistrée entre octobre 1991 et octobre 1992, aux mêmes stations.

Les valeurs de la salinité des eaux ont varié pendant l'année 1992 entre 24,57 et 39,26%. Les eaux de la station 3 ont accusé des différences notables de salinité d'une sortie à l'autre, avec une influence fluviale plus accentuée aux mois de mai et de juillet; on note ainsi la plus basse salinité au mois de juillet (24,57%). Bien que le cours d'eau soit à son plus bas niveau de l'année, il sembleraît que les courants sont à l'origine de cette baisse de salinité, en dirigeant les eaux estuariennes vers notre point de prélèvement. Aux autres stations, les valeurs de la salinité varient entre 36,49 et 39,26% et évoluent généralement de la même manière; on enregistre ainsi une légère baisse de la salinité des eaux au mois d'octobre en raison des pluies.

3.2 Evolution des paramètres chimiques et biotiques

3.2.1 Les orthophosphates

Les teneurs mises en évidence en orthophosphates varient entre 0,0 et 1,4 $\mu mol\ l^{-1}$. Un enrichissement général des eaux en orthophosphates a été enregistré en périodes printanière et automnale. La station 2 (Port) était la plus riche en 1991,

sauf en août où la station 3 la remplace (fig. 2A). En 1992, les valeurs maximales sont enregistrées pendant le mois d'avril aux trois stations.

3.2.2 Les nitrates

La concentration des nitrates dans les eaux étudiées a varié entre 0,05 et 17,4 µmol l^{-1} (fig. 2B). Les valeurs les plus élevées ont été enregistrées à la station 3 en raison d'une influence estuarienne plus marquée, comme l'indique les valeurs de la salinité (mai et juillet 1992); cette influence de salinité n'a pas été relevée au cours de l'été 1991.

3.2.3 L'ammonium

Les teneurs de l'eau en ammonium ont varié pendant notre période d'étude entre 0,04 et 4,46 µmol l^{-1}. La plus forte valeur a été relevée à la station 3, en juillet 1992, en raison d'une influence estuarienne plus prononcée (comme pour les nitrates; voir plus haut). D'une manière générale, des valeurs relativement élevées ont été enregistrées entre mai et août à la même station (fig. 2C). Aux stations 1 et 2, les variations marquées en concordent mieux avec le comportement des autres paramètres chimiques et biotiques; la différence en teneur de ce paramètre, entre 1991 et 1992, est très faible. Après une baisse quasi générale de la concentration d'ammonium en septembre, une recrudescence de celle-ci a été observée en octobre dans les eaux de trois stations.

3.2.4 Les nitrites

Les teneurs des eaux en nitrites ont varié entre 0,0 et 0,7 µmol l^{-1}. Les eaux de la station 3 sont aussi les plus chargées en nitrites, plus particulièrement en juillet 1992. Les eaux de la station 1 sont, par contre, les plus pauvres (fig. 2D). Dans les eaux de la station 2, les valeurs sont souvent les plus élevées en 1991 (surtout en octobre), et sont intermédiaires entre les stations 1 et 3 en 1992.

3.2.5 La chlorophylle a

Les valeurs de la chlorophylle a sont presque indétectables en 1991 sauf à la station 2 où on a deux pics, l'un en mai et l'autre en août (1,1 µg l^{1}). En 1992, les concentrations de chlorophylle a mesurées dans les eaux étudiées ont varié entre 0,0 et 2,15 µg l^{-1}. Les plus hautes valeurs sont relevées, comme en 1991, à la station 2, avec deux maxima: le premier situé en avril-mai et le deuxième en septembre. De même, deux maxima de chlorophylle ont été observées dans les eaux de la station 3 (juillet et septembre-octobre). A la station 1, on enregistre des valeurs moyennes en chlorophylle a en avril-mai pour atteindre un maximum en juillet (1,2 µg l^{-1}) et diminuer par la suite (fig. 3).

Ces résultats témoignent de la présence de deux poussées phytoplanctoniques pendant notre période d'étude dans l'ensemble des eaux étudiées. Nos résultats indiquent par ailleurs une activité biologique plus prononcée en 1992 qu'en 1991, plus particulièrement aux stations 1 et 3.

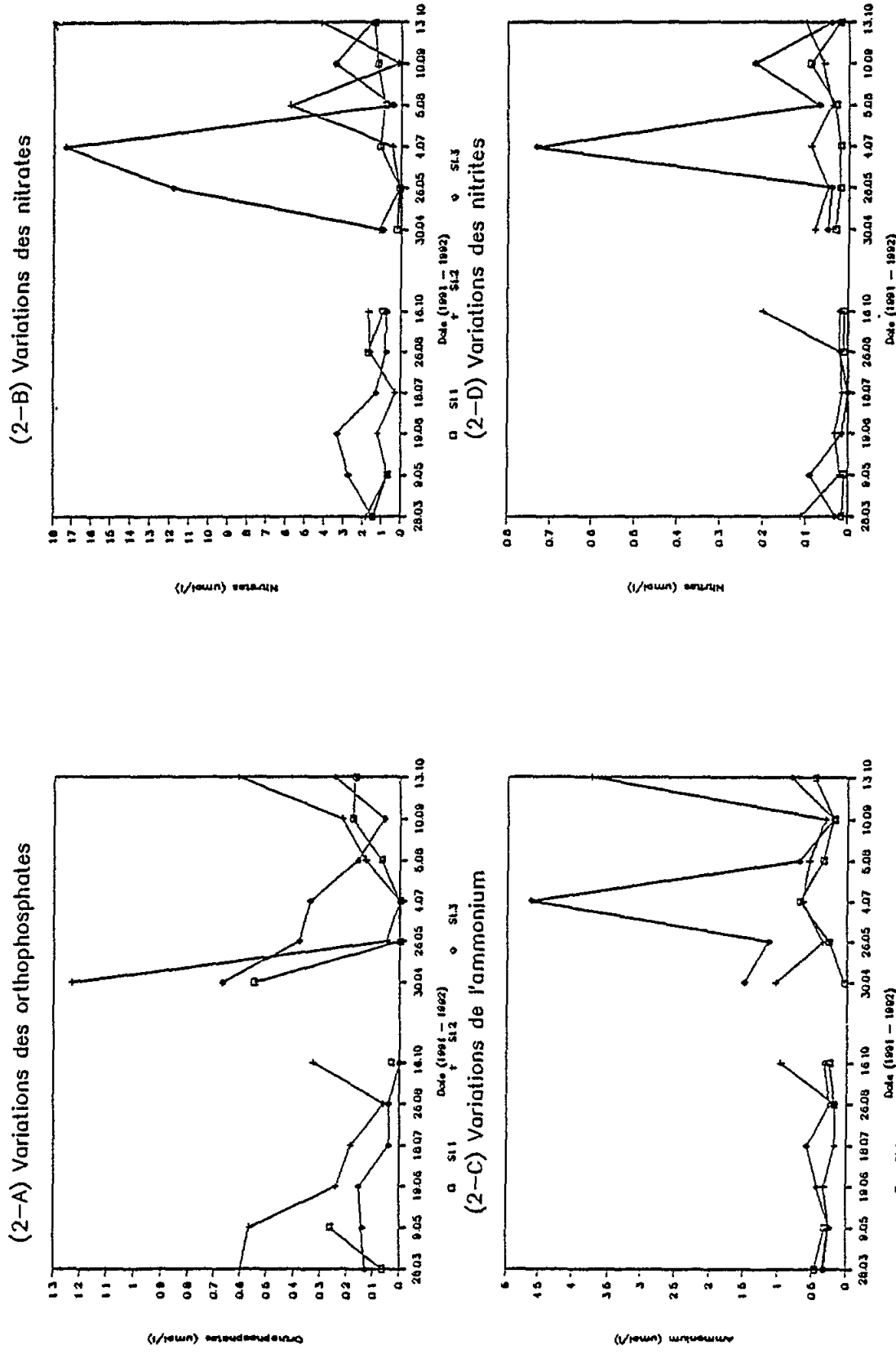

Fig. 2 Variations des concentrations de phosphates, de nitrates, d'ammonium et de nitrites exprimées en μmol l^{-1}, dans les eaux étudiées

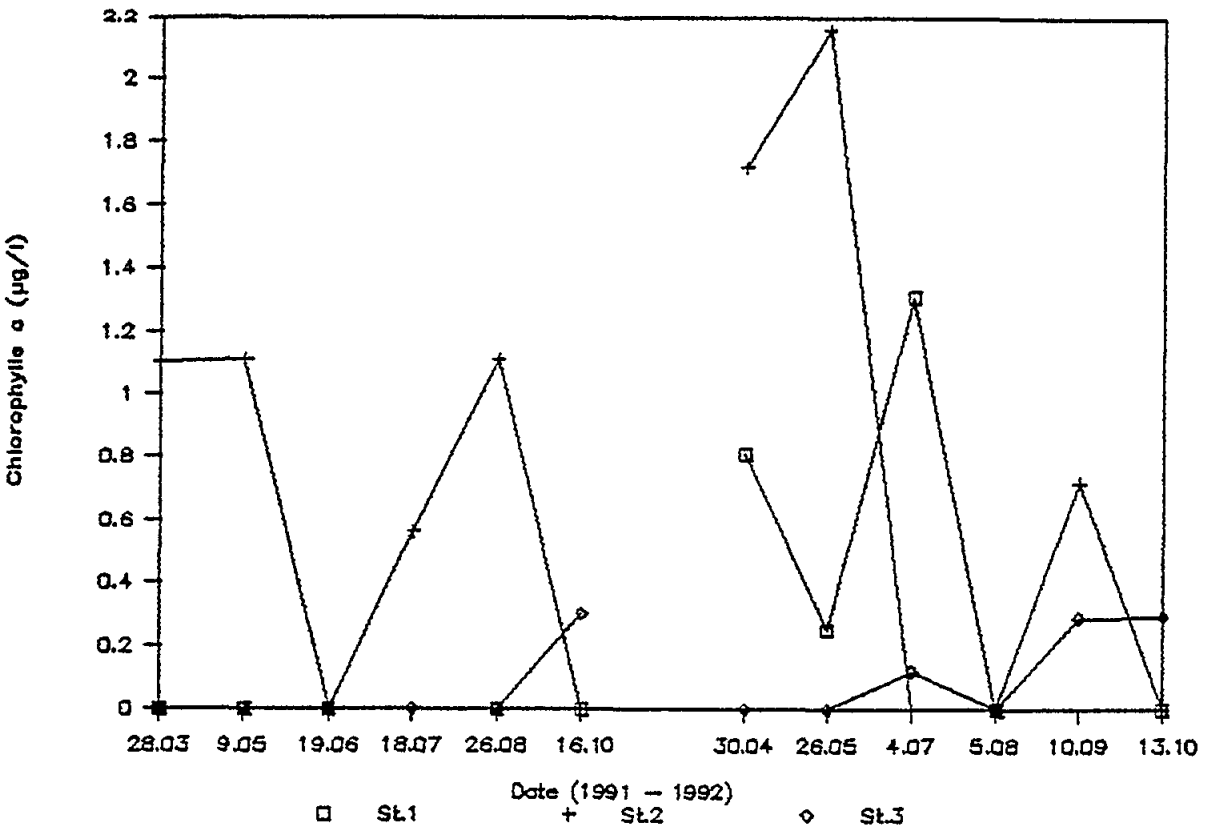

Fig. 3 Variations de la chlorophylle a, exprimée en $\mu g\ l^{-1}$, dans les eaux étudiées

3.3 Le zooplancton

Malgré le fait que la zone étudiée soit limitée à la région côtière, nous avons trouvé un nombre relativement important d'espèces zooplanctoniques; plus de 112 espèces dont 54 copépodes ont été définis, dans les échantillons de 1991 et 1992. A part les copépodes, dix autres groupes ont été présents dans les échantillons de 1991, et 15 en 1992. Le tableau 1 montre la composition spécifique des différents groupes zooplanctoniques rencontrés dans nos pêches de 1991 seulement.

En ce qui concerne l'abondance, la biomasse et les flux de matières, nous nous contentons de traiter les données de 1991 seulement.

L'étude de l'abondance totale du zooplancton (en individus m^{-2}) dans la couche 0-5 m, a montré que la station 2 est la plus riche en nombre d'individus parmi les stations étudiées avec deux maxima: le premier en juin avec 18000 i/m^2 et le deuxième, qui est moins important, en octobre (10700 i/m^2). Aux stations 1 et 3, le nombre maximum ne dépasse pas 4000 i/m^2 avec deux maxima aussi en juillet et août. Les résultats indiquent une grande dominance des copépodes dans la plupart des échantillons; ils représentent entre 65 et 97% (souvent plus de 80%) de l'abondance totale du zooplancton.

Tableau 1

Composition spécifique des différents groupes zooplanctoniques présents dans les stations étudiées en 1991

COPÉPODES	**SIPHONOPHORES**
Acartia clausi	Cylophyes appendiculata
A. discaudata	Abylopsis tetragona
A. grani	A. eschscholtzi
Calanus minor	
C. tenuicornis	**CHAETOGNATHES**
C. gracilis	Sagitta setosa
Candacia armata	S. inflata
Clausocalanus furcatus	S. friderici
C. arcucornis	S. serratodentata
Ctenocalanus vanus	S. minima
Eucalanus elongata	S. bunctata
E. attenuatus	
E. monachus	**LARVES DE CRUSTACÉS**
Eucalanus nasutus	Nauplii des copépodes
Paracalanus parvu	Zoé d'euphasiacés
P. grassus	Metanauplius de Meganoctiphanes norvegica
Centropages kroyeri	Calyptopsis de M.n
Copilia quadrata	Metazoède de Porcellina
Euchaeta marina	Zoé de Partunus
Corycaeus clausi	Portunus puber
C. typicus	Erifia spenifrons
C. flaccus	
C. brehmi	**CLADOCÈRES**
Isias clavipes	Evadne spinifera
Euterpina acutifrons	E. tergistina
Microstella rosea	E. nordmani
Phaenna spenifera	
Oncaea curta	**OSTRACODES**
O. media	Cypridina cestania
Oithona plumifera	
O. nana	**AMPHIPODES**
Pleurommama abdominalis	Hyperia latissima
Lucicutia flavicornis	Anchytomera blossevillei
Labidocera sp.	
Temora stylifera	**APPENDICULAIRES**
Ascomyzon parvum	Oikopleura dioica
Cymbasoma longispinosum	O. longicauda
Sapphirina opalina	O. albicans
PTÉROPODES	**THALIACÉS**
Limacina inflata	Thalia democratica
L. bulloides	Salpa fusiformis
Creseis acicula	Doliolum sp.
MÉDUSES	**POLYCHÈTES + LARVES**
Aglaura hemistoma	Naupasa isochaeta
Clytia noliformis	Polydora ciliata
Obelia sp.	Nerine foliosa
Phialidium hemisphaericum	Disoma multisetosum
Podocoryne carnea	Pygospio elegans
Jeunes meduses	

Les appendiculaires et les ptéropodes occupent la deuxième place après les copépodes pendant les quatres premières sorties et aux trois stations. Les autres groupes tels que les méduses, les cladocères, les chaetognathes et les larves de crustacés sont plus ou moins importants.

Les espèces dominantes en nombre sont Clausocalanus arcuicornis, C. furcatus, Paracalanus parvu, Euterpina acutifrons, Oithona nana, Calanus minor aux stations 1 et 3, Acartia grani et Acartia discaudata à la station 2 pour les copépodes. En ce qui concerne les autres groupes, elles sont: Obelia sp. (méduses), Limacina inflata (ptéropodes), Oikopleura dioica (appendiculaires), Caligus rapax (larves de décapodes).

3.3.1 Evolution de la biomasse zooplanctonique (mgN m^{-2})

3.3.1.1 Biomasse totale du zooplancton

L'évolution de la biomasse totale du zooplancton, dans la couche 0-5 m, montre deux maxima aux stations 2 et 3, entre mars et octobre 1991 (fig. 4). Le premier maximum (le plus élevé pour les trois stations) a lieu en juin par rapport à ce qu'on observe généralement dans le bassin Levantin (Lakkis, 1971); la plus forte biomasse se trouve à la station 2 (36 mgN m^{-2}). Le deuxième maximum se situe en octobre; il est plus faible que celui de juin pour la station 2 et plus élevé pour la station 3. Tandis que l'évolution de la biomasse à la station 1 n'indique qu'un seul maximum en juin avec 15 mgN m^{-2}.

3.3.1.2 Biomasse des copépodes

En plus de leur dominance en nombre, les copépodes sont aussi le groupe le plus important en biomasse; ils constituent entre 13,6 et 78,7 % et entre 31,8 et 91% (souvent plus de 50%) de la biomasse totale de zooplancton en poids sec et en azote respectivement. Leur pourcentage le plus faible se trouve à la station 3 (33 à 63,5% en azote). La différence entre le pourcentage en poids sec et celui en azote est dû à la différence dans le rapport N/PS entre les différents groupes.

Compte tenu de leur dominance, l'évolution de la biomasse des copépodes, durant la période d'étude en 1991, ressemble beaucoup à celle du total du zooplancton surtout pour les stations 2 et 3 (fig. 5) avec deux maxima: le premier en juin avec 32 et 5 mgN m^{-2} aux stations 2 et 3 respectivement, le deuxième maximum en octobre. La station 1 n'indique qu'un seul maximum en août avec 10 mgN m^{-2} qui est la plus grande valeur enregistrée dans les trois stations durant ce mois.

Les espèces les plus importantes au point de vue de la biomasse sont: Paracalanus parvu, Clausocalanus arcuicornis et C. furcatus qui étaient dominantes en nombre. D'autres espèces ont été caractérisées par une biomasse parfois plus importante, malgré leur faible abondance comme: Euchaeta marina, Eucalanus monachus, Calanus minor, Centropages kroyeri et Oithona plumifera. Il y a, par ailleurs, des espèces abondantes en nombre mais dont la biomasse est très faible comme Euterpina acutifrons, Acartia grani et Acartia discaudata.

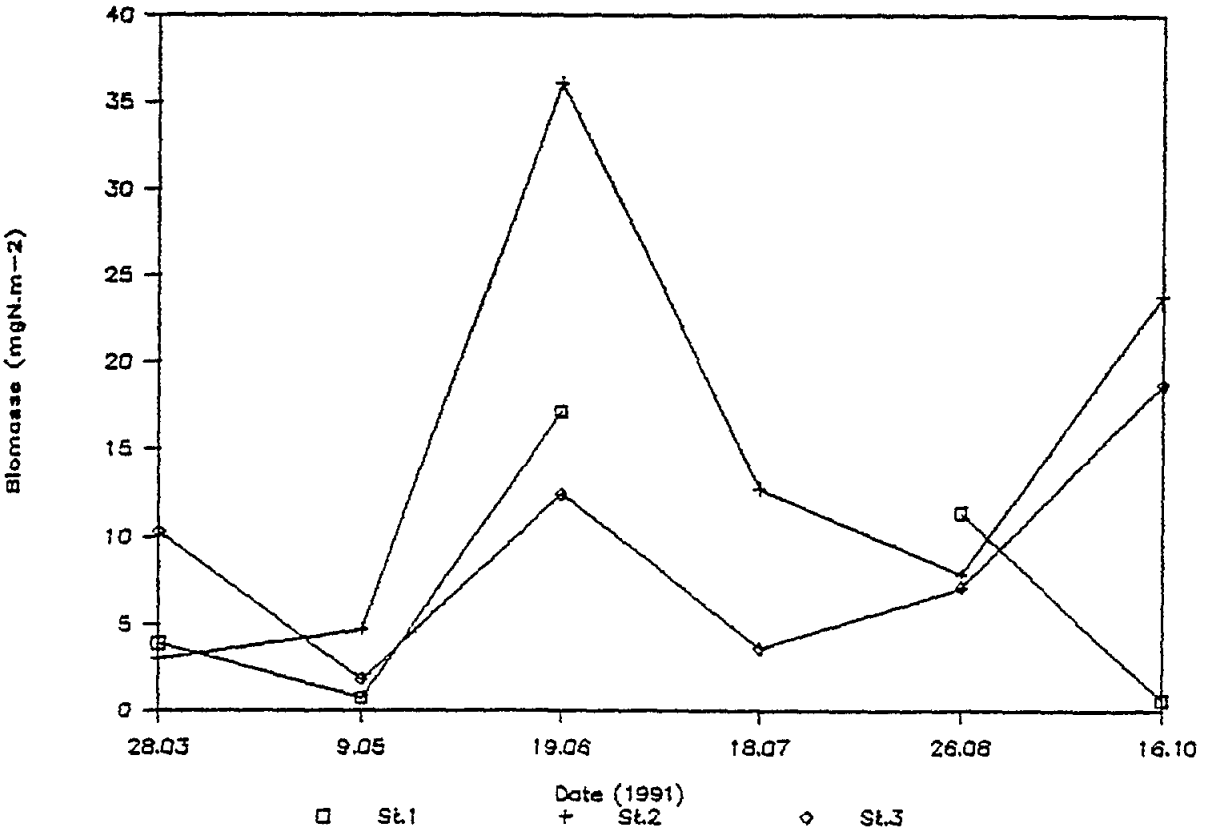

Fig. 4 Evolutions de la biomasse (mgN m^{-2}) du total du zooplancton dans les stations étudiées (1991)

3.3.1.3 Ptéropodes

L'évolution de la biomasse de ce groupe varie beaucoup d'une station à l'autre (fig. 6A). A la station 1, on constate une biomasse relativement élevée seulement en juin. A la station 2, un maximum de biomasse se situe en juillet; une deuxième poussée, plus faible, est remarqué en octobre. Deux maxima sont enregistrés à la station 3; le premier en juin et le deuxième en octobre; ce dernier est le plus important pour toute la période d'étude et l'ensemble des stations étudiées (11,5 mgN m^{-2}).

3.3.1.4 Méduses

Il ressort de la figure 6B que la biomasse de ce groupe est presque nulle à la station 3 et très faible à la station 1 (0,25 mgN m^{-2}). A la station 2, la biomasse des méduses est relativement importante en mai et surtout en octobre (5,5 mgN m^{-2}).

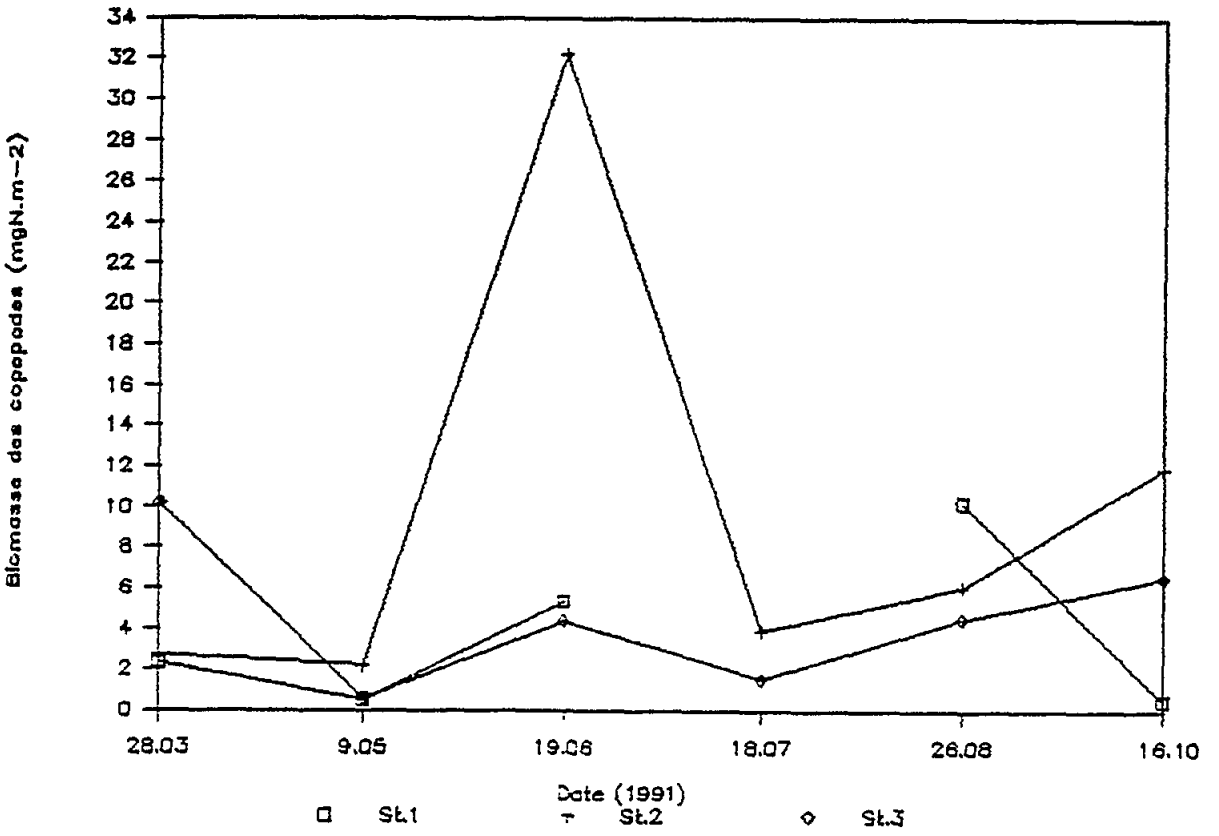

Fig. 5 Evolution de la biomasse (mgN m^{-2}) des copépodes dans les stations étudiées en 1991

3.3.1.5 Siphonophores

Nous n'avons compté aucun individu de ce groupe entre juillet et octobre; leur maximum de biomasse ne dépasse pas 1,7 mgN m^{-2} à la station 1, et il est inférieur à 1 mgN m^{-2} à la station 2 en juin. A la station 3, le maximum se situe en mai avec 1,03 mgN m^{-2} (fig. 6C).

3.3.1.6 Chaetognathes

Leur biomasse est presque nulle entre mars et mai, le maximum se situe en juin aux trois stations (fig. 6D), et la station 3 est la plus riche.

La biomasse en azote des autres groupes, comme les appendiculaires et les larves de crustacés, n'est pas très importante malgré leur présence en bon nombre dans tous les échantillons.

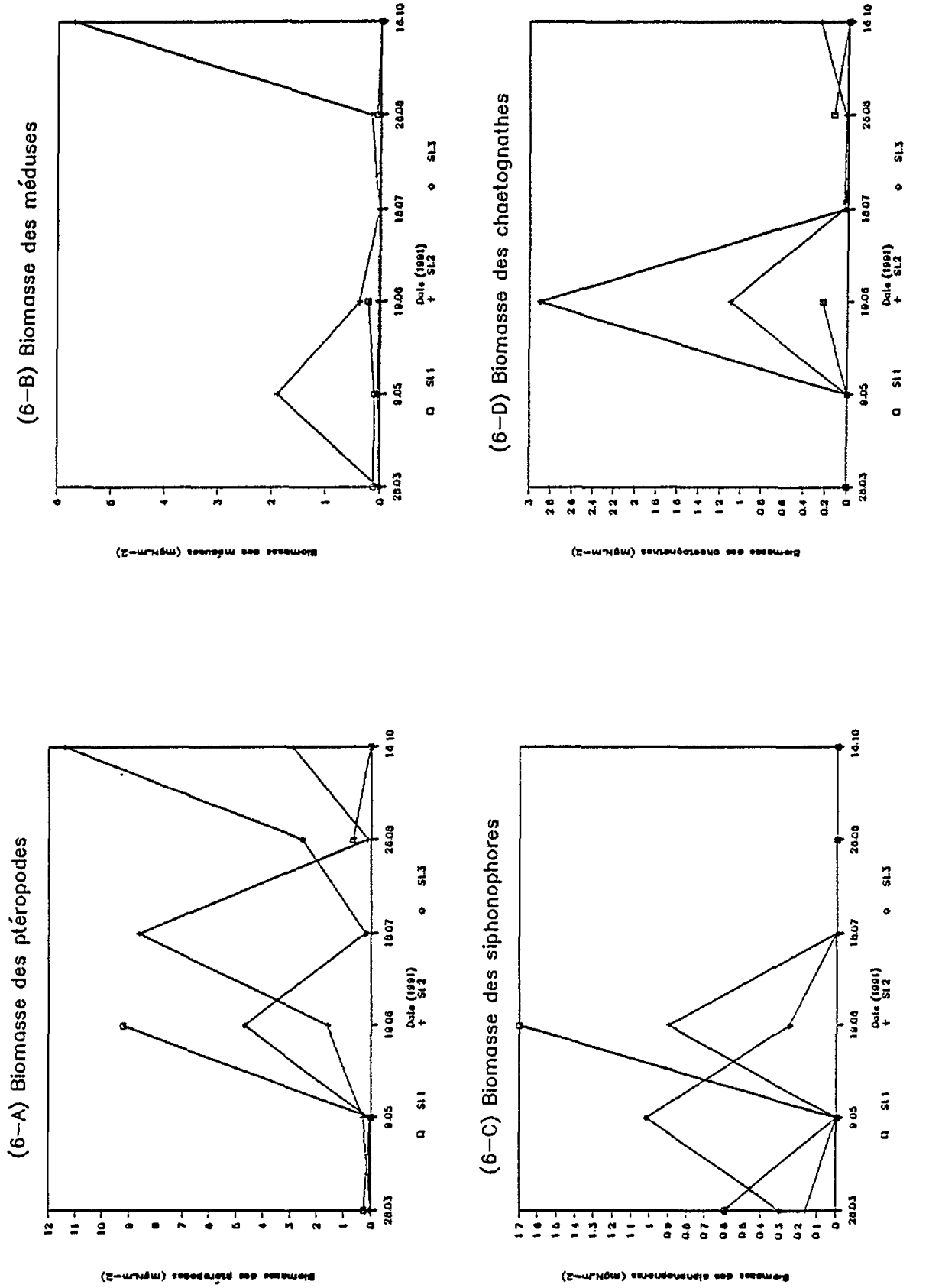

Fig. 6 Evolution de la biomasse de quelques groupes zooplanctoniques, exprimée en mgN m^{-2}

3.3.2 Evolution du flux de la production secondaire (PrII) en mgN m^{-2} j^{-1}

3.3.2.1 Production du total du zooplancton

Le flux de la production totale de zooplancton montre une évolution semblable à celle de la biomasse, sauf pour la station 1 (fig. 7A). Nous constatons deux maxima pour les stations 2 et 3; le premier est en juin avec 2,4 mgN m^{-2} j^{-1} à la station 2 et le deuxième en octobre avec 1,2 et 0,8 mgN m^{-2} j^{-1} (stations 2 et 3). A la station 1, la production en juin et en août est presque la même; ceci est dû aux groupes ayant un fort taux de croissance. La ressemblance de l'évolution de la biomasse et de la production II est due à la dominance d'un seul groupe: les copépodes.

3.3.2.2 Production des copépodes

Le flux de la production secondaire des copépodes représente entre 50 et 96% de la production totale de zooplancton (souvent plus de 80%). L'évolution spatio-temporelle de ce flux ressemble beaucoup à celles de la biomasse de ce groupe et de la production totale de zooplancton (fig. 7B). Le maximum de la production II ayant lieu en juin à la station 1 explique la stabilité de la production totale de zooplancton entre juin et août; ceci est dû au fort taux de croissance exponentielle des copépodes; la station 2 est toujours la plus productive.

Pour les flux dûs aux autres groupes, nous citons la production des ptéropodes qui varie entre 0,1 et 0,41 mgN m^{-2} j^{-1} et celle des siphonophores entre 4,3 - 15 µgN m^{-2} j^{-1}.

3.3.2.3 Flux de l'excrétion

Comme pour le flux de la production secondaire, le flux de l'excrétion totale montre un maximum en juin aux trois stations (fig. 7C) avec une valeur maximale de 0,34 mgN m^{-2} j^{-1} à la station 2 et un autre maximum en octobre aux stations 2 et 3. Le flux dû aux copépodes est le plus important et constitue entre 60 et 96,6% du flux de l'excrétion totale. La participation des autres groupes dans ce flux ne dépasse pas 0,001 mgN m^{-2} j^{-1} dans la plupart des échantillons, sauf pour les ptéropodes et les amphipodes qui excrétent respectivement entre 0,0001 - 0,034 et 0,0036 - 0,001 mgN m^{-2} j^{-1}.

3.3.2.4 Flux de la mortalité

L'évolution de ce flux pour le total du zooplancton ressemble aux autres flux, surtout celui de la production secondaire (fig. 7D). Le maximum est enregistré en juin à la station 2 (1,7 mgN m^{-2} j^{-1}). Le flux dû aux copépodes représente 45% (station 3, mai) et 95,5% (station 2, mars) du flux total et varie entre 0,0003 et 1,6 mgN m^{-2} j^{-1}. Les flux dûs aux autres groupes, comme les ptéropodes, les siphonophores et les méduses, sont quelques fois importants, comme c'est le cas à la station 3 (octobre) où 0,285 mgN m^{-2} j^{-1} sont dûs aux ptéropodes et 0,022 mgN m^{-2} j^{-1} aux siphonophores et le cas de la station 2 (octobre) où 0,142 mgN m^{-2} j^{-1} sont dû aux méduses.

3.3.2.5 Efficacité de transfert entre herbivores et carnivores

Cette efficacité représente le rapport entre la production de zooplancton carnivore et la production de zooplancton herbivore. Les valeurs obtenues varient entre 0,1 et 32%; elles se situent en général dans la fourchette communiquée par Slobodkin (1962). Cette efficacité était faible après le mois de juin sauf à la station 2 en octobre. Les faibles valeurs de cette efficacité peuvent être expliquées par l'hypothèse que nos échantillons ont été pris dans la couche superficielle où les herbivores sont dominantes.

3.4 Les métaux lourds

3.4.1 Dans l'eau de mer

3.4.1.1 Le cadmium (Cd)

Les concentrations du cadmium dans les eaux étudiées varient entre 0,09 et 0,8 µg l^{-1}. A l'exception d'une seule valeur enregistrée en octobre 1991 à la station 3, ces teneurs sont comprises entre 0,09 et 0,4 µg l^{-1} (fig. 8A). Les variations spatio-temporelles de teneur en cadmium sont relativement faibles, particulièrement en 1992. Les eaux des stations 1 et 3 sont plus chargées en ce métal que celles de la station 2 en 1991 et aux deux premières sorties en 1992.

3.4.1.2 Le cuivre (Cu)

Les variations spatio-temporelles du cuivre sont relativement importantes (fig. 8B). Les concentrations de ce métal varient entre 0,5 et 5,7 µg l^{-1}, dans les eaux étudiées. Les eaux de la station 3 étaient les plus chargées en cuivre en 1991, particulièrement en octobre. En 1992, les stations 1 et 2 sont les plus riches. De faibles valeurs sont enregistrées en période automnale (1992), aux trois stations.

3.4.1.3 Le plomb

Les concentrations du plomb dans les eaux étudiées varient entre 0,49 et 2,83 µg l^{-1} (fig. 8C). Les résultats obtenus montrent que les variations spatio-temporelles des concentrations en plomb ont été très faibles en 1991. Par contre, les teneurs en ce métal ont montré des variations accentuées en 1992, où les eaux de la station 2 étaient beaucoup plus chargées en ce métal que celles des stations 1 et 3. Les plus fortes valeurs sont enregistrées en juillet aux trois stations.

3.4.1.4 Le zinc

Comme pour les autres métaux, les concentrations en zinc sont, en général, plus élevées en 1992 qu'en 1991. Ces concentrations varient entre 0,42 et 9,3 µg l^{-1} dans les eaux étudiées (fig. 8D). Les plus fortes valeurs sont enregistrées à la station 3, sauf en juillet 1992 où les eaux de la station 2 étaient les plus chargées en ce métal. La station 1 était souvent la plus pauvre. Nous constatons aussi que les concentrations du zinc ont leur maximum en période post-estivale et diminuent beaucoup en août et septembre 1992.

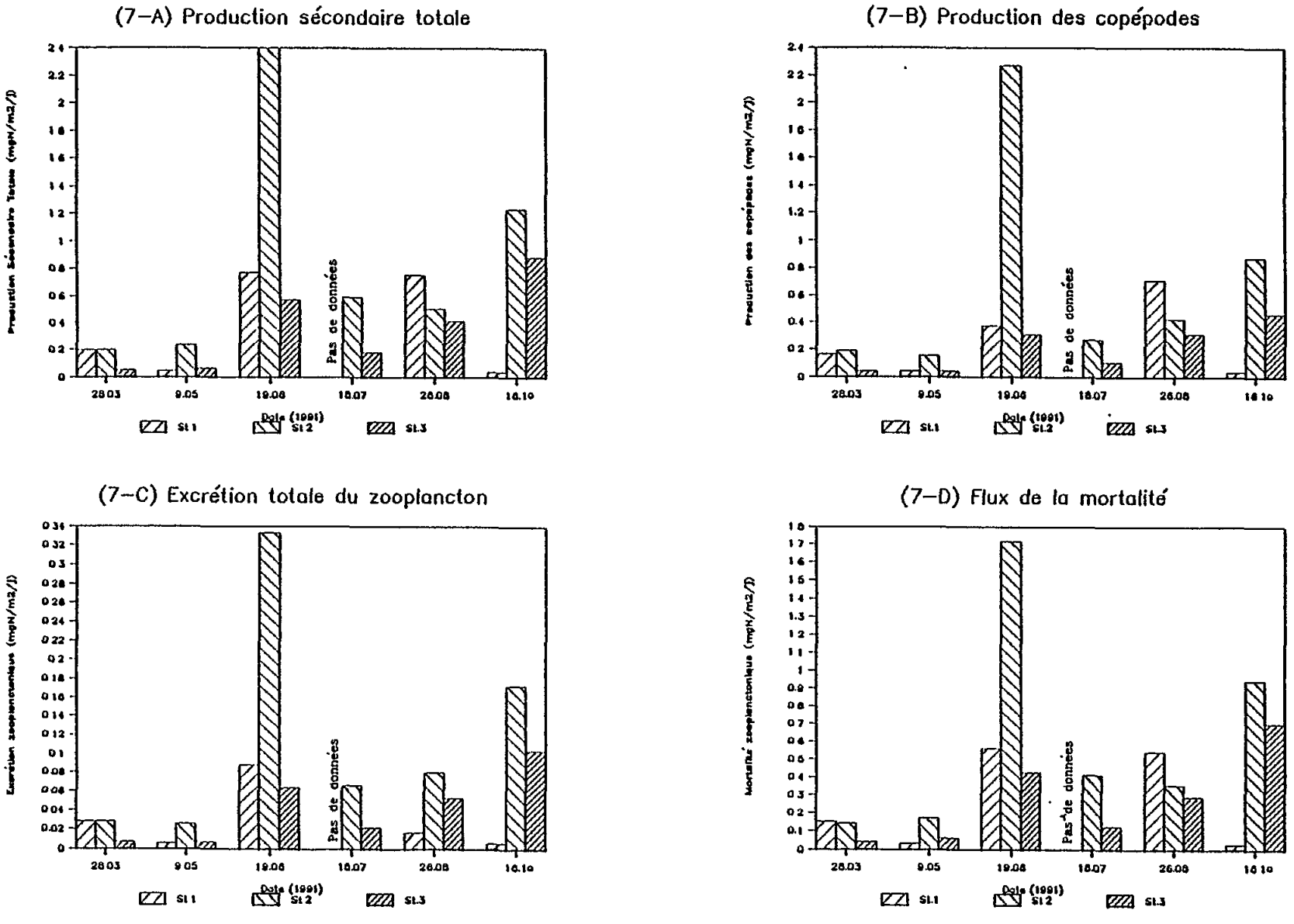

Fig. 7 Evolution de quelques flux de matière, dans les stations étudiées

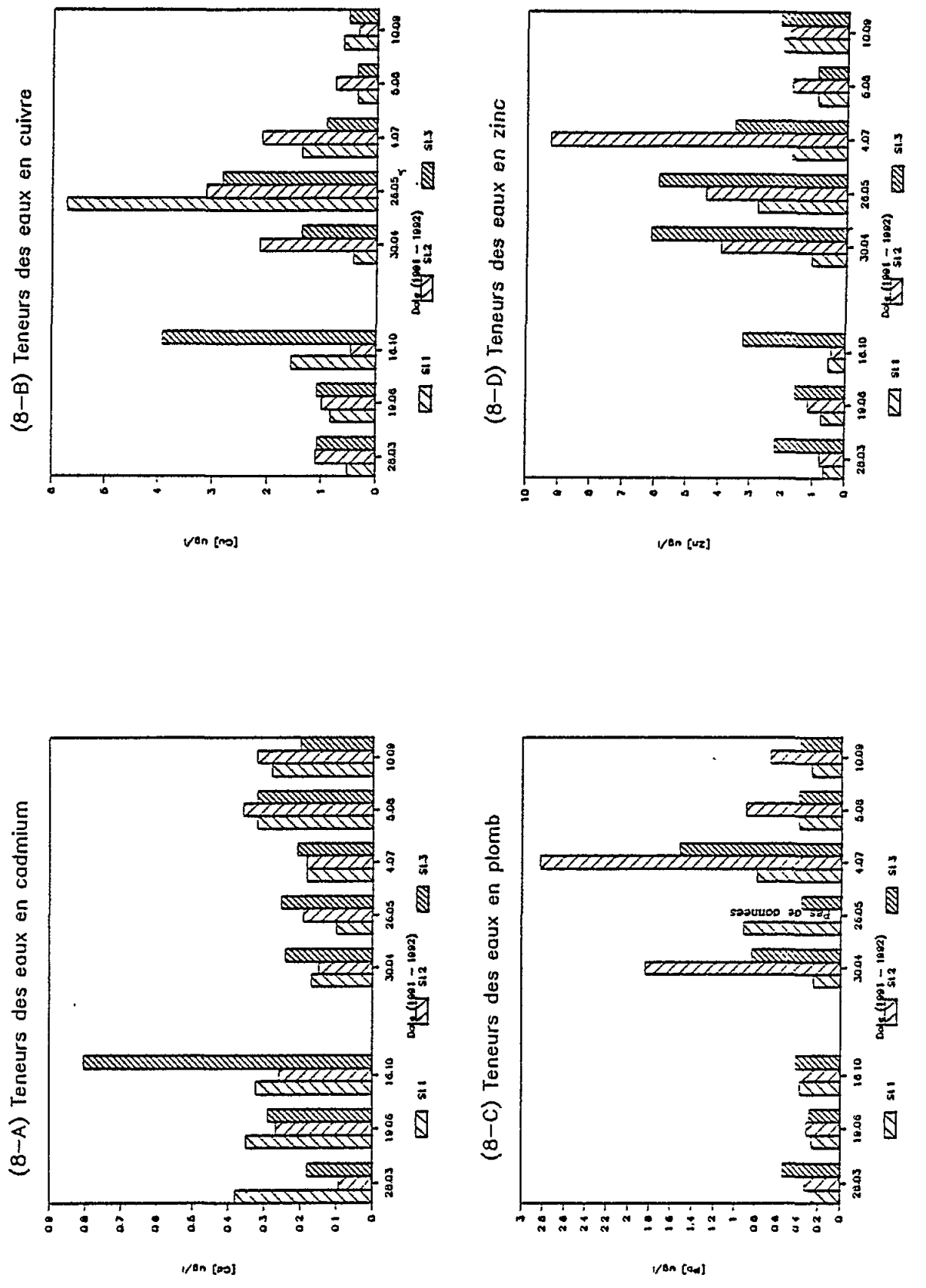

Fig. 8 Variations de la teneur des eaux étudiées en quelques métaux lourds à l'état de traces

3.4.2 Dans le zooplancton

Les concentrations des métaux lourds ont été mesurées dans tous les groupes zooplanctoniques ayant une biomasse suffisante, pour effectuer l'analyse. Le tableau 2 donne les résultats des analyses des métaux lourds effectuées sur les différents groupes zooplanctoniques, exprimés en µg de métal par gramme de poids sec.

3.4.2.1 Dans les copépodes

Vu la dominance du groupe des copépodes au sein du zooplancton, les concentrations des métaux concernés ont été le mieux étudiées dans ce groupe où nous avons mesuré les concentrations des quatre métaux (cuivre, zinc, plomb et cadmium) dans tous les échantillons prélevés aux trois stations en 1992 et à deux occasions seulement en 1991.

3.4.2.2 Le cadmium (Cd)

Les concentrations du cadmium dans les copépodes ont été indetectables dans les échantillons de 1991 et dans la plupart de ceux de 1992. Les copépodes échantillonnés en juillet et août 1992 contenaient des teneurs très faibles de ce métal aux stations 2 et 3 et deux concentrations notablement élevées à la station 1 (fig. 9A). 90% des teneurs enregistrées étaient comprises entre 0,0 et 5 µg g^{-1} poids sec.

3.4.2.3 Le cuivre

Le cuivre, qui est, jusqu'à un certain point, un élément nécessaire à la vie du plancton, était présent en concentrations relativement importantes dans les échantillons des copépodes analysés. Ces concentrations varient entre 5 et 103 µg g^{-1} poids sec. Les plus fortes valeurs sont enregistrées à la station 1 en septembre et à la station 3 en mai et en août; les copépodes de la station 2 sont les plus pauvres en ce métal (fig. 9B).

3.4.2.4 Le plomb

Les concentrations du plomb dans les copépodes étudiés varient entre des valeurs indetectables et 217 µg g^{-1} poids sec. Les plus fortes valeurs sont relevées dans les copépodes échantillonnés à la station 3 (fig. 9C). Les teneurs en ce métal ont été très faibles aux stations 1 et 2 en 1991; elles ont augmenté fortement en 1992 pour arriver à >100 µg g^{-1} poids sec (station 1, juillet).

3.4.2.5 Le zinc

Les résultats obtenus indiquent de fortes concentrations du zinc dans les copépodes. Ces concentrations varient entre 123 et 4860 µg g^{-1} poids sec (fig. 9D). Les valeurs les plus fortes sont enregistrées en période estivale. Les copépodes de la station 2 accumulent le plus ce métal par comparaison aux copépodes des autres stations.

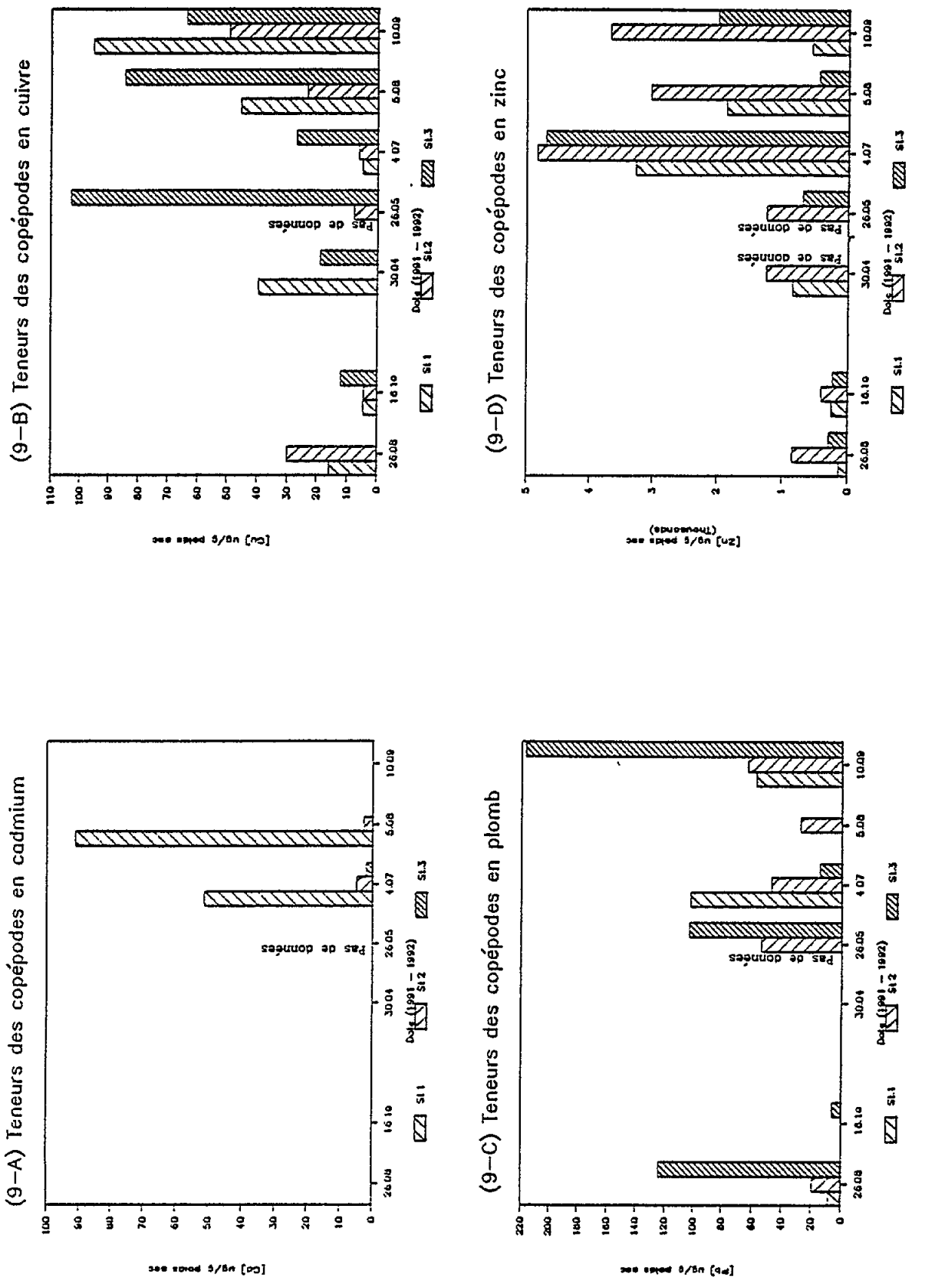

Fig. 9 Teneurs des copépodes en quelques métaux lourds à l'état de traces

3.4.2.6 Dans les autres groupes zooplanctoniques

En plus des copépodes, nous avons mesuré les métaux dans certains groupes zooplanctoniques ayant des biomasses suffisantes pour faire les analyses. Les groupes concernés sont: les ptéropodes et les larves des crustacés qui sont des herbivores, ainsi que les chaetognathes et les isopodes qui sont carnivores. Le tableau 2 montre les principaux résultats obtenus pour tous les groupes zooplanctoniques étudiés.

Tableau 2

Teneurs de quelques groupes zooplanctoniques en métaux lourds étudiés, exprimées en µg g^{-1} poids sec

Nom du Groupe étudié	Station 1				Station 2				Station 3			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
Copépodes												
26.08.1991	ND	16	8	123	ND	30	19	840	ND	ND	124	280
16.10.1991	ND	5	ND	244	ND	4	ND	391	ND	12	6	225
30.04.1992	ND	40	ND	847	ND	ND	ND	1245	ND	19	ND	NM
26.05.1992	NM	NM	NM	NM	ND	8	54	1239	ND	103	103	690
04.07.1992	51	5	102	3284	5	6	47	4836	2	27	14	4690
05.08.1992	91	45	ND	1856	3	24	28	3042	ND	85	ND	439
10.09.1992	ND	95	57	557	ND	39	63	3685	ND	64	217	1984
Larves des Crustacés												
26.08.1991	NM	NM	NM	NM	ND	14	ND	1395	NM	NM	NM	NM
26.05.1992	NM	NM	NM	NM	NM	NM	NM	NM	ND	ND	192	NM
04.07.1992	ND	14	ND	NM	ND	ND	ND	1104	NM	NM	NM	NM
05.08.1992	NM	NM	NM	NM	ND	8	ND	1679	NM	NM	NM	NM
Ptéropodes												
10.09.1992	NM	NM	NM	NM	NM	NM	NM	NM	ND	35	166	408
Chaetognathes												
30.04.1992	ND	ND	ND	1785	NM	NM	NM	NM	NM	NM	NM	NM
04.07.1992	ND	ND	73	1081	NM	NM	NM	NM	NM	NM	NM	NM
05.08.1992	NM	NM	NM	NM	NM	NM	NM	NM	ND	82	163	1970
Isopodes												
04.07.1992	NM	NM	NM	NM	NM	NM	NM	NM	ND	ND	14	1245

NM: Non mesuré
ND: Non détecté

Nous constatons que les concentrations de ces quatre métaux, dans ces groupes, sont relativement moins importants que chez les copépodes.

5. DISCUSSION ET CONCLUSION

Les concentrations de sels nutritifs que nous avons relevées dans les eaux étudiées se situent, en général, dans l'intervalle de variation généralement observé dans le bassin Levantin (Lakkis et Zeidane, 1987). S'agissant des métaux lourds, des concentrations relativement fortes ont été déterminées dans les eaux et dans le zooplancton. Ces fortes valeurs pourraient être dues à la nature écologique des stations étudiées. Des valeurs plus élevées que les nôtres sont cependant enregistrées dans les eaux de certaines zones sujettes aux activités humaines, comme l'indique les travaux de Grancini et al. (1976) en mer Adriatique, Alpha et al. (1982) le long du littoral sicilien, Scoullos et Dasenakis (1982) dans le golfe de Gera (Grèce), Fytianos et Vasilikiotis (1982) au nord de la Grèce.

En comparant l'abondance totale du zooplancton en surface (0 m) entre 1991 et 1992, nous constatons une augmentation très marquée de cette abondance entre 1991 et 1992. La station 3 est devenue plus riche que la station 2 en 1992. Par ailleurs et durant les deux ans, nous avons remarqué une faible abondance entre mars et mai aux trois stations.

La comparaison de nos résultats avec ceux qui ont été obtenus, dans le bassin Levantin ou d'autres bassins de la Méditerranée orientale indique que:

- Le nombre d'espèces identifiées (112 espèces) durant les périodes printanière et estivale de 1991 et 1992 est proche du nombre donné par Lakkis et Zeidane (1987) dans les eaux libanaises voisines. Pourtant, nos résultats sont préliminaires pour les eaux syriennes.

- Le maximum d'abondance se produit en retard par rapport aux observations faites communément en Méditerranée orientale (Lakkis, 1971 et 1973). Les valeurs maximales, 5400 i m^{-3} (1991) et 10000 i m^{-3} (1992), sont plus élevées de celles communiquées par Lakkis et Zeidane (1987) et par Pancucci-Papadopoulou et al. (1992), mais elles sont inférieures à celles communiquées par Lakkis (1971) et par Dowidar (1985).

- L'abondance des copépodes dans nos échantillons varie entre 65 et 97% de l'abondance totale de zooplancton. Elle se situe dans la fourchette donnée par El Maghraby et Dowidar (1973) et Pancucci-Papadopoulou et al. (1992). Les pourcentages de l'abondance des autres groupes sont proches de ceux communiquées par Regner et al. (1985).

- En ce qui concerne les valeurs de la biomasse zooplanctonique, elles ont varié, pendant la période d'étude en 1991, entre 6 et 462 mg PS m^{-2}. Ces valeurs sont comparables avec celles relevées pour la Méditerranée orientale (Delalo, 1966; Pasteur et al., 1976; Benovic, 1977).

Les concentrations du cadmium obtenues dans le zooplancton varient entre des valeurs indetectables et 4,7 µg g^{-1} poids sec, pour les stations 2 et 3, considérées comme polluées. Ces valeurs se situent dans la fourchette communiquée par Fowler (1985) pour des spécimens individuels zooplanctoniques dans des zones côtières, et par Hardstedt-Roméo (1982) pour le zooplancton du large soumis à la pollution par des eaux usées. 10% de l'ensemble des valeurs enregistrées (2 mesures à la station 1) ont dépassé largement les valeurs indiquées par d'autres auteurs et ont représenté jusqu'à 18 fois le reste de nos valeurs; ceci pourrait être dû à une pollution éventuelle des échantillons analysés pour ce métal.

La plupart des teneurs en cuivre enregistrées dans les différents groupes zooplanctoniques (83% des mesures), se situent dans la fourchette communiquée par Roméo (communication personnelle) et par Krishnaswami et al. (1985). Les plus grandes valeurs, dépassant les concentrations de ces auteurs, ont été trouvées à la station 3; ceci peut s'expliquer par l'influence des eaux fluviales chargées en polluants industriels et urbains.

Les concentrations du plomb, dans les échantillons pris aux stations 1 et 2, sont comparables à celles données par Greig et al. (1977) pour un échantillon mixte de zooplancton. La majorité des valeurs enregistrées à la station 3 sont beaucoup plus élevées. Nous pouvons retenir les explications déjà avancées dans le cas de cuivre, pour ces fortes teneurs en plomb (voir plus haut).

En ce qui concerne le zinc, nous avons déterminé des concentrations relativement élevées par rapport à celles relevées dans la bibliographie. Ceci pourrait être lié aux fortes teneurs des eaux étudiées en ce métal.

6. REMERCIEMENTS

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THE ROLE OF THE GREEN ALGAE ULVA IN THE CYCLING OF COPPER IN MARINE COASTAL ECOSYSTEMS

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A B S T R A C T

The bioaccumulation of Cu and its effects on the growth of Ulva sp. were tested during laboratory experiments with Cu concentrations 10 to 100 times the natural levels. The uptake of Cu by the tissues was more rapid and almost complete during the first 6 days of the experiments. The metal content was higher in algal discs remained in higher ambient Cu concentrations. Release of the metal accumulated was observed in all the discs; it was related to the initial metal concentration of the solution as well as to the duration of exposure. The metal content of the tissues after a 30-day period of recovery, was proportional to the metal accumulated and considerably higher in discs remained in Cu contaminated solutions for more than 15 days, showing that Ulva tissues retain an important amount of Cu after an episode of pollution. The effect of Cu on the growth of Ulva discs was more significant in elevated Cu concentrations (20 to 100 times the natural levels).

In situ experiments using benthic chambers indicated that degradation of increased Ulva biomass resulted in enrichment of the exchangeable sediment fraction in Cu and increase of the dissolved Cu near the sediment surface.

1. INTRODUCTION

Green algae of the genus Ulva are commonly the dominant macroalgal species in eutrophicated marine coastal areas. Ulva sp. grow abundantly in spring - summer period, contributing substantially to the biomass, and playing an important role in the cycling of mineral elements in the littoral ecosystems.

In environments with high nutrient concentrations Ulva sp. exhibit increased growth rates followed by nitrogen accumulation in the thalli and depletion of the dissolved inorganic nitrogen (Viaroli et al., 1992). The surface area:volume (SA:V) ratio of the Ulva thallus is higher in comparison with other algal species, resulting in elevated rates of nutrient uptake (Rosenberg and Ramus, 1984).

Besides an indicator of eutrophication (Ho, 1981), Ulva sp. could be used as a bioindicator species of metal pollution. It has been shown that Ulva tissues collected from urban areas consistently contained higher levels of all the metals, apart

from Cd, than that from the rural areas (Ho, 1990). It seems that Ulva sp. have developed adequate protective mechanisms within the cells by bonding metal ions with active sites of the proteins like the amino, carboxyl and sulfate functional groups. The heavy metal bioaccumulation by various algal species has been tested by a number of researchers. However, the accumulation rate or the correlation between the metal concentration in the water and in the algal tissues is not yet well known.

Since benthic macroalgae are of major importance as primary producers in coastal waters (Mann, 1973) as well as metal concentrating agents, their metal content is passed directly into the next link in the food chain. However, this macroalgal biomass is largely unconsumed and thus decomposed providing the environment with great amounts of organic matter and biogenic particles that may be transferred to the sediment. Some preliminary results of our experiments (Scoullos and Caberi, 1991) indicated that an important portion of the accumulated metals was bound to structural components of the algal cell walls. As a result, metals are not entirely released in the seawater during algae degradation but they remain bound to the organic suspended matter.

In shallow coastal areas growth and decomposition of increased biomass of Ulva plants result in the depletion of dissolved oxygen (Viaroli et al., 1992) and the appearance of anoxic conditions on the sediment surface. Sediments constitute one of the most concentrated sinks of trace metals. Within sediments, metals may partition among several different types of binding sites, according to physical and chemical characteristics of the sediment. Partitioning plays an important role in determining the biological significance of sediment bound metals and in determining metal exchange between sediments and water (Luoma and Bryan, 1981). Nevertheless, the role of the increased Ulva biomass in eutrophicated areas on the cycling of trace metals between the water column and the sediment surface, is not investigated.

Most of the experiments on the metal bioaccumulation by the green macroalgae have been performed using relatively high dissolved metal concentrations which could rarely appear even in polluted marine ecosystems. In this study we were interested in the effects of relatively low metal concentrations on the bioaccumulation rate, the growth and the chemical composition of the green algae Ulva sp. that dominate in coastal areas of Saronicos gulf and especially in the industrialised area of the Elefsis gulf. Furthermore, we tried to understand the role that increased biomass of Ulva plants could play in the cycling of trace metals in this particular area.

The uptake and release of heavy metals have been studied during field experiments by employing four mesocosms installed in a shallow embayment within the gulf of Elefsis (Loutropyrgos, Greece). Results of the experiments have been also reported by Scoullos and Caberi (1991). One of the most important characteristics of these field experiments were the large fluctuations of the dissolved metal concentrations in the enclosures.

In order to determine more precisely the rates of bioaccumulation and growth of Ulva sp., a number of laboratory experiments were designed, in which initial metal concentrations in the test cultures were maintained constant. The uptake and release of heavy metals as well as the growth of the tissues were tested in three

different metal concentrations on the basis of sampling every three days, using three replicates of discs cut from Ulva thalli transferred from the studied area. The metal release from the tissues was determined by removing the discs every three days into uncontaminated seawater.

For the investigation of the potential role of Ulva biomass in the precipitation or the dissolution of trace metals from the sediment surface, a series of field experiments in the same coastal area of Loutropyrgos, were carried out. About eight benthic chambers were placed firmly on the sea bottom and at the end of a ten-month period, trace metal concentrations were determined in sediment samples taken within the chambers. Particulate and dissolved metals in seawater, sampled near the sediment surface, were also determined.

The present paper focuses on the role of Ulva plants on the fate of copper in the marine environment. Copper is one of the essential micronutrients and also necessary for a wide range of metabolic processes, although at elevated levels and in a readily soluble form, it becomes toxic to biological systems (Flemming and Trevors, 1989).

2. MATERIALS AND METHODS

Marine plant material consisting mainly of Ulva rigida (C.Ag.) atoms (Bliding, 1963), was collected from Loutropyrgos bay in the gulf of Elefsis. The plants were carefully cleaned from the epiphytes and the suspended materials. The algae were transferred to the laboratory in a cooler filled with seawater of the same area.

For the study of the metal uptake about 300 discs, with a diameter of 25 mm, were cut from mature individuals of Ulva rigida, the microscopic physiological condition of which was tested before the experiments (Panayotidis, pers. comm.). Discs were cut from the expanded region of the fronts avoiding the thin areas within 3 cm of the margin and the thicker areas near the attachment cells. The cell height and the thallus thickness in this part of the thallus is uniform within individuals as well as between individuals of a similar size (Steffensen, 1976). All test cultures were maintained at room temperature 20 ± 2 EC, 14:10 light:dark, under white fluorescent light at 5000 lux. Algae discs remained in Cu solutions of 15, 30 and 150 ppb of seawater transported from the area studied, for 2, 4, 6, 15 and 30 days. Control discs were kept under the same conditions for 30 days.

Before running the uptake tests, culture vessels were filled with the desired Cu concentration solutions from the reservoir bottles. After addition of the discs, the solution in each test vessel was replaced every day so that excessive bacterial growth and nutrient depletion, as well as decrease of the dissolved metal concentration due to algae uptake or to adsorption onto the plastic vessel walls, could be avoided. The metal content of the samples from each exposure time was determined in three replicates with one disc of Ulva per replicate, by Flame or Graphite Furnace Atomic Absorption Spectrophotometry after treatment with concentrated nitric acid (Sperling, 1977).

The uptake rates of Ulva discs were calculated as specific uptake rate (SUR; % uptake per day) using the formula:

$$SUR = \frac{100\,[\ln (C_t/C_{control})]}{t}$$

where: C_t = Cu concentration of the discs on day t and $C_{control}$ = Cu concentration of discs in the control experiment.

In order to test recovery from exposure to Cu, three replicates of the discs which remained in 15, 30 and 150 ppb Cu for 2, 4, 6, 15 and 30 days, were transferred into filtered uncontaminated seawater from the studied area, for 2, 6 and 15 days and the Cu content of the thallus was measured to determine the amount of the metal released. For a further comparison of the behaviour of Ulva rigida to elevated Cu concentrations during field experiments and laboratory ones as well, the concentration factors as the $Concentration_{tissue}$ / $Concentration_{seawater}$ ratios were calculated.

The diameter of seven discs, expressed as the mean value of two vertical disc diameters, was measured every day during the experiments. The growth of discs under test conditions was calculated as specific growth rate (SGR; % increase per day) using the formula:

$$SGR = \frac{100\,[\ln (D_2/D_1)]}{t}$$

where: D_1 = initial disc diameter and D_2 = disc diameter on day t.

Apart from the experiments of ecotoxicology, microscopic observations on the physiology of the algae tissues were made.

In order to understand the impact of Ulva biomass on the metal concentration of the sediments, a series of field experiments, with a duration of about ten months, were carried out in the same coastal area of Loutropyrgos, like all the above described experiments. Three plastic cylinders, which are characterised as benthic chambers, with a volume of about 50 l, were placed firmly on the sediment surface at a depth of 4 m. Two kilos wet weight of Ulva rigida plants were left within each chamber until complete degradation. Three other plastic cylinders were placed on the same area and for the same period without containing any algae, in order to be used as blanks. The upper end of all the chambers was closed in a way that only water could enter. The dissolved oxygen, pH and temperature were measured regularly inside the chambers as well as in the surrounding environment. At the same time, the cylinders were cleaned up from benthic algae and other organisms attached on the walls and on the upper end preventing the water circulation. Another plastic cylinder with only its upper end open, was attached firmly on the sediment surface within which two kilos wet weight of Ulva plants were added for the same time period, so that the suspended matter deriving only from the algal degradation could be estimated.

Monitoring the algae condition inside the benthic chambers, we observed complete degradation of the biomass after almost ten months. At that time, three replicates of sediment cores were taken from each chamber, since cores preserve an undisturbed water - sediment interface. Each core was sliced into subsamples of 1 cm thickness. All chemical treatments were performed on the <63μm sediment fraction, which was separated by wet sieving through nylon screen. In addition, seawater samples were taken from the chambers as well as samples of suspended matter near the sediment surface. In all these samples Cu concentrations were determined. Dissolved Cu was analysed using the method of Riley and Taylor (1968) modified by Scoullos and Dasenakis (1984). Particulate Cu was determined by filtering water samples through 0.45μm Millipore filters and treating the filters with concentrated nitric acid.

For a better understanding of the fate of Cu in the sediments, a five step sequential leaching procedure was followed (Tessier et al., 1979). With these sequential extractions we obtained results on Cu partitioning into five sediment fractions: exchangeable (NaOAc, pH 8.2), bound to carbonates (NaOAc, pH 5), bound to Fe - Mn oxides (NH_2OH . HCl), bound to organic matter and sulfides (H_2O_2) and residual (HF, $HClO_4$). Cu concentration of all samples was finally determined by employing Graphite Furnace and Flame Atomic Absorption Spectrophotometry.

3. RESULTS AND DISCUSSION

Cu uptake, as plotted against duration of exposure to 15 ppb Cu (Fig. 1) and 150 ppb Cu (Fig. 2), was more rapid the first 4 and 6 days, respectively, but slowed considerably afterwards. A slight decrease of the metal accumulated is observed after the 6th day of the experiments in both Cu solutions with a further increase after the 15th day. It seems that a saturation of the binding sites in the algal cells is taking place with increasing exposure time followed though by the simultaneous growth of the discs which has as a result the continuous increase of the accumulated metal content.

In Tables 1 and 2 the uptake as well as the Specific Uptake Rate (% uptake per day) of Ulva rigida tissues are presented. The % uptake per day is higher in discs which remained in the 150 ppb Cu solution it is however decreasing with exposure time more rapidly than in discs from the 15 ppb Cu solution. The increased algae uptake could be attributed to the effect of Cu on the protein production of the algal cells, since it is known that organisms accumulate Cu with the synthesis of a small molecular weight copper-binding protein, which has a striking sequence homology to the zinc - and cadmium - containing metallothioneins (Lerch, 1980). The rapid decrease of the % uptake of the tissues in the higher Cu concentrations shows the toxic effects of Cu resulting also to lesser growth rates of the discs observed in the 150 ppb Cu solution.

It is noteworthy that there is a good correlation between the specific uptake rates of Ulva discs remained in 15 ppb and 150 ppb Cu (corr. coeff. r=0.96 for N=5), indicating that Ulva plants' reaction to Cu in the two different Cu solutions does not differ significantly, as far as the bioaccumulation is concerned.

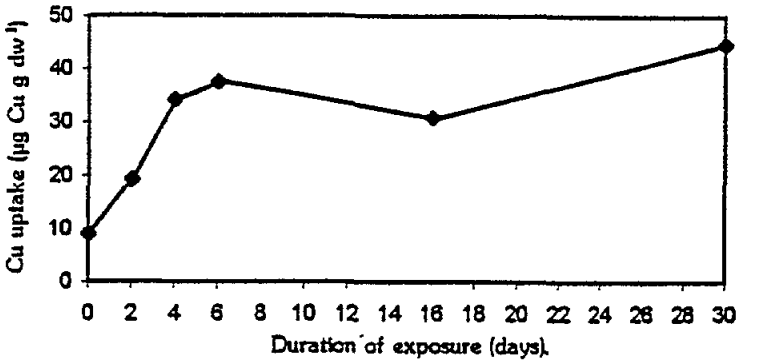

Fig. 1 Rate of Cu uptake by Ulva rigida discs exposed to 15 ppb Cu. Five discs sampled at each time interval

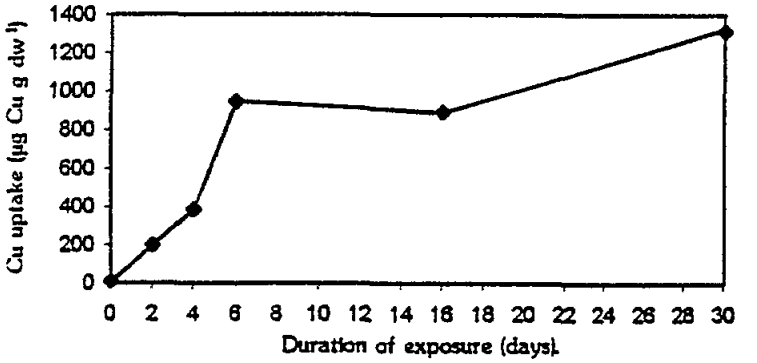

Fig. 2 Rate of Cu uptake by Ulva rigida discs exposed to 150 ppb Cu. Three discs sampled at each time interval

The uptake rates could not be calculated from the field experiments because it was difficult to define, for a given moment, the exact metal concentration of water (Scoullos and Caberi, 1991). However, measurements of the Cu content of Ulva tissues in various Cu concentrations and for the same duration of exposure, in the enclosures during the field experiments showed that Cu accumulation is proportional to the concentration of the dissolved metal in the medium (corr. coeff. range from 0.945 to 0.999 for N=4). It is interesting that the same phenomenon is observed during the laboratory experiments with 15, 30, and 150 ppb Cu solutions, regardless the higher metal accumulated by Ulva discs during the latter experiments.

Table 1

Cu uptake, uptake rates, concentration factors and growth rates for Ulva discs remained in 15 ppb Cu for 30 days. Five discs sampled at each sampling day

Sampling days	Cu uptake µg g^{-1} dw		Specific Uptake Rate (SUR) % uptake day^{-1}	Concentration Factor C_{tissue}/C_{water}		Specific Growth Rate (SGR) % growth day^{-1}	
	Control	15 ppb Cu	15 ppb Cu	Control	15 ppb Cu	Control	15 ppb Cu
2	10.45	19.26	42.1	6970	1280	4.40	4.22
4	12.27	34.16	35.4	8180	2280	4.56	3.62
6	7.77	37.43	25.1	5180	2500	3.68	3.03
15	4.39	30.88	8.8	2950	2060	3.25	2.49
30	6.58	44.70	5.6	4390	2980	2.68	2.29

Table 2

Cu uptake, uptake rates, concentration factors and growth rates for Ulva discs remained in 150 ppb Cu for 30 days. Three discs sampled at each sampling day

Sampling days	Cu uptake µg g^{-1} dw		Specific Uptake Rate (SUR) % uptake day^{-1}	Concentration Factor C_{tissue}/C_{water}		Specific Growth Rate (SGR) % growth day^{-1}	
	Control	150 ppb Cu	150 ppb Cu	Control	150 ppb Cu	Control	150 ppb Cu
2	7.54	201.2	150.6	5030	1340	4.40	0.64
4	8.41	384.4	91.5	5600	2560	4.56	0.41
6	11.01	947.1	76.0	7350	6270	3.68	0.41
15	10.01	891.2	30.0	6670	5940	3.25	0.21
30	13.89	1316.4	16.3	9260	8780	2.68	0.05

Concentration factors ($C_{tissue}/C_{seawater}$) calculated from the laboratory experiments (Tables 1 and 2) increased with increasing exposure time and metal concentration in the water as well. It should be noted however, that in the 15 ppb Cu solution concentration factors are constant after the 4th day of exposure. In Table 3 a comparison of the concentration factors calculated from field experiments with those of the above described experiments is presented. Concentration factors calculated from field experiments are lower and rather constant with time of exposure but also with increasing ambient Cu concentrations, showing that Ulva plants could be used as monitoring organisms for Cu pollution (Seeliger and Edwards, 1977). The higher values of the ratios during laboratory experiments, where a single metal is added in the test solution, is a result of the absence of other metals, like Zn, that act antagonistically to Cu (Seferlis et al., 1993).

Table 3

Comparison of the Concentration Factors ($C_{tissue}/C_{seawater}$) calculated using data obtained from field experiments with mesocosms and laboratory ones

Sampling days	FIELD EXPERIMENTS Dissolved copper concentrations		
	13.4 ppb	31.1 ppb	116.5 ppb
6	1190	1350	540
9	250	370	1690
12	1000	1440	820
15	1440	1390	3250
18	370	2590	
24	1630	1230	
	LABORATORY EXPERIMENTS Dissolved copper concentrations		
	15 ppb	30 ppb	150 ppb
2	1280		1340
4	2280		2560
6	2500	5970	6270
15	2060	4520	5940
30	2980	7300	8780

As far as the Cu release of Ulva discs removed in uncontaminated seawater is concerned, the results of the laboratory experiments agree with those obtained from the field. From Figures 3 and 4, it becomes obvious that discs remained in higher Cu concentrations, release a higher proportion (70 - 80%) of the metal accumulated during the first 6 days of the experiment, than the ones remaining in lower Cu concentrations (about 60%). However, we observe that, after 15 days in unpolluted seawater, Cu content of the "recovered" discs from the 15 ppb Cu solution is similar to the natural levels, whereas discs from the 150 ppb solution retain a significant amount of Cu. Another interesting observation on the recovery of Ulva plants after Cu pollution of the seawater is that discs remained in various Cu concentrations for more than 15 days tend to retain in their tissues, after 15 days in clean seawater, about 50% of the initial metal accumulated.

A feature that also differentiates discs maintained in different Cu concentrations (15, 30 and 150 ppb Cu) is the specific growth rate (SGR). As it is shown in Figure 5, there is a rather slow decrease of the SGR with time in the control experiment and in the test cultures as well, but there is a more significant decrease of the SGR with increasing Cu concentration of the solution. Specific growth rates of discs that remained in 15 ppb Cu do not differ significantly (student t-test for p=0.05) in contrast with discs which were kept in 150 ppb Cu, showing that Ulva growth is not affected by the 15 ppb concentration of Cu (about ten times the natural levels).

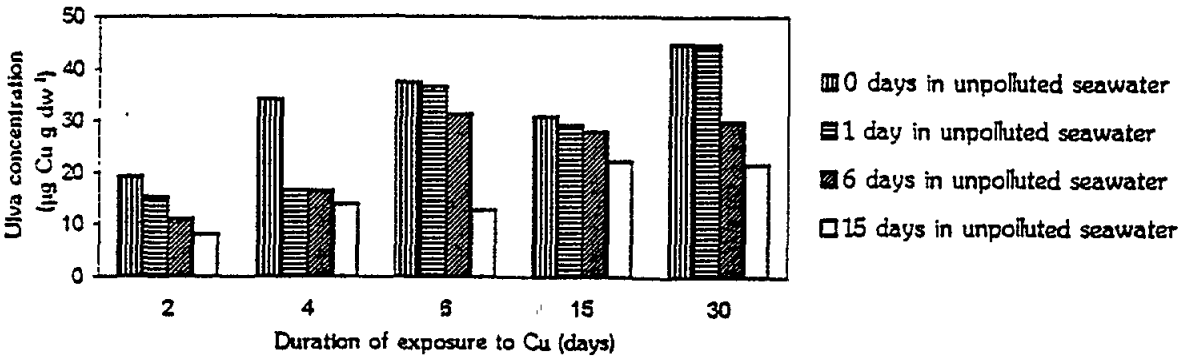

Fig. 3 Cu concentrations of dry Ulva rigida tissues after exposure to 15 ppb Cu for 2, 4, 6, 15 and 30 days and removal in unpolluted seawater for 1, 6 and 15 days

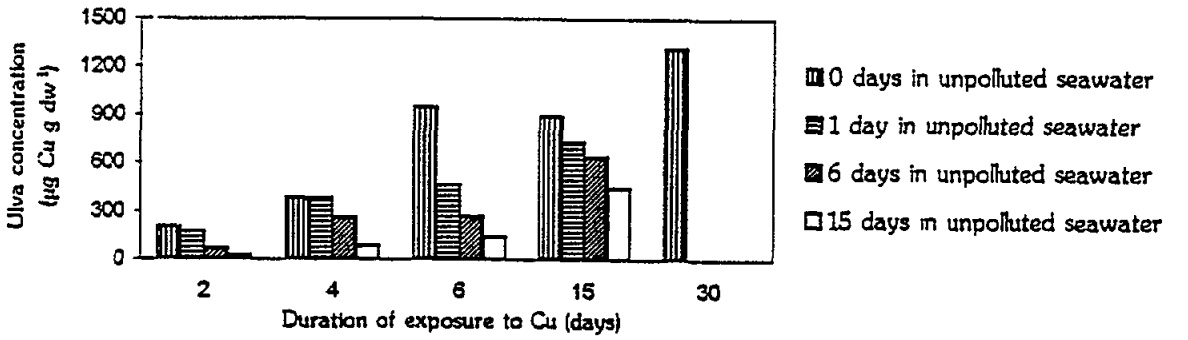

Fig. 4 Cu concentrations of dry Ulva rigida tissues after exposure to 150 ppb Cu for 2, 4, 6, 15 and 30 days and removal in unpolluted seawater for 1, 6 and 15 days

Cu concentrations of the five sediment fractions, and the percentage of Cu bound to each fraction to the total Cu concentration of the sediment are presented in Table 4. In the same table the mean value of the first 5 cm of each sediment core is also calculated. The table shows that there are some rather small differences in the partition of Cu between the two benthic chambers, although they are not statistically significant. A slight increase of the % Cu in the exchangeable fraction as well as in the reducible fraction, where Cu is bound to Fe-Mn oxides or hydroxides, is observed

in the chamber with the algal biomass (Core B). However, the relatively low percentage of Cu in this fraction determined in the first centimetre of the sediment core could be attributed to the decreased dissolved oxygen of the seawater in the chamber. According to Foerstner (1983), under reducing conditions the sorbed heavy metals on Fe and Mn hydroxides and oxides are readily mobilized.

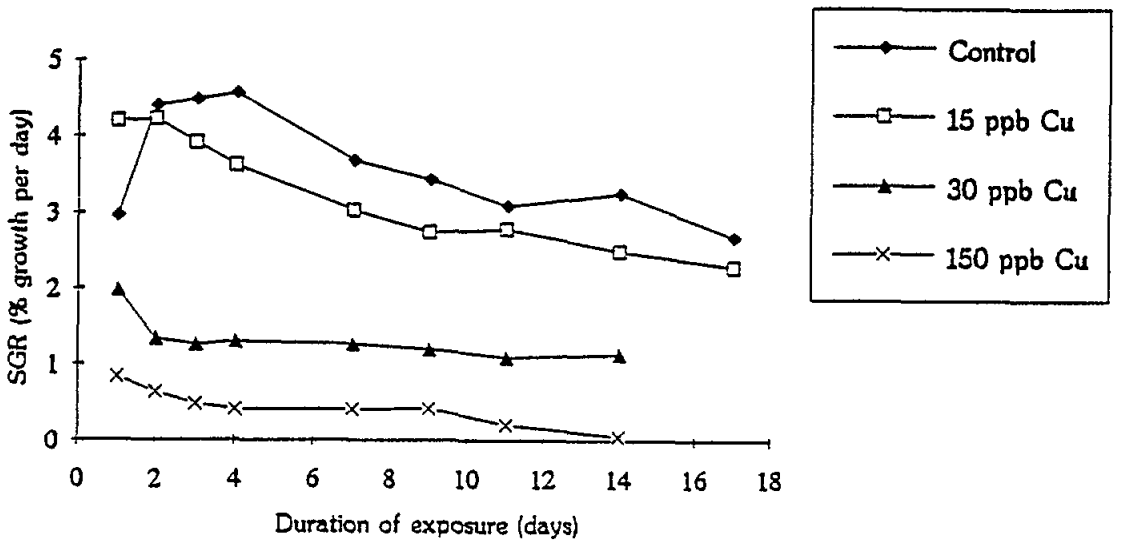

Fig. 5 Specific growth rates of Ulva discs during 17 days in various Cu concentrations, seven discs measured each day

With the exception of the residual fraction, a significant proportion of the total metal is found in the organic / sulfide fraction due to the strong affinity of Cu for complexation with organic binding sites. Calmano et al. (1988), showed that among various types of substrates, Cu was bound mainly to algal cell walls. Assuming that chamber B, within which degradation of the algal biomass took place, was enriched in Ulva cell wall pieces, we can explain the elevated percentage of Cu determined in the surface sediment (0-2cm) of this core.

The more important observation from this experiment is the difference between the dissolved and the particulate Cu concentrations measured in the two chambers. In chamber B, dissolved Cu concentration was relatively very high (28.1 $\mu g\ l^{-1}$) in comparison with chamber A (0.95 $\mu g\ l^{-1}$) and the surrounding environment (about 1.5 $\mu g\ l^{-1}$). On the contrary, particulate Cu concentration in chamber B was lower (7.16 $\mu g\ g^{-1}$) than in chamber A (179.7 $\mu g\ g^{-1}$). Taking into account the fact that during the experiment, and especially the last two months, dissolved oxygen decreased in chamber B in comparison with chamber A and the natural environment (unpublished data), followed by a slight decrease of pH values, the above mentioned results could not be unexpected.

Table 4

Distribution of Cu in sediment fractions (µg g^{-1}). Sediment samples were analysed in three replicates.
SD: standard deviation. Core A: Benthic chamber without algae
Core B: Benthic chamber with Ulva biomass

Depth cm	Exchangeable fraction			Carbonate fraction			Fe - Mn oxides fraction			Organic/sulfidic fraction			Residual fraction			Total
Core A	Mean Conc. µg g^{-1}	SD	% in fraction	Mean Conc. µg g^{-1}	SD	% in fraction	Mean Conc. µg g^{-1}	SD	% in fraction	Mean Conc. µg g^{-1}	SD	% in fraction	Mean Conc. µg g^{-1}	SD	% in fraction	µg g^{-1}
0-1	1.36	0.18	5.9	1.78	0.18	7.7	2.88	0.06	12.5	5.18	2.76	22.4	11.90	1.27	51.5	23.10
1-2	1.45	0.28	6.5	1.85	0.16	8.3	2.88	0.10	12.8	5.47	1.67	24.4	10.80	1.56	48.1	22.45
2-3	1.30	0.08	6.0	1.73	0.12	7.9	3.10	0.05	14.2	5.70	0.67	26.1	9.97	0.31	45.7	21.79
3-4	1.35	0.14	6.9	1.77	0.11	9.0	2.86	0.09	14.6	5.34	1.22	27.1	8.35	1.34	42.5	19.67
4-5	1.41	0.17	6.8	1.90	0.08	9.1	2.97	0.18	14.2	5.91	0.82	28.4	8.65	2.05	41.5	20.84
mean 0-5cm	1.37		6.4	1.81		8.4	2.94		13.7	5.52		25.7	9.93		45.9	21.57
9-10	1.20	0.03	7.5	1.72	0.08	10.8	3.45	0.40	21.6	4.52	0.54	28.4	5.03	1.32	31.6	15.92
Core B	Mean Conc. µg g^{-1}	SD	% in fraction	Mean Conc. µg g^{-1}	SD	% in fraction	Mean Conc. µg g^{-1}	SD	% in fraction	Mean Conc. µg g^{-1}	SD	% in fraction	Mean Conc. µg g^{-1}	SD	% in fraction	µg g^{-1}
0-1	1.63	0.10	7.5	1.70	0.10	7.8	2.96	0.20	13.6	5.53	0.28	25.4	9.91	0.95	45.6	21.74
1-2	1.39	0.04	8.1	1.58	0.04	9.2	2.92	0.19	17.1	4.69	0.33	27.4	6.53	1.16	38.2	17.11
2-3	1.40	0.07	8.2	1.59	0.06	9.4	3.04	0.32	17.9	4.51	1.11	26.5	6.43	2.21	37.9	16.97
3-4	1.38	0.09	7.4	1.60	0.07	8.5	3.26	0.47	17.4	4.38	0.63	23.4	8.10	1.31	43.3	18.71
4-5	1.29	0.05	7.9	1.56	0.05	9.6	3.27	0.20	20.1	4.20	0.47	25.9	5.91	0.26	36.5	16.22
mean 0-5cm	1.42		7.8	1.61		8.9	3.09		17.2	4.66		25.7	7.38		40.3	18.15
9-10	1.31	0.08	7.5	1.60	0.07	9.1	3.00	0.12	17.2	4.20	0.27	24.0	7.37	0.77	42.2	17.49

4. CONCLUSIONS

The bioaccumulation of Cu and its effects on the growth of Ulva sp. were tested during laboratory experiments with culture solutions of Cu concentrations 10 and 100 times the natural levels. The uptake of Cu by the tissues was more rapid and almost complete during the first 6 days of the experiments. The metal content of the tissues was higher in algal discs that remained in higher ambient Cu concentrations. Release of the metal accumulated was observed in all the discs, it was related though with the initial metal concentration of the solution as well as the duration of exposure. The metal content of the tissues after a 30-day period of recovery, was proportional to the metal accumulated and considerably higher in discs that remained in Cu contaminated solutions for more than 15 days, showing that Ulva tissues retain an important amount of Cu after an episode of pollution. In contrast to field experiments, the concentration factors calculated from laboratory experiments using single metal solutions are not constant over the range of Cu concentrations tested and thus, according to Seeliger and Edwards (1977), Ulva could not be used as a monitoring organism for Cu. The effect of Cu on the growth of Ulva discs was more significant in elevated Cu concentrations (20 to 100 times the natural levels).

The role of increased Ulva biomass in the cycling of Cu in eutrophicated coastal areas was studied during in situ experiments using benthic chambers. The most important observation from these experiments was, on the one hand, the depletion of the dissolved oxygen, the decrease of pH values and the enrichment in Cu bound to the exchangeable sediment fraction of the samples taken from the chamber where algal degradation took place. On the other hand, the algal degradation resulted in the increase of dissolved Cu, either in the form of free ion or most probably as soluble humic complexes or colloidal complexes. These findings are very important as far as the bioavailability of Cu is concerned. Metals bound to the exchangeable sediment fraction are available to benthic organisms that live in the sediments, but also they are easily mobilized since this sediment fraction is very sensible to changes of the redox conditions (Foerstner, 1983). Furthermore, free Cu ions as well as Cu complexed with organic ligands could be very toxic to organisms as they enter easily through the cell membranes (Nelson and Donkin, 1985). However, Cu speciation in the dissolved phase near the water - sediment interface should be more extensively investigated.

5. ACKNOWLEDGEMENTS

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MONITORING OF Hg, Cd, Cu, Pb, Zn, Co, Be AND V AT A DEEP WATER COAL FLY ASH DUMPING SITE

by

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ABSTRACT

Mercury, cadmium, copper, lead, zinc, cobalt, beryllium and vanadium as well as manganese, aluminum and iron were determined in the sediments collected at 12 stations of a deep water coal fly ash dumping site and the results compared to the natural background levels in the area. Presence of coal fly ash in the sediments caused changes in the natural concentration of most of the metals analyzed. The levels of mercury, beryllium and aluminum were higher than the natural concentration while the concentrations of lead, cobalt, vanadium, manganese, zinc and iron were lower than the average natural levels. Cadmium concentrations were not affected. These changes were attributed to the mixing of sediment with coal fly ash rather than to chemical changes in the sediments due to leaching/adsorption processes.

1. INTRODUCTION

The Maor David power station at Hadera, on the Mediterranean coastline of Israel, produces c.a. 400,000 tons of coal fly ash (CFA) per year. Of this, about 60% is utilized, e.g. in the manufacture of cement and building blocks, and in road construction and landscaping. Until 1988 the excess ash was piled on land adjacent to the power station. However, this was not considered a permanent solution, both because the land available for this disposal was fully utilized and also because two new power units were planned in the area. Scarcity of suitable land, and the possible effects of land disposal on the quality of ground water, which is a valuable and fully utilized resource in Israel, brought the Israel Electric Corporation (IEC), operator of the power station, to propose the disposal of this ash at sea. The IEC was granted a permit to dump 1,000,000 tons of coal fly ash over two years at a deep water site off the Israeli coastline. The dumping site of c.a 210 km^2 area (Fig. 1) is located 70 km west of the Hadera power station at a water depth of 1,400 m, beyond the continental shelf and far from fishing grounds. Conclusions drawn from studies elsewhere (Bamber, 1984; Norton, 1985) were taken into consideration when the decision was made to allow the disposal at sea but not at near shore locations. Disposal of CFA, using a 1,500 ton capacity split-bottom hopper barge, started in April 1988 and continued for two years. As part of the permit requirements, a monitoring programme was initiated in order to assess the influence of the coal fly ash on the marine environment. It was found (Golik and Krom, 1990; Kress et al., 1993a) that the CFA descended to the bottom in 4 hours at velocities ranging from 5 to 50 m/min,

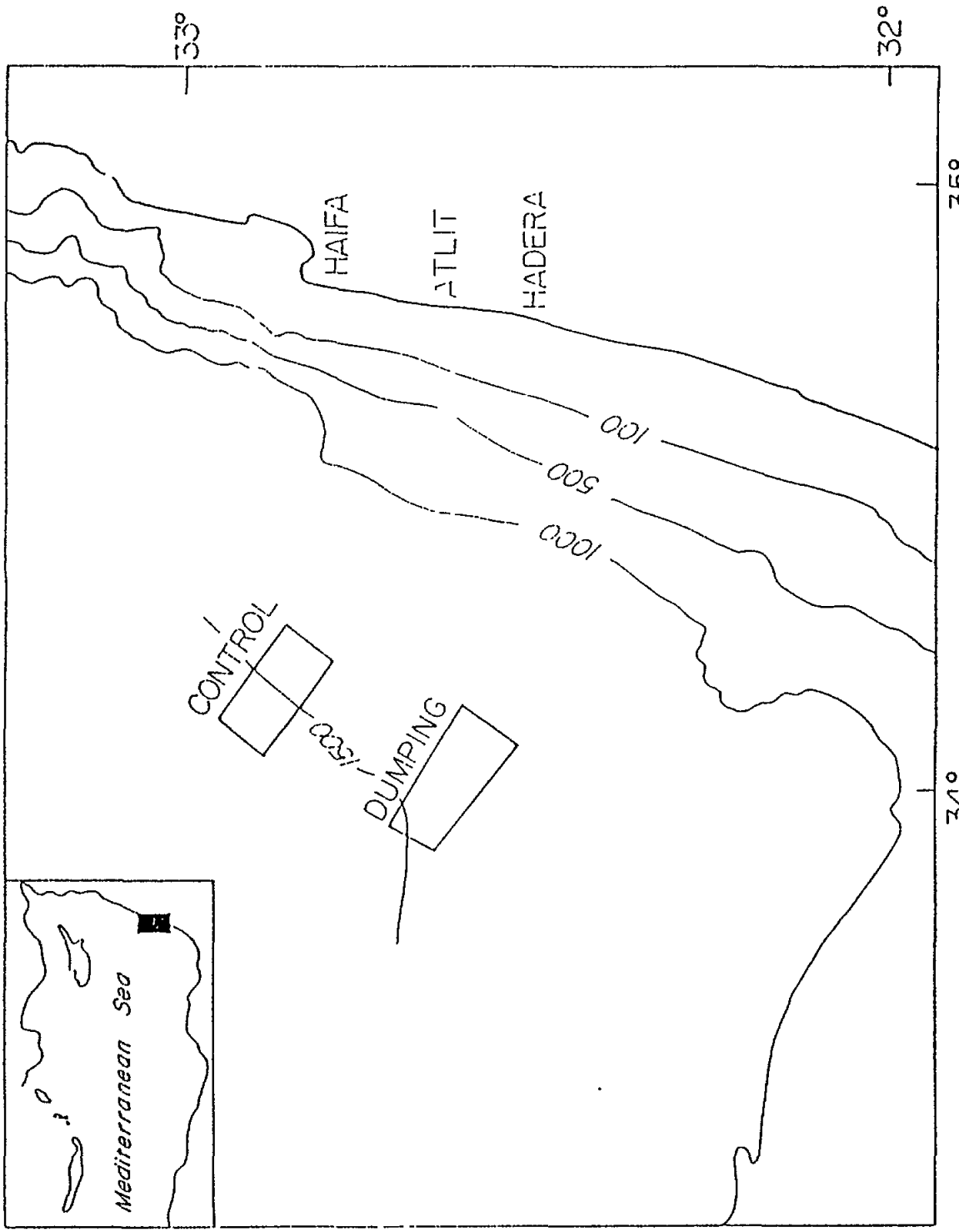

Fig. 1 Location of the coal fly ash dumping site and the control site off Atlit

with minimal effect on the water column. Therefore, the monitoring programme focused on the benthic compartment: sediments and fauna (Kress et al., 1991, 1993a, 1993b).

Coal fly ash, the product of coal combustion, is composed mainly of oxides of silicon, aluminum, iron and calcium but also contains trace amounts of heavy metals that are listed in the annexes of the Land based Sources (LBS) and the Dumping Protocols of the Barcelona Convention. CFA can cause physical changes to the substrate at the disposal site but the main ecological concern related to the disposal of coal fly ash inland or at sea is the possible release of heavy metals to the environment. Leaching of heavy metals from coal fly ash has been found in laboratory experiments under different experimental conditions (Kress, 1993; Rose et al., 1985; Van der Sloot and Nieuwendijk, 1985, among others).

This report lists the concentration of mercury, cadmium, copper, lead, zinc, cobalt, beryllium and vanadium as well as manganese, aluminum and iron found in sediment samples collected at the dumping site in 1992 and compares these findings to those from a control site as well as to the findings of surveys conducted at the two sites in previous years.

2. METHODS

2.1 Study area and sampling

The survey at the coal fly ash dumping site took place on December 30, 1992 on board the R/V Shikmona. Twelve stations were sampled (P1-P12, see figures 1-2 and Table 1). Five stations (AT1-AT5) at a control site were sampled on January 27, 1993 (Fig. 1, Table 2). Both areas were sampled in previous years: the dumping site in November 1988 before dumping started (stations HAD2, HAD5 and HAD7), in May 1990 one year after the commencement of ash disposal (stations B1-B4) (Table 3), and in May 1991 after two years of dumping (stations P1-P11) (Table 1); the control site was sampled in June 1989, (stations AT1-AT2) and June 1990 (stations B1AT-B2AT) (Table 3).

Sediments were sampled using an Ocean Instruments, Inc. model BX 700Al box corer. Small sub-cores were taken from the box corer using hollow plexiglass cylinders and frozen immediately. In addition, surface sediments scraped from the upper 3 cm were placed in plastic containers and frozen.

Representative samples of CFA from the Hadera power station (referred hereafter as HFA) were obtained from the IEC for chemical analysis.

2.2 Laboratory procedures

The sediment sub-cores collected during the 1993 and 1992 surveys were extruded and the upper layer (1 cm) taken for analysis. Fe, Mn and Al were analyzed also in the second layer (1-3 cm). The samples were lyophilized (freeze dried) and digested using concentrated HNO_3 in high pressure decomposition vessels for the

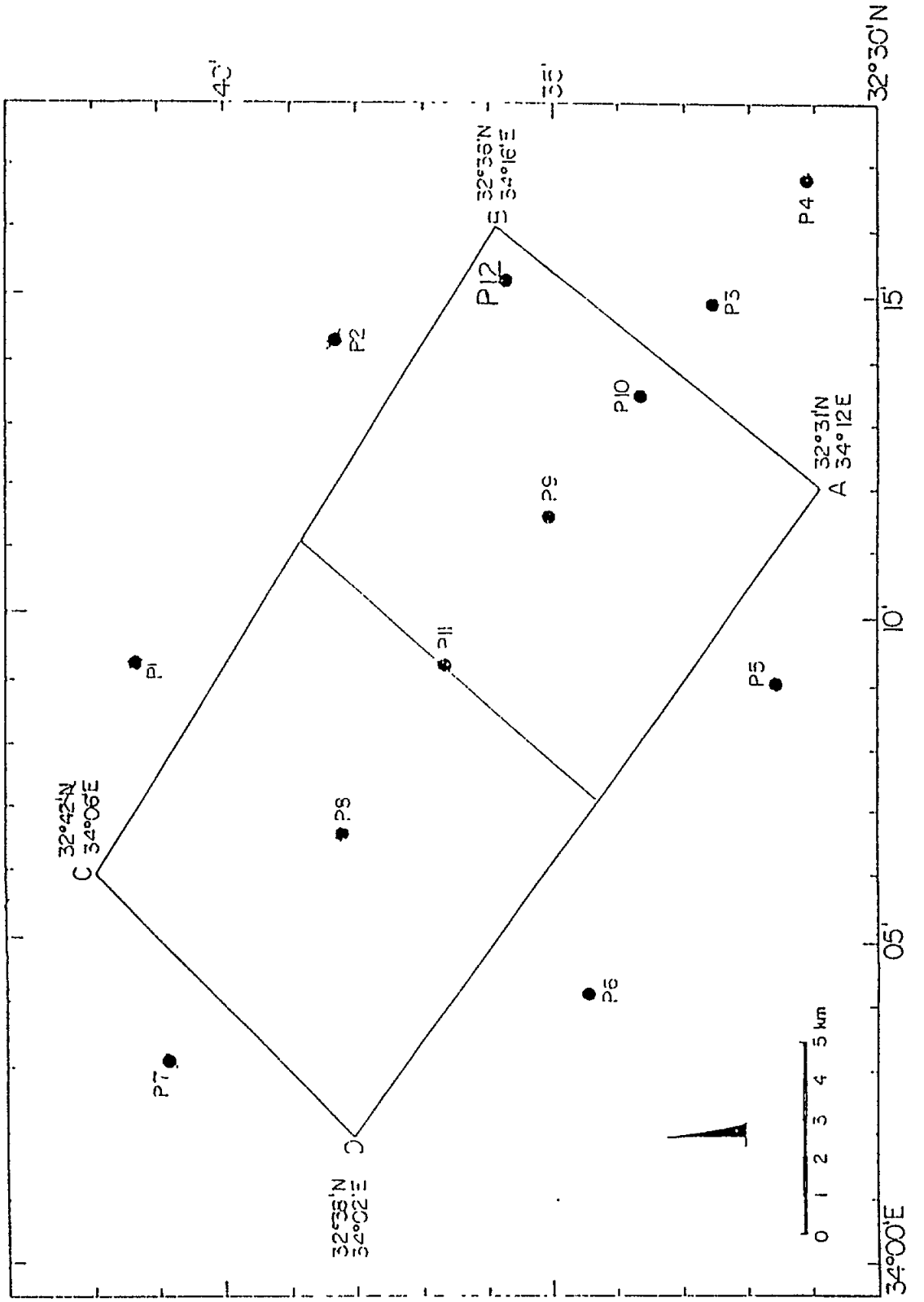

Fig. 2 Map of station locations at the fly ash dumping site (May 1991 and December 1992)

determination of Hg, Cd, Pb, Zn, Co, Be and V. Details of this procedure are reported in Hornung et al. (1989). Cu, Zn, Mn, Al and Fe were determined after digestion of the samples with a mixture of HF and aqua regia (ASTM, 1983). Zn was determined by both methods in order to intercompare the results. Duplicates of each sample were analyzed and the average concentration reported. Accuracy and precision of the methods were determined by analyzing a series of known certified standards together with the samples and are reported in Tables 4-5. All the metals, except Hg and Be were determined by flame atomic absorption spectroscopy using a Perkin Elmer 1100B or an Instrumentation Laboratory IL 951 AA spectrophotometer equipped with a D_2 arc background correction. Be was determined by graphite furnace atomic absorption using a Perkin Elmer 1100B equipped with an HGA 700 and Hg was determined by cold vapour atomic absorption on a Coleman 50A mercury analyzer system.

Table 1

Sampling locations at the coal fly ash dumping site.
(May 1991 and December 1992)

Station No.	Latitude N	Longitude E	Depth m
P1	32E41.37´	34E09.24´	1416
P2	32E38.31´	34E14.13´	1402
P3	32E32.55´	34E14.86´	1350
P4	32E31.17´	34E16.85´	1300
P5	32E31.59´	34E09.00´	1382
P6	32E34.40´	34E04.23´	1430
P7	32E40.80´	34E03.09´	1485
P8	32E38.20´	34E06.50´	1446
P9	32E35.04´	34E11.44´	1394
P10	32E33.66´	34E13.38´	1369
P11	32E36.60´	34E09.26´	1427
P12	32E35.81´	34E15.26´	1376

In the previous surveys, conducted during 1988-1991, the sediment analyzed was scraped from the surface of the box corer sample and therefore the samples represented the upper 3 cm layer. The analytical methods employed were as in the present study, except for Cu that was measured after HNO_3 digestion. Be, Co and V were not analyzed in the 1988-1991 surveys.

Table 2

Sampling locations for sediment at the deep sea control site off Atlit (January 1993)

Site	Latitude N	Longitude E	Depth m
AT1	32E49.49´	34E21.50´	1431
AT2	32E50.52´	34E19.40´	1456
AT3	32E52.00´	34E17.01´	1493
AT4	32E53.51´	34E14.52´	1520
AT5	32E55.00´	34E12.00´	1570

Table 3

Sampling locations at the coal fly ash dumping site (May 1990) and the control site (June 1990)

Station no.	Latitude N	Longitude E	Depth m
Dumping site			
B1	32E38.00´	34E08.00´	1417
B2	32E40.00´	34E04.50´	1474
B3	32E35.30´	34E11.70´	1399
B4	32E32.70´	34E16.60´	1319
Control site			
B1AT	32E51.40´	34E18.01´	1465
B2AT	32E49.20´	34E22.20´	1410

Heavy metals in coal fly ash were determined after digestion with a mixture of HF and aqua regia (ASTM, 1983), except for Be, Co and V that were determined after digestion with concentrated HNO_3 (Hornung et al., 1989). All the metals, except Cd and Be were determined by flame atomic absorption spectroscopy using a Perkin Elmer 1100B or an Instrumentation Laboratory IL 951 AA spectrophotometer equipped with a D_2 arc background correction. Cd and Be was determined by graphite furnace atomic absorption. Accuracy and precision of the methods are reported in Tables 4-5.

In this report we will distinguish between the concentration found by the methods used and the total concentration calculated from the % recovery of the methods.

Table 4

Quality control for heavy metal determination in sediments and coal fly ash.
(Digestion in HNO_3)

Standard reference material	Element (ppm)						
	Cd	Zn	Pb	Be	Co	V	Hg
MESS-1(NRCC)							
certified	0.59±0.1	191±17	34.0±6.1	1.9±0.2	10.8±1.9	72.4±17	
found	0.61	197	31.8	1.59	9.19	55.5	
% recovery	103	103	94	84	85	77	
Estuarine sediment 1614 (NIST)							
certified	0.36±0.07	138±6	28.2±1.8	1.5*	10.5±1.3	94±1	
found	0.30±0.01	139±5	26.7±0.02	1.51±0.02	8.16±0.05	82.0±2.3	
% recovery	83	101	95	100	78	87	
Coal fly ash 1633a (NIST)							
certified	1.00±0.16	220±10	72.4±0.4	12*	46*	297±6	
found	0.53±0.02	157±6	30.2±0.3	6.65±0.11	17.0±0.3	133±0.2	
% recovery	53	71	42	55	37	45	
BEST-1(NRCC)							
certified							0.092±0.009
found							0.095±0.004
% recovery							103

* Non certified value

Table 5

Quality control for heavy metal determination in sediments and coal fly ash (n=4). Digestion in HF mixture

Standard reference material	Mn (ppm)	Al (%)	Fe (%)	Cu (ppm)	Zn (ppm)	Cd (ppm)
MESS-1 (NRCC)						
certified	513±25	5.84±0.20	3.05±0.17	25.1±3.8	191±17	0.59±0.1
found	518±10	5.84±0.14	2.57±0.05	28.9±0.7	192±12	0.46±0.01
% recovery	101	100	84	115	101	78
Estuarine sediment 1614 (NIST)						
certified	375±20	6.25±0.2	3.35±0.10	18±3	138±6	0.36±0.07
found	353±6	6.12±0.18	3.10±0.05	18.9±1.1	122±0.2	0.18±0.02
% recovery	94	98	93	105	88	42
Coal fly ash 1633a (NIST)						
certified	179±8	14.3±1.0	9.4±0.1	118±3	220±10	1.00±0.16
found	173±5	14.4±0.3	8.00±0.17	117±6	232±2	0.82±0.02
% recovery	97	101	85	99	105	82

2.3 Determination of the amount of coal fly ash in the sediments

The amount of fly ash in the sediments was determined both by measuring the CFA layer thickness in the sub-cores (fly ash can be easily distinguished from the natural brown sediment by its contrastant silver grey color) and by a chemical method, using the measured concentration of Mn and Al in the samples. The method is based on the different concentrations of Mn and Al in deep sea sediments and in HFA (see Table 6) and assumes simple mixing of the two substances. Details are presented in Krom and Galil (1990) and Kress et al. (1993a). Briefly, the measured concentrations of Mn and Al in the samples are plotted on a single mixing diagram where deep sea sediment and HFA are the two end members and the amount of fly ash in the samples is calculated from the regression lines (Fig. 3). The method has an uncertainty of ca. 10% due to the variability in the determination of the end members concentration.

Table 6

Average heavy metal concentrations in deep sea sediments collected at the control area (January 1993) and in representative samples of coal fly ash from the Maor David power station at Hadera

	HNO_3 digestion							HF digestion				
	conc., ppm							conc.,ppm			conc., %	
	Hg	Cd	Pb	Zn	Be	Co	V	Cu	Zn	Mn	Al	Fe
Atlit (1993), average*	0.067	0.11	44.7	131	3.15	25.6	102	76.5	133	1811	7.54	5.76
Std	0.014	0.01	8.0	21	0.05	2.7	2	1.5	8	28	0.23	0.07
CV (%)	21	9.1	17.9	16.0	1.7	10.4	2.0	2.0	6.0	1.5	3.1	1.2
Hadera fly ash												
Jul-Dec 1989	-	0.58#	28.7	85.4	6.81	19.5	155	87.9	108	412	14.0	3.57
Jul-Dec 1991	-	0.55#	30.8	70.5	7.54	19.0	150	94.6	97.0	424	14.6	3.32
Values corrected for incomplete recovery												
Jul-Dec 1989	-	0.71	68.3	120	12.3	53	352	88.8	102	425	13.9	4.20
Jul-Dec 1991	-	0.67	73.3	98.7	13.6	51	335	95.6	91.9	437	14.5	3.91

* Average of 4 stations: AT1, AT2, AT4 and AT5.
\# HF digestion

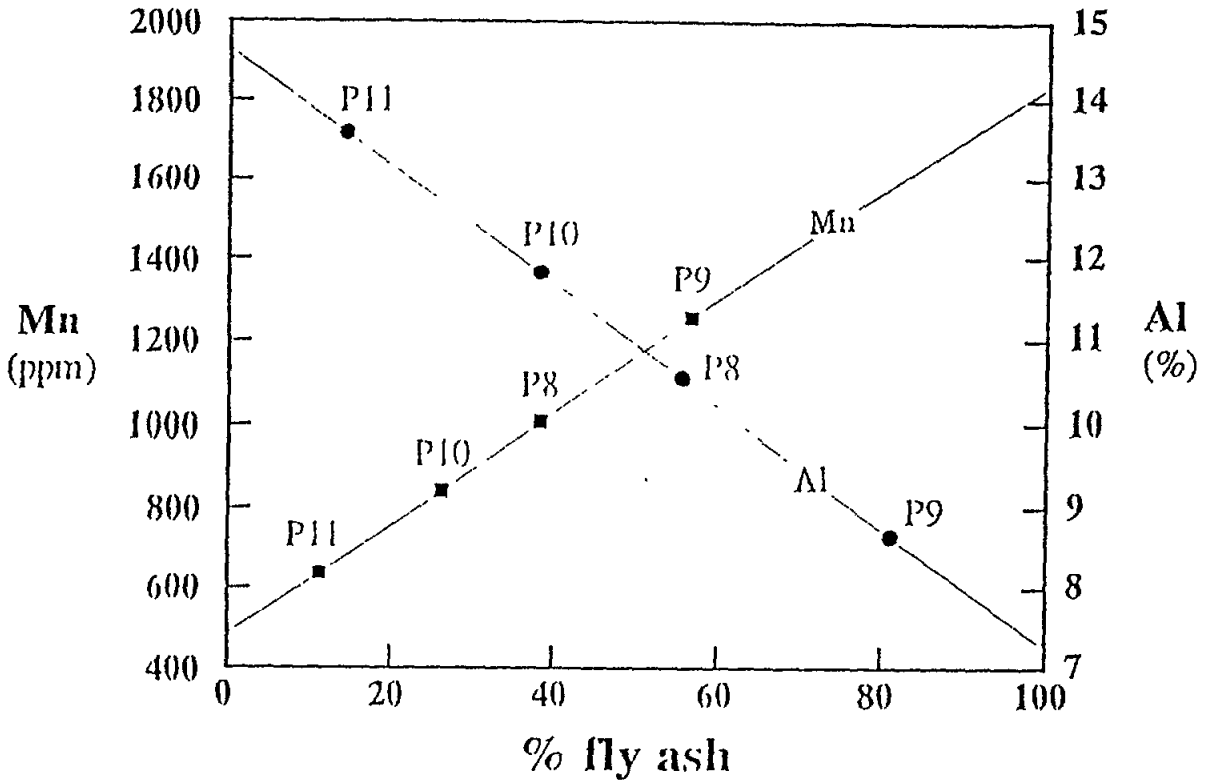

Fig. 3 Mixing diagram for Hadera fly ash and deep sea sediment. The concentrations of Mn (squares) and Al (circles) found at stations P8-P11 (May 1991) are plotted

3. RESULTS AND DISCUSSION

3.1 Visual examination

The surface sediment collected at the disposal site was a mixture of natural sediment and CFA. Samples P8 and P9 contained a layer of fine grained CFA deeper than 1.0 cm, and samples P10 and P12 CFA layers 0.5 and 0.3 cm thick, respectively.

Further evidence for the presence of CFA on the seabed came from benthic trawl hauls in the area (Kress et al., 1993a, 1993b) which contained CFA in various forms from a powdery state to aggregates (1-3 cm in diameter) and even to boulders up to 80 cm long.

3.2 Heavy metals in the sediments

The concentrations of mercury, cadmium, copper, lead, zinc, cobalt, beryllium, vanadium, manganese, aluminum and iron in the sediment samples collected at the dumping site in 1992 and at the control area in 1993 are summarized in Tables 7-9. Table 10 presents the amount of fly ash in the samples, calculated from both Mn and Al experimental concentrations.

Table 7

Heavy metals concentrations (ppm) in the upper layer of the sediments collected at the fly ash dumping site (30 December 1992 - 1 January 1993)

Sample	Hg	Cd	Cu*	Pb	Zn*	Zn	Be	Co	V
P1	0.047	0.09	76.4	47.9	88.8	106	2.47	23.5	105
P2	0.068	0.20	66.1	50.7	95.3	105	2.48	27.3	105
P3	0.066	0.07	76.1	51.1	83.6	131	2.67	26.9	103
P4	-	0.14	65.0	52.2	88.4	98.2	2.34	25.7	103
P5	0.117	0.08	69.5	47.5	84.4	96.2	2.61	21.8	103
P6	0.050	0.11	70.5	35.2	82.6	94.6	2.91	26.1	101
P7	0.075	0.14	73.6	51.6	84.5	105	2.99	25.3	101
P8	0.117	0.10	74.4	34.0	64.9	49.8	6.65	19.7	89.7
P9	0.290	0.12	82.0	30.8	81.3	66.8	5.93	17.6	89.2
P10	0.170	0.08	72.3	39.3	83.9	83.6	5.63	18.8	89.9
P11	0.120	0.15	73.5	45.2	97.3	126	3.63	24.8	97.2
P12	0.124	0.09	74.9	45.7	133	122	3.92	23.9	96.1

* Digestion in HF mixture

Comparison between the amount of fly ash (or thickness of CFA layer) determined at sampling stations P1-P12 and the locations of the dumping events showed a good correspondence (Fig. 4); the thickest layer of CFA (ca. 1cm) was found where many dumping events were concentrated (station P9).

In order to assess the effect of CFA on the sediments at the dumping site we first determined the natural background concentrations in the undisturbed deep sea sediments of the area and in representative samples of Hadera fly ash. These values were then compared to the experimental values found at the dumping site. The metal levels in the sediments sampled at the control area in 1993 were averaged and the values taken as reference background levels. The data for station AT3 were

Table 8

Heavy metals in sediments collected at the fly ash dumping site (December 1992).
(1) denotes the upper 1 cm layer of the sediment, and
(2) the layer between 1 and 3 cm

Sample	Mn ppm	Al %	Fe %
P1(1)	1861	7.61	5.43
P1(2)	1759	7.10	5.98
P2(1)	1572	7.68	5.18
P2(2)	1965	7.66	5.82
P3(1)	1514	7.74	5.56
P3(2)	1948	7.63	5.94
P4(1)	1378	7.17	5.21
P4(2)	1882	7.93	6.16
P5(1)	1674	7.10	5.31
P5(2)	1735	7.66	5.94
P6(1)	1885	7.30	5.53
P6(2)	1829	7.60	5.98
P7(1)	1818	7.03	5.40
P7(2)	1843	7.17	5.69
P8(1)*	833.8	11.0	4.50
P8(2)	2025	7.37	5.63
P9(1)*	710.5	12.6	3.73
P9(2)	1981	7.99	5.70
P10(1)*	1046	10.4	4.59
P10(2)	1960	7.86	5.93
P11(1)**	1815	7.46	5.80
P11(2)	1946	7.58	5.79
P12(1)*	1429	8.24	5.01
P12(2)	1780	7.81	5.93

* Fly ash detected visually and chemically
** Fly ash detected visually

omitted because the concentrations found were lower than for the other stations, results as yet not understood. The average heavy metal concentrations for the control area, as well as standard deviation and coefficient of variance are summarized in Table 6. Table 6 presents also the heavy metal concentrations in representative samples of Hadera coal fly ash determined by the methods used and the total concentrations calculated from the recovery for each element (Tables 4-5). Mercury concentration in HFA is 0.30 ± 0.05 ppm (Dr. A. Metzger, IEC, personal communication). Comparison between the concentrations in HFA and natural deep sea sediments showed that the total concentrations of Mn and Fe in HFA were lower than the concentrations found in the sediments while the total concentrations of Hg, Cd, Be, Co, V and Al were significantly higher in HFA. Pb, Cu and Zn concentrations were similar in both substances.

Table 9

Heavy metals in surface sediments collected at a deep sea control site off Atlit (27-28 January 1993)

Digestion in HF mixture

Sample	Mn (ppm)	Al (%)	Fe (%)	Cu (ppm)	Zn (ppm)
AT1	1810	7.33	5.78	77.2	130
AT2	1817	7.51	5.65	75.5	141
AT3	1578	6.72	5.01	66.8	108
AT4	1771	7.39	5.75	74.6	121
AT5	1849	7.92	5.85	78.5	141

Digestion in HNO_3

Sample	Hg (ppm)	Cd (ppm)	Pb (ppm)	Zn (ppm)	Co (ppm)	Be (ppm)	V (ppm)
AT1	0.053	0.12	53.3	126	25.5	3.21	98.8
AT2	-	0.11	50.9	123	22.7	3.07	103
AT3	0.081	0.15	47.3	133	26.6	2.77	102
AT4	-	0.13	41.6	108	24.3	3.18	104
AT5	-	0.09	33.0	166	29.9	3.15	103

From the results of the dumping site samples presented in Tables 7-10 it can be seen that at stations P8-P10 and P12, where significant amounts of fly ash were detected, the concentrations of mercury, beryllium and aluminum were higher than the background concentrations while the concentrations of lead, cobalt,

vanadium, manganese, zinc (by HNO_3) and iron were lower than the average natural levels. At the other stations (P1-P7 and P11) the concentrations found were not significantly different (within two standard deviations) from the background levels, except for mercury, that was higher than natural also at stations P5 and P11. Cadmium concentrations at all stations were similar to the natural levels. Copper at most stations and zinc (by HF) at all stations of the dumping site were lower than those measured at the control site.

Table 10

Coal fly ash content (%) in surface sediment samples collected at the dumping site off Hadera (30 December 1992 - 1 January 1993)

Station	Calculated from Mn concentration	Calculated from Al concentration
P1	5	9
P2	24	10
P3	28	11
P4	37	1
P5	17	-1
P6	3	3
P7	8	-2
P8	74	70
P9	83	99
P10	60	60
P11	8	6
P12	34	20

The deviation of Hg, Mn, Al and Fe concentrations from the natural background levels in the samples affected by the presence of CFA was due to the different concentrations of the metals in the two matrices: deep sea sediment and fly ash (see Table 6). However, the differences between the measured and background values for Pb, Zn (by HNO_3), Be, Co and V were not due only to the different concentrations found in the two materials but mainly a result of the different recoveries of the metals from the two matrices (Tables 4-5). For example, the total concentration of zinc in HFA and deep sea sediments were not significantly different but the recoveries by HNO_3 were 71% and 102%, respectively. Therefore, the values found for zinc at the stations where CFA was present were lower than the natural ones. A different example is Co. The total concentration of cobalt was higher in HFA than in deep sea sediments (52 and 25.6 ppm, respectively) therefore, a higher than natural concentration was expected for example in station P9, where 91% of the sample consisted of CFA. However, the concentration found was lower than the

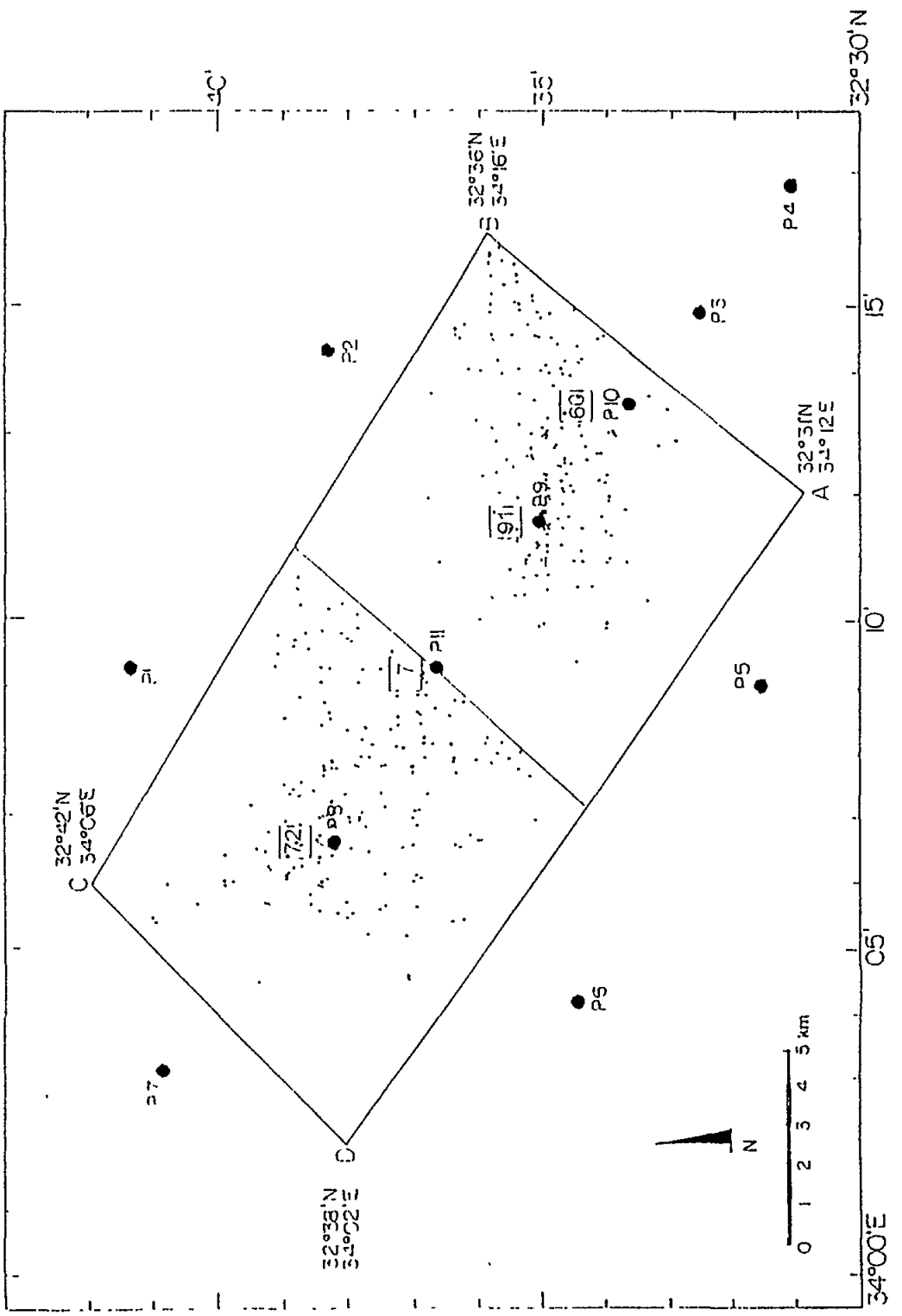

Fig. 4 Fly ash content at the dumping site stations, 3½ years after dumping (December 1992). The percentage of ash in the samples is presented in the boxes and the small circles represent the locations of the dumping events

natural levels, a result of the different Co recoveries: 37% in CFA and 82% in sediments (Table 4).

In 1992, the sediment layer between 1 and 3 cm depth in the subcores was also analyzed and the concentrations of Al, Mn and Fe determined. The concentrations found were similar to the natural background concentrations for the area (Table 8) with the following average values: Mn- 1988 ± 93 ppm, Al- 7.61 ± 0.27% and Fe- 5.87 ± 0.15%. This finding, together with the fact that the samples sampled in the vicinity of the stations containing fly ash (stations P1-P7 and P11) exhibited natural background concentrations lead us to assume that the different concentrations found in the sediments with CFA were a reflection of the mixing of sediment with CFA rather than chemical changes in the sediments due to chemical leaching/adsorption processes.

3.3 Comparison to the results from previous years

The results of the survey conducted at the dumping site in 1991, two years after the start of the disposal operations are presented in Table 11. Table 12 summarizes the results of two surveys to the dumping site (1988 and 1990) and two surveys to the control area off Atlit (1989 and 1990). Be, Co and V were not measured.

Table 11

Heavy metals in surficial sediments collected at the fly ash dumping site (May 1991)

Stn. no.	Elements (ppm)					Elements (%)	
	Cd	Pb	Cu	Zn	Mn	Fe	Al
P1	0.10	48.4	67.1	90.4	2047	5.95	7.60
P2	0.09	48.3	70.8	95.4	2087	6.27	8.05
P3	0.10	46.4	65.2	91.8	1952	5.92	7.73
P4	0.16	47.9	69.6	106.3	2002	6.16	8.08
P5	0.11	47.1	69.0	90.9	1998	5.98	7.98
P6	0.07	46.0	72.9	92.3	1914	5.71	8.06
P7	0.19	31.4	69.5	82.5	1925	5.74	6.54
P8	0.14	36.5	60.2	62.1	1109	5.17	10.03
P9	0.02	31.9	41.8	50.8	731	3.38	11.27
P10	0.10	38.9	56.7	73.1	1365	5.16	9.21
P11	0.16	42.8	68.5	84.3	1719	5.52	8.19
L8					500	3.23	13.82

L8 = fly ash collected from beam trawl

Table 12

Trace element concentration in sediments (upper 3 cm) from the dumpsite prior to dumping (November 1988), one year after dumping commenced (May 1990), and at the control site (Atlit). Mercury was below detection limit (<0.005 ppm) for all sediment samples

Stn. no.	Date	Location	Cd (ppm)	Pb (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (%)	Al (%)
HAD2	Nov. 88	Hadera	0.19	37.8	68.2	79.7	1985	5.32	7.95
HAD5	Nov. 88	Hadera	0.21	35.5	67.2	74.9	1917	5.23	7.73
HAD7	Nov. 88	Hadera	0.26	34.9	68.9	72.0	1995	5.43	7.66
B1	May 90	Hadera	0.23	39.7	64.4	90.7	1751(743)	4.90	
B2	May 90	Hadera	0.22	37.4	66.6	97.9	1880(1824)	5.25	7.30
B3	May 90	Hadera	0.20	36.7	61.8	91.5	1645(995)	4.85	
B4	May 90	Hadera	0.20	41.4	65.4	105.6	1842(1629)	5.57	
AT1	Jun. 89	Atlit	0.13	36.4	61.3	98.9	-	5.33	8.11
AT2	Jun. 89	Atlit	0.16	37.8	65.7	93.3	-	4.92	7.97
B1AT	Jun. 90	Atlit	0.24	44.9	63.9	101.5	1897	5.34	
B2AT	Jun. 90	Atlit	0.20	42.6	63.6	102.3	1860	5.38	

In parenthesis: concentration in the upper 0.5 cm

In 1991, the amount of CFA in the sediments at stations P8-P11 was determined chemically to be 47, 69, 32 and 13%, respectively (Kress et al., 1993a), in good agreement with the results of the 1992 survey. The same changes in concentrations detected in 1992 at the stations affected by CFA can be seen in 1991 at stations P8-P11: a decrease in Mn, Fe, Zn and Pb concentrations and increase in Al concentration compared to the natural background levels. At the other stations (P1-P7), where fly ash was not detected, the concentrations were similar to those measured at the control area. However, it must be emphasized that in order to compare between the different surveys it was necessary to correct the results and take into account the differences in recoveries due to the use of different methods (e.g. Cu determination) and moreover, the between years variations in the recoveries of the same method.

The averages of the measured and calculated total concentrations in the sediments collected at the control site in 1989, 1990 and 1993 as well as the average concentrations in the sediments not affected by CFA but collected at the dumping site (1988, 1990-1992) are presented in Table 13. These results were compared to the natural area in 1993. It can be seen that the average value for Al, Pb, Cu, Cd, Mn and Fe were not significantly different while the average value for Zn in the control area in 1993 was higher than in the previous years while the average value for Mn

Table 13

Average heavy metal concentrations in deep sea sediments off Hadera and Atlit

	conc. %		conc. ppm						
	Al	Fe	Mn	Cu	Cu*	Zn	Zn*	Cd*	Pb*
Hadera									
1992, n=3	7.14±0.11 (7.21)	5.41±0.09 (6.08)	1792±88 (1828)	71.2±1.7 (64.7)	-	83.8±0.9 (88.2)	98.6±4.6 (96.7)	0.11±0.02	44.8±7.0 (47.2)
1991, n=6	7.92±0.18 (7.47)	6.00±0.18 (6.06)	2000±57 (1961)	-	69.1±2.5 (77.6)	-	94.5±5.5 (95.4)	0.11±0.03	47.4±0.9 (49.4)
1990, n=2	-	5.41±0.16 (5.88)	1821±21	-	66.0±0.6 (77.6)	-	102±4 (105)	0.21±0.01	39.4±2.0 (40.2)
1988, n=3	7.78±0.12 (7.34)	5.33±0.08 (6.27)	1966±35 (1927)	-	68.1±0.7 (74.0)	-	75.5±3.2 (80.3)	0.22±0.03	36.1±1.3 (41.0)
Atlit									
1993, n=4	7.54±0.23 (7.62)	5.76±0.07 (6.47)	1811±28 (1848)	76.5±1.5 (69.5)	-	133±8 (140)	131±21 (128)	0.11±0.01	44.7±8.0 (47.1)
1990, n=2	-	5.36±0.02 (5.83)	1879±19	-	63.8±0.2 (75.0)	-	102±0.4 (105)	0.22±0.02	43.8±1.2 (44.7)
1989, n=2	8.04±0.07 (7.58)	5.13±0.20 (6.03)	-	-	63.5±2.2 (69.0)	-	96.1±2.8 (102)	0.15±0.02	37.1±0.7 (42.2)

* Digestion in HNO_3. Values in parenthesis are corrected for % recovery

is lower. The differences in Zn were probably analytical. The relative standard deviation (rsd) for the average Zn concentration found at the control area in 1993 was 16% as opposed to a maximal rsd of 6% during the other surveys. Therefore, the natural background values for Zn in the sediments of the region should be 97.4 ± 8.5 ppm and not 131 ± 21 ppm as found in 1993.

4. CONCLUSIONS

a) The presence of coal fly ash in the sediments can be easily detected visually. CFA was found in a powdery state, and as aggregates (1-3 cm diameter) and boulders (maximum length found: 80 cm).

b) Natural background levels of the metals measured were determined from sediments collected at a control site located at the same water depth as the dumping site (see Table 6).

c) Beryllium, cobalt and vanadium, substances listed in the annexes of Land Based Sources (LBS) and the Dumping Protocols of the Barcelona Convention, were determined for the first time in the deep sea sediments of the Israeli Mediterranean coastline.

d) Presence of fly ash in the sediments caused changes in the natural concentration of the metals. The levels of mercury, beryllium and aluminum were higher than the average natural concentrations while the levels of lead, cobalt, vanadium, manganese, zinc (by HNO_3) and iron were lower. Cadmium concentrations were not affected.

e) The changes in the natural concentrations of the metals in the sediments influenced by fly ash were probably a result of mixing between deep sea sediments and coal fly ash rather than chemical changes in the sediments due to leaching/adsorption processes.

f) There was good agreement between the results three and a half years after the commencement of the dumping operations and the results, at the same stations, after two years of dumping. There was also good correspondence between the location of the dumping events and the amount of CFA found at the bottom.

g) The between years comparison of the results was performed after the individual recoveries (calculated from the analysis of international standard reference materials) were taken into account.

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EXPERT SYSTEM FOR PHYTOPLANKTON CLASSIFICATION

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A B S T R A C T

Multimedial taxonomic databases and expert system software make the setting up of a modern identification system a very innovative undertaking. The combination of various fields of computer technical and scientific expertise open up an entirely new field of datasharing and environmental troubleshooting. There exists a great need for quickly accessible information of bloom forming, toxic and indicative plankton species (eutrophication, pollution). This work describes a multimedia information system that covers the taxonomy of marine organisms among the proctist taxa including environmental data and video images of organisms as observed under the optical and scanning electron microscopes.

A data and image bank has been built from observations of field and laboratory phytoplankton samples under optical and scanning electron microscopes and bibliography. The system combines, at the present time, information from more than 2000 taxa including 450 different views of about 150 marine phytoplankton species. The database programme is used to explore combinations of taxonomic and morphological characters for all species.

The knowledge base of the system has been developed through matrices organized with a database programme. The data matrices are composed of variables visible under the optical inverted microscope adequate for unambiguous identification of each species. After testing the matrices, such combinations are translated into expert system rules.

These new developments greatly facilitate the identification of the most common phytoplankton species by specialists and non-specialists alike.

1. INTRODUCTION

Although there is general consensus in accepting the major importance that species identification has for the assessment of biodiversity in specific marine systems (IUBS), particularly in the development of environmental impact studies, there has been a decrease in interest for taxonomic studies, during the last decades, to the benefit of ecosystem flux studies. This has resulted in a decline in the number of scientists experts in the identification of the taxa present in natural mixed communities (Nature, August 1990).

Aquatic organisms, particularly those forming the plankton communities, play an important role not only as the basis for fisheries and general ecosystem management but also because they constitute the major pathway for carbon and nutrient removal from the surface down to deeper layers where their potential for interfering with the radiative balance of the Globe (greenhouse effect) is minimised.

It is well known that not all the species have the same efficiency in converting inorganic carbon and nutrients into organic matter. When nutrients are present in excess of the natural concentrations, eutrophication sets on and produces such noxious effects as anoxia and extracellular substances which not only endanger the living resources but reduce the potential use of the coastal zone for recreation.

A number of plankton species also play a very important role in the transmission of phycotoxins to molluscs and other higher organisms used for human consumption. Several of these species are commonly found in coastal and open sea waters but seem to proliferate in zones with dense aquaculture operations thus creating a real hazard to the consumers and important losses to the operators.

Phytoplankton plays a very central role in the processes controlling eutrophication of the aquatic environment. Phytoplankton species identification, often required for environmental monitoring and assessment, is becoming a task that few specialists around the world are capable of carrying out. The question becomes even more critical when environmental studies are carried out by technicians lacking a proper knowledge of systematics. Yet the need exists for a proper identification, at least to the level of genus, of phytoplanktonic organisms in a large number of environmental studies carried out around the world.

The number of species that may be handled in any individual study is not less than three to five hundred, covering fifty to one hundred different genera. The original descriptions of genus and species are scattered over a large number of books and identification keys not always easy to access and certainly difficult to consult while the sample is under the microscope. Very often, identification of genera and species is done through morphological characters of individuals or groups of individuals under the microscope. The shape and the size, as well as other morphological features give clues to the person carrying out the analysis. Technological advances make taxonomic, biological and environmental information accessible to scientists and technicians alike even those located away from the *taxonomical cathedrals* as someone has called marine stations with centennial libraries.

Digitized video images may be stored in data banks for further use as templates for the identification of other images. The possibility of comparing live high-resolution microscope images with other *frozen* computer stored images, is technically feasible. However, the amount of storage required for a single image in black and white(may be more than 250 Kbytes if the image is kept with some degree of colour or hues of grey) make data handling a crucial step in the above process.

The general goal of the project was the development of a knowledge base to assist marine biologists in the identification of phytoplankton organisms, including those responsible for eutrophication and toxic blooms. The data included in the

knowledge base, mostly morphological information collected as alphanumeric and video graphic files on computer compatible media, were obtained in field studies by means of microscopic observations and from the specialised bibliography. An expert system was to be developed to assist the biologist in retrieving the morphological and other identification data based on identification matrices.

The project concentrated on building up of expertise on marine phytoplankton species and indicators of environmental changes, focusing on the production of a software package containing the taxonomic information available in the literature and in developing an operational system capable of assisting marine biologists in the identification of phytoplankton organisms obtained in field studies through the compilation of information stored in a multimedia database containing both numeric, textual and pictorial information (drawings, photographs, electron microscope images, video, etc.). A prototype expert system was developed for fast identification of phytoplankton organisms and retrieval of the taxonomic information available through morphological and other features. The taxonomic information on the Mediterranean phytoplankton, was checked through its use by various independent experts thus contributing to the consolidation of the knowledge base.

2. MATERIALS AND METHODS

The taxonomic knowledge base developed consists of tables including taxa hierarchically classified according to Class, Order, Suborder, Family, Genus, Species, Varieties and Forms. The synonyms, when existing, were also included in the table. The classification scheme followed was that provided by the Atlas of Phytoplankton edited by A. Sournia (Sournia, 1986; Ricard, 1987; Chrétiennot-Dinet, 1990) and other authors (Round et al., 1990; Chrétiennot-Dinet et al., 1993) with modifications when required following the nomenclature according to the criteria given by the International Code of Botanical Nomenclature (I.C.B.N.) (Greuter, 1988). Ecological and bibliographic information have also been included.

An Inventory of taxa quoted in the Northwestern Mediterranean has been compiled (Velásquez and Cruzado, 1995, 1996) using two different sources of information:

- taxa deriving from microscopic observations (following the Uttermohl technique) carried out in over 1000 water samples obtained in the NW Mediterranean Sea.

- taxa quoted in scientific references published since 1883 (see list of references)

The observations were made in the Gulf of Lions, the Catalan and the Balearic Seas (see Fig. 1) and include those made by the author in the course of various cruises between 1979 and 1992 (see Table 1). Figure 1 shows the extension of the area covered by these studies (Cruzado and Velásquez, 1990; Velásquez and Cruzado, 1990). The bibliographic references consulted cover the work of taxonomists from the NW Mediterranean since Gourret (1883) and include review papers,

especially the exhaustive work carried out by M. Travers (1975) which provided most of the taxa including synonyms, and by Margalef and Estrada (1981), as well as a number of unpublished reports and personal communications. Whenever possible, original sources have been consulted. A number in chronological order (in brackets after each taxon) was assigned to each of the bibliographic references on which this work is based (Table 2).

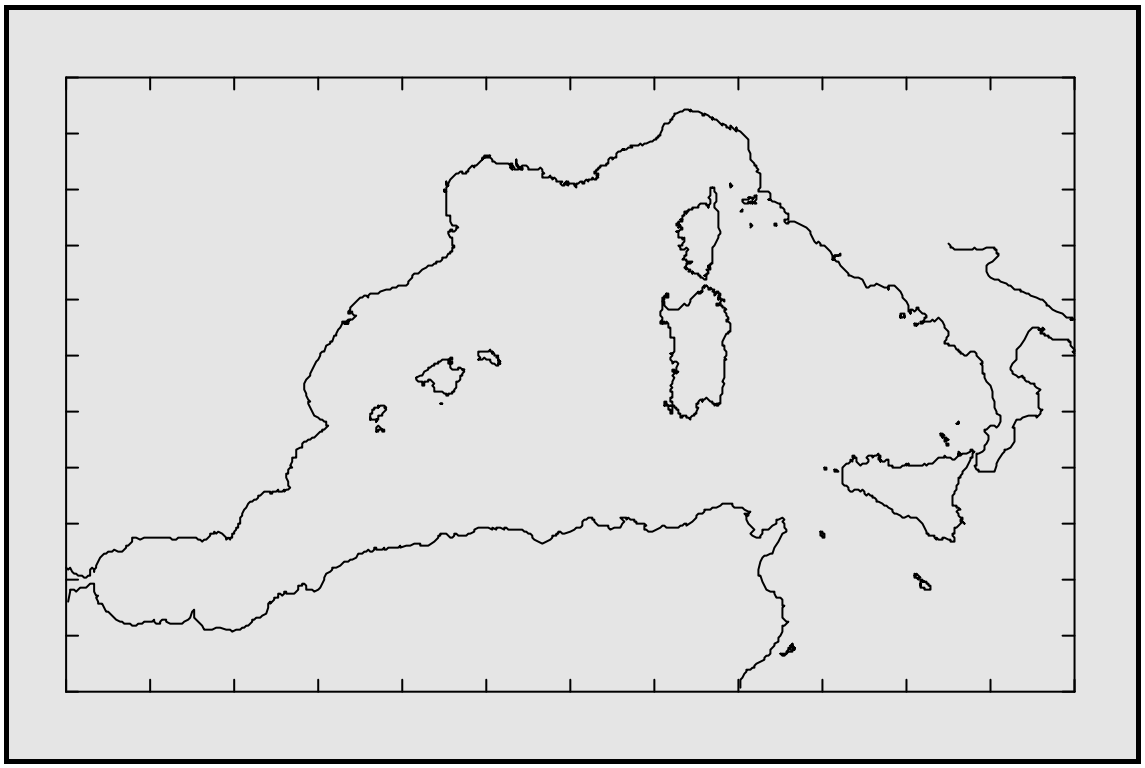


Fig. 1 Area in the western Mediterranean Sea covered by the present study

Table 1

Oceanographic cruises included in the present work

Cruise	Date	Year	Project	Funding
CARON 85	25-28 Feb.	1985	CARON	CAICYT
PELAGOLION III	10-15 Mar.	1988	PICS	CSIC/CNRS
PANACHE I	10-15 Mar.	1988	FRONTAL	CNRS
PANACHE II	6-19 Nov.	1988	FRONTAL	CNRS
DISCOVERY	12-26 Dec.	1988	EROS 2000	CEC
CYBELE	12-19 Apr.	1990	EROS 2000	CEC
TYRO	20 Nov. - 5 Dec.	1991	EROS 2000	CEC

Table 2

Key to the references in the Inventory

0.	Travers, M. (1975)	17.	Jacques, G. (1967, 1969)
1.	Massutí, M. (1930)	18.	Margalef, R. (1969)
2.	Dangeard, P. (1932)	19.	Blanc, F. et al. (1969)
3.	Margalef, R. (1945a)	20.	Margalef, R. (1971)
4.	Navarro, F.P.; Bellón Uriarte, F. (1945)	21.	Blanc, F. et al. (1975)
		22.	Bourgade, B. (1977)
5.	Margalef, R. (1945b)	23.	Estrada, M. (1979)
6.	Margalef, R. (1951)	24.	Estrada, M. (1980)
7.	Morales, E. (1952)	25.	Kim, K.T. (1980)
8.	Balle, P. (1953)	26.	Arfi, R. et al. (1982)
9.	Morales, E. (1956)	27.	Margalef, R.; Estrada, M. (1991)
10.	Margalef, R. (1957)	28.	Delgado, M. (1987)
11.	Margalef, R.; Morales, E. (1960)	29.	Palau, M. et al. (1991)
12.	Herrera, J.; Margalef, R. (1961)	30.	Estrada, M. (1991)
13.	Margalef, R.; Herrera, J. (1963a)	31.	Delgado, M. (Personal Comm.)
14.	Margalef, R.; Herrera, J. (1963b)	32.	Estrada, M. (Personal Comm.)
15.	Margalef, R. (1964)	33.	Margalef, R. (Personal Comm.)
16.	Margalef, R. (1965)	34.	Velásquez, Z.R. (this work)

Many species have been cited by the original authors with names different from those accepted at present. An effort has been made to adapt the original citations to the most recently accepted systematics and, whenever possible, synonyms have been indicated (by an **=** sign). Some of the taxa may not reflect the state of the art with regard to the ever-changing taxonomical and systematic knowledge and some difficulties have been encountered with regard to the systematic classification of some of the genus cited by the various authors.

Most of the taxa included in the inventory refer to organisms observed in the marine planktonic domain. However, genus that may have a benthic habitat and also those that appear in the estuarine areas have been included. An attempt was made, in the case of the Diatoms, to classify the ecological characteristics of the genus. The difficulty to define the term **planktonic** (vs. benthic and/or epiphytic), particularly in the estuarine and littoral areas (ecotones) has been acknowledged by Ricard (1987). In such habitats, the local populations are often formed by allochtonous organisms originating in either continental or oceanic systems and may be due to organisms showing **tychoplanktonic** characters. Accordingly, the habitat(s) has been represented in the scheme by a letter (***P***=Planktonic; ***B***=Benthic; ***E***=Epiphytic; ***T***=Tychoplanktonic). Those genera not showing any of the above letters are considered only Planktonic. It must be noted, however, that individual species, within any given genus, may show one or other behaviour.

A large number of images obtained, mostly by the author, from observations under the inverted optical and the scanning electron microscopes were digitized either with a scanner or with a video camera directly coupled to the inverted microscope. The NTSC signal was sent to a PC (Velásquez et al., 1991) providing a 512x512 B/W image of 256 levels of grey (see Fig. 2). The images were saved on magnetic media and incorporated into the main multimedia database (FOXPRO) together with the taxonomic and morphological characteristics of the species presented in the images.

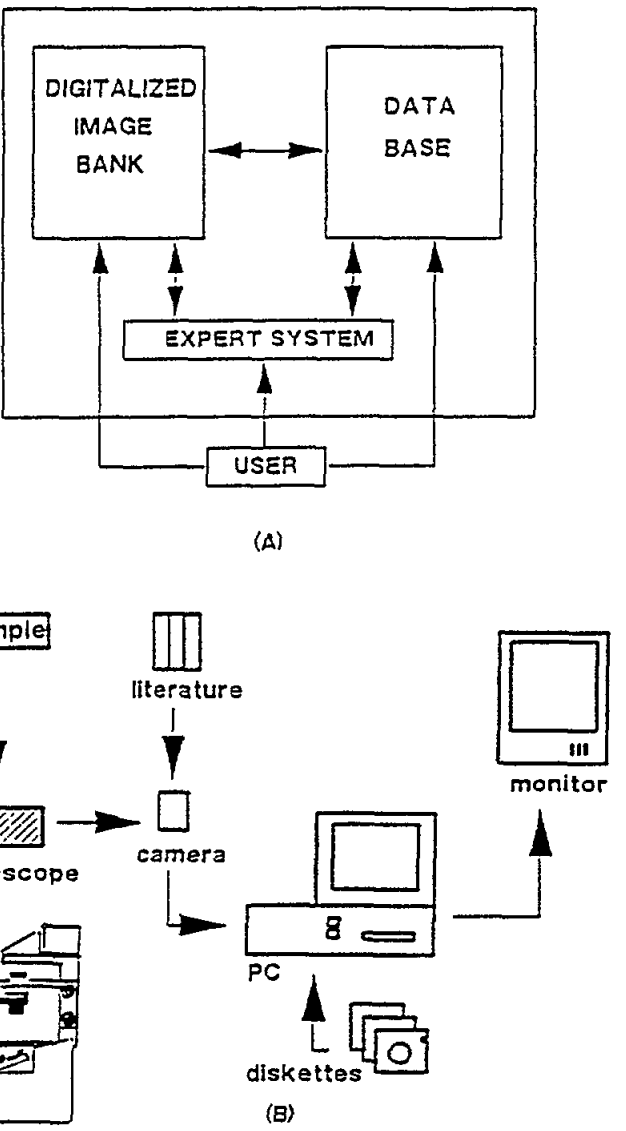


Fig. 2 Schematic diagram of (A) the database, and (B) the image capturing system

A computer database was developed in order to facilitate the organization of many phytoplankton records, including bibliographic references, optical and electron microscope images as well as descriptive and ecological information. The data constitute the knowledge base for an expert system at present at the pilot stage (Velásquez et al., 1991).

The entire system runs on a Quadra MacIntosh computer with an 2 Gbyte external hard disk. Practically no knowledge of the internal structure is required to access all the data with a number of functions accessible through push buttons on the screen (see Figures 3 and 4). Various images may be available for one single species while a number of species may not have any image. The system is completely open to the introduction of new information and is thus fully upgradable.

3. RESULTS AND DISCUSSION

As a result of the activities carried out within the project, the following specific products were developed:

- A classification scheme covering all the Classes, Orders, Suborders, Families and Genus of the marine phytoplankton including the most up-to-date corrections.

- A taxonomic inventory of all marine phytoplankton cited in the Northwestern Mediterranean Sea.

- A prototype expert system geared to the identification of phytoplankton species working on matrices of morphological and other data obtained from the study of each family or group of taxa.

- A running software package (based on MacIntosh) interconnected with a taxonomic and image database and with the expert system, .

The Classification Scheme

A classification scheme was developed for the organization of the taxonomic knowledge base (Annex I). The scheme consists of eighteen classes, each corresponding to one Class, with genus hierarchically classified according to Order, Suborder and Family. The scheme follows the classification suggested by the Atlas of Phytoplankton edited by A. Sournia (Sournia, 1986; Ricard, 1987; Chrétiennot-Dinet, 1990) with modifications (Chrétiennot-Dinet et al., 1993) when required following the nomenclature according to the criteria given by the International Code of Botanical Nomenclature (I.C.B.N.) (Greuter, 1988). Ecological and bibliographic information, when available, have also been included.

Six classes have not been cited in the Northwestern Mediterranean Sea (Inventory). A comparison of the number of Orders, Families and Genus in the 12 Classes that have been cited with those given in the Classification Scheme (Atlas) is shown in Figure 5 and Table 3. Most of the Orders and Families are present in the NW Mediterranean phytoplankton although a number of Genus have never been observed.

About 55 % of the species cited in the Inventory belong to the Class Bacillariophyceae and 39 % to the Class Dinophyceae. Follow in importance the Class Prymnesiophyceae (Haptophyceae) with close to 5 % and two other Classes Dictyochophyceae and Prasinophyceae represent about 1 % of the species cited.

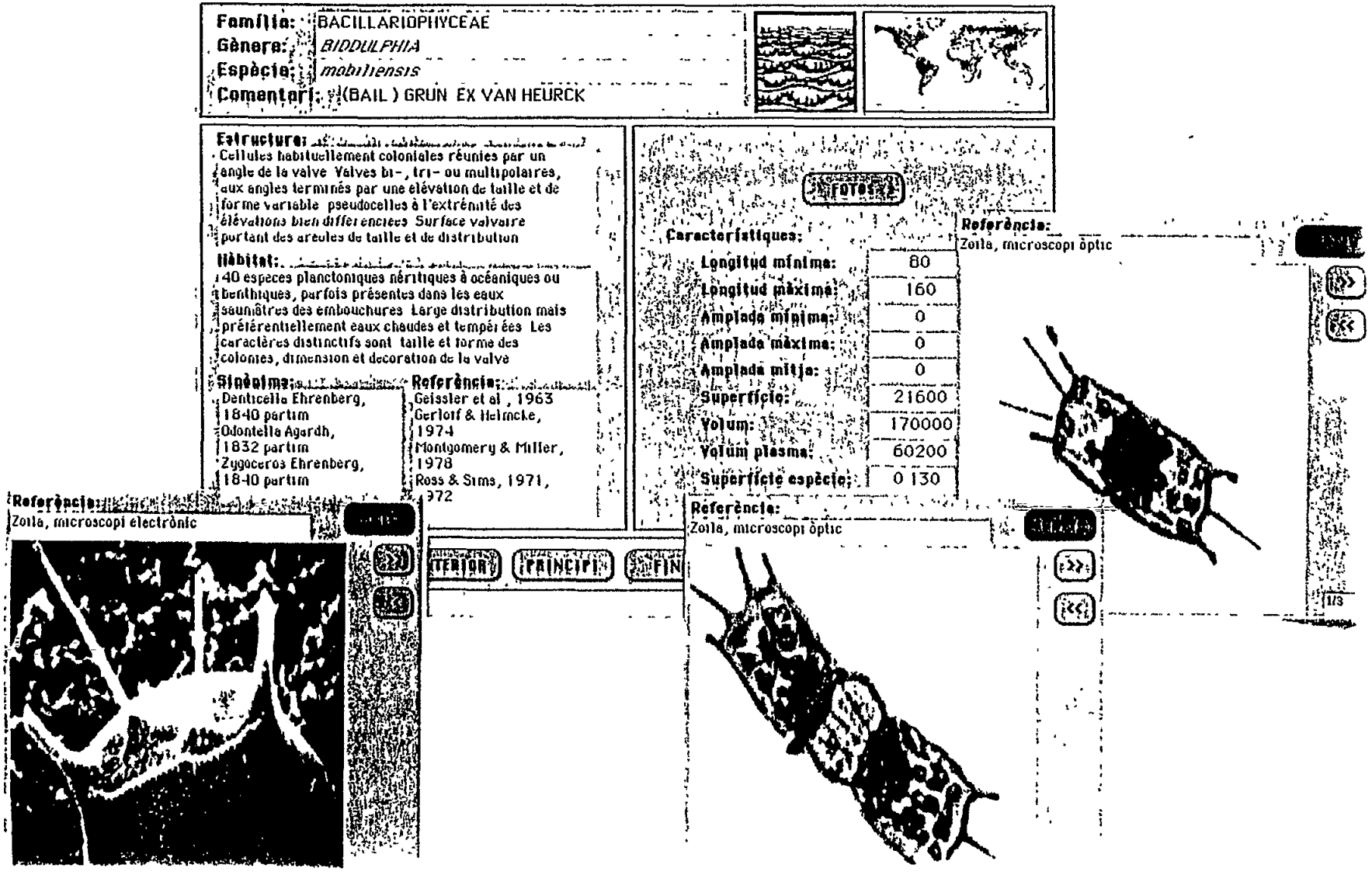


Fig. 3 Layout of the Graphics User Interface for species Biddulphia mobiliensis

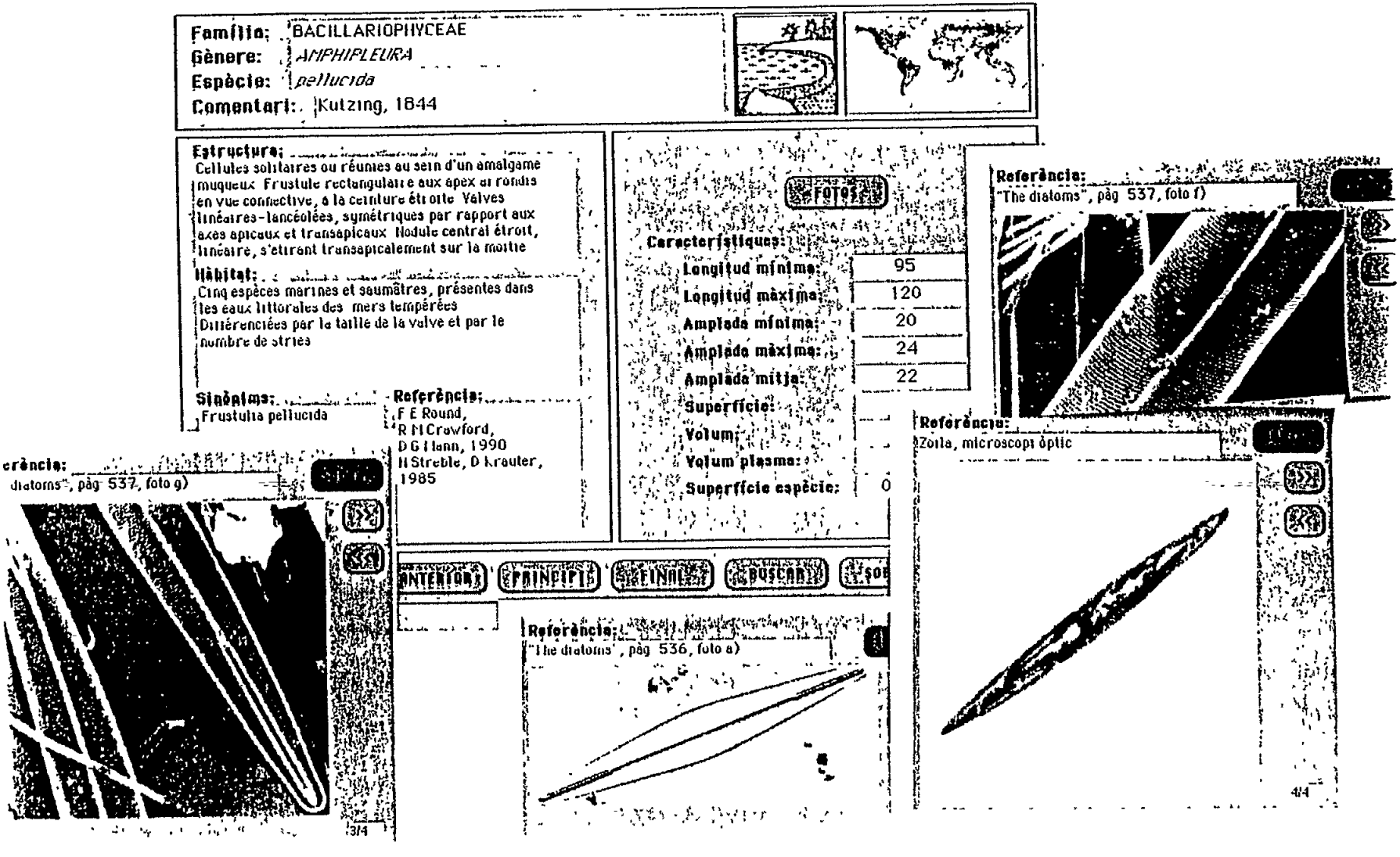
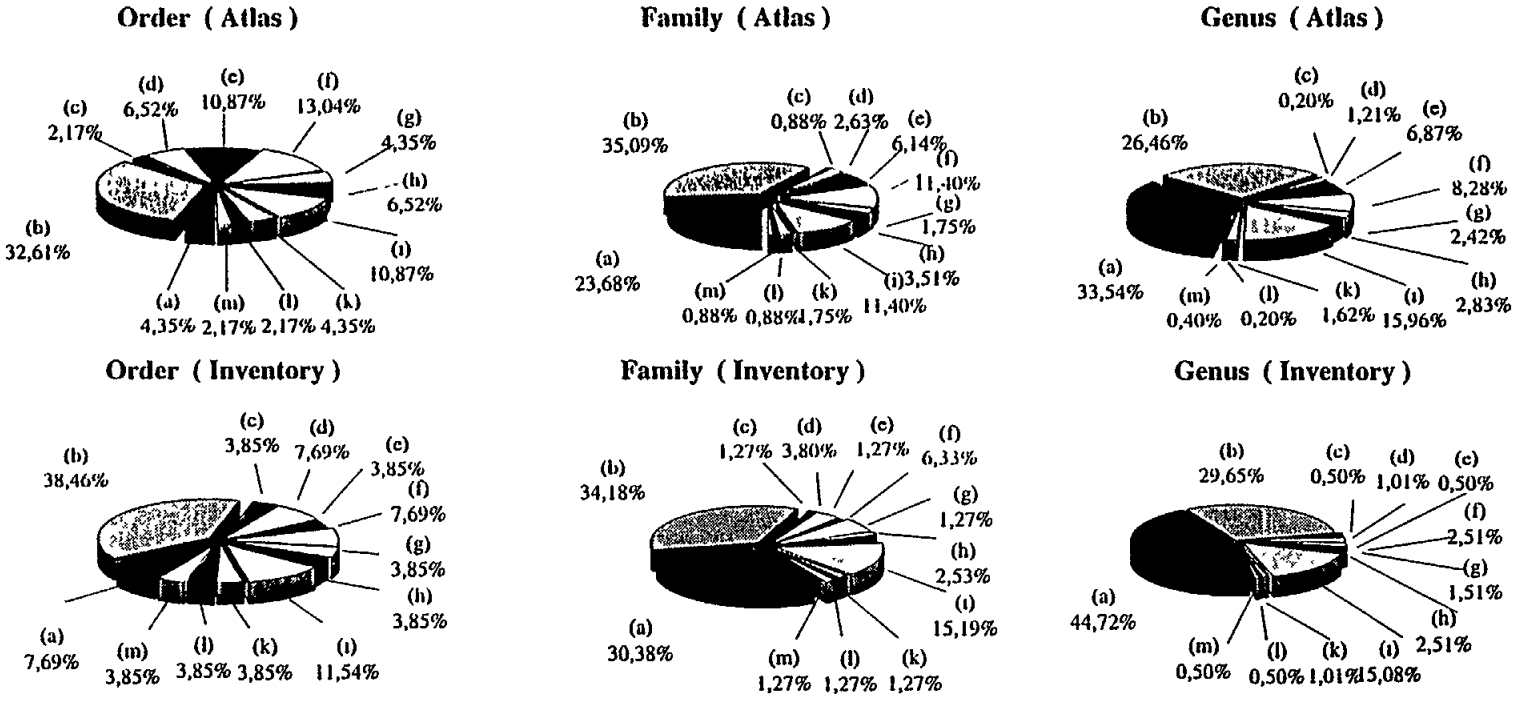


Fig. 4 Layout of the Graphics User Interface for species Amphipleura pellucida



(a) Bacillariophyceae
(b) Dinophyceae
(c) Dictyochophyceae
(d) Cyanophyceae
(e) Chlorophyceae
(f) Chrysophyceae
(g) Chryptophyceae
(h) Prasinophyceae
(i) Prymnesiophyceae (Haptophyceae)
(k) Euglenophyceae
(l) Charophyceae
(m) Micromonadophyceae

Fig. 5 Comparison of the number of taxa included in Sournia's Atlas and those observed in the NW Mediterranean Sea

Table 3

Comparison of the number of taxa included in Sournia's Atlas
and those observed in the NW Mediterranean Sea

Class	Order		Family		Genus		Species	Synonyms	Varieties
	atlas	inventory	atlas	inventory	atlas	inventory			
Bacillariophyceae	2	2	27	24	166	89	729	66	192
Dinophyceae	15	10	40	27	131	59	520	67	89
Dictyochophyceae	1	1	1	1	1	1	16	4	13
Cyanophyceae	3	2	3	3	6	2	2	3	
Chlorophyceae	5	1	7	1	34	1	1	1	8
Chrysophyceae	6	2	13	5	41	5	5		
Chryptophyceae	2	1	2	1	12	3	3	7	
Prasinophyceae	3	1	4	2	14	5	18	21	
Prymnesiophyceae (Haptophyceae)	5	3	13	12	79	30	66	54	
Euglenophyceae	2	1	2	1	8	2	2	1	
Charophyceae	1	1	1	1	1	1	1		
Micromonadophyceae	1	1	1	1	2	1	1	1	

The Inventory

Compilation of bibliographic references on the occurrence of phytoplankton in the NW Mediterranean Sea was carried out for this project consulting various libraries in the Western Mediterranean (Barcelona, Palma de Mallorca, Banyuls-sur-Mer, Villefranche-sur-Mer, etc.). Whenever it was required, the taxonomic information was revised. An inventory of species citations in the above references as well as our own observations was prepared and gave rise to a database containing the species citations in the above references as well as the author's own observations (see Annex II). This activity ended up with two papers (Velásquez and Cruzado, 1995, 1996). The following is a summary of the phytoplankton investigations from which the inventory is a compilation.

Early investigations on the taxonomy of phytoplankton were carried out by Gourret (1883) with his studies on the Peridinean in the Gulf of Marseilles. This author made a historical systematic description of 68 different species of Dinoflagellates. Peragallo and Peragallo (1908) provided, almost at the same time, a list of diatom species found in the Gulf of Lions and, between 1905 and 1937, Pavillard carried out numerous works in the Gulf of Lions (Pavillard, 1909; 1916a,b,c) and off Monaco (Pavillard, 1934; 1936; 1937) identifying 55 species of Peridinians and 50 species of Diatoms. Dangeard (1927) published the systematics of phytoplankton collected during the cruise Sylvana in the NW Mediterranean Sea between February and June of 1913. The same author (Dangeard, 1932) published the systematics of the species observed near Banyuls sur Mer between 1925 and 1932 including 97 species of Peridineans, 53 species of Diatoms and one of Coccoliths. Bernard (1938) published a quantitative study of the annual cycle of nanoplankton off Monaco and Banyuls sur Mer. Lecal (1952) studied the Coccoliths of the Western Mediterranean Sea.

The early works of Navarro and Massutí (1929) and of Massutí (1930, 1944) in the Catalan Sea and in the bay of Palma de Mallorca were reviewed by Navarro and Bellón-Uriarte (1945) who included in their catalog of the Balearic Sea 908 species of planktonic and benthic algae with the exclusion of the Diatoms. On the other hand, Massutí and Margalef (1950) provided an excellent guide for the identification and systematic classification of the Mediterranean phytoplankton species.

Margalef (1951) reviewed the early work carried out by Dangeard and Bernard in the French Catalonia and his own work in Blanes and Castellón. Margalef (1969, 1971) and Margalef and Estrada (1987) also compiled the species found by various authors working along the Catalan coasts in locations such as the bays of Blanes (Margalef, 1945b), Cadaqués (Margalef, 1945a) and Palma de Mallorca (Balle, 1953, 1954, 1959; Navarro and Massutí, 1940), the ports of Barcelona (Morales and Arias, 1965; Margalef and Herrera, 1966) and Mahó (Massutí, 1948) and coastal areas off Castelló (Herrera and Margalef, 1957, 1961; Margalef and Herrera, 1963b, 1964; Margalef, 1969), Barcelona (Margalef and Ballester, 1967; Margalef and Castellví, 1967; Margalef, 1969, 1971; Estrada, 1979, 1980, 1987) and the Ebro delta (López and Arté, 1972). López (1955; 1966) studied the systematics of the genus Ceratium, Blasco (1970a,b) of Chaetoceros didymus and Hemidiscus hardmanianus Grev. and Castellví (1963) of Skeletonema costatum.

On the other hand, Margalef described two new species of Dinoflagellate Scaphodinium mirabile nov. gen. nov. sp. (Margalef, 1963) and Ceratoperidinium yeye nov. gen. nov. sp. (Margalef, 1969).

All the above works are a valuable help for the study of local systematics but do not deal with the ecological interrelationships of the different species. Halim (1960) was perhaps the first to make a qualitative and quantitative study of the ecological cycle of the dinoflagellates in Villefranche sur Mer. Devèze (1959) reported a study carried out in the Gulf of Marseilles during the period 1955-1956 with 58 species of Dinoflagellates, 57 species of Diatoms and 5 belonging to other classes that had been identified by Margalef. Fenaux (1958) published a contribution to the knowledge of Kofoidinium velelloides Pavillard. Cachon and Cachon-Enjumet (1964) described two new species from plankton sampled off Villefranche sur Mer. Nival (1965) published work on the cycle of Dyctiocha fibula. Minas et al. (1968) on the COMEXO laboratory-buoy located in the bay of Villefranche sur Mer, cited 43 systematic units of Diatoms during their study of the microplankton and environmental conditions. Leger (1971) published a paper on the phytoplankton collected from that same buoy, this time anchored offshore, in the Gulf of Lions. Sournia (1972) introduced 4 new Dinoflagellates while Balech (1976) published work done on Protoperidinium of the Gulf of Lions, Jacques and Soyer (1977) contributed to the knowledge of the free Dinoflagellate Pseliodinium vaubanii (Sournia).

Until the beginning of the 1960s, all the work was carried out with samples obtained by towing a fine-meshed net. A. Travers (1962) and M. Travers (1962) simultaneously used the traditional net sampling and the new Utermöhl technique to study the phytoplankton of the Gulf of Marseilles between 1961 and 1962. As a result of their parallel work with both techniques, A. Travers (1962) published a systematic inventory including 203 Diatom and 156 Dinoflagellate species while M. Travers (1962) focused on the quantitative aspects and both authors made an important contribution to the methodology and ecology of the phytoplankton of the Mediterranean Sea. This work was followed by the publication of an inventory of Protists of the Gulf of Marseilles (M. Travers, 1975) constituting perhaps the most exhaustive work ever done on the subject that has been used as the basis for our work.

Since 1975, *in extenso* inventories and checklists of phytoplankton species identified are not included in the scientific literature, the authors referring only to the most frequently observed species or to those offering a special ecological interest. Nevertheless, taxonomic information may be found in various publications (Estrada, 1979; Delgado, 1986; Descy and Willems, 1991) referring to the phytoplankton communities studied along both the Spanish coasts (Catalan and Balearic seas) and the French coasts (Gulf of Lions, Gulf of Marseilles).

A number of early papers have collected and synthesized the knowledge available. Massutí and Margalef (1950), in the book *Introducción al estudio del fitoplancton marino* offered a valuable guide for the identification and systematic classification of some important marine phytoplankton species because of their abundance. Tregouboff and Rose (1957) handbook of planktonology of the western

Mediterranean, based on 27 years of studies in the region, contains 227 plates and 2200 figures. Sournia (1973; 1978; 1982; 1990) compiled a catalog of species and taxa of recent marine dinoflagellates. Rampi and Bernhard (1978) produced an identification key for the Mediterranean pelagic diatoms with 28 genera and 126 species. More recently, Delgado and Fortuño (1990) published an atlas of Mediterranean phytoplankton containing 45 plates with electron micrographs of diatoms. Recently, Margalef (1995) has prepared an account of his observations across the Catalan Sea in the summers of 1993 and 1994. However, no attempt has been made so far to compile a checklist of the phytoplanktonic flora of the northwestern Mediterranean following modern taxonomic criteria. Not all the authors having made contributions to the knowledge of phytoplankton of the NW Mediterranean are referred to here as their works had been included in one or another of these reviews. On the other hand, other authors contributors to the present work are referred to in the inventory.

The Expert System

Knowledge acquisition is defined, in a narrow sense, as the extraction of knowledge from knowledge sources, transformation of them into an internal representation form and maintenance of the knowledge base under a given framework of knowledge representation. Knowledge sources are mainly, but should not be, restricted to human experts. A variety of knowledge about species identification lies scattered in different forms. The integration of knowledge sources (surface knowledge, deep knowledge) which report from human experts and specialised literature is an important task of knowledge engineers. The use of test cases should enable the refinement of the knowledge base and detection of conflicts as well as the verification of its consistency and completeness.

Building of species identification keys for use by expert systems is the critical point in the process of development of the system. Formal representation of the taxonomist's knowledge should be backed by information originated in an integrated data management system to form the knowledge base by a process of inference. To do so, the reasoning process that includes the oriented dialogue used for the booting of the system should be accompanied by a proper administration of data and images to be offered to the user during the development of the system consultations. In the process devoted to the user, the adequate assistance level is incorporated to provide the deepest possible explanatory capacity to the phytoplankton system. This characteristic increases the reliability and facilitates the use of the system favouring the acquisition process and the maintenance of the knowledge, in the domain of the specialist.

One of the main tasks in building expert systems is the identification of experts and definition of a user model. The expert model consists of the identification of the expert domain, of problem solving strategies, the qualitative and quantitative analysis of the expertise, the identification of criteria for system assessment and so on. The user model consists of the identification of usage domain, of leadership of interactions, the necessity of explanation function, the extension of a system by itself and so on. The concept of user initiative in consultation is important. Another important task is the development of a flexible and appealing user interface. A

number of tools are today available for such a purpose being capable of merging on the same screen textual information, numerical data, still and live video images and other graphical information required for the system to be of use to scientists facing species identification.

Most of the utilities concerning the identification procedure involve the use of the image collection (more than 500 images from more than 200 species at present being available) as an aid to the microscope observer. The live video image displayed on a color video monitor may be, at any time (one key stroke) swapped with images from the collection, searching through them according to shapes, genus or simply species menus. The live image may also be frozen in one part of the monitor screen. The images are displayed on the remaining of the screen. The images are usually overlaid with morphological characteristics displayed as text and graphics assisting in the process of taxonomic identification of the organism observed on the microscope.

However, for those cases in which visual observation is insufficient for species identification, the system begins operation requesting information related to the morphological characters required for identification of the taxa. The characteristics on which the knowledge base is founded (see Tables 3 and 4 as examples) are selected with the assistance of the data base system. For example, of the 12 characteristics initially defined for the genus Coscinodiscus, only six were retained because of the degree of redundancy that existed among them. Between one and six questions allow a complete resolution of the 16 taxa contained in the genus Coscinodiscus. Similarly for the genus Prorocentrum.

Each question the Expert System asks the user is accompanied by a HELP menu and a video image informing the user of the various options and on their appearance in images from the collection obtained with the microscope video unit. Some more difficult cases may require the user to go to the scanning electron microscope. However, the very small features often used for identification purposes have so far been avoided. Further development may require a second step to be included in the identification procedure with the features only visible under the SEM.

The easy access to all the plankton and environmental data at once and the possibility for combining searches and correlations to be established between the various variables allows quick and easy production of custom-made files containing such combinations of variables as may be required for multivariate analysis. A small collection of well sorted out statistical and other techniques may be easily adapted for the work within the structure of the data base system accompanying the Expert System.

Several attempts have been made to transfer taxonomic knowledge to computer compatible format and prototype Expert Systems have been developed to assist the biologist in the identification process. However, the work proposed here will not only cover a wider range of marine organisms but will also include environmental data and video images of organisms as observed under the microscope. Such a system should also represent a breakthrough by the fact that all the information will be available to remote users via electronic mail.

Table 4

Major taxonomic features used for identification of species in genus Coscinodiscus

Species	Arealo rows	Central area	Shape	Concave	Thin areoles	Chromatophores
C. centralis	radial	rosette	convex	no	no	numerous small plates small plates
C. concinnus	radial	rosette	convex	no	yes	numerous small, rounded, in all citoplasm
C. concinnus	radial	free area	convex	no	yes	numerous, small, rounded, in all citoplasm
C. curvatulus	sectors	no special structure	flat	no	no	
C. excentricus	curved tangential	no special structure	flat	no	no	small numerous, flat
C. granii	radial	rosette	flat	yes	yes	oval or rounded, in all citoplasm
C. gigas	radial	free area	flat	yes	no	
C. lineatus	straight tangential	no special structure	flat	no	no	numerous plates
C. marginatus	irregular mesh	no special structure	flat	no	no	numerous, small, rounded
C. nodulifer	radial	papillae	flat	no	no	
C. nitidus	free areolae	no special structure	flat	no	no	
C. oculus-iridis	radial	rosette	flat	yes	no	numerous, rounded
C. perforatus	radial	rosette	flat	no	no	numerous, rounded central and marginal region
C. radiatus	radial	no special structure	flat	no	no	cocciform central and marginal region
C. stellaris	radial	star-like	convex	no	yes	numerous, small, rounded
C. thorii	radial	rosette	convex	no	yes	in central and marginal region
C. wailessi	radial	free area	flat	yes	yes	
C. perforatus	radial	free area	flat	no	no	numerous, rounded central and marginal region

The linkage between images and data base techniques for the specific purpose stated is a novel feature that no other presently available system has. Adding identification aids for the numerous variations of some of the more frequent species as text and graphic attributes is also a value added factor of the product. These new developments should greatly facilitate the identification by non specialists of the most common phytoplankton species.

Systematisation of attributes (morphological measurements and other features such as chloroplasts, flagella, etc.) into major groups leading to an adequate level of detail for univocal identification at least to the level of genus. Construction of identification matrices for the genus Coscinodiscus and Prorocentrum in order to facilitate optimizing the number of morphological characters used for identification (Tables 4 and 5). Translation of the matrix into rules for the Expert System. Testing of the knowledge base. A paper by Velásquez et al. (1991) was the result of this activity.

The Data Base

A software package has been developed to assist the user in the identification of species and in the systematisation of data in the databank. The package was geared to work in parallel with the direct video images from the microscope and the image and data bank (Figure 2). A graphic user interface was developed for a MacIntosh Quadra (Figures 2 and 3) computer including the taxonomic knowledge base and the phytoplankton/oceanographic databank. The data base includes a list of all known taxa at the level of genus and contains fields referring to the bibliographic references from which the systematic classification was derived, to the morphological descriptions (when available) and the ecological preferences (habitat, etc.).

The data base also includes entries at the highest level of specification (species, variety, form) which derive from direct observation in Mediterranean samples or cited in the bibliography. These entries contain fields with the published reference or the identification of the sample in which the taxon was observed. In the latter case, a link is established to the oceanographic data corresponding to the sample. Such data include basic oceanographic parameters and cell counts for the various taxa observed.

Whenever available, digital images of the taxon have been included in the database which are displayed in a separate window. An important feature is that most of the images were taken directly from the microscope when studying the field samples and show some of the uncertainties inherent to the method, far from the *perfect* images often shown in books and identification keys. A number of scanning electron microscope images have also been included.

The system was tested during the various phases in the development by exposing it to different users not always skilled phytoplanktologists.

Table 5

Major taxonomic features used for identification of species in genus Prorocentrum

Species	Form	Theca	Depression	Teeth	Spines	Spinules	Length		Width	
							min	max	min	max
P. micans	lanceolate	yes	assymetric	0	yes	yes	40	60	30	50
P. gracile	lanceolate	yes	assymetric	0	yes	no	50	64	18	23
P. compressum	ovate	yes	symetric	2	no	no	30	50	15	25
P. balticum	round	yes	symetric	2	no	yes	16	19	0	0
P. minimum	round	yes	symetric	0	yes	yes	14	18	14	18
P. lima	ovate	yes	symetric	0	no	no	30	60	20	28
P. triestinum	lanceolate	yes	assymetric	0	yes	no	18	22	6	11
P. rostratum	lanceolate	no	assymetric	1	no	yes	18	30	6	12
P. aporum	ovate	no	symetric	0	yes	no	30	32	21	26
P. cassubicum	ovate	yes	assymetric	0	no	no	0	0	22	25
P. nanum	round	yes	symetric	2	no	no	8	104	6	12
P. scutellum	heart-shape	yes	assymetric	0	yes	no	35	57	30	45
P. dentatum	lanceolate	no	assymetric	1	no	yes	15	30	7	12
P. mexicanum	ovate	yes	symetric	1	no	no	30	38	20	25
P. ovum	ovate	yes	symetric	0	no	no	14	14	10	10
P. cordatum	ovate	yes	symetric	0	no	yes	6	24	5	20
P. maximum	heart-shape	yes	symetric	0	yes	yes	25	50	20	38
P. vaginulum	lanceolate	yes	assymetric	2	yes	no	20	40	6	15
P. arcuatum	lanceolate	yes	assymetric	1	yes	no	50	70	30	30
P. rotundatum	round	no	symetric	2	no	no	72	92	0	0
P. magnum	round	yes	symetric	0	no	no	72	92	0	0
P. dactylus	lanceolate	no	symetric	0	no	no	65	70	16	16

4. CONCLUSIONS

The checklist used for building the data base is the result of more than one hundred years of work of a considerable number of phytoplankton taxonomists and ecologists working in the NW Mediterranean Sea. Some of these scientists have made a very substantial contribution to the Taxonomy and Ecology of phytoplankton. A number of species were first identified in samples from this region and their original description is still being used by marine biologists around the world.

The greater precision introduced in the taxonomy of diatoms by the electron microscopy observations challenges the original identification thus making some taxa to appear and other disappear. The analysis of the references gives support to such a statement although a purely statistical, superficial assessment, could give birth to the idea of species extinction and appearance. In this regard it may be concluded that:

- The taxonomic identification of phytoplankton in the Northwestern Mediterranean has been mostly done before 1967.

- Very few species have been added since the consolidated checklist published by Travers for the Gulf of Marseilles. The most recent additions are mainly unpublished work.

- The classification keys used by the various authors are far from being homogeneous particularly since the electron microscope techniques were introduced in the taxonomical work.

- As a consequence, a large number of synonyms have appeared when many genus and species names in use since the early days were changed, producing certain degree of confusion to the non-specialized biologist.

- The knowledge base of which the present checklist is part should allow a wider acceptance of the morphological criteria used for identification purposes.

The system described should make easily available not only the most widely accepted morphological criteria for identification of microplankton taxa but the actual images as observed by any operator under the inverted optical microscope in circumstances that are far from ideal. Thus, with the assistance of the system, any mediumly trained operator should be in condition of identifying most of the phytoplankton taxa visible under the inverted microscope.

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