MEDITERRANEAN ACTION PLAN

MED POL

UNITED NATIONS ENVIRONMENT PROGRAMME

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
(GENERAL FISHERIES COUNCIL FOR THE MEDITERRANEAN)

RESEARCH ON THE EFFECTS OF POLLUTANTS ON MARINE ORGANISMS AND THEIR POPULATIONS (MED POL IV)

RECHERCHE SUR LES EFFETS DES POLLUANTS SUR LES ORGANISMES MARINS ET LEURS PEUPLEMENTS (MED POL IV)

FINAL REPORTS OF PRINCIPAL INVESTIGATORS
RAPPORTS FINAUX DES CHERCHEURS PRINCIPAUX

MAP Technical Reports Series No. 4

UNEP

Athens, 1986

For bibliographic purposes this volume may be cited as:

UNEP/FAO: Research on the Effects of Pollutants on Marine Organisms and their Populations (MED POL IV). MAP Technical Reports Series No. 4. UNEP, Athens 1986.

Pour des fins bibliographiques, citer le présent volume comme suit:

PNUE/FAO: Recherche sur les effets des polluants sur les organismes marins et leurs peuplements (MED POL IV). MAP Technical Reports Series No. 4. UNEP, Athens 1986.

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This volume is the fourth issue of the Mediterranean Action Plan Technical Reports Series.

This Series will collect and disseminate selected scientific reports obtained through the implementation of the various MAP components: Pollution Monitoring and Research Programme (MED POL), Blue Plan, Priority Actions Programme, Specially Protected Areas and Regional Oil Combating Centre.

Ce volume constitue le quatrième numéro de la série des Rapports techniques du Plan d'action pour la Méditerranée.

Cette série permettra de rassembler et de diffuser certains des rapports scientifiques établis dans le cadre de la mise en oeuvre des diverses composantes du PAM: 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 de lutte contre la pollution par les hydrocarbures.

INTRODUCTION

The United Nations Environment Programme (UNEP), in co-operation with the relevant specialized United Nations Agencies (FAO, WHO, WMO, IOC), presented to the Intergovernmental Meeting of Mediterranean countries (Barcelona, 1975) a proposal for a Co-ordinated Mediterranean Pollution Monitoring and Research Programme (MED POL).

MED POL was approved and UNEP was requested to implement the Programme, consisting of seven pilot projects, in close collaboration with the relevant specialized United Nations Agencies.

Its pilot phase (MED POL-Phase I) was designed as the precursor of a long-term programme for pollution monitoring and research in the Mediterranean (MED POL-Phase II) to be carried out according to the provisions of the legal component of the Mediterranean Action Plan.

The pilot projects approved at the 1975 Barcelona Meeting as parts of MED POL-Phase I were:

MED POL I: Baseline Studies and Monitoring of Oil and Petroleum Hydrocarbons in Marine Waters

MED POL II: Baseline Studies and Monitoring of Metals, particularly Mercury and Cadmium, in Marine Organisms

MED POL III: Baseline Studies and Monitoring of DDT, PCBs and Other Chlorinated Hydrocarbons in Marine Organisms

MED POL IV: Research on the Effects of Pollutants on Marine Organisms and their Populations

MED POL V: Research on the Effects of Pollutants on Marine Communities and Ecosystems

MED POL VI: Problems of Coastal Transport of Pollutants

MED POL VII: Coastal Water Quality Control

Subsequent to the 1975 Barcelona Meeting, several other projects were added or considered as collaterals to MED POL to broaden the scope of the programme and to provide the necessary support to it. They were:

MED POL VIII: Biogeochemical Studies of Selected Pollutants in the Open Waters of the Mediterranean

MED POL IX: Role of Sedimentation in the Pollution of the Mediterranean Sea

MED POL X: Pollutants from Land-Based Sources in the Mediterranean

MED POL XI: Intercalibration of Analytical Techniques and Common Maintenance Services

MED POL XII: Input of Pollutants into the Mediterranean Sea through the Atmosphere

MED POL XIII: Modelling of Marine Systems

Participants in the pilot projects were national research centres designated by the States participating in the Mediterranean Action Plan.

The co-ordination of the MED POL-Phase I (1975-1981) was carried out by UNEP as a part of the Mediterranean Action Plan (MAP).

The following United Nations Co-operating Agencies were responsible for the technical implementation of various pilot projects :

- The Food and Agriculture Organization of the United Nations (FAO) through the General Fisheries Council for the Mediterranean (GFCM) (MED POL II, III, IV and V),
- The United Nations Educational, Scientific and Cultural Organization (UNESCO) (MED POL IX and XIII),
- The World Health Organization (WHO) (MED POL VII and X),
- The World Meteorological Organization (WMO) (MED POL XII),
- The International Atomic Energy Agency (IAEA) (MED POL VIII and XI) and
- The Intergovernmental Oceanographic Commission (IOC) of UNESCO (MED POL I and VI)

This volume of the MAP Technical Reports Series is the collection of final reports of the Principal investigators who participated in the pilot project : "Research on the Effects of Pollutants on Marine Organisms and their Populations (MED POL IV)".

INTRODUCTION

Le Programme des Nations Unies pour l'environnement (PNUE), en coopération avec les organismes spécialisés compétents des Nations Unies (FAO, OMS, OMM, COI), a présenté à la Réunion intergouvernementale des pays méditerranéens (Barcelone, 1975), une proposition de Programme coordonné de surveillance continue et de recherche en matière de pollution dans la Méditerranée (MED POL).

Le MED POL a été approuvé, et il a été demandé au PNUE de mettre en oeuvre le programme qui se compose de sept projets pilotes, en étroite collaboration avec les organismes spécialisés compétents des Nations Unies.

Sa phase pilote (MED POL - Phase I) a été conçue comme le prélude d'un programme à long terme de surveillance continue et de recherche en matière de pollution dans la Méditerranée (MED POL - Phase II) à mettre en oeuvre conformément aux dispositions de l'élément juridique du Plan d'action pour la Méditerranée.

Les projets pilotes approuvés à la Réunion intergouvernementale de Barcelone, en 1975, dans le cadre de la Phase I du MED POL, comprenaient:

MED POL I: Etudes de base et surveillance continue du pétrole et des hydrocarbures contenus dans les eaux de la mer

MED POL II: Etudes de base et surveillance continue des métaux, notamment du mercure et du cadmium, dans les organismes marins

MED POL III: Etudes de base et surveillance continue du DDT, des PCB et des autres hydrocarbures chlorés contenus dans les organismes marins

MED POL IV: Recherche sur les effets des polluants sur les organismes marins et leurs peuplements

MED POL V: Recherche sur les effets des polluants sur les communautés et écosystèmes marins

MED POL VI: Problèmes du transfert des polluants le long des côtes

MED POL VII: Contrôle de la qualité des eaux côtières

A la suite de la Réunion de Barcelone de 1975, plusieurs autres projets ont été adjoints ou considérés comme subsidiaires au MED POL en vue d'étendre la portée du programme et de lui assurer l'appui indispensable. Ce sont:

MED POL VIII: Etudes biogéochimiques de certains polluants au large de la Méditerranée

MED POL IX: Rôle de la sédimentation dans la pollution de la mer Méditerranée

MED POL X: Polluants d'origine tellurique dans la Méditerranée

MED POL XI: Inter-étalonnage des techniques d'analyse et services communs d'entretien

MED POL XII: Polluants d'origine tellurique dans la Méditerranée

MED POL XIII: Modélisation des systèmes marins

Les participants aux projets pilotes étaient des centres nationaux de recherche désignés par les Etats prenant part au Plan d'action pour la Méditerranée.

La coordination de MED POL - Phase I (1975-1981) a été assumée par le PNUE dans le cadre du Plan d'action pour la Méditerranée.

Les organismes coopérants des Nations Unies qui étaient chargés de l'exécution technique des divers projets pilotes sont les suivants:

- Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO) par l'entremise du Conseil général des pêches pour la Méditerranée (CGPM) (MED POL II, III, IV et V).

- Organisation des Nations Unies pour l'éducation, la science et la culture (UNESCO) (MED POL IX et XIII).

- Organisation mondiale de la santé (OMS) (MED POL VII et X).

- Organisation météorologique mondiale (OMM) (MED POL XII).

- Agence internationale de l'énergie atomique (AIEA) (MED POL VIII et XI), et

- Commission océanographique intergouvernementale (COI) de l'UNESCO (MED POL I et VI).

Ce volume de la série des Rapports techniques du PAM rassemble les rapports finaux des chercheurs responsables qui ont participé au projet pilote intitulé: "Recherche sur les effets des polluants sur les organismes marins et leurs peuplements (MED POL IV)".

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No final report was submitted by the Centre.

Research Centre: Institute of Oceanography and Fisheries
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Principal Investigator: H.H. SALEH

The Principal Investigator submitted a copy of a scientific paper entitled: Effect of the polluted water pumped by Tabia pumping station on Tilapia zillii Gerv. (Not published).

Centre de Recherche : Station marine d'Endoume et
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MARSEILLE
France

Chercheur principal : G. BELLAN

Période couverte par le rapport : janvier 1977 - mars 1980

INTRODUCTION

Les recherches effectuées à la Station marine d'Endoume et pouvant être rattachées à MED IV, ont sérieusement débuté en 1969. Elles sont basées pour l'essentiel, sur l'action de détergents issus de la pétroléochimie sur divers invertébrés marins. D'autres recherches, réalisées dans d'autres équipes de la Station marine d'Endoume, ont essentiellement pour but d'apprécier l'impact de l'élévation de la température et ne seront pas prises en compte dans le présent rapport. Les recherches que nous avons conduites avec les détergents sont à relier à celles, plus systématiquement orientées sur le côté "pratique", conduites par l'Institut scientifique et technique des Pêches Maritimes (ISTPM).

CONSIDERATIONS METHODOLOGIQUES

Sélection des espèces :

Les espèces suivantes ont été choisies :

Polychètes : Capitella capitata, Scolelepis fuliginosa, Nereis caudata, Nereis succinea, Nereis diversicolor.

Mollusques : Mytilus galloprovincialis, Cardium glaucum, Venerupis aureus (Tapes aureus), Abra ovata.

Crustacés : Idothea balthica basteri, Sphaeroma serratum, Jassa falcata, Hyale spp., Echinogammarus stocki.

Echinodermes : Paracentrotus lividus.

Quelques-unes seulement seront prises en considération dans ce bref rapport. On a choisi ces espèces en fonction de leur intérêt écologique (rôle qu'elles jouent au sein des Communautés et Ecosystèmes). Ce choix date, pour l'essentiel, du début des années 1970 et n'a pu être révisé ultérieurement. Parmi les espèces obligatoires, il n'a été retenu que Mytilus galloprovincialis encore que peu de recherches aient été réalisées, dans le cadre de MED POL, avec cette espèce.

Polluants utilisés :

70 détergents cationiques, anioniques et non ioniques appartenant aux familles chimiques les plus utilisées ont été utilisés, mais un seul métal lourd : le chlorure mercurique ($HgCl_2$). On a choisi d'étudier les détergents compte tenu de leur importance dans le milieu marin : on les retrouve toujours au voisinage des concentrations urbaines littorales, au débouché des fleuves, ils peuvent être présents très loin en mer (jusqu'à des dizaines de kilomètres de la côte) et sont susceptibles d'être stockés dans des sédiments (complexe

gonflant des argiles). Leur action néfaste sur la faune et la flore marine est patente.

METHODOLOGIE

Les animaux sont, en général, récoltés dans le milieu naturel (il y a eu quelques élevages au Laboratoire de Polychètes) et mis à stabuler pendant une semaine. En règle générale, les bioessais ont été de type "statiques". Plus récemment un système "flux continu" a été mis au point et est devenu pleinement opérationnel.

Des données ont été acquises en utilisant des détergents marqués au tritium. Cette technique permet de suivre le transfert du polluant depuis le milieu d'expérience jusqu'aux organes où il est stocké. On peut apprécier ce transfert non seulement sur le plan qualitatif (localisation du détergent) mais aussi sur le plan quantitatif (quelle proportion est fixée). Il est prématuré d'en tenir compte dans ce rapport.

RESULTATS

Mytilus galloprovincialis

Métaux lourds : Chlorure de mercure mercurique ($HgCl_2$).

Des stress combinés avec les variations de température ont montré qu'il y avait accroissement de la sensibilité de l'espèce avec l'augmentation simultanée de la température (synergie). Au contraire, des tests combinés avec la diminution de la salinité (passant de 30°/•• à 25°/••) montrent qu'il y a diminution de la toxicité (antagonisme) du $HgCl_2$ lorsque la salinité diminue. Toutes autres conditions expérimentales étant identiques, on note une augmentation de la sensibilité de M. galloprovincialis au $HgCl_2$ lorsque les expériences ont lieu en milieu statique (avec renouvellement périodique du milieu) par comparaison à ce qu'on observe lors d'expériences en milieu ouvert (flux continu).

Autres polluants : Divers détergents ont apporté des données tout à fait analogues.

Autres organismes marins : Venerupis (Tapes) aureus.

Métaux lourds : Chlorure de mercure mercurique $HgCl_2$.

Des stress combinés avec les variations de température ont montré qu'il y avait synergie du polluant avec l'augmentation de la température. Au contraire, qu'il y a antagonisme lorsque l'on combine l'action du $HgCl_2$ avec la diminution de la salinité (jusqu'à 25°/••). Toutes autres conditions expérimentales étant identiques, les mortalités chez V. aureus sont identiques, que l'on utilise un "flux continu" ou que les expérimentations soient conduites en "statique" avec renouvellement périodique du milieu expérimental.

Détergents : CL 50-96 h (mg/l) de détergents à différentes températures

		V. aureus	M. galloprovincialis
Syntopon C	125°C	5.41	5.70
	17°C	3.39	4.75
(nonyl phénol)	23°C	2.50	2.50
Plurafac RA43	13°C	1.3	
	17°C	1.	
(alcol à chaîne droite oxyéthylénée)	23°C	0.35	

Action de baisses fortes de salinité :

Il n'y a pas de mortalité (au bout de 15 jours d'expérience) pour des salinités supérieures ou égales à 25°/•• (sans introduction de polluants). Il y a un brusque accroissement de la mortalité pour des salinités plus basses (salinité léthale : 20°/••).

Taux de Mortalité

S°/••	Durée	4 jours	6 jours	10 jours	15 jours
21°/••		35%	40%	45%	60%
20°/••		45%	70%	100%	

Un écart de salinité de 1°/•• au niveau des salinités léthales ou sub-léthales accroît fortement la mortalité.

Scolelepis fuliginosa

Détergents :

L'essentiel des recherches a porté sur les actions de synergie ou d'antagonismes de divers détergents en fonction de variations de la salinité. En règle générale, la toxicité des détergents est accentuée (synergie) pour des salinités nettement inférieures à la normale (18 à 22°/••), ou nettement supérieures à la normale (44 à 50°/••). Elle est, au contraire, atténuée (antagonisme) pour des salinités plus proches des normales (25°/••, 30°/••, 42°/••). Si l'on veut entrer davantage dans le détail, et en fonction de la toxicité propre des détergents, on constate :

- que pour des salinités non léthales (25, 30 et 42°/••), il y a atténuation de la toxicité des détergents moyennement toxiques lauryl phosphate, oxyéthyléné acide, lauryl sulfate oxyéthyléné neutralisé à la soude, sulfate d'alcool laurique neutralisé à l'ammoniaque, mélange d'anioniques Syntaryl A 990;

- que ces mêmes salinités accentuent la toxicité de détergents fortement toxiques (alkyl aryl oxyéthyléné, alkyl phénol oxyéthyléné) ou faiblement toxiques (ester phosphorique d'alcool linéaire, acide oléique condensé sur 14 molécules d'oxyde d'éthylène). La concentration du détergent dans le milieu influe aussi. Par exemple,

le lauryl phosphate oxyéthyléné acide, provoque pour une salinité de 20°/••, un antagonisme à faibles et fortes concentrations et une synergie à moyenne concentration.

Capitella capitata.

Détergents :

Les résultats sont similaires à ceux obtenus avec S. fuliginosa.

Echinogammarus stocki

Des stress cumulés ou successifs ont été créés en combinant plusieurs facteurs altéragènes fréquemment rencontrés dans le milieu naturel et plus particulièrement en milieu estuarien. Ainsi, l'espèce étudiée a été soumise à des variations de salinité et/ou de température, simultanées ou successives et cela, en présence de chlorure mercurique ou à diverses concentrations de détergents (non ionique alkyl aryl oxyéthyléné-Syntopon C). Les expériences ont été réalisées en circuit ouvert avec renouvellement constant de la solution. L'évolution de sensibilité des espèces a été étudiée en fonction des variations de temps léthaux obtenus (TL10, TL50, TL90).

Métaux lourds : Chlorure de mercure mercurique ($HgCl_2$).

La sensibilité d'E. stocki, mis en présence de $HgCl_2$ est variable suivant les temps de stabulation précédant l'expérience. Cette variabilité peut aller dans le sens d'une augmentation de la sensibilité en fonction du temps de stabulation ou d'une diminution selon les concentrations employées. Pour une même concentration de $HgCl_2$ et pour des conditions similaires de stabulation, les réponses fournies par divers lots de E. stocki sont identiques. La variabilité des réponses obtenues en fonction du temps ou des conditions particulières de stabulation peut donc être attribué à une homogénéité de la population.

Détergents :

Les niveaux de synergie température/détergents varient en fonction de la température beaucoup plus qu'en fonction de la toxicité du détergent.

Idothea baltica basteri

Détergents :

A court terme, la toxicité croissante des détergents peut s'établir ainsi : anioniques, non ioniques, cationiques. Parmi les détergents anioniques, les moins toxiques sont les alkyl aryl sulfonates, les plus toxiques les phosphates et les sulfates d'alcool laurique. Pour les détergents non ioniques ce sont les alcools à chaîne droite et les alkyl aryl polyoxy-éthylénés qui sont les plus toxiques, alors que les condensats, esters, amines acides, se révèlent être les moins toxiques. A long terme, l'exposition à des détergents non ioniques a des conséquences multiples chez Idothea et affecte l'organisme à tous les niveaux.

Il apparaît, tout d'abord que les concentrations moins toxiques ne sont pas

nécessairement les plus basses. L'activité du produit passe par un minimum qui varie selon le produit. Un nombre élevé de cas de mortalité se situe au moment de la mue.

Des phénomènes de synergie et d'antagonisme entre l'action du détergent et une augmentation de température peuvent agir sur le taux de mortalité. Chez les jeunes, la mortalité ne suit pas une loi normale. Le rendement de la nourriture consommée semble meilleur chez les animaux exposés que chez les témoins.

Le taux d'hydratation des animaux semble constant malgré les variations de concentration. On observe des allongements de la période intermue.

En fonction du sexe et du paramètre considéré (température, produit, température + produit), par rapport aux témoins, la taille des individus exposés peut, soit ne présenter aucune variation significative, soit présenter des variations significatives dans le sens d'une augmentation ou d'une diminution de taille, le taux de croissance se trouvant modifié.

La reproduction est la fonction la plus perturbée par l'exposition aux détergents. Si l'animal est prépubère, il y aurait un retard de maturation important, alors que s'il est adulte, il pourrait y avoir une adaptation en fonction de la concentration. Chez les mâles, il peut y avoir un blocage de la division cellulaire. On aurait des individus stériles. Pour les femelles, la fécondité est variable suivant les conditions d'élevage. On observe une baisse de la fécondité due soit à un blocage de la ponte, soit à un avortement de la femelle. Les jeunes issus d'animaux exposés présentent dans une proportion non négligeable des individus malformés. Ces affectations tératogènes concernent les antennes, les pattes, le pléotelson, les valves. Elles sont par ailleurs observées à la suite de la mue chez des juvéniles normaux exposés à des concentrations plus ou moins fortes de détergent. Au niveau histologique, en dehors des gonades mâles où l'on observe un blocage de la division cellulaire dans les premiers stades de la prophase, on peut noter au niveau du tube digestif la présence de cellules hypertrophiées et de tumeurs. La glande de mue, en fonction de la durée d'exposition, peut être soit hypertrophiée, soit hypotrophiée. Ceci est confirmé par l'histoautoradiographie. Au niveau biochimique, il a été possible d'acquérir des connaissances sur les protéines de l'hémolymphe d'Idothea.

Sous l'influence du détergent, on observe des variations du taux des différentes fractions d'hémocyanine et en fonction de la concentration une modification dans les proportions relatives des différentes fractions pouvant résulter d'un blocage ou d'une dissociation d'une des fractions.

Pour les femelles, d'autres phénomènes peuvent se produire dont une résorption plus poussée des protéines de l'ovocyte. Des comptages opérés à l'aide d'un produit marqué montrent que le détergent se retrouve dans tous les organes. Le tube digestif et les caecums peuvent éliminer le produit d'une manière plus ou moins importante, ce qui n'est pas le cas de la gonade et du système nerveux. Il y a une importante élimination au moment de la mue. Cependant, il reste un reliquat qui augmente d'une mue sur l'autre.

Pour les hypothèses évoquées pour tenter d'interpréter les altérations diverses se manifestant à long terme, il apparaît que de nombreux mécanismes affectés ont en commun des problèmes de synthèse de protéines (glande de mue,

gonade). Au total, l'ensemble conjugué de toutes ces atteintes, qui constituent des facteurs "directs" agissant sur l'espèce, aurait pour effet de faire baisser le taux de renouvellement de l'espèce et pose le problème de son maintien.

Paracentrotus lividus

Métal lourd : Chlorure de mercure mercurique.

Les résultats de trois séries d'expériences ont permis d'établir les valeurs minimales et maximales des pourcentages de réussite.

F= fécondation, D= 1° division cellulaire, G= gastrulation, E= echinopluteus.

Concentration mg/l	F	D	G	E
0	99	99	96	95
0,01	99	98	77,5	70
0,03	99	97,5	70	44
0,05	99	97	66	4
0,10	98	91	30	0
0,20	95	83	0	0
0,30	62,5	70	0	0
0,40	60	51	0	0
0,60	30	20	0	0
0,80	20	0	0	0
1	0	0	0	0

Les valeurs des concentrations efficaces 50% sont comprises dans les gammes de concentration du tableau ci-dessous.

Stade	F	D	G	E
% de réussite	60-30	51-20	66-30	70-44
Concentration	0.40-0.60	0.40-0.60	0.05-0.10	0.01-0.03
CE 50 observées	0.46	0.40	0.07	0.03

DISCUSSION DES RESULTATS

Le but de MED POL IV est de mesurer avec précision, pour l'ensemble de la Méditerranée les actions d'un certain nombre de polluants sur un petit nombre d'espèces (à caractère commercial assez accusé) et sur leurs populations. On pourrait en déduire les capacités de résistance de ces espèces à des niveaux

donnés (et reconnus par ailleurs) de polluants. Les recherches de l'Equipe de la Station marine d'Endoume travaillant dans le cadre de MED POL IV, avaient été définies d'emblée et dès 1969, comme devant tendre à expliquer le comportement de telle ou telle espèce caractéristique de biocoenose ou de niveau trophique vis à vis d'un stress quelconque, auquel serait soumis un milieu naturel quelconque. Il s'agissait de mettre en évidence (du moins de le tenter) les mécanismes qui conduisent aux diverses modalités de déséquilibre au sein de Communautés ou d'Ecosystèmes. Aussi ont été privilégiées les études à long terme, notamment sur le cycle de développement des espèces, les études de synergies ou d'antagonismes de polluants entre eux ou avec des facteurs du milieu susceptibles d'être modifiés "artificiellement" dans le milieu naturel, et plus généralement, les notions de stress et de sensibilité des espèces vis à vis de ces stress ou les unes par rapport aux autres. Cette voie d'approche ne peut être, évidemment, envisagée qu'en liaison étroite avec des études sur le milieu, par exemple avec des études du type de celles conduites dans le cadre de MED POL V.

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Période couverte par le rapport : Mars 1977 - mars 1981

INTRODUCTION

L'Institut Benaki a eu son premier contact avec le problème de la toxicité des pesticides sur les organismes marins à l'occasion du projet pilote MED POL IV. Jusqu'alors (1977), le Laboratoire de Toxicologie des Pesticides s'occupait essentiellement de la toxicité des pesticides sur les animaux à sang chaud.

Etant donné que la pêche occupe, en Grèce, une activité économique très importante et que des pesticides sont utilisés dans l'agriculture en quantités énormes, les recherches sur la toxicité des pesticides envers les organismes marins devraient aboutir à :

a) éliminer les pesticides très toxiques pour les organismes marins des programmes pour la protection des cultures élaborées par le Ministère de l'Agriculture;

b) éliminer aussi les pesticides qui se décomposent difficilement ou s'accumulent (sont adsorbés) dans le corps de certaines espèces, comme c'est le cas de certains herbicides qui sont utilisés dans les rizières, constituant ainsi un danger pour la mer.

CONSIDERATIONS METHODOLOGIQUES

Sélection des espèces : Furent sélectionnées les espèces suivantes :

Mugil sp., Maena smaris, Sparus auratus, Dicentrarchus labrax, Murex trunculus, Murex brandaris, Pagurus sp., Palaemon sp. Ces organismes sélectionnés vivent dans des eaux missalées (Mugil sp., Palaemon sp.) ou près des côtes (Murex sp., Pagurus sp). Les deux premières espèces se trouvent en abondance dans les embouchures des canaux de drainage de terres agricoles.

Polluants analysés :

Paraquate (Herbicide), Diméthoate (Insecticide), Chlorpyriphos (Insecticide), Fenthion (Insecticide), Molinate (Herbicide), Propanil (Herbicide), Mecoprop (MCPP) (Herbicide).

METHODOLOGIE

Pour les tests de toxicité : milieu statique, avec changement d'eau et redosage toutes les 8 heures pour les organophosphorés (insecticides) et toutes les 24 heures pour les herbicides. Inhibition de la cholinestérase : méthode électrométrique, basée sur la mesure de l'acidité, provoquée par l'hydrolyse de l'acétylcholine a été utilisée ainsi que l'inhibition de l'enzyme par les esters organophosphorés, mesurée après une incubation du

substrat (acétylcholine) avec l'enzyme (cerveau) à 37°C pendant deux heures. Accumulation des pesticides dans le corps des organismes.

Paraquate : méthode de A. Calderbanc and S.H. Yuen : Analyst, 90, 99 (1965)
Esters organophosphorés : méthode de W.E. Dale, and J.W. Miles : J. Aoac, 59 (1), 165 (1976).

RESULTATS

Mugil cephalus (et divers poissons Maena smaris, Sparus auratus et Dicentrarchus labrax).

Paraquate :

a) Toxicité aiguë - Pas de mortalité chez Mugil cephalus en 3 mois, à 0.01; 0.05 et 0.1 ppm. A 1 ppm la TL50 est de 16 jours et à 10 ppm de 1 h.

b) Etude histologique - Les poissons, exposés aux concentrations fortes, morts dans les 24 heures, présentaient les signes d'une inflammation intense. Leur peau était gonflée, muqueuse, avec des hématomes, les branchies furent endommagées et couvertes de mucus, le foie et les reins gravement atteints par des hémorragies et des nécroses, et le tube digestif distendu, plein de gaz et présentant des ulcères hémorragiques multiples. Ces symptômes moins graves mais plus différenciés furent aussi observés chez les poissons après une exposition de 15 jours à 1 ppm. Ainsi furent observées une dégénérescence hydropique prononcée du foie, stase biliaire et sanguine, dégénérescence hyaline et nécrose tubulaire des reins, et, érosions mucosales du tube digestif. Pourtant, les organes les plus gravement atteints furent les branchies : amincissement du filament branchial, destruction des parois épithéliales des lamelles secondaires et distension de l'extrémité des filaments branchiaux, accompagnée d'une fibrose de la région basale distendue; ces derniers symptômes furent aussi observés chez le crustacé et le gastéropode.

c) Accumulation du paraquate - Le paraquate détecté dans le corps du poisson fut trouvé en grandes quantités dans l'appareil digestif et la peau; dans les muscles les quantités étaient faibles. Le Tableau I ci-dessous montre les concentrations du paraquate dans différents tissus de Mugil sp. après une exposition de 15 jours à 1 ppm.

Tableau I

Organe	Concentration* en µg/g (Moyenne de 5 individus)
Muscles	0.192 ± 0.067
Ovaires	0.230 ± 0.141
Peau	4.742 ± 1.784
Système digestif	6.083 ± 1.872

* Furent analysés des tissus individuels.

Organophosphorés (dimethoate et chlorpyriphos) :

a) Inhibition de la cholinestérase du cerveau - Le seuil de concentration de diméthoate efficace est 1 ppm pour une exposition continue de 24 heures; bien qu'il existe une relation nette entre l'inhibition et la hauteur de la concentration, la durée de l'exposition jouerait un rôle important. Le chlorpyriphos étant beaucoup plus toxique pour les poissons que le diméthoate, les concentrations utilisées étaient de l'ordre de ppb; ainsi la concentration-seuil est celle de 5 ppb et le temps d'exposition le plus convenable est celui de 24 heures. On constate aussi que l'inhibition commence très tôt, deux heures d'exposition étant suffisantes surtout pour les concentrations élevées; son action est plus rapide que celle de diméthoate (Tableaux II et III).

Relation entre l'inhibition de la cholinestérase (ChE) du cerveau et les quantités du diméthoate accumulées chez Mugil sp : Cinétique de deux phénomènes.

Les poissons furent exposés au diméthoate à 5 ppm pendant 24 heures. Au bout de ce temps l'activité cholinestérasique et la concentration du toxique furent mesurées dans différents tissus. Ensuite, les poissons furent transportés dans de l'eau de mer propre et les mesures de l'activité enzymatique et de la concentration furent répétées 2, 4, 6, 8, 24 et 48 heures après la fin de l'exposition au toxique; pour le calcul de l'activité cholinestérasique les mesures continuèrent jusqu'à 720 heures.

Comme on peut constater sur les tableaux II et III, l'inhibition cholinestérasique du cerveau et les concentrations du diméthoate dans les tissus augmentent par rapport au temps de l'exposition.

A l'aide du tableau IV on constate aussi que la concentration dans les différents tissus décroit rapidement après leur transport dans l'eau propre; la détoxification commence juste deux heures après, et, au bout de 24 heures, les quantités détectées étaient au niveau du seuil de la précision de l'appareil (chromatographe); ce qui fait penser à l'élimination et la détoxification presque complète du toxique. Au contraire (Tableau V) l'inhibition de la ChE persiste longtemps; 24 heures après le transport des poissons dans l'eau propre, elle diminue seulement de 8,1%, au bout de 96 heures elle est encore au niveau de 50% et sa récupération n'est complète qu'après 720 heures.

CONCLUSIONS

Pour détecter la pollution de la mer par les produits organophosphorés, l'inhibition de la cholinestérase, outre sa valeur incontestée en tant que critère de toxicité, apparaît plus tôt que les premiers symptômes d'intoxication et peut servir comme méthode plus précise et plus simple que l'analyse chimique. A l'encontre de l'analyse chimique qui est basée sur la détection des quantités d'un toxique rapidement dégradé et éliminé d'un organisme, la mesure de l'activité de la ChE a l'avantage d'être basée sur un effet qui persiste assez longtemps après l'élimination du toxique.

Tableau II

Quantité de Diméthoate dans différents tissus de Mugil sp. par rapport au temps écoulé après une exposition de 24 heures à 5 ppm (en p.p.m. du poids frais de tissu)

Tissu	Heures d'exposition		
	4	24	48
Muscles	0,77 ± 0,08	1,54 ± 0,38	1,48 ± 0,45
Tube digestif	0,71 ± 0,07	2,32 ± 0,45	---
Branchies	0,63 ± 0,07	1,62 ± 0,33	2,72 ± 0,70
Peau	1,04 ± 0,18	1,77 ± 0,61	7,61 ± 1.04

Tableau III

Inhibition de la cholinestérase du cerveau de Mugil sp. après une exposition au Diméthoate à 5 ppm et à temps différents

Temps d'exposition (en heures)			
2	4	24	48
8,2 ± 1,4	20,8 ± 1,8	87,1 ± 3,6	88,8 ± 4,3

Tableau IV

Concentrations du diméthoate dans différents tissus de Mugil sp. exposé en 5 ppm pendant 24 heures et ensuite transporté dans un milieu non intoxiqué

Tissu	Quantités de diméthoate détectées (en p.p.m. du poids frais du tissu)					
	A la fin de l'exposition (24h)	Pendant la détoxification				
		2h	4h	6h	8h	24h
Muscles	1,76 ± 0,45	--	0,93 ± 0,12	0,75 ± 0,10	0,73 ± 0,15	0,12 ± 0,03
Tubes dig.	2,22 ± 0,22	1,49 ± 0,49	0,93 ± 0,30	0,57 ± 0,06	0,58 ± 0,37	0,14 ± 0,06
Branchies	2,00 ± 0,35	1,40 ± 0,62	0,68 ± 0,12	0,40 ± 0,04	0,28 ± 0,04	0,05 ± 0,05
Peau	1,73 ± 0,09	0,84 ± 0,07	1,07 ± 0,36	0,76 ± 0,13	0,32 ± 0,12	0,08 ± 0,06

Tableau V

Inhibition (en %) de la cholinestérase du cerveau de Mugil sp. esposé en 5 ppm de diméthoate pendant 24 heures et ensuite transporté dans un milieu non toxique

Durée de la détoxification	Inhibition de la cholinestérase
2	84,9 ±5,3
4	84,1 ±4,0
8	80,5 ±4,1
24	78,0 ±6,8
48	71,6 ±10,4
72	66,3 ±2,1
96	50,7 ±10,1
168	48,3 ±1,8
336	27,4 ±4,9
720	0

Herbicides (Molinate, Propanyl) :

Tableau VI

Toxicité de deux herbicides sur Mugil (mortalité en pourcentage) par rapport au temps d'exposition

Herbicide et concentration	Nombre d'animaux	Temps d'exposition (en heures)							
		1	6	24	30	32	46	48	94
Molinate									
1 ppm	6	0	0	0	33.3	0	0	66.6	100.0
3 ppm	6	0	100.0						
5 ppm	6	0	100.0						
Propanyl									
5 ppm	5	0	0	0	0	20.0	20.0	20.0	80.0
10 ppm	5	0	0	60.0	80.0	100.0			
20 ppm	5	50.0	100.0						

Mollusques (Murex trunculus, Murex brandaris)

Paraquate :

a) Toxicité aiguë - Pas de mortalité aprés 1 mois à 0.01, 0.05 et 0.1 ppm. A 1 ppm, la TL50 est de 18 jours, à 10 ppm de 24 heures.

b) Accumulation chez Murex et Pagurus est présentée ci-dessous:

Tableau VII

Concentration du paraquate dans le corps de M. brandaris et Pagurus sp. après une exposition de 3 jours à differentes concentrations

Concentrations (ppm)	Quantités de paraquate en µg/g	
	M. brandaris*	Pagurus sp.*
10.0	2.82 ± 0.83	---
5.0	2.24 ± 1.01	14.63 ± 3.72
2.5	---	9.21 ± 2.45
1.0	1.46 ± 0.47	3.16 ± 0.89

* Chaque échantillon comprenait 20 individus.

Crustacés (Pagurus sp., Palaemon sp.).

Paraquate :

a) Mortalité aiguë - Pas de mortalité au bout de 1 mois à 0.01, 0.05 et 0.1 ppm. TL50 est de 10 jours à 1 ppm et 36 à 10 ppm.

b) Accumulation chez Pagurus sp. (Tableau VII). Ce crustacé accumule 3 à 6 fois plus de paraquate que Mugil et Murex.

Herbicides (Molinate, Propanyl, Mecoprop) :

Palaemon sp. est moins sensible que Mugil cephalus. Le propanyl est le plus toxique, le Mecoprop, le moins. Le tableau VIII ci-après montre la toxicité de trois herbicides sur Palaemon (mortalité en pourcentage) par rapport au temps d'exposition.

Tableau VIII

Herbicide et concentration en ppm.	Temps d'exposition (en heures)										Nbre. d'animaux
	24	48	72	96	120	168	192	216	240	264	
Molinate											
5	1.7	3.4	6.9	10.3	19.0	53.4	53.6	67.2	69.0	74.1	58
10	7.0	12.3	26.3	72.0	87.7	100.0					57
20	18.0	58.0	100.0								50
Propanyl											
1	0	0	0	0	0	19.0	26.2	28.6	33.3	38.1	42
4	0	3.4	32.2	50.8	72.8	100.0					59
8	7.7	25.0	57.7	78.8	86.5	100.0					52
Mecoprop											
20	0	2.4									42
50	4.5	9.1	9.1	11.4	11.4	56.8	56.8	59.1	65.9	65.9	44
100	25.0	32.7	50.0	88.5	100.0						52

DISCUSSION DES RESULTATS

Les expériences effectuées du 1/3/77 au 1/3/80 appartiennent à deux groupes bien distincts. Le premier comprend les expériences relatives aux problèmes posés par l'utilisation des herbicides à grande échelle et la pollution des côtes à proximité des embouchures de drainage des champs de culture qui en résulte; le second comprend des expériences sur les dangers résultant de la pollution de la mer par les insecticides organophosphorés après un accident.

Les expériences du premier groupe ont démontré que les herbicides possèdent une action toxique non négligeable sur tous les organismes examinés, vivant dans les eaux dessalées ou près des côtes, et par conséquent, près des embouchures de canaux de drainage. Mais le vrai danger n'est pas l'action directe des herbicides, manifestée par la mort des organismes marins, il dérive de l'accumulation biologique. Les crustacés accumulent de grandes quantités d'herbicide; or ces animaux constituent une partie du zooplancton très importante et jouent un rôle prépondérant dans la chaîne trophique.

Le second groupe comprend des expériences relatives à la détection de la pollution de la mer par les esters organophosphorés à la suite d'un accident (p.e. naufrage d'un bateau chargé de quelques tonnes de chlorpyriphos dans le Golfe de Navarin en 1978). La méthode mise au point présente un double avantage : d'une part, elle met en évidence le fait que l'effet sur l'activité cholinestérasique persiste plus longtemps que les quantités du toxique détectables par l'analyse chimique, et d'autre part, l'utilisation du cerveau de certains poissons est une source de cholinestérase permettant la détection de la pollution à des niveaux très bas.

L'intérêt que présentent ces deux problèmes nous incite à continuer nos expériences dans les objectifs que nous venons de citer.

PUBLICATIONS

L'inhibition de la cholinestérase du cerveau de différentes espèces de poisson, comme critère de la pollution marine par les insecticides organophosphorés. Meded. Rijksuniv. Landbouwwetensch. Gent 45/4, 923-927 (1980).

Toxicity of paraquat to three marine organisms. Bull. Envirnm. Contam. Toxic 25, 283-288 (1980).

Etude comparative de la toxicité du diméthoate sur Mugil sp. et de son accumulation dans ses tissus: Bul. Inst. Phytopath. Benaki: 13 (2): 188 - 194.

Toxicité comparée de trois herbicides, utilisés aux cultures de riz, sur deux espèces marines vivant dans les eaux mi-salines. (En préparation).

Action of temperature on dimethoate's toxicity to marine organisms with emphasis on the inhibition of AcheE. (En préparation).

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Period of Reporting: 1977 - 1981

INTRODUCTION

The Laboratory has performed different kinds of research on systematic plankton ecology and has been interested in the study of plankton communities, particularly in polluted zones in the Saronicos gulf.

The basic idea of this study is the toxic effects of some heavy metals on the representatives of two distinct populations of the copepod Acartia clausi.

METHODOLOGICAL CONSIDERATIONS

Acartia clausi

Acartia clausi is a calanoid copepod common in the Mediterranean. Frequent but not abundant in the Aegean Sea, it increases in numbers in the Saronicos gulf (gulf of Athens), representing 50% of the total numbers of copepods (Moraïtou-Apostolopoulou, 1974). This species also has a very clear regional distribution. Inside the heavily polluted Elefsis Bay (at the north of the Saronicos gulf) it forms very dense populations, being almost the exclusive component of the very rich zooplanktonic community during the winter.

To test the toxicity of various metals we used specimens belonging to two populations: one in the heavily polluted Elefsis Bay and another living in a relatively non-polluted area situated about 25 km S.E. of Elefsis Bay (in the case of chromium we used specimens only from the polluted area). Recently a series of experiments was performed with the copepod Tisbe holothuriae. The elaboration of the results obtained is still proceeding.

The metals used in the toxicity test were: Cu, in the form of $CuSO_4 . 5H_2O$, Cd, in the form of $CdCl_2 . 2H_2O$, Cr, in the form of Na_2CrO_4 and CrO_3 (hexavalent chromium) and $Cr(NO_3)_3 . 9H_2O$ (trivalent chromium).

METHODOLOGY

Acute toxicity:

The LC_{50} 48 h values (concentration of a toxicant lethal to 50% of the test animals after 48 hours of exposure) was determined by static bioassays.

Acartia was collected by horizontal planktonic hauls (WP2 plankton sampler). The plankton samples were transported immediately to the laboratory, and Acartia was sorted under a binocular microscope. All experiments were performed in rooms of constant temperature. The sea-water used had previously been filtered and autoclaved. The test solutions were prepared by diluting stock solutions of metal salts in order to obtain the final concentrations of metal ions. For every concentration and for each of the two

populations, 20 mature and apparently healthy females were put individually into glass jars containing 50 ml of solution. The jars were covered with aluminium foil sheets. Survivors and dead animals were counted every 24 hours and the dead animals removed. An animal was thought to be dead when it failed to respond to mechanical stimulation.

Sub-lethal effects:

Capture of animals, preparation of medium etc., were as described for acute toxicity testing.

In these series of experiments we exposed the animals to low (sub-lethal) concentrations of the same toxicants, in order to find the results of long-term exposure to low doses on the various physiological processes (longevity, egg production, respiration, feeding). In the longevity and fecundity experiments, for each concentration of the toxicant and for each one of the two populations, 20 mature females were placed individually in 50 ml of solution in glass containers. No food was added in these series of experiments. The containers were checked daily for survivors and the eggs produced were counted under a binocular microscope. The toxic solution was changed on alternate days.

The feeding experiments were run in 500 ml Erlenmeyer flasks filled with 400 ml of sea-water and containing 10 mature females of Acartia. Five flasks were used for each concentration of toxicant and for each one of the two regions. Four species of phytoplankton grown in monospecific cultures were used as food. Air was introduced permanently to the container as a trickle of bubbles to avoid sedimentation of phytoplanktonic cells. In order to obtain the same food concentration in all jars, the four species of phytoplankton were mixed thoroughly and the same volume of the mixture was added in all jars. The quantity of food consumed by the copepods (ingestion rate) was calculated from the difference between the concentration of phytoplankton cells after the addition of food and 24 hours later, corrected by a factor given by the control mortality. The density of phytoplankton cells was estimated by counting on haemotocymetric counting cells (type Malassez). Oxygen consumption was measured by polarography using a pH meter (Radiometer type) equipped with a gas monitor giving partial pressures of dissolved oxygen directly. The electrode (type Clark) had a platinum cathode. The principle of this method is described by Kanvischer (1959). The animals were placed individually in 2 ml syringes filled with 1,5 ml oxygen-saturated water. The syringes were hermetically sealed and kept in rooms of constant temperature.

For acute toxicity, the treatment of the results was carried out according to Bliss's modified mathematical method. For sub-lethal effects, all results were tested statistically by the paired t-test.

RESULTS

Acartia clausi (2 populations)

Acute toxicity:

Copper proved the most and chromium the least toxic metal, while cadmium presented an intermediate toxicity.

In all cases the elevation of temperature resulted in an abrupt increase of sensibility to the metals tested (Cd,Cr).

The LC_{50} 48 h of chromium were also found to vary with the form of chromium compound and the annual generation to which the specimens belonged. A significant difference in the tolerance of all metals between the two populations of Acartia (the one living in the heavily polluted area and the other living in the relatively non-polluted area) is that in all cases the Acartia living in the polluted area proved more tolerant. The LC_{50} 48 h of the metals are given in the following table.

Table I

Form of the metal	Area polluted	Area non-polluted	T °C	mg/l LC_{50} 48 h
$CuSO_4 \cdot 5H_2O$	+			0.82 ± 0.0026
"		+		0.03 ± 0.0044
$CdCl_2 \cdot 2H_2O$	+		14	1.50 ± 0.038
"		+	14	1.20 ± 0.028
"	+		22	0.74 ± 0.023
"		+	22	0.60 ± 0.043
Na_2CrO_4	+		14	16.99 ± 2.38)Autumn
"	+		18	11.47 ± 3.87)generation
"	+		22	8.83 ± 2.10)
"	+		14	16.37 ± 0.18 Winter generation
"	+		14	12.26 ± 2.62 Summer generation
CrO_3	+		14	19.27 ± 2.8 Winter generation

Trivalent chromium in the form of $Cr(NO_3)_3 \cdot 9H_2O$, precipitated to the bottom proved to be non-toxic for Acartia.

Sub-lethal effects:

Copper: In the range of copper concentrations 0.001 to 0.01 mg/l, all tested activities of the animals coming from the clean region seem affected. Feeding activity, longevity and fecundity showed a progressive reduction with the increase of copper doses. On the other hand oxygen consumption rates showed a continous increase in the same range of concentrations.

The pollution-adapted population of Acartia seems more resistant to sub-lethal copper stress. Although longevity and respiration were affected in all concentrations used (the reaction was of the same type as for the animals of the polluted area), ingestion rate was not affected at 0.001 mg/l, decreasing at higher concentrations. The fecundity of the pollution-adapted population is higher than that of the clean area, and shows a slight increase at 0.001 and 0.0025 mg/l, dropping approximately to the fecundity level of the control animals at 0.01 mg/l

Table II

Mean ingestion rate, egg production, oxygen consumption per animal of Acartia clausi in different Cu concentrations

Concentration of Cu (mg $l^{-1}H_2O$)	Ingestion rate (cells $24h^{-1}$)		Egg production (in 3 days)		Oxygen consumption ($\mu l.O_2 20h^{-1}$)	
	Polluted area	Clean area	Polluted area	Clean area	Polluted area	Clean area
0	25600	25550	5.25	3.12	0.010	0.006
0.001	24950	14440	6.0	1.0	0.018	0.009
0.0025	-	-	7.06	-	-	-
0.005	12290	3065	-	0.28	0.022	0.019
0.01	-	-	5.69	0	0.030	0.024

Cadmium: All tested cadmium concentrations reduce the longevity of Acartia in comparison with the mortality of the controls. Generally the mean longevity declines with increasing Cd concentrations.

The oxygen consumption of the two populations of Acartia after exposure to cadmium differs markedly: in the pollution-adapted population a continuous increase of O_2 consumption was observed with increasing concentrations of cadmium in the range of Cd concentrations tested. The oxygen consumption of the animals of the non-polluted area seems to be unaffected between 0.2 and 0.6 mg. On the contrary, at the 0.8 mg/l concentration, an abrupt increase in respiratory rates was observed.

Table III

Oxygen consumption (µl O_2/cop./24 h) of the two populations of Acartia after exposure to cadmium

Conc. of Cd (mg/l)	polluted	non-polluted
0	0.032 ± 0.016	0.025 ± 0.017
0.2	0.037 ± 0.005	0.025 ± 0.0058
0.4	-	0.026 ± 0.014
0.6	0.038 ± 0.012	0.025 ± 0.026
0.8	0.043 ± 0.012	0.044 ± 0.015
1.0	0.059 ± 0.913	

Chromium: The exposure of Acartia to sub-lethal concentrations of cadium resulted in a reduction of its longevity proportional to the chronium concentrations used. Furthermore, when Acartia was exposed to low Cr concentration it demonstrated a decrease in feeding capacity and increase in respiratory rates proportional to the concentration of metal.

Table IV. Ingestion and respiratory rates of Acartia exposed to sub-lethal Cr (in the form of Na_2CrO_4) concentrations.

Cr^{6+} concentration mg/l	Ingestion rates cells/cop./24 h 14 ± 0.5°	22 ± 0.5°C	Respiratory rates mlO_2/copepod/24 h
0	11.392	10.162	0.96
1		5,715	0.99
2	8,218		1.10
3		4,646	
4	5,960		1.22
6	5,500	3,625	1.23
8			1.43

During 1981 two research projects were realized:

a) Impact of chromium on the populations dynamics of Tisbe holothuriae

The impact of different chromium concentrations (0-control-; 0,5; 1; and 2mg/lt) on the populations dynamics of the harpacticoid copepod Tisbe holothuriae has been studied. The studied parameters were: the longevity of the F_2 (parent) and F_3 (offspring) generation, the number of egg sacs produced by F_2 females, the interval between the formation of two successives sacs, the percentage of egg sac absortion and the numbers of F_3 offsprings.

All tested chromium concentrations affect the longevity of both the F_2 and F_3 generation, the latter being much more sensitive. No inhibition of the ability of egg sac formation has been noticed. On the contrary the development of egg sacs was strongly infuenced by chromium and an increased percentage of abortion was observed in direct relationship with chromium concentrations. The number of F_3 offspring decreased with increasing chromium concentrations.

b) Differentiation of the sensitivity to copper and cadmium in different life stages of the harpacticoid copepod Tisbe holothuriae, Humes

In this research we have studied the acute toxicity of copper and cadmium, two of the most toxic heavy metals, to various life stages of the benthic marine copepod Tisbe holothuriae, Humes. The following was found:

1) Copper was more toxic to all life stages of Tisbe than cadmium; and

2) the more sensitive life stage of Tisbe to both copper and cadmium was the one day old nauplii and the lower LC_{50} 48h values were found for this life stage: (0.3142 mgCu/l and 0.5384 mgCd/l (see Table V below).

Table V. LC_{50}(48h) in mg ions/l. of Cu in the form of $CuSO_4.5H_2O$ and Cd in the form of $CdCl_2.2H_2O$ to various life stages of Tisbe holothuriae

Life stage	Metal	
	Cu	Cd
One day old nauplii	0.3142 ± 0.0052	0.5384 ± 0.0062
Five day old nauplii	0.3415 ± 0.0004	0.645 ± 0.0092
Ten day old nauplii	0.5289 ± 0.0011	0.9061 ± 0.0066
Females with ovigerous bands	0.4473 ± 0.0021	0.9166 ± 0.0056
Females with ovigerous sacs	0.4281 ± 0.0027	0.8727 ± 0.0166

The resistance of Tisbe to copper and cadmium progressively increased with larval age and the more resistant larval stage was the ten day old copepodids (0,9061 mgCd/l and 0,5289 mgCu/l. However the increase of Tisbe resistance to two metals was not extended to the adult stages tested. The Tisbe with ovigerous bands were more resistant to metals than those bearing ovigerous

sacs. In the case of copper, females with ovigerous bands were more sensitive than the 10 day old copepodids, while for cadmium they demonstrated a slightly different (higher) resistance.

Females with ovigerous sacs showed an increased sensitivity and for both metals were more sensitive than the 10 day old copepodids. The more resistant life stage of Tisbe was the 10 day old copepodids for copper, while in the case of cadmium the 10 days copepodids and females with ovigerous bands showed the higher resistance to cadmium (0.906 for the 10 day old copepodids and 0,916 for the females bearing ovigerous sacs).

DISCUSSION OF RESULTS

Two important points emerge from these results:

1. The indication of the possibility of some marine organisms developing adaptation to pollution conditions expressed as a higher tolerance to different toxicants. Similar adaptations have been demonstrated by other authors in marine polychets.

2. Usually even very low doses of heavy metals affect the physiological processes of the animals. This is important because it demonstrates that even low concentrations can destroy animal populations, as higher doses do, by affecting their life processes.

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Principal Investigator: E. NEVO

Period of Reporting: June 1978 - March 1980

INTRODUCTION

The objective of this research is to study the direct effects of various pollutants on the genetic structure of marine organisms in laboratory controlled experiments.

The significance of this study may involve both theoretical and practical aspects. Theoretically, it might shed light on the selective processes operating on the genetic systems of marine organisms. Practically, the results may suggest the use of some marine organisms as (a) biological monitors for the levels of specific marine pollutants, and (b) as biological control organisms for the elimination of pollutants. During the years 1975-1978, the Institute of Evolution conducted preliminary studies on (a) thermal pollution (Nevo et al., 1977), and (b) chemical pollution (Nevo et al., 1978). These preliminary studies, which are relevant to MED POL were conducted before we signed the agreement with FAO (GFCM)/UNEP. Since the agreement was signed, the Institute conducted its research primarily in specifically polluted and controlled laboratory environments with the major aim of unveiling differential mortality of genotypes and thereby achieving both practical goals mentioned.

METHODOLOGICAL CONSIDERATIONS

Selection of the species:

Palaemon elegans was chosen to study the effects of mercury salts for the following reasons:

(a) P. elegans is a widespread East Atlantic species, also common along the Mediterranean coasts where it constitutes one of the prevailing species mainly in the rocky shores, rock pools and lagoons.

(b) The biology, including morphology, sex ratio, reproduction and behaviour of this species are known in great detail.

(c) P. elegans is a generalist species adapted to heterogeneous spatial and temporal environments, so it is expected to be genetically variable and therefore suitable for our study.

(d) P. elegans is easily raised and bred in laboratory aquaria; hence we can follow the effects of pollutants for several generations.

(e) Large samples are easily collected and can be kept at standardized laboratory conditions; our work is heavily dependent on these conditions.

(f) Palaemon is one of the organisms recommended by the FAO (GFCM)/UNEP mid-term expert consultation on the co-ordinated project on pollution in the Mediterranean, (May 1977).

Two Molluscs were also selected (Cerithium scabridum and Monodonta turbinata) and preliminary results obtained.

Pollutants analysed:

In the Palaemon study a mercury salt ($HgCl_2$) was used in concentrations of 0.02-0.40 ppm.

With Gasteropods, the following were used:

(i) mercury salt ($HgCl_2$);

(ii) nonionic detergents (Berol 716) and monyl phenol ethylen (Maolophen 89);

(iii) crude oil (dissolved in DMSO), and

(iv) synergistic influence of oil and detergent.

METHODOLOGY

Palaemon elegans:

Controlled and standardized laboratory experiments were conducted in 10 aquaria, 80 litres each (70 x 30 x 40 cm). Sea-water was brought repeatedly throughout the study from a well 30 m deep of the Shikmona National Institute of Oceanography. Water in the aquaria was kept at stable conditions (22°C; pH = 8.3), and aereated throughout the experiments. Palaemon elegans was collected near the Shikmona Mediterranean coast from rocky pools, and introduced into the aquaria in quadrangular cages (25 x 25 cm), subdivided into small interconnected cells (5 x 5 cm) so that water current could pass freely through all cells. Each prawn was put separately into a single cell to prevent aggression and preying. No food was given throughout the experiments which lasted 1-10 days depending on the $HgCl_2$ concentration.

Experiments involved both control and tests. If testing was conducted simultaneously, several tests were compared with the same control. The only difference between control and test was the addition of the pollutant to the test. Both control and test each included 25 prawns. All together we tested 3682 prawns in the aquaria (1538-controls; test - 2144). The survivors of the experiments were deep-frozen (-80°C), and later analysed electrophoretically.

Electrophoretic analysis:

Soft tissues of whole animals were homogenized and studied by starch gel electrophoresis. Out of 30 assayed loci we decided to concentrate in this study only on the enzyme phosphoglucomutase (Pgm). The reason was that this system was highly polymorphic, involving 5 alleles and hence 15 genotypes. Thus we could find differential mortality of genotypes correlated with the pollutant concentration. In all 1406 experiments for Pgm (703 control and 703 test) were analysed. A total of 5 alleles was found: S^-,S,M, F, F^+ for Slow, Medium, and Fast migrations from the origin.

Gasteropods:

All procedures concerning electrophoresis are the same as those described for P. elegans. However, in this study more genetic polymorphism is tested for potential sensitivities to the effect of the pollutants.

RESULTS

Palaemon elegans. The 5 alleles discovered in our study (Pgm^{S-}, Pgm^{S}, Pgm^{M}, Pgm^{F}, Pgm^{F+}) recombine into 15 genotypes, 5 homozygotes and 10 heterozygotes. The effects of the mercury salt ($HgCl_2$) on the differential mortality of the 15 genotypes are given in Table I. The following results were indicated:

(a) General mortality rate.

General mortality rate in all 71 experiments (30 controls and 41 mercury tests) is significantly correlated with the $HgCl_2$ concentration ($r=0.66$, $P<0.00001$). The correlation between mortality rate and $HgCl_2$ concentration is even stronger in concentrations lower than 0.20 ppm. In the higher concentrations (0.24 - 0.40 ppm $HgCl_2$) an unexplained high variance in mortality rate was found which caused a decrease in the over-all correlation.

Some data about the survivorship (in % as means) are given in Table I.

Table I

Survivorship of Palaemon elegans under the effects of the mercury salt

$HgCl_2$ in ppm	≤0.03	0.035 -0.04	0.05- 0.06	0.07- 0.08	0.12	0.18	0.24- 0.26	0.30	0.40
1 day			80		0		8	58	31
3-6 days	71	70	63	65	57	33	60	20	48
7-9 days	87	67					52		
10-11 days	66		23						

(b) Differential mortality patterns of homozygotes and heterozygotes.

The percentage of heterozygotes among the survivors of the $HgCl_2$ polluted environment varied from indifference (0.02-0.08 ppm) to ascending superiority (0.12-0.24 ppm) and descending to inferiority (0.26-0.40 ppm). Obviously, a mirror image is reflected by the frequency of the homozygotes among the survivors; i.e. homozygotes increase both in low and high concentrations of $HgCl_2$.

(c) Genetic patterns:

(i) Allele frequencies. No significiant correlations were found between the frequencies of each of the five alleles and the $HgCl_2$ concentrations.

The maximal correlation was r = -0.19 of the fast allele, F. The frequency of the slow allele, S, reflects the convex curve displayed by the heterozygote frequency.

The major analysis and findings relate to genotype rather than allele frequencies.

(ii) Genotype frequencies. Out of the 15 possible genotypes the major significant results relate to the five genotypes.

F^+M, FM, SS, MS, and MM, and primarily to the MS heterozygote.

- The F^+M heterozygote, although a rare genotype, decreases almost significantly with increasing $HgCl_2$ concentration (r=-0.23, P=0.056).

- The FM heterozygote decreases significantly with increasing $HgCl_2$ concentration (r=-0.32, P=0.006).

- The MS heterozygote displays a concave-shaped curve similar to, and probably responsible for, the arched curve of all heterozygotes.

- The MM homozygote displays a significant depression in the concentration range 0.12-0.24 ppm. Its high values were found both at low, but most remarkably, at high concentration.

- The SS homozygote appears to be largely invariant throughout the concentration range from 0.00 to 0.40 ppm $HgCl_2$.

Gasteropods (all pollutants).

(i) In both species, 30 gene loci have been screened and successfully resolved electrophoretically for their optimal scoring.

(ii) The LD 50 of all four pollutants for both species has been determined. Details of experiments and results can be obtained by writing to Dr. Batia Lavie, who is conducting this research at the Institute of Evolution.

DISCUSSION OF RESULTS

The most significant and important findings concern the relationship of the different patterns of MS heterozygote and MM homozygote. The MS increases significantly in proportion in the tests conducted at the concentration range 0.02-0.18 (sign test, 22 pluses vs 6 minuses, P=0.004). Within the range of 0.0-0.12 ppm, the correlation was r=0.52, P<0.00005.

The frequency of MS decreases significantly in high $HgCl_2$ concentrations (0.30-0.40 ppm).

In other words, the MS heterozygotes are presumably selectively superior in the $HgCl_2$ concentration range of 0.02-0.20 ppm. In contrast the MM homozygote appears to be selectively superior in low, but most importantly, in high (0.26-0.40 ppm) concentrations.

We thus found evidence of differential survival, hence differential fitness, of genotypes in the mercury-polluted environment. Note that the MS heterozygote is fitter at the intermediate concentration range whereas the MM homozygote appears to be the only selectively superior genotype in the high $HgCl_2$ range of 0.40 ppm which is 13333 times higher than the concentration of the control sea-water of the Shikmona 30 m deep well (0.00003 ppm).

These results suggest a theoretical as well as a practical conclusion.

(a) Theory: A genetic polymorphism can be preserved in nature due to the different fitnesses of its genotypes in an ecologically subdivided environment into different niches (the $HgCl_2$ different concentrations in our experiment). Furthermore, heterozygosity (the MS heterozygous in our experiment) is presumably the solution nature offers to broad niches, whereas homozygosity (the MM homozygous in our experiment) is presumably the solution for narrow niche environments (the high $HgCl_2$ concentration in the tests).

In other words, heterozygosity promotes generalism, while homozygosity promotes specialism.

(b) Practice: The first applied objective, indicated in the introduction, was to find biological monitors for the levels of specific marine pollutants not only reflecting short-term situations but rather tracing the ongoing long-term processes in the marine environment. Such a monitoring system may be an indispensible gauge to the genetic changes populations undergo before their final extermination as a result of pollution. The findings, primarily those of the differential pattern of the MM and MS genotypes, suggest that a monitoring system based on genetic population changes is not only theoretically commendable but, most importantly, practically feasible.

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Principal Investigator: Z. YARON

The final report was not received, but from a previous report the following summary information can be given for the work performed from June to October 1979.

INTRODUCTION

Cortisol is the main corticosteroid hormone in fish. It functions both as a glucocorticoid, controlling protein and carbohydrate metabolism and as a mineralocorticoid, controlling osmoregulation in marine or in SW-adapted fish. Cortisol is secreted by the interrenal tissue under the stimulation of ACTH and is metabolized mainly by the liver.

In our laboratory a system has been developed in which cortisol secretion by superfused interrenal tissue of Sarotherodon aureus could be stimulated by a pulse of ACTH at physiological levels. The system was used to study the nature of the corticotropic stimulation in fish and it revealed that this stimulation is mediated by c-AMP as a second messenger.(Ihan and Yaron, J. Endocr. 86, 269, 1980)

Organochlorines, such as o,p'DDD and p,p' DDE inhibited the increase of cortisol output in response to ACTH whether administered to the donor fish or when given in the superfusion medium for, at least, 5 h (Figs. 1,2,3). (Ihan and Yaron , J. Endocr. 87, 185, 1980)

The suppressive effect of organochlorines on interrenal function has been chosen as a model for investigating the sublethal effects of these xenobiotic compounds in Mediterranean fish. The present report is basec on a series of experiments performed during 3 months along the lines established in this model with 2 species of mullets (Mugil cephalus and Liza ramada).

METHODOLOGICAL CONSIDERATIONS

Fish raised from fingerlings collected in Mediterranean estuaries were purchased from the fish farms along the coast. L. ramada were acclimatized in freshwater while M. cephalus were adapted to SW, both at 24 °C, in the laboratory. Head kidneys containing the interrenal tissue were excised, pooled when necessary to obtain about 1 g, and superfused for 5 h in Eagles Basal Medium or Hank's salts only, both containing bovine serum albumin (50 mg%) and 4mM $NaHCO_3$. Flow rate was 0.6 ml/min. Medium was collected every 15 min before stimulation by ACTH (porcine) and every 5 min thereafter. Temperature was 26° ± 2°. Cortisol was measured in aliquots of the superfused medium by RIA according to the laboratory routine (Terkatin-Shimony et al.G Comp. Endocrinol. 40, 143, 1980),

o,p'DDD dissolved in dimethyl formamide (DMF) was added to the medium in the experimental superfusion, while in control superfusions the orgnochlorine was omitted.

RESULTS

Liza ramada. The pattern of the control superfusion differed somewhat from that observed in Tilapia. The spontaneous cortisol output by the interrenal of Liza was high and did not reach a low baseline. This required many superfusion experiments in order to adapt the superfusion system to the new species. In recent experiments the response in the control superfusion to a 5 min pulse of ACTH (3mU) was prominent: a 4-fold increase in cortisol output. (Fig.4). Addition of o,p'DDD (70 mg/l) suppressed the output of cortisol by the superfused interrenals in response to ACTH (Fig.5).

Mugil cephalus. The results of superfusion experiments of interrenals taken from SW-adapted fish are still inconclusive. Nevertheless, tissue taken from fish during adaptation to SW (2 days) responded to ACTH (3mU) in superfusion in accordance with the model (Fig.6). Addition of o,p'DDD (50 mg/l) to the medium suppressed the response to ACTH (Fig.7).

Since the actual activities have commenced only recently, it seems too early to make appraisals. However, the basic idea of the participation in MED POL lies in the opportunity to contribute our knowledge and experience in fish endocrinology to a somewhat unrelated - but nonetheless important - field of marine ecology. Experiments designed to reach the minimal effective dose of o,p'DDD and other organochlorines in mullets and other Mediterranean fish are in progress.

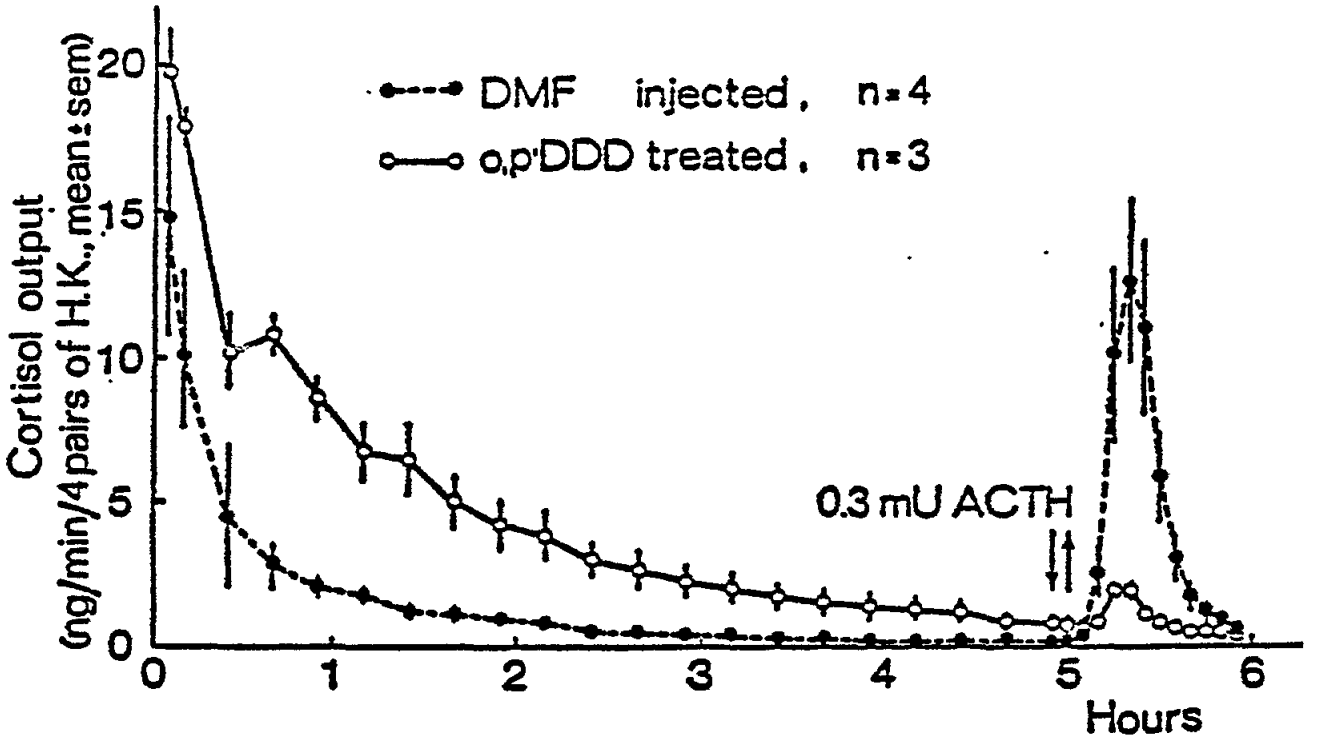

Fig. 1. The response to a 5 min pulse of ACTH (0.1 mU/ml) of interrenal tissue taken from o,p'DDD-treated fish (50 mg/Kg) and from DMF injected controls (1 ml/Kg). The organochlorine, or the vehicle only, were injected i.p. 24 h prior to sacrifice. The tissue was superfused with Eagle's basal medium containing $NaHCO_3$ (4 mM) and BSA (50 mg/%), pH 7.6. Flow rate was 0.6 ml/min.

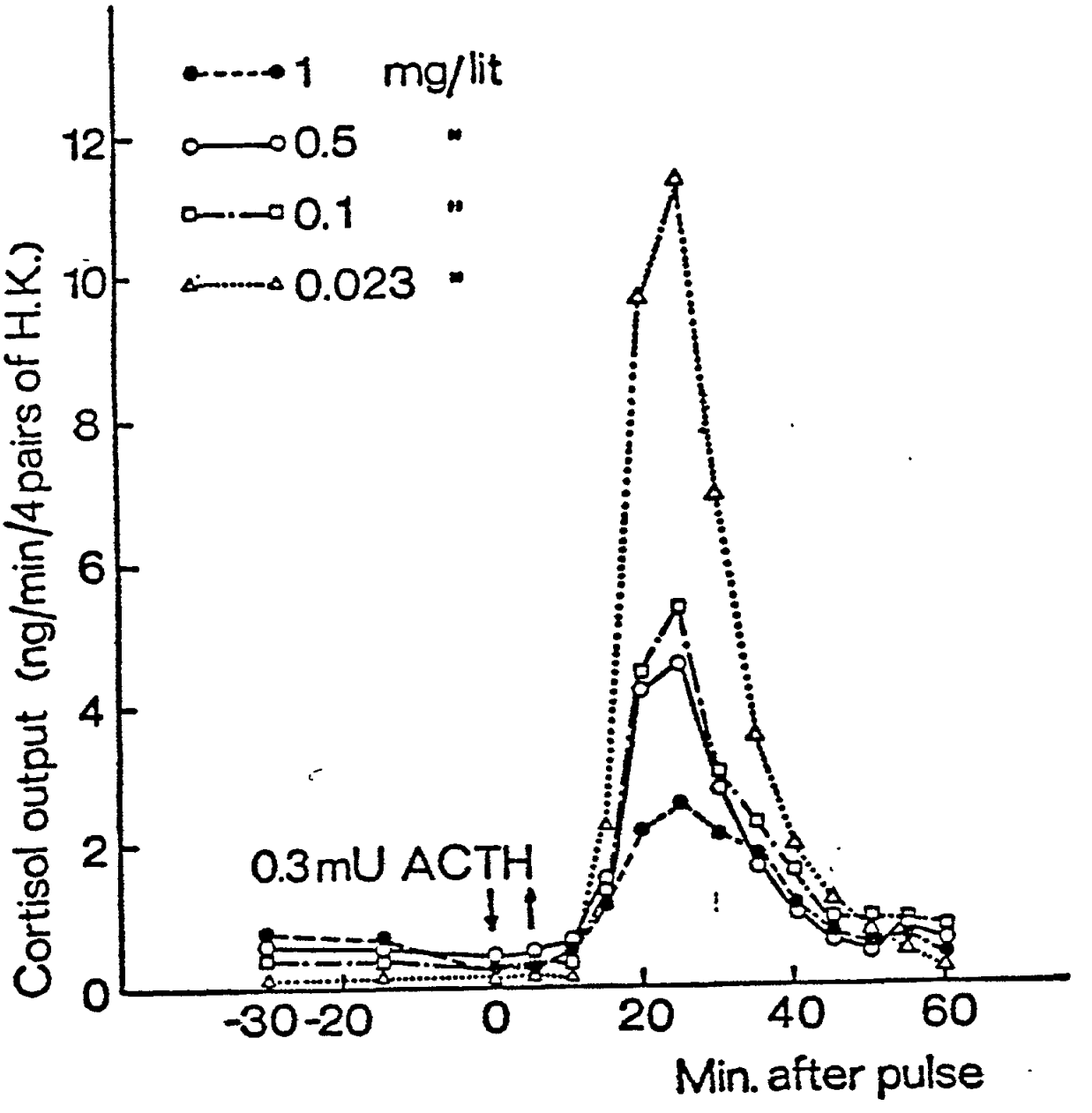

Fig. 2. The response to ACTH of superfused interrenal tissue taken from fish injected with various doses of o,p'DDD 24 h previously. Other details as in Fig.1.

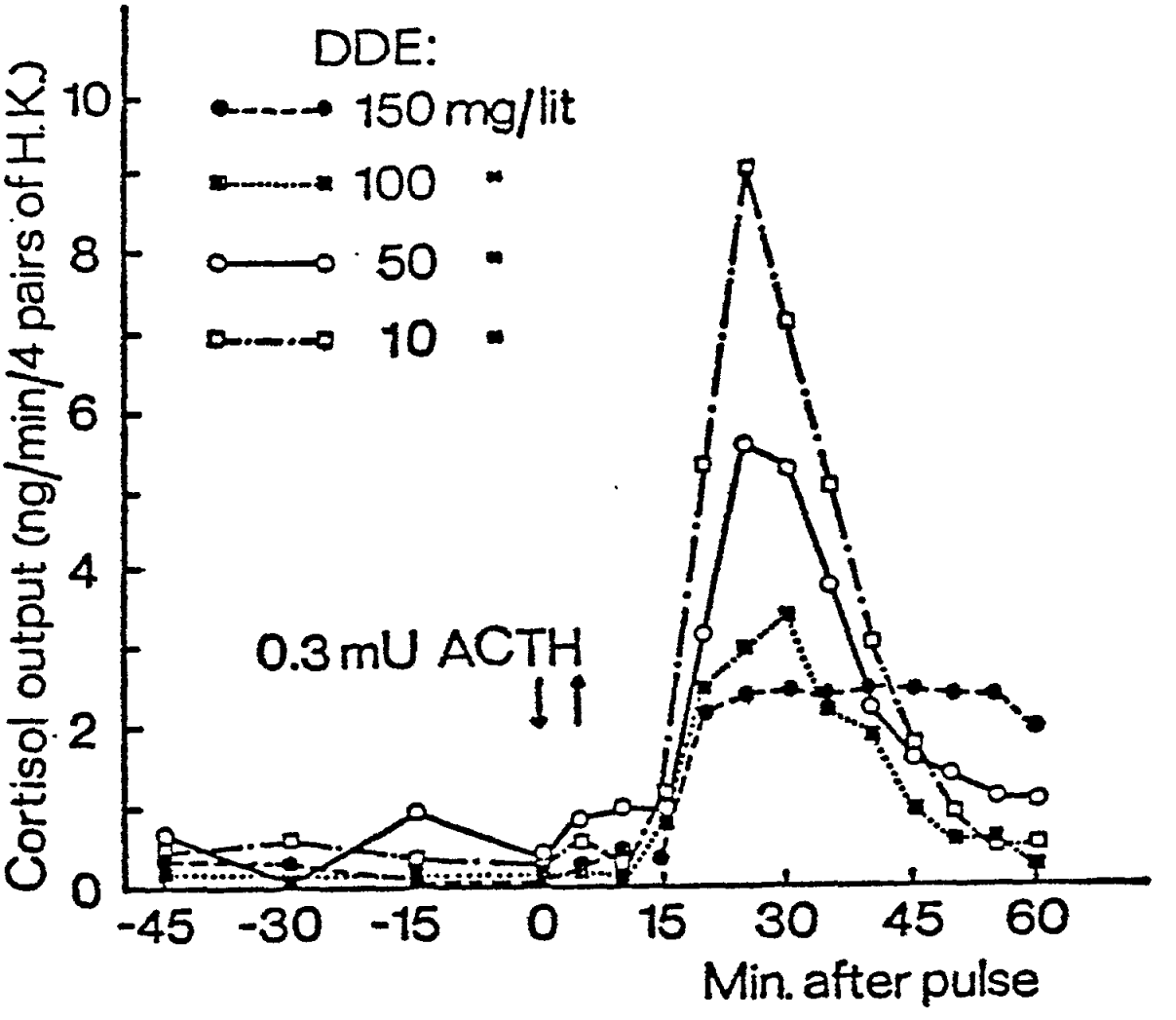

Fig. 3. The effect of various concentrations of p,p'DDE, in the superfusion medium, on the response of interrenal tissue to ACTH. Other details as in Fig. 1.

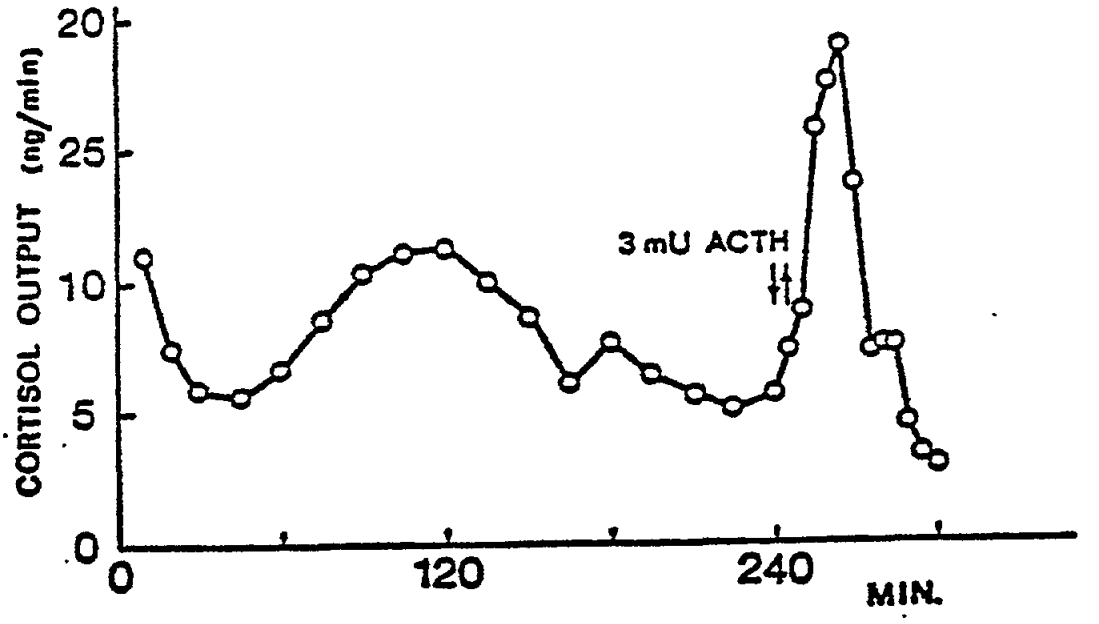

Fig. 4. Cortisol output in response to ACTH by superfused interrenal tissue of untreated Liza ramada raised in freshwater (control)

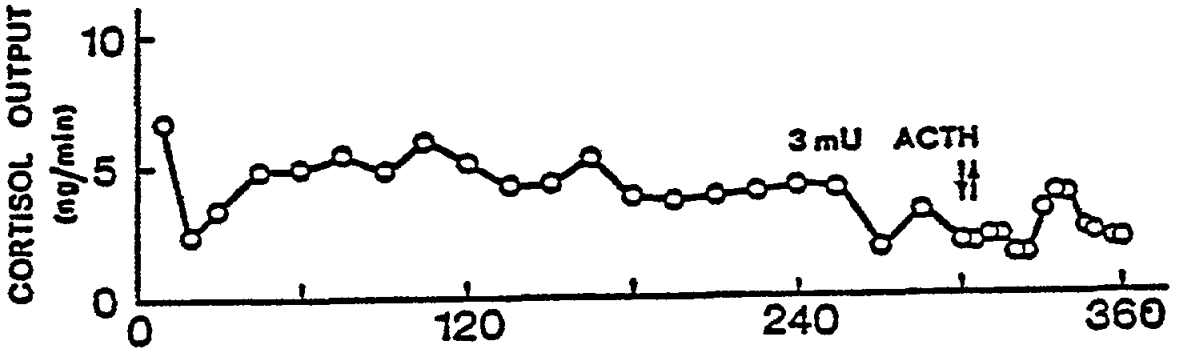

Fig. 5. Cortisol output in response to ACTH by superfused interrenal tissue of Liza ramada raised in FW. The medium contained 70 ppm of o,p'DDD. Note that the cortisol output in response to ACTH was totally abolished.

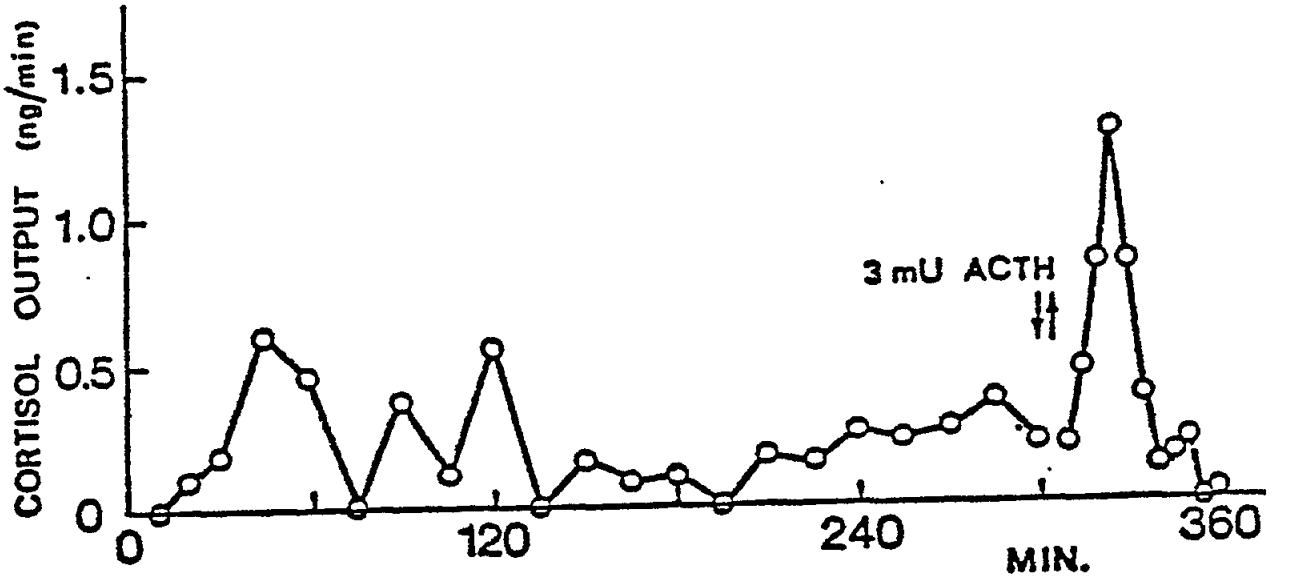

Fig. 6. Cortisol output in response to ACTH by superfused interrenal tissue of Mugil cephalus adapted to SW. (control)

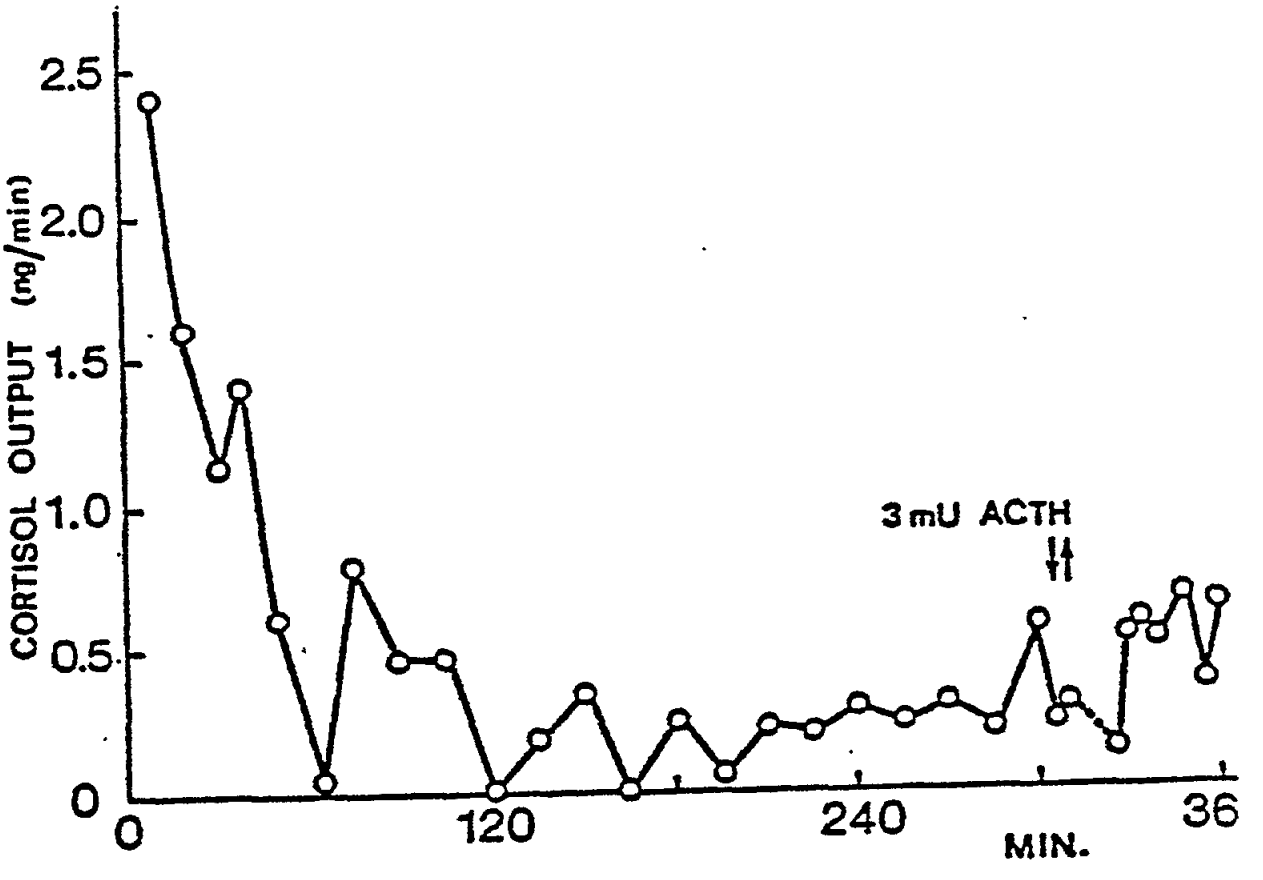

Fig. 7. Cortisol output in response to ACTH by superfused interrenal tissue of Mugil cephalus adapted to SW. Note that the response to ACTH was totally abolished.

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Principal Investigator: L. DALLA VENEZIA

Period of Reporting: 1 July 1978 to March 1980

INTRODUCTION

In 1977 the Research Centre commenced its contribution to MED POL with the signature of the agreement with MED POL III.

Because our test organisms have no economic significance, our research has only indirect importance for fishery activities, but they nevertheless represent one of the lowest levels of the marine food chain.

METHODOLOGICAL CONSIDERATIONS

Selection of the species:

Tisbe bulbisetosa (Copepoda Harpacticoida). This species was selected because:

(a) it has a short life cycle, and can be easily used for long-term experiments;

(b) it can be reared in the laboratory;

(c) we have been using it in several previous experiments (already published), on short and long-term effects of other pollutants and it will be possible to make comparisons between the effects of different pollutants on the same species.

Pollutant analysed:

The polychlorinated biphenyl (PCB) mixture marketed under the name of Aroclor 1254 (Monsanto Company, USA) was utilized for experiments. The actual concentration in experimental media was determined by gas-chromatographic analysis (see below).

METHODOLOGY

Chemical part:

The suspension was prepared by adding 10 ul of Aroclor 1254 standard solution in ethanol and 0.2 ml of Corexit 7664 (Esso) to 1000 ml of filtered sea-water. This mixture was shaken in a separatory funnel for a few minutes, in order to obtain an initial concentration of 100 µg/l of Aroclor. Samples of the aqueous phase were withdrawn daily for 30 days following preparation and analysed by gas-chromatography to determine the actual concentration and to examine the trend of PCB with time. 20 ml of stock suspension were repeatedly extracted with n-hexane. All extracts were combined, dried over anhydrous sodium sulphate, concentrated under reduced pressure to 2 ml then

injected into a Hewlett Packard 5750 gas-chromatograph, equipped with a Ni^{63} electron capture detector. Quantification was based on comparison of peak heights with those obtained for a standard solution of Aroclor 1254.

Biological part:

Individuals of Tisbe bulbisetosa, used as test organisms, were derived from populations reared in the laboratory in glass jars containing about 3 l of sea-water of 35 ± 2°/•• salinity. The jars were kept in a room of constant temperature at 18 ± 1°C with a light cycle of 12 h.

Animals were fed small fragments of boiled wheat and Ulva sp.

(a) Toxicity experiments: A solution was prepared containing 100 µg/l PCB and 0.2 ml/l Corexit in sea-water. Other test solutions were then prepared by dilution to obtain Aroclor concentrations of 10, 1 and 0.1 µg/l.

Adult female and male Tisbe bulbisetosa were exposed to each concentration in 20 ml culture dishes, with five animals of each sex per dish. Dishes with animals in clean sea-water served as controls. No deaths were observed after one week.

(b) Acclimation experiments: Each group of experiment (a) was transferred to a new culture dish containing 500 µg/l Aroclor suspension and 0.2 ml/l Corexit.

(c) Fecundity experiments: About 40 ovigerous female Tisbe bulbisetosa, randomly chosen from stock cultures, were distributed in eight 100 ml jars containing a 100 µg/l Aroclor suspension. Another 40 ovigerous females were distributed in the same number of jars containing clean sea-water, with the purpose of following eight "polluted" populations and eight control populations. At the second generation it was observed that the density was low in "polluted" populations, at which point all ovigerous F_1 females were immediately transferred into 10 µg/l Aroclor suspension.

The last test suspension was prepared as in experiment (a), by dilution of 100 µg/l Aroclor and 0.2 ml/l Corexit.

F_2 females, from both "polluted" and clean sea-water, were coupled with F_2 males of the same origin in 20 ml culture dishes (one pair per dish). Each ovigerous female was transferred to a 20 ml culture dish and followed daily until hatching of the first egg-sac. Nauplii were counted and the female was transferred to a second jar; this procedure was followed for all subsequent egg-sacs.

(d) Survival of nauplii: Groups of 20 newly hatched nauplii Tisbe bulbisetosa were put in 20 ml culture dishes containing both "polluted" and clean sea-water. The following nominal test suspensions were used:

100 µg/l Aroclor 1254 + 0.2 ml/l Corexit 7664

10 µg/l Aroclor 1254 + 0.2 ml/l Corexit 7664

1 µg/l Aroclor 1254 + 0.02* ml/l Corexit 7664

---

* because it was prepared by dilution from the previous one.

0.2 ml/l Corexit 7664

clean sea-water

For each treatment 240 individuals (12 jars) were tested.

(e) Coupling: Adult males and females, coming from nauplii of previous experiments, were allowed to couple, in 100 ml jars, containing the same five treatments, listed above. The number of ovigerous females for each treatment was counted and the ratio ovigerous females/total females calculated.

Because of the small size of the test organisms, it was not possible to determine their PCB content.

RESULTS

Tisbe bulbisetosa. Polychlorinated biphenyl (Aroclor 1254):

Chemical part:

Variation of Aroclor with time is not significant when mixed with Corexit 7664.

The decrease of concentration when Aroclor is not mixed with Corexit is as low as 25% in 10 days and less than 20% after 20 days.

Biological part:

(a) Toxicity experiments. No mortality was noted in adult Tisbe bulbisetosa exposed up to 100 µg/l PCB.

(b) Acclimation experiments. The cumulative mortality in 500 µg/l PCB is much higher in males than in females: previous exposure to different concentrations of pollutant (or clean water) seems to have no significant effect on sensitivity, either positive (due to acclimation), or negative (due to impairment).

(c) Fecundity experiments. The results are summarized in the following table.

	No. of ♀♀	Average Number of Sacs/♀	s	Average Number of Nauplii/♀	s
"Polluted" females (10 µg/l Aroclor+ 0.2 ml/l Corexit)	21	3.90	2.47	61.57	56.52
Control females	30	4.27	2.05	69.33	50.83

Differences between control and treated females in terms of average number of sacs and average number of nauplii are not significant. The difference in Minimum Generation Interval under different conditions is, on the contrary, significant:

	Minimum Generation Interval
"Polluted" animals (10 µg/l Aroclor + 0.2 ml/l Corexit)	24.67 ± 3.40 days
Control animals	22.10 ± 1.94 days

Kolmogoroff-Smirnov test: significant at P = 0.01

The duration of the entire biological cycle is lengthened in "polluted" conditions; thus in polluted and in control media females produced on average 0.93 and 1.26 nauplii per day, respectively.

(d) Survival of nauplii. It must be noted that gas-chromatographic analyses, made "a posteriori" gave the following initial concentrations:

instead of 100 µg/l ..... 80 µg/l

" 10 µg/l ..... 16 µg/l

" 1 µg/l ..... 1.6 µg/l

Results of survival are summarized in the following table:

	Aroclor 1254/Corexit 7664			Corexit 7664	Sea-water
	80 µg/l	16 µg/l	1.6 µg/l	0.2 ml/l	Controls
Surviving (No.)	28	47	92	108	152
Surviving (%)	11.67	19.58	38.33	45.00	63.33
Females (No.)	16	28	49	55	78
Males (No.)	12	19	43	53	74
Sex ratio	1.33	1.47	1.14	1.04	1.05

Since 0.2 ml/l Corexit 7664 had some adverse effect on nauplius (while it is completely non-toxic to adults), we preferred to apply G-test for significance to Aroclor+Corexit values against Corexit, rather than against controls, that is between the following pairs of treatments:

(80 µg/l Aroclor 1254 + 0.2 ml/l Corexit 7664) and 0.2 ml/l Corexit
G = 69.01 P<0.001

(16 µg/l Aroclor 1254 + 0.2 ml/l Corexit 7664) and 0.2 ml/l Corexit
G = 36.19 P<0.001

(1.6 µg/l Aroclor 1254 + 0.02 ml/l Corexit 7664) and controls
G = 30.33 P<0.001

0.2 ml/l Corexit 7664 and controls
G = 16.34 P<0.001

(e) Coupling. Males and females, coming from the previous experiment, coupled normally in all five treatments and the percentage of ovigerous females was near to 100% in all treatments. This aspect however needs further investigation to determine whether egg-sacs were fertile at two higher concentrations.

DISCUSSION OF RESULTS

Chemical part:

The main aim of the analytical work was to control the stability with time of the added PCB. Results indicate that PCB concentration in natural sea-water is significantly reduced within a few days. Our opinion, verified in preliminary experiments, is that the PCBs in standing sea-water samples concentrate on glass-water surfaces, reducing the actual aqueous concentration. The presence of the surfactant Corexit 7664 reduces this unwanted effect and consequently makes the suspension more stable with time. In previous experiments 0.2 ml/l Corexit solution, used to obtain sufficiently stable suspensions of Kuwait crude oil, seemed to be non-toxic in biological assays with adult Tisbe bulbisetosa. The same concentration was demonstrated to be sufficient to stabilize suspensions of PCBs in sea-water.

In experiment (a) the test suspensions with nominal concentrations of 10, 1 and 0.1 µg/l and in experiment (c) the concentration of 10 µg/l of Aroclor, were obtained by diluting the 100 µg/l stock suspension. By adopting this approach, Corexit concentration was lowered and consequently its capacity to stabilize the PCB suspension was reduced. In fact, concentrations determined by analysis at the end of the experiments were 1.7 to 1.9 µg/l, instead of 10 µg/l.

In experiment (d), on the contrary, the 10 ug/l PCB medium contained 0.2 ml/l Corexit: concentrations determined at the end of the experiments were 5.6 to 6.0 µg/l.

Biological part:

When we started this research, we had four aims:

(i) to determine the average number of nauplii produced by a female;

(ii) to determine the survival of nauplii;

(iii) to determine the mortality of adult males and females; and

(iv) to determine the occurrence of coupling, in each case under "polluted" and control conditions.

As regards points (i), (iii) and (iv), the differences between the treatments appeared insignificant. The only highly significant difference is that of point (ii): under the effect of a pollutant probably a very strict selection occurs at the nauplius stage, so that the surviving individuals can tolerate the adverse conditions as well as control individuals tolerate the normal conditions. In this way, it is possible, in our opinion, to explain the lack of a significant pollutant effect upon nauplius production and frequency of coupling.

Research Centre: Gruppo Ricerca Oceanologica-Genova (CROG), Istituto di Idrobiologia e Pescicoltura, Università di Genova, Italy

Principal Investigator: M. ORUNESU (A. VIARENGO)

Period of Reporting: June 1978 to March 1980

INTRODUCTION

Bivalve molluscs, particularly mussels and oysters have been used in recent years as biological indicators of the pollution of the coastal marine environment and of the estuaries.

Besides checking mussels on a world scale with regard to the tissue concentrations of heavy metals, hydrocarbons, pesticides, etc., it has been deemed necessary to conduct intense studies aimed at evaluating the effects that the accumulation of polluting substances can exert on the physiological and biochemical activities of the animals. Based on these considerations, the G.R.O.G. (Gruppo Ricerca Oceanologica-Genova) has undertaken interdisciplinary research for the purpose of studying the biological effects exerted by heavy metals (particularly copper) on mussels. The research was carried out bearing in mind two directives:

(i) to check if Cu^{2+} can alter the protein metabolism and the ATP (adenosintriphosphate) content in the mussel tissues, causing a condition which could hinder the normal development and the ponderal growth of the animal; and

(ii) to see if Cu^{2+} can induce in the mussel tissues the synthesis of particular proteins - thioneins and chelatins - which can complex the metal, exerting in this way a protective function. The synthesis of these proteins could therefore supply an indication as to the presence of heavy metals in the environment. Another important item of information it could furnish, is to give an indication of the animal's capacity to reduce the cytotoxic effects exerted by copper or by other heavy metals.

METHODOLOGICAL CONSIDERATIONS

Selection of the species:

Specimens of Mytilus galloprovincialis Lam. 4-6 cm long, collected at Palmaria (La Spezia), were used. Before use, the mussels were maintained for at least 5 days in an aquarium with aerated recirculating artificial sea-water (La Roche et al., 1970), at 14-15°C. During the experiments both the water (one liter/animal) and the metal (at a final concentration of 0.08 ppm) were changed daily.

Pollutants analysed:

Cu (Cu^{2+})

METHODOLOGY

Uptake of amino acids:

Two hours before removing the soft parts from the shells, 7.5 µCi/l of the ^{14}C-labelled amino acid mixture were added to the tanks containing the mussels. Tissues were homogenized with 2 volumes of 0.25 M sucrose. To evaluate the uptake of amino acids, aliquots of 50 µl were spotted on to Whatman GF/C glass fibre filter discs (25 mm diameter) and dried for 10 hrs at 37°C. The radioactivity present in the homogenates, which reflects the uptake of amino acids, was measured in a Packard liquid scintillation spectrometer.

Rate of protein synthesis:

Two hours before use, each mussel received 2 µCi of ^{14}C leucine by injection into the posterior adductor muscle. Tissues were removed and homogenized as described above. Aliquots of 25 µl of the homogenates were used to analyse protein content and aliquots of 100 µl were employed to evaluate the incorporation of the labelled precursor into proteins as described by Yu & Feigelson (1970).

Radioactivity values, due to the incorporation of the labelled amino acid into the protein fraction, were expressed per mg of tissual proteins and corrected for the amino acid uptake in the tissue.

Assay of proteins:

Proteins from the tissues were precipitated in 0.2 N perchloric acid and the pellet assayed according to the Lowry method (Lowry et al., 1951) using Serum Albumin, Fraction V, as standard.

Measurement of radioactivity:

Radioactivity was measured in a Packard 2425 liquid scintillation spectrometer. Each sample was counted in 5 ml of toluene containing 5 g/l of PPO for a time interval sufficient to assure a counting error no greater than 5%.

Assay for ATP:

The tissues were quickly removed from the mussels, frozen in liquid nitrogen and then weighed.

Each tissue was homogenized with two volumes of ice-cold 7% (w/v) trichlorocetic acid, as described by Wijsman (1976).

After centrifugation, the resulting supernatant was diluted as needed with 20 mM tris-HCl, pH 7.4 and measured for ATP content, as described by Cheer (Cheer et al., 1974), using luciferin-luciferase.

Copper concentration in mussel tissues:

The copper concentration was determined by atomic absorption spectrophotometry as indicated by Capelli (Capelli et al., 1978).

Isolation of the gill Cu^{2+}-binding proteins:

Gills were removed from the soft part of the mussels and homogenized in 1 vol of 10mM K-phosphate pH 7.8 containing phenylmethyl sulfonyl fluoride at the final concentration of 5 mg/l; most of the tissue debris was removed by centrifugation at 20,000 x g for 10 min. The supernatant was heated at 60°C for 10 min and then centrifuged at 25.000 x g for 10 min. The final supernatant was applied directly to a 1.1 x 110 cm Sephadex G. 75 column and eluted at 6 ml/h with 10 mM tris-HCl, pH 8.6, containing 0.01% NaN_3 as antimicrobial agent. Aliquots of 3 ml were collected and analysed for absorbance at 280 mm. Copper concentration was measured using a Perkin Elmer 560 atomic absorption spectrophotometer.

Incorporation of labelled ^{35}S cystein:

The radio-labelled precursor (2 µCi/animal) was injected into the posterior adductor muscle 7 hrs before death. The gill preparations were dialysed overnight against 20 mM K-phosphate, pH 7.8, to remove all free amino acids and then chromatographed on a Sephadex G-75 column as described in Methodology above. Fractions of 3 ml were collected and analysed for absorbance at 280 nm. Aliquots of 1.5 ml were used for the determination of ^{35}S radioactivity. Radioactivity was measured in a Packard liquid scintillation spectrometer, model 2425.

Each sample was counted in 10 ml of instagel for enough time to assure a counting error no greater than 5%.

Evaluation of sulphydryl group concentration:

The fractions 18-23 (30 ml) containing 12.000 molecular weight proteins, were pooled and concentrated to a volume of 3 ml by membrane filtration (employing Diaflo 0.5 UM filters and an Amicon mod. 52 units.). The concentrated solution was assayed for the determination of sulphydryl group using a slight modification of the Ellman method (Ellman, 1959). The reaction mixture contained 200 µl of 100 mM Na-phosphate buffer, pH 7.3, 30 µl of 1mM EDTA, pH 7.23, 30 µl of Ellman's reagent (39.6 mg of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) in 10 ml of phosphate buffer, pH 7), 200 µl of distilled water, 600 µl of sample. Absorbance was measured at 412 nm, 10 min after Ellman's reagent addition.

RESULTS OF EFFECTS

The studies aimed at checking the effects of Cu^{2+} on protein metabolism and on the tissue concentrations of ATP have been conducted on samples of Mytilus galloprovincialis Lam., kept for 1, 3 and 7 days in artificial sea-water containing 0.08 ppm of Cu^{2+}. It was noticed that Cu^{2+} accumulates rapidly in the tissues of the animal, reaching within 7 days values of 34-40 µg/g in the gills, 10-12 µg/g in the digestive gland and 3-4 µg/g in the mantle (Fig. 1). It was also demonstrated that the uptake of amino acides from the environment by the three tissues is decreased to 30-45% after 3 days and to about 5-10% after 7 days of exposure to the metal (Fig. 2). The data summarized in Fig. 3 demonstrate that the rate of protein synthesis, as evaluated from the incorporation of labelled amino acids into the proteins of the gills, mantle and digestive gland, was significantly decreased (to about 50-70% of controls) after 7 days of exposure to the metal. With regard to the

concentration of ATP, it was noticed that after 7 days of exposure to the metal, a considerable decrease (from 30 to 40%) of this high-energy compound took place in all the tissues examined (Table below).

Tissues of *Mytilus galloprovincialis*	days of exposure to Cu^{2+} (0.08 ppm)				
	-	3		7	
Gills	0.89	0.81	(- 9%)	0.64	(-28%)
Digestive Gland	1.41	1.23	(-13%)	0.99	(-30%)
Mantle	2.60	2.10	(-20%)	1.77	(-32%)

The ATP content in the tissues examined is expressed as umoles of ATP/g wet weight. The values shown are the means of at least 4 determinations.

The results obtained demonstrate that Cu^{2+} can influence the protein metabolism, reducing the rate of two correlated processes, namely the uptake of amino acids from the environment and the rate of protein synthesis, which both require a high availability of ATP.

The modifications observed could therefore depend, at least, upon the decreased concentration of tissue ATP.

The results obtained suggest that Cu^{2+} can exert a detrimental effect on the growth and survival of mussels.

As far as the second aspect of the problem is concerned, our data indicate that Cu^{2+}, when present in sea-water at a concentration of 0.08 ppm, induces in the gills of the mussels the rapid biosynthesis (within the 48th hour) of proteins capable of binding the metal.

These proteins, which were separated on a Sephadex G-75 column and identified on the basis of their high copper content, have a molecular weight of about 11.000-12.000, similar to that of thioneins and chelatins.

As can be seen from Fig. 4, the concentration of these proteins (and of the copper associated with them which permits their identification) is very low in the gills of the controls, but increases more than 20 times after only 48 hours of exposure to the metal (Fig. 5).

These results could indicate that copper stimulates the synthesis of low molecular weight copper-binding proteins in the gills of mussels. Further studies with ^{35}S labelled cysteine as a precursor amino acid strongly support this assumption. Actually, it has been found that the incorporation of ^{35}S cysteine into the 12.000 molecular weight fraction from the gills of copper-exposed mussels is 7-10 times greater than in controls (Fig.6). In addition, in some preliminary work it has been shown that the sulphydrylic concentration in the 12.000 molecular weight fraction obtained from the gills

of mussels exposed for 48 hrs to copper is increased (Fig.7). Taken together, our results demonstrate that copper ions rapidly induce the synthesis of low molecular weight, sulphydryl-rich, copper-binding proteins in the gills of the mussels. The induction of these thionein-like proteins not only indicates the presence of Cu^{2+} in the environment, but probably also represents a detoxification mechanism against the cytotoxic effects of the metals.

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PUBLICATIONS

The results obtained through MED POL IV have been presented in manuscripts:

VIARENGO, A., PERTICA, M., MANCINELLI, G., CAPELLI, R., and ORUNESU, M. Effects of Copper on the Uptake of Amino Acids, on Protein Synthesis and on ATP Content in Different Tissues of Mytilus galloprovincialis Lam. Marine Environmental Research. 4, 145-152 (1981)

VIARENGO, A., PERTICA, M., MANCINELLI, G., PALERMO, S., and ORUNESU, M. Rapid Induction of Copper-Binding Proteins in the Gills of Metal Exposed Mussels. COMP. BIOCHEM. PHYSIOL., 67 C, 2, 215-218 (1980)

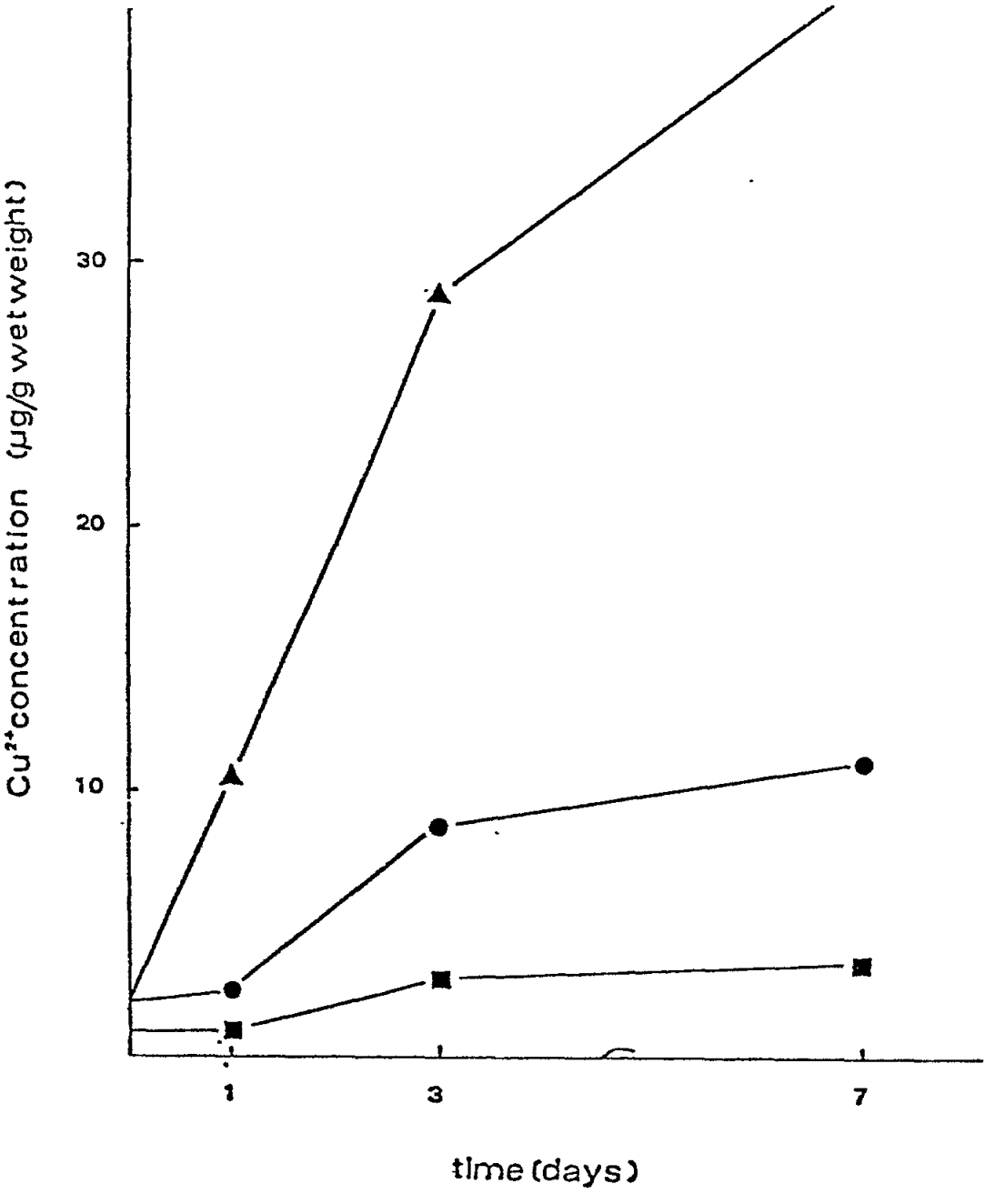

Fig. 1. Copper concentration in the gills (▲——▲), digestive gland (●——●) and mantle (■——■) of mussels exposed to the metal (0.08 ppm) from 1 to 7 days. The values shown in the figure are the means of at leat 4 determinations

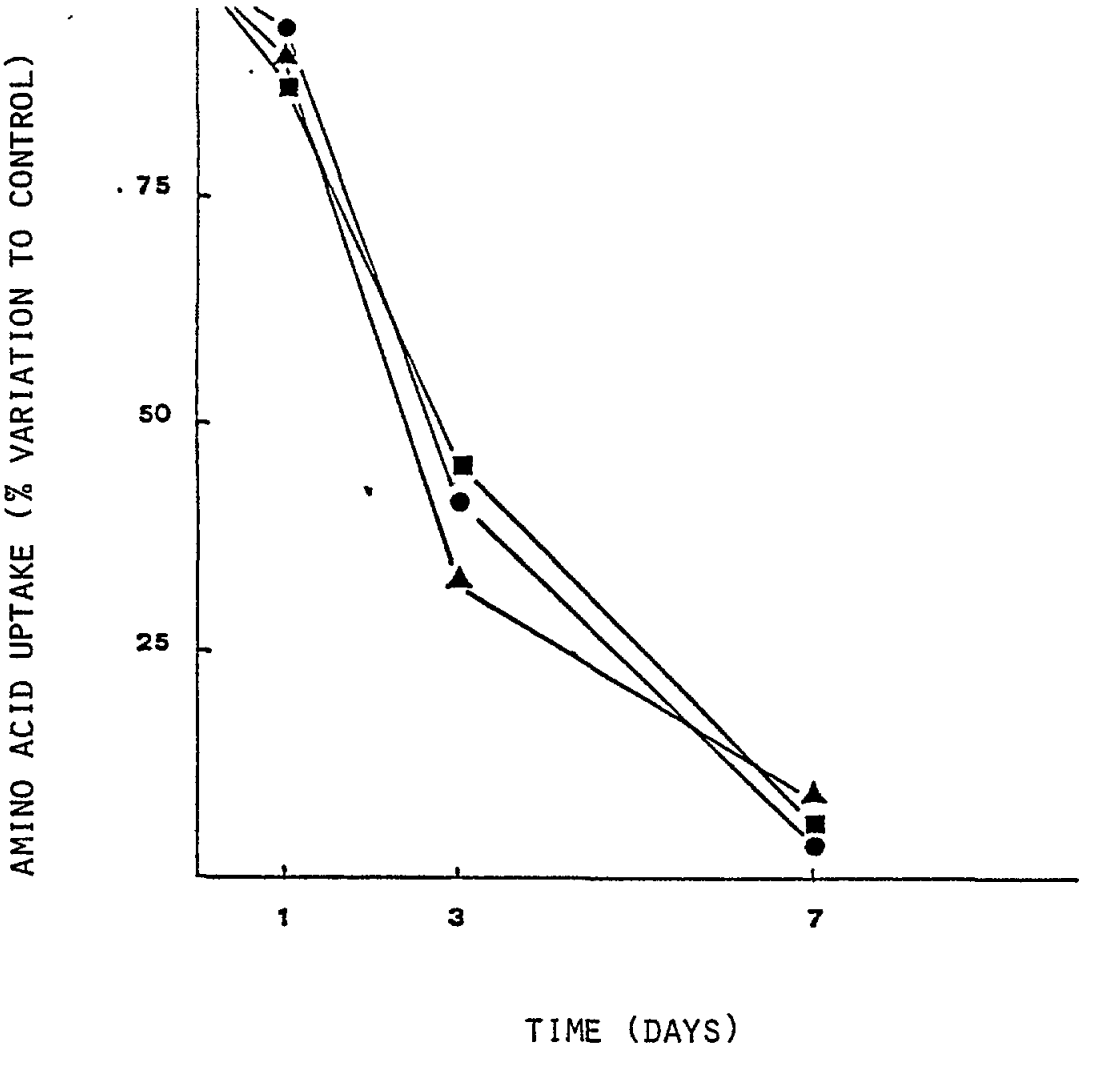

Fig. 2. Uptake of ^{14}C labelled amino acids by the gills (▲——▲), digestive gland (●——●) and mantle (■——■) of mussels exposed to Cu^{++} (0.08 ppm) for different periods of time. The mussels were exposed, at the indicated times, to 7.5 µCi of ^{14}C labelled amino acids for two hours and then the radioactivity present in the examined tissues was detected as described in Methods. The amino acid uptake is expressed as percentage of the response of the control group. The control values for the amino acid uptake (expressed as cpm/100 µl of homogenate) by the gills, digestive gland and mantle are 38.382, 18.696 and 3.129, respectively. The values represent the means of at least 4 experiments.

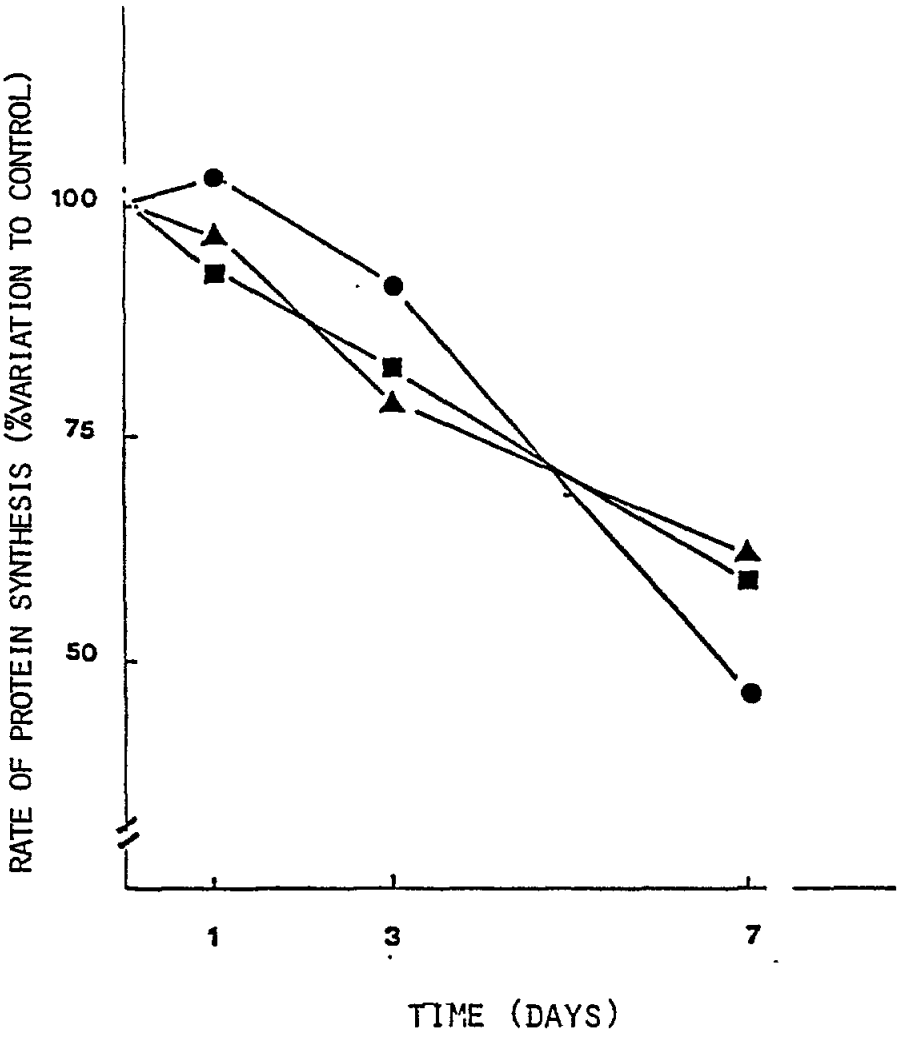

Fig. 3. Rate of protein synthesis in the gills (▲——▲), digestive gland (●——●) and mantle (■——■) of mussels exposed to Cu^{++} (0.08 ppm) for different periods of time. Two hours before death each mussel was injected with 2 µCi of ^{14}C leucine into the posterior adductor muscle. The rate of protein synthesis has been evaluated as described in Methods and is expressed as percentage of the response of the control group. The control values of the rate of protein synthesis, expressed as cpm per mg protein/cpm per 100 µl of homogenate, were as follows: gills = 0.332, digestive gland = 0.179 and mantle= 0.144. The values shown are the means of at least 4 experiments.

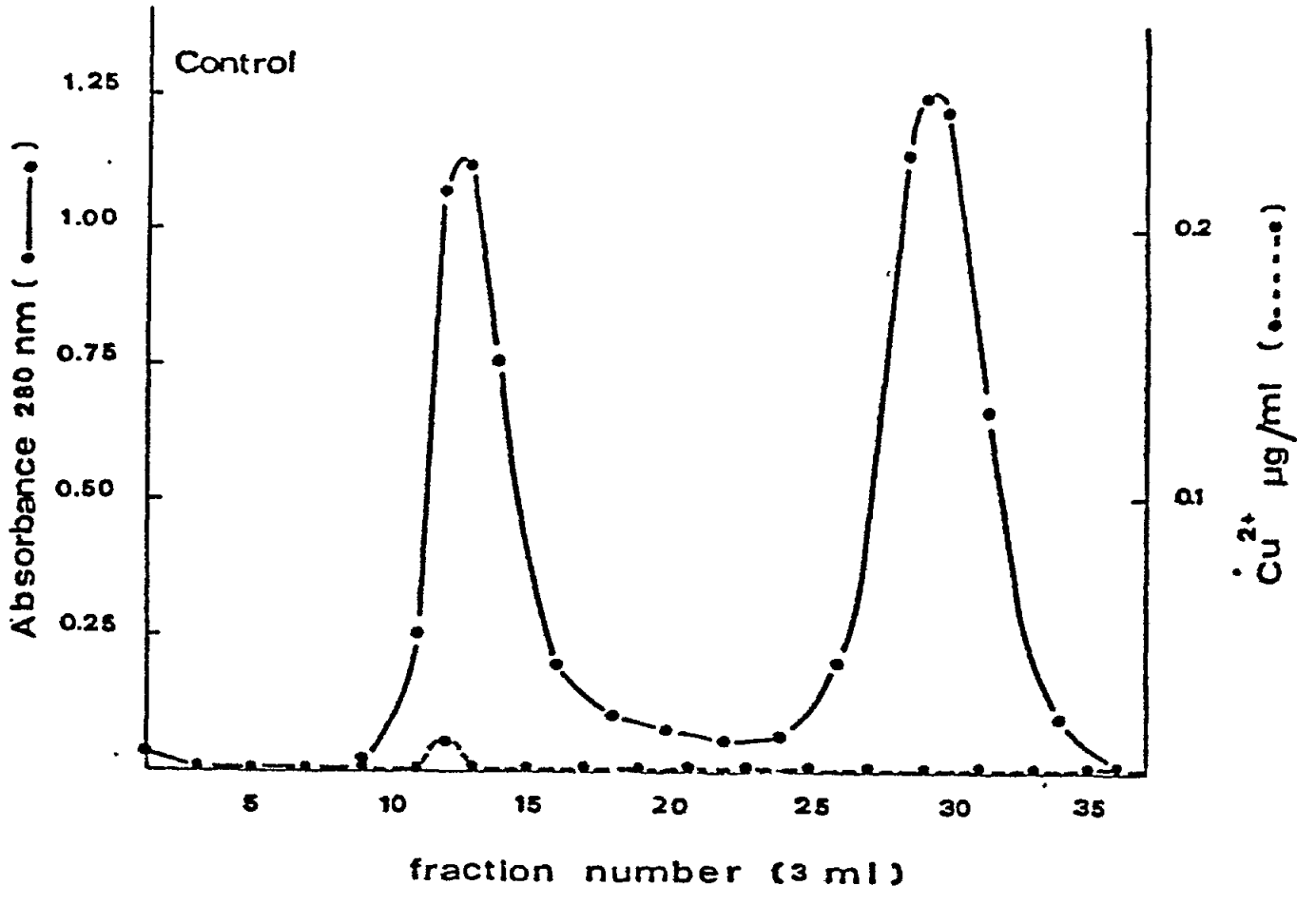

Fig. 4. Sephadex G-75 chromatography of gill preparations from control mussels. The experimental conditions were those described under Methods. Absorbance at 280 nm (●——●) and copper content (●- - - -●) were monitored.

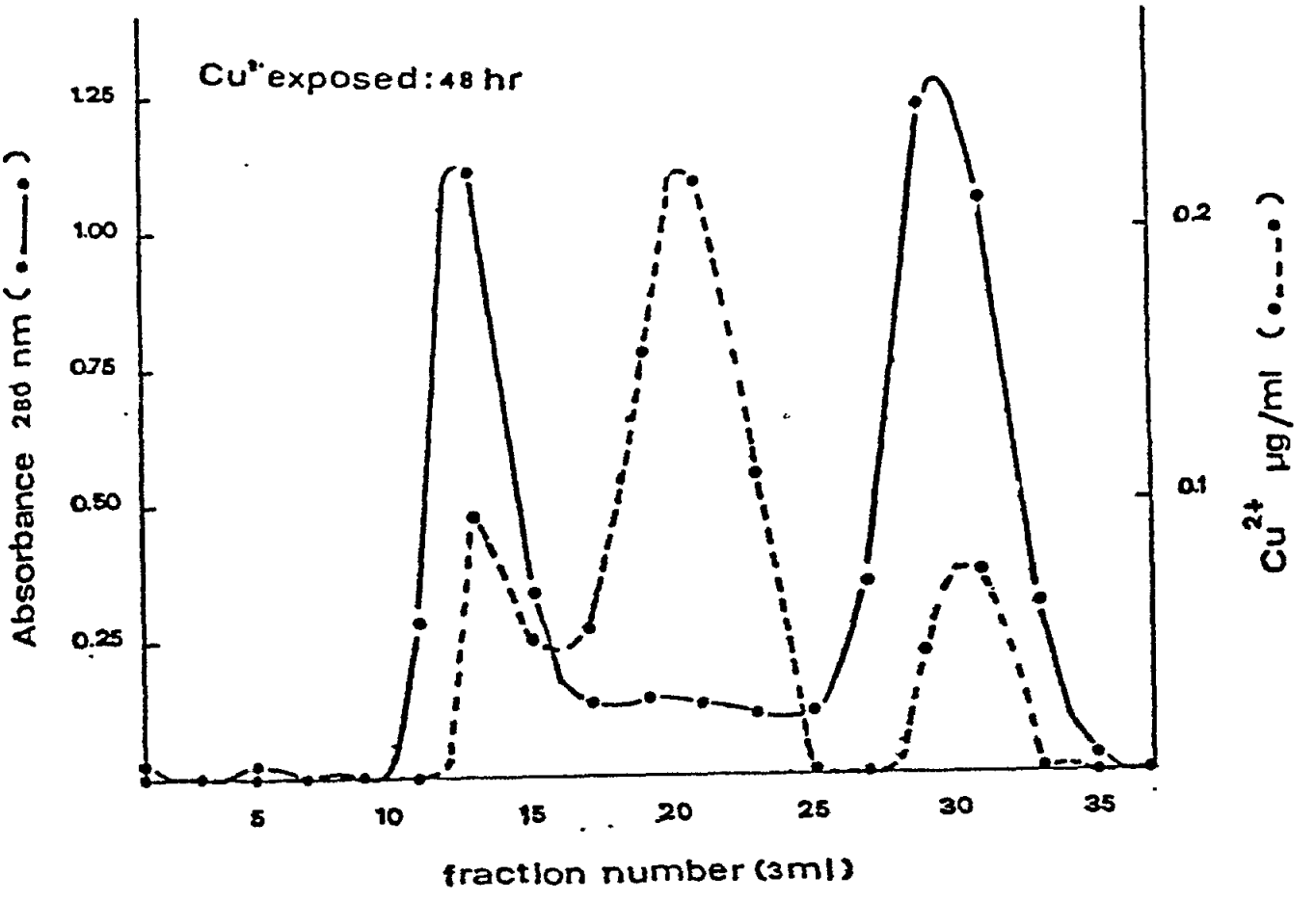

Fig. 5. Sephadex G-75 chromatography of gill preparations from mussels exposed to Cu^{2+} (0.08 ppm) for 48 hrs. The experimental conditions were those described under Methods. Absorbance at 280 nm (●——●) and copper content (●- - -●) were monitored.

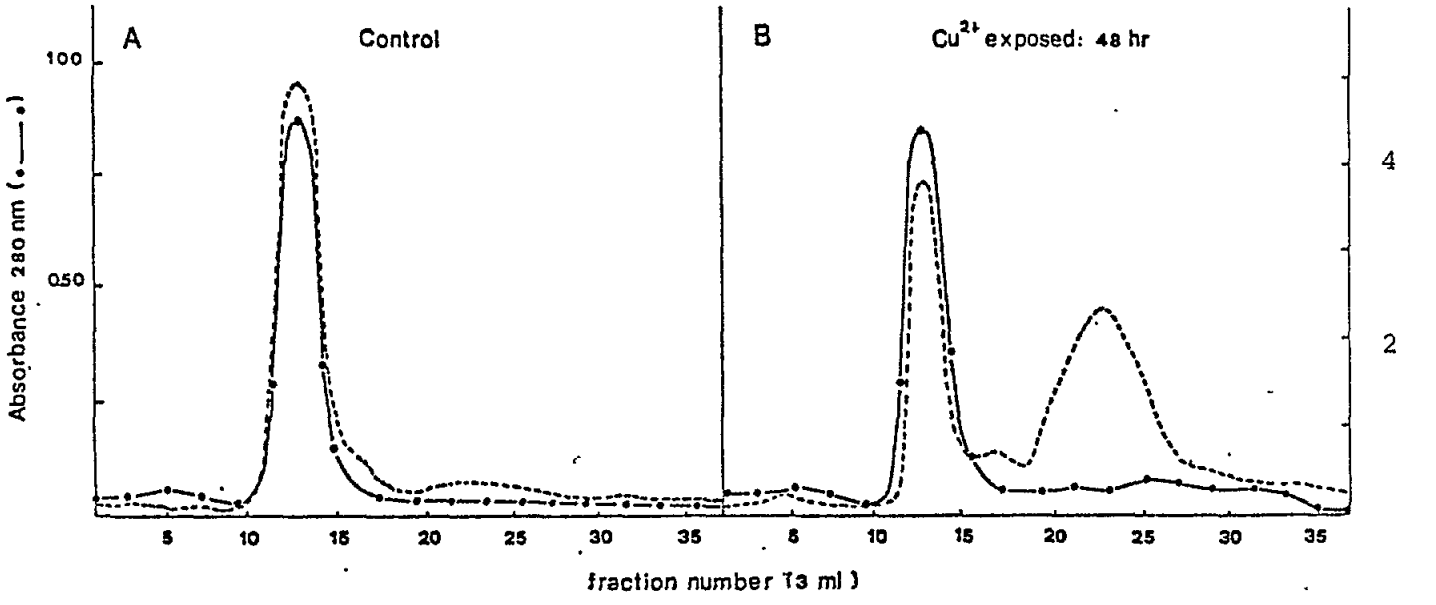

Fig. 6. ^{35}S cystaine incorporation into soluble proteins from the gills of the controls and 48 hrs Cu^{2+} exposed mussels. The raidiolabelled precursor (2 µCi/animal) was injected into the posterior adductor muscle 7 hrs before death. The gill soluble fractions were dialyzed overnight against 10 mM potassium phosphate pH 7.8 to remove all free amino acids and then chromatographed on a Sephadex G-75 column as described in Methods. Fractions of 3 ml were collected and analysed for absorbance at 200 nm (•——•). Aliquots of 1,5 ml were used for the determination of ^{35}S radioactivity (- - - - - -)

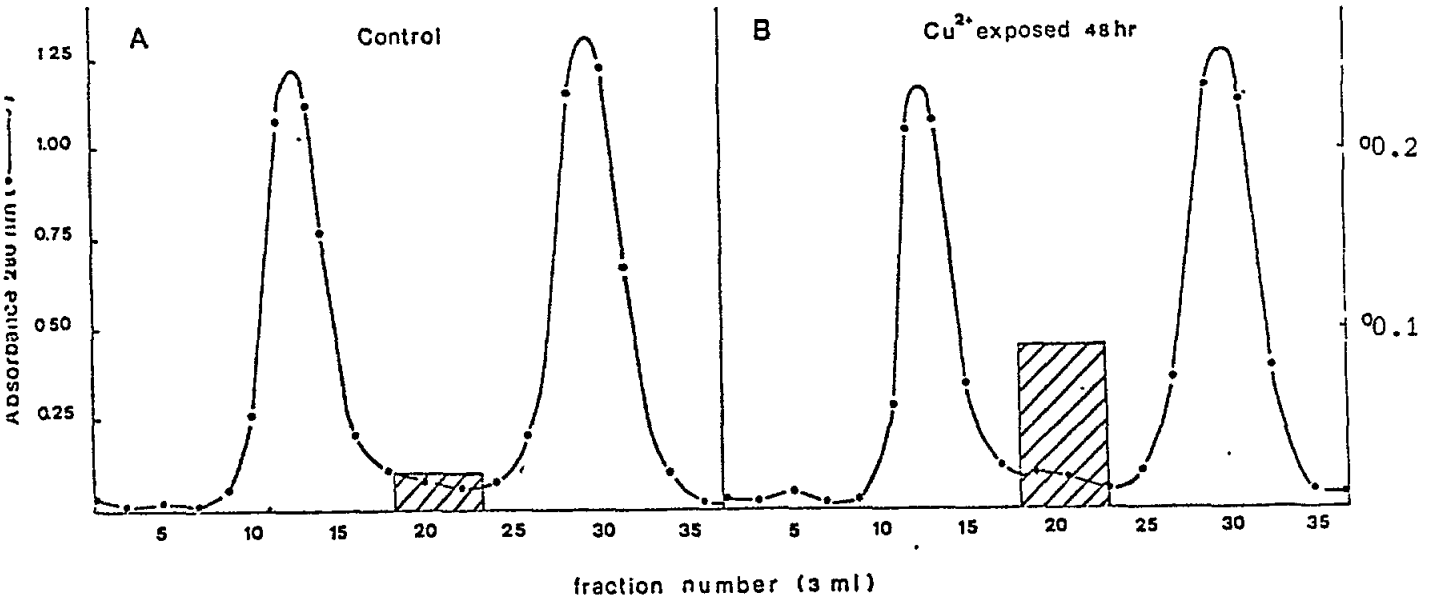

Fig. 7. Comparison of relative sulphydryl group concentration in the fractions 18 to 23 from controls (Panel A) and 48 hrs Cu^{2+} exposed mussels (Panel B). The fractions 18-23 (18 ml) were pooled and concentrated to a volume of 3 ml by membrane filtration. The concentrated solution was assayed for the determination of sulphydryl groups with DTNB reagent (5,5'-dithiobis (2-nitrobenzoic acid))

Research Centre: University of Malta,
MSIDA,
Malta

Principal Investigator: L.J. SALIBA (January 1976 - May 1980)
V. AXIAK (May 1980 - March 1981)

Period of Reporting: January 1976 to March 1981

INTRODUCTION

Research work on the effects of pollutants on marine organisms and their populations was commenced by the Research Centre in mid-1972, and a regular programme, involving both research by full-time staff members, and research performed by postgraduate students for their M.Sc. degree thesis, has been in progress ever since. This research is geared to provide the following data:

- toxicological data on lethal and sub-lethal effects of pollutants in general (e.g. heavy metals) to assess potentialities of acquaculture under local conditions;
- toxicological data on oil and oil dispersant mixtures to enable the most effective control of oil slicks on sea and on beaches compatible with preservation of marine life;
- measurable sub-lethal effects of the most common pollutants to develop simple bioassay techniques for monitoring purposes.

METHODOLOGICAL CONSIDERATIONS

Selection of the species:

Bacillariophyta: Phaeodactylum tricornutum

Crustacea: Artemia salina (all stages), Palaemon elegans (adults, larvae), Orchestia mediterranea (adults).

Mollusca: Monodonta articulata (adults), Monodonta turbinat (adults), Murex trunculus (adults), Patella lusitanica (adults).

Echinodermata: Arbacia lixula (adults), Paracentrotus lividus (adults).

Osteichthyes: Coryphaena hippuris (adults), Boops boops (adults), Mugil cephalus (adults).

Pollutants analysed:

(a) Heavy metals:

Mercury: sulphate, acetate, chloride, nitrate;

Cadmium: sulphate, acetate, chloride, nitrate;

Copper: sulphate, acetate, chloride, carbonate.

(b) Pesticides: Aldrin, Dieldrin, DDT, Permethrin.

(c) Crude Oil: Bunker C. Abu Dhabi Crude

(d) Oil Dispersants: Petrocon, Emken Spillwash LT, Fleetex BD, Kraken MC 563, Servo CD 200, Ardrox SR 61, Perolin PK 868, Shell Dispersant LTX, Rochem OSR, Allexhem 33, Climel 968, Nomar SOS, Servo CD 1000, Shell Dispersant X, TC 1000, Tretol, Shell S22 LT, Oil Slick Solvent (OSS), Lankromvl OSD, Moaslick Premium, Emken, Slickgone LTD, BP 100X, Symperonic OSD20.

METHODOLOGY

- For all experiments, sea-water was collected from Marsaxlokk Bay (S.E. Coast of Malta) or St. Andrews (N.E. Coast of Malta). No industrial or other effluents in vicinity. Salinity 37.5°/oo. Sea-water sterilized for 12 hours at 70°C passed through 0.45 um oxoid filter before use.

- Artemia salina reared from commercially obtained eggs. Phaeodactylum tricornutum obtained as culture from U.K. All other animals collected from coastal waters or beaches in adult stage.

- Test concentrations prepared by dissolving pollutants in sea-water to give required concentation in ppm (mg/l or ml/l). For heavy metal salts, concentration always calculated in terms of metal ion on basis of atomic weight. All toxicity and behavioural experiments conducted in static conditions. Concentrations (e.g. solutions) renewed at appropriate intervals.

- Studies on immersion/emersion behaviour in Mondonta articulata and M. turbinata carried out by use of aktograph described in paper by Saliba & Vella (1977).

  Oxygen consumption measured by method described in same paper.

- Behavioural experiments on Arbacia lixula utilized spring balance to determine stress required for dislodgement of animal from substrate.

  Behavioural experiments on Paracentrotus lividus and Monodonta turbinata involved direct observation.

- Growth and multiplication of Phaeodactylum tricornutum carried out by culturing under aseptic conditions in nutrient medium, and performing cell counts by use of a Coulter Counter.

  Primary production measured by analysis for chlorophyll-a, method as described by Strickland and Parsons (1972), obtaining extraction coefficient by absorbance readings at 665, 645, 630 and 750 nm. Following initial samplings pollutants added, held for 48 hours in thermostatically-controlled room with 12-hour photoperiod of 223 Lux (Lumens/m^2). Samples lifted after 24 and 48 hours.

- Effects of Permethrin on fish muscle enzymes carried out in vitro. LDH, MDH, SDH and COX studied in homogenates of red and white muscle after centrifugation of neceic and cellular debris. PK partially purified from white muscle. Kinetic parameters of enzymes determined spectro-photometrically.

  Effects on haemocyanin in Murex trunculus observed by draining and diluting haemocyanin, adding pesticide concentrations, and reading at 280 nm and 346 nm.

RESULTS

Artemia salina

Mercury:

Mercuric sulphate, acetate, and chloride reduced percentage of hatched eggs by 50% - 95% at 1.0 ppm. Reductions were also evident at concentrations down to 0.1 ppm.

Acetate appeared to exercise the greatest inhibition, sulphate the least. Exposure to 0.1, 0.005 and 0.0025 ppm mercury (as sulphate, acetate and chloride) did not result in growth differentiation, but all concentrations caused considerable larval mortality. Acute toxicity studies on 2-week old larvae with all three salts, using concentrations of 0.1, 0.5 and 0.25 ppmHg ion, gave LT_{50} values ranging from 13.5 hours to 26.5 hours. Mercuric sulphate was the most toxic, mercuric acetate the least.

Cadmium:

The percentage of hatching was reduced by 20% to 60% at concentrations of 1.0 ppm cadmium ion, in the three salts tested - sulphate, acetate and chloride. Lesser reductions were observed at lower concentrations down to 0.05 ppm. Sulphate appeared to be the most toxic salt, acetate the least. Exposure to cadmium sulphate, acetate and chloride showed slight growth inhibition in larvae, and extensive mortality as compared with controls. Acute toxicity studies on 2-week-old larvae with all three salts at 1.0, 0.5 and 0.025 ppm Cd ion gave LT_{50} values ranging from 50 to 120 hours, indicating that cadmium salts have no acute toxic effect as compared to mercury salts.

Copper:

Cupric sulphate caused no significant inhibition of hatching at 0.5 and 1 ppm, but inhibited hatching by 85% at 5 ppm. Exposure of larvae to cupric chloride, acetate, sulphate and carbonate, at concentrations of 0.1, 0.05 and 0.025 ppm inhibited growth rate, the degree of inhibition being directly proportional to the concentration. In acute toxicity tests with cupric chloride, acetate, and sulphate, at concentrations between 1 and 10 ppm Cu ion, LT_{50} values ranged from 10 to 35 hours. Cupric acetate was the most toxic, cupric chloride the least. Differences in mortality between larvae acclimated in low concentrations (0.1 - 0.025 ppm) and those reared in sea-water showed that (except with copper acetate), previous exposure results in an increased degree of tolerance.

Oil Dispersants:

Tests with 13 oil dispersants on Artemia salina adults gave 24-hour LC_{50} values between 1 and 500 ppm, and 48 hour LC_{50} values between 0.5 and 250 ppm.

Crude Oil:

Larval mortality on exposure to the water-soluble fractions of surface and sunken crude oil was studied. After 8 days, mortalities in surface and sunken oil WSFs were 80% and 65% as compared to 37% in sea-water controls.

Orchestia mediterranea

Oil Dispersants:

Tests with 18 oil dispersants on Orchestia mediterranea adults gave 24-hour LC_{50} values between 25 ppm and 7200 ppm, and 48-hour LC_{50} values between 15 ppm and 200 ppm.

Palaemon elegans

Copper:

Acute toxicity tests with copper sodium citrate on newly hatched larvae of Palaemon elegans gave 24 hour LC_{50} values of 86 ppm (20°C) and 46 ppm (23°C), and 48-hour LC_{50} values of 25 ppm (20°C) and 25 ppm (23°C). Similar tests with mercuric sulphate, acetate and chloride on adults gave 24 hour LC_{50} values between 0.6 and 1.5 ppm, and 48-hour LC_{50} values between 0.3 and 0.5 ppm (all at 20°C).

Patella lusitanica

Mercury:

Acute toxicity tests with mercuric chloride, acetate and nitrate on adults gave 24-hour LC_{50} values between 38 and 48 ppm, and 48-hour LC_{50} values between 20 and 28.5 ppm. Mercuric acetate was the most toxic.

Cadmium:

Acute toxicity tests with cadium chloride, acetate, sulphate and nitrate on adults gave 24-hour LC_{50} values between 39 and 60 ppm, and 48-hour LC_{50} values between 21.5 and 29 ppm. Cadmium sulphate was the most toxic, followed by cadmium acetate.

Monodonta articulata

Mercury:

Acute toxicity studies on adults with mercuric sulphate acetate and chloride gave a 24-hour LC_{50} value of 8 ppm and a 48-hour LC_{50} value of 6 ppm for sulphate. Values for acetate and chloride were over 10 ppm. Initial toxic symptoms observed were the retraction of the snail inside the shell. Mercuric sulphate was the most toxic of the three salts. Effects of the three salts on immersion/emersion behaviour and interface activity showed an increase in total emersion periods over 24 hours and a decrease in interface activity, as the concentration increased between 0.03 and 1.0 ppm. Tests on effects of the three salts on acquatic oxygen consumption showed a progressive decrease, starting at 0.01 ppm. Sulphate gave the greatest effect, acetate the least.

Cadmium:

Acute toxicity studies with the acetate chloride and sulphate showed that with cadmium sulphate the 24-hour LC_{50} value is over 10 ppm, and the 48-hour LC_{50} value is 8 ppm. With acetate and chloride, both 24- and 48-hour LC_{50} values were above 10 ppm. Toxic symptoms observed were the same as for mercury. Effects on immersion/emersion ratio and interface activity at 24-hour exposures to concentrations of 0.01 and 1.0 ppm showed a general increase in both the emersion and interface periods, which was independent of the concentration. None of the three salts, at concentrations between 0.01 and 1.0 ppm, appeared to affect aquatic oxygen consumption.

Monodonta turbinata

Crude oil:

Acute toxicity studies on the effects of surface and sunken crude oil on adults showed LT_{50} values as being 68 hours for 20 ppm toxicity, decreasing with lesser concentrations, in the case of sunken crude oil, and 120 hours for 20 ppm of surface crude oil. This experiment cannot, however, be seen in the same light as other acute toxicity tests, as the oil was not uniformly distributed throughout the sea-water. Toxicity symptoms observed were inversion of the snail, as compared with retraction in M. articulata on exposure to mercury and cadmium. Sublethal effects studied included effects on gregarious behaviour-exposure to oil causing the animals to remain solitary, as opposed to their normal clustering. Effects of crude oil on immersion/emersion behaviour increased the percentage of time spent at the interface from 5% to 56%, immersion time being moderately, and emersion time drastically, reduced. Fractional distillation of the oil, and exposure to different fractions, showed that the interface period increased with rise in boiling point, up to that over 300°C.

Dispersant - Oil Mixtures:

Acute toxicity studies of various proportions of dispersants and oil (250 ppm dispersant: 250 ppm oil, 50 ppm dispersant: 250 ppm oil and 25 ppm dispersant: 250 ppm oil. These ratios and concentrations were used both for the lethal and sublethal studies) as well as of dispersants alone, showed that increased mortalities were recorded with increase in dispersant concentration. Toxicity of the dispersant-oil mixtures was greater than that of either oil or dispersant alone. Thus for Petrocon International the LT 50 in hours recorded on exposure to dispersant/oil mixture (1:1), oil alone (250 ppm) and dispersant alone (250 ppm) were: 26.4, 76.8 and 37.2 respectively. On increase in dispersant concentrations in the dispersant-oil mixtures, two sublethal effects were recorded, namely: a general increase in total emersion periods over 24 hours as compared to exposure to surface oil in immersion/emersion activity of snails, and a decrease in acquatic oxygen consumption.

Heavy metals:

Acute toxicity studies of mercuric sulphate at different temperatures gave 24-hour LC_{50} values of 2 ppm at 30°C, 91 ppm at 25°C, 98 ppm at 20°C, and 100 ppm at 15°C. The mortality data was analysed by a 2 way ANOVA (temperature X concentration) and the synergistic effect was found to significant. AAS analysis indicated no appreciable loss of mercury salts from the test solutions over the exposure period of 24 hours. Similar toxicity studies using cadmium sulphate gave 96-hour LC_{50} values of 2 ppm at 30°C, 3 ppm at 25°C, 3 ppm at 20°C, and 10 ppm at 15°C. Again a 2 way ANOVA showed that, concentration, temperature and the interaction between them were all significant.

Murex trunculus

Pesticides:

Results obtained indicated that up to 5 ppm, aldrin and dieldrin have no apparent effect on the copper binding property of haemocyanin.

Since this is involved in oxygen binding, this infers that these pesticides show no effect on oxygen transport in animals having haemocyanin as the oxygen carrier.

Arbacia lixula

Mercury:

Acute toxicity studies on adults with mercuric sulphate gave 24-hour LC_{50} values of 1.5 pmm Hg, 48-hour LC_{50} values of 0.5 ppm Hg, and 72-hour LC_{50} values of 0.35 ppm Hg. Adults subjected to concentrations of 0.1 to 0.5 ppm Hg showed cytdysis, resulting in release of pigment to the external medium. This release was found to be concentration-dependent. It could not be determined whether the pigment involved was echinochrome, spinochrome or melanin. At the same concentrations, the power of adhesion of the tube-feet is also quickly lost, and at lower concentrations, down to 0.5, the adhesive powers are affected, and reduced. The degree of reduction is proportional both to concentration and to time of exposure.

Paracentrotus lividus

Mercury:

Acute toxicity tests on adults with mercuric sulphate, chloride and acetate gave 24-hour LC_{50} values between 0.5 and 1.5 ppm, and 48-hour LC_{50} values between 0.4 and 0.8 ppm. Mercuric chloride was the most toxic.

Cadmium:

Acute toxicity tests on adults with cadmium chloride, sulphate, nitrate and acetate gave 24-hour LC_{50} values between 5.0 and 6.0 ppm, and 48-hour LC_{50} values between 2.2 and 3.5 ppm. Cadmium nitrate appeared to be the most toxic, followed by cadmium acetate.

Crude oil:

Adults to whose tests crude oil was applied, when placed in sea-water with the oral side uppermost, took a much longer time to right themselves than normal control animals.

Coryphaena hippuris, Boops boops and Mugil cephalus

Pesticides:

(Permethrin): At concentrations of 1 and 5 ppm, there appeared to be no effect on substrate affinity. In the fish muscle enzymes studied in vitro, the pesticide raised the maximum velocity of pyruvate kinase and malate dehydrogenase, and suppressed the maximum velocity of succinate dehydrogenase and cytochrome oxidase. On average, there was no effect on lactate dehydrogenase. Soluble and insoluble forms of acethylcholinesterase from the brain were found to be unaffected.

Phaeodactylum tricornutum

Mercury:

Experiments on the effects of 4 mercuric salts (chloride, acetate, sulphate and nitrate) on growth and multiplication gave no meaningful results, the variations recorded having no bearing either on the presence of the pollutant or on its concentrate. This is ascribed to possible instrument malfunction, and the experiment could not in fact be repeated for this reason. Exposure of Phaeodactylum tricornutum to low concentrations of the same four salts (0.01 to 3.0 ppm) showed considerable decreases in chlorophyll-a production after 24 hours, even at the lowest concentration of 0.01 ppm. The decrease, both in absolute terms, and relative to untreated controls, was accentuated after 48 hours, and inhibition was proportional to the concentration. Of the four salts, mercuric acetate was the least toxic.

Cadmium:

Exposure to cadmium acetate, chloride, sulphate and nitrate at concentrations between 5 and 75 ppm cadmium ion resulted in decreased growth and multiplication as compared to the controls. Some differences between the four salts were observed though these were not very marked. At 5 ppm, increased cell counts were recorded at 24 and 48 hours, but these were less than those in the sea-water controls, and the differences became more at 48 hours. From 25 ppm upwards, cell numbers decreased with exposure, the decrease being directly proportional both to exposure period and to concentration. Exposure to the same four salts at the same range of concentrations showed decreases in chlorophyll-a production both after 24 and after 48 hours, as compared to the controls. Inhibition was directly proportional to concentration, and no apparent differences between the four salts were observed.

DISCUSSION OF RESULTS

Heavy metals:

The results of both acute toxicity and sub-lethal tests showed that mercury is more toxic to marine life (on the basis of the species studied) than other metals. Sensitivity varied among the species tested, but in all cases, the LC_{50} or LT_{50} values for mercury were much lower than those for cadmium, and other metals (such as copper, lead, iron and zinc) which had been tested in previous work. In particularly sensitive organisms, no apparent difference between the various salts of mercury were recorded, but in the more resistant, such as Monodonta articulata, differences between the salts started to become obvious. This supports the theory that the form of the metal, as well as the metal ion itself, is a factor to be considered in assessing the toxicity of a pollutant. The sub-lethal effects recorded show that simple bioassay techniques for determination of relatively low concentrations of mercury in sea-water could possibly be developed, using Monodonta articulata, Paracentrotus lividus, Arbacia lixula or Phaeodactylum tricornutum as the test organism. It is not considered that cadmium offers such possibilities, as the concentrations required to produce readily observable and/or measurable effects are much higher. Copper offers some possibilities, principally in connection with growth-rate inhibition, but these effects could easily be masked.

Crude oil:

The sub-lethal effects of pollution by crude oil showed that these can be significant where coastal species are concerned. In the case of actual oil on the surface, species moving through the air/water interface, such as Monodonta, appear to be the most susceptible. The effects of water-soluble fractions as shown by the results obtained should be seen in the light of the fact that the experiments were conducted in static systems, and conditions would be much different in the sea itself.

Oil dispersants:

Acute toxic effects of the several dispersants tested on Artemia salina and Orchestia mediterranea showed a wide range of toxicity. These tests are useful in demonstrating the relative effect of different dispersants under Mediterranean conditions. Although modern techniques for assessing dispersant toxicity compare oil/dispersant mixtures with oil alone, in practice, in oil pollution control at sea, dispersants are applied even in areas where no oil is actually present (especially when the oil is in isolated patches) and a large amount of dispersant does not encounter oil at all. The absolute toxicity of dispersants, i.e. on their own, is therefore also necessary as a subject of study. On the whole, the toxicities recorded for the dispersants were higher than those officially claimed in the countries of origin (mainly northern Europe).

Dispersant - oil mixtures:

Studies on Monodonta turbinata indicate greater toxic effects of such mixtures than of oil or dispersants alone.

Pesticides:

Studies on these were limited to a few biochemical effects, and results indicated that enzyme systems are subject to some alterations following exposure to organochlorine pesticides.

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Centre de recherche:	Institut Scientifique de Pêches Maritimes CASABLANCA Maroc
Chercheur principal:	A. N'HILA

L'institut a singé l'accord de coopération le 23 janvier 1978 mais il était dans l'impossibilité de participer au projet pilote et, en conséquence, il a annulé son contrat le 23 février 1980.

Centro de investigación: Instituto de Investigaciones Pesqueras
CADIZ
España

Investigador principal: R. ESTABLIER

INTRODUCCION

El Instituto de Investigaciones Pesqueras de Cádiz viene realizando estudios sobre la acumulación de diversos metales (Cu, Fe, Mn, Zn, Hg y Cd) por organismos marinos desde el año 1967. Habiéndose estudiado también la acumulación cuantitativa de Cu, Fe, Mn y Zn en diversos órganos del ostión (Crassostrea angulata) y Ostra (Ostrea edulis) y la concentración letal media del Cu, Zn y Cd sobre la C. angulata.

Asímismo se han realizado estudios sobre la actividad de las valvas (registrada con un quimógrafo) de la C. angulata cuando estos moluscos son sometidos a diferentes concentraciones de metales.

CONSIDERACIONES METODOLOGICAS

Selección de especies:

Las especies que se han utilizado en los bioensayos han sido las siguientes:

Peces: Lisa (Mugil auratus), Dorada (Sparus aurata), Róbalo (Dicentrachus labrax) y Pez Sapo (Halobatrachus didactylus)

Crustáceos: Langostino (Penaeus kerathurus) y Camarón (Palaemonetes varians).

Moluscos: Choco (Sepia officinalis)

En los bioensayos se han utilizado estas especies por ser las únicas de las cuales se podían obtener ejemplares vivos y también por su posibilidad de ser cultivadas y obtener huevos y larvas.

Contaminantes estudiados:

En los experimentos se han utilizado los siguientes metales pesados y sus derivados: cloruro de mercurio ($HgCl_2$), metilmercurio (CH_3HgCl), cloruro de cadmio ($CdCl_2.H_2O$) y sulfato de cobre ($CuSO_4.5H_2O$).

METODOLOGIA

Los análisis de metales se han realizado por espectrofotometría de absorción atómica.

Los resultados de los efectos biológicos producidos por la acumulación en diversos órganos de los distintos metales estudiados se han dado en los ensayos con cantidades subletales por las alteraciones histológicas producidas y en los bioensayos de toxicidad letal por los resultados obtenidos en la determinación de la concentración letal media (LC_{50})

RESULTADOS DE LOS EFECTOS Y COMENTARIOS

Un resumen de los resultados obtenidos tanto en las experiencias de toxicidad letal como en las de acumulación y efectos histopatológicos se dan a continuación:

Toxicidad letal:

a) Para las larvas y post-larvas de Penaeus kerathurus en experiencias hechas sobre 24 horas se han obtenido los siguientes valores medios de CL_{50} (expresados en mg/l):

	Hg (CH_3HgCl)	Hg ($HgCl_2$)	Cd ($CdCl_2.H_2O$)	Cu ($CuSO_45H_2O$)
Nauplius	0.0054	0.0052	0.937	0.103
Protozoea I	0.0046	0.0082	-	0.077
Protozoea II	0.0049	0.0075	1.305	0.081
Protozoea III	0.0035	0.0047	1.270	0.107
Mysis I	0.0071	0.0098	1.270	0.098
Mysis II	0.0098	0.0092	1.230	0.092
Mysis III	0.0071	-	-	-
Post-Larvas (P_1-P_3)	0.0220	-	1.640	-
Post-Larvas (P_4-P_6)	0.0469	-	4.890	1.470

b) Para las larvas de Sparus aurata (de 1 a 3 días), los valores del CL en experiencias realizadas con una duración de 24 horas han sido los siguientes: Hg ($HgCl_2$) 0.35 mg/l, Cd ($CdCl_2.H_2O$) 2.80 mg/l y Cu ($CuSO_4.5H_2O$) 0.27 mg/l.

c) Para las larvas de Sepia officinalis (de 1 a 2 días) los valores medios de la CL_{50} correspondientes a experiencias realizadas con una duración de 24 horas han sido los siguientes: CH_3HgCl 17.0-19.0 µg/l, Hg ($HgCl_2$) 23.7-28.0 µg/l, Cd ($CdCl_2.H_2O$) 6.0-8.0 mg/l y Cu ($CuSO_4.5H_2O$) 0.17 mg/l. De las experiencias realizadas sobre la influencia del Cu y Cd sobre la eclosión de los huevos de S. officinalis de 1 día se ve que, con respecto al Cd, no se observa ningún desarrollo de los huevos por encima de los 0.8 ppm, consiguiéndose el 50% de eclosión con concentraciones comprendidas entre las 0.4 y 0.8 ppm. En relación con el Cu, se ha podido observar que con concentraciones de 20 a 40 ng/l se producen eclosiones superiores a las del testigo. Con 80-160 ng/l disminuye sensiblemente el porcentaje de eclosión y se observa un acortamiento del período de incubación.

Acumulación y efectos histopatológicos:

a) Halobatrachus didactylus. Hg ($HgCl_2$):

8 ejemplares fueron tratados con 0.1 mg/l de Hg durante 10, 20 y 35 días. Se ha estudiado la acumulación de Hg y los efectos histopatológicos en la sangre, hígado, riñón y bazo. La acumulación máxima de Hg después de 35 días de tratamiento se ha detectado en el hígado (70.86 mg/kg de peso húmedo) seguido del riñón (39.84 mg/kg) del bazo (37.50 mg/kg) y de la sangre (3.69 mg/kg). Las alteraciones citohematológicas e histopatológicas más destacadas han sido: discreta eritroanisocitosis, eritrohipocromemia aislada y tendencia a la fragmentación con formación de eritroplastidos. En el hígado hay tumefacción, vacuolización en muchas zonas y aumento del número de núcleos. En el riñón se aprecia una progresiva vacuolización con despolarización de los núcleos y acúmulo de detritus eosinófilo que obstruyen la luz de los tubos renales.

b) Halobatrachus didactylus. Cd ($CdCl_2.H_2O$):

Se han realizado tres experiencias manteniendo 3 ejemplares en cada una con 50 mg/l de Cd durante 96 horas. Los valores medios de acumulación de Cd referidos a peso húmedo han sido los siguientes: músculo 0.15 mg/kg., sangre 1.20 mg/kg., hígado 5.21 mg/kg., riñón 12.79 mg/kg., intestino 39.05 mg/kg. Las alteraciones citohematológicas más importantes han sido; alteraciones de los eritrocitos, anisocitosis, anisocromemia, vacuolización, microcitosis, picnosis, poikilocitosis y signos de cariorreis. Las alteraciones histopatológicas más importantes han sido: en el intestino, pérdida de orientación de los núcleos del epitelio de las vellosidades, hipercromatismo, vacuolización y signos de necrosis. En el hígado, aspecto reticulado travecular y aumento del número de núcleos. En el riñón, núcleos a veces sin polarización basal, hipocromatismo, luces pequeñas o dilatadas llenas de un material amorfo eosinófilo y signos de degeneración tubular.

c) Dicentrachus labrax. Hg ($HgCl_2$):

10 ejemplares se trataron con 0.1 mg/l de Hg durante 18, 39, 48 y 62 dias. Se han realizado análisis de mercurio en el músculo, hígado, intestino, riñón, bazo, branquias y sangre. La acumulación máxima de mercurio después de 62 días de tratamiento se ha encontrado en el hígado (329.25 mg/kg de peso húmedo), seguido del riñon (176.50 mg/kg) y el bazo (125.00 mg/kg). Asímismo se ha observado que la acumulación de Hg en todos los órganos estudiados aumenta con el tiempo de exposición.

Las alteraciones citohematológicas observadas han sido: eritroanisocitosis, eritromacrocitosis, con modificaciones considerables de la morfología de los núcleos de las células sanguíneas. Se han apreciado también modificaciones histopatológicas en las branquias, hígado, riñón e intestino.

d) Mugil auratus. Hg ($HgCl_2$):

15 ejemplares se trataron con 0.10 mg/l de Hg durante 10, 24, 35, 46 y 56 días. Se ha visto que la acumulación máxima de Hg después de 56 días de tratamiento se produce en el hígado (101.23 mg/kg de peso húmedo) seguido de los ciegos pilóricos (20.90 mg/kg) e intestino (19/70 mg/kg).

Las alteraciones histopatológicas observadas han sido: en el hígado modificaciones de los cordones y vacuolización de los elementos del parénquima y en el intestino aumento del espesor del epitelio, desorganización en la situación de los núcleos, gran vacuolización y aumento de las células de corión.

e) Mugil auratus. CH_3HgCl:

12 ejemplares se han tratado con 0.008 mg/l de Hg durante 15, 30 y 45 días. la acumulación máxima de Hg después de 45 días se ha observado en el hígado (28.90 mg/kg de peso húmedo) seguido por el bazo (19.10 mg/kg), riñón (16.72 mg/kg) y los ciegos pilóricos (11.00 mg/kg).

Se ha observado que las alteraciones histopatológicas están en relación con el tiempo de exposición y la mayor acumulación de esta forma del mercurio. Los efectos más manifiestos se han detectado a nivel de hígado y riñón, branquias, intestino y estómago. En el hígado se observa tumefacción, gran vacuolización y aumento del número y grosor de los capilares con desorganización del parénquima. En el riñón hay extensa vacuolización con degeneración tubular, hemorragias y signos de glomerulonefritis.

f) Sparus aurata. Cu ($CuSO_4.5H_2O$):

6 ejemplares se trataron con 0.20 mg/l de Cu durante 77 días. La máxima acumulación de Cu se ha encontrado en el hígado (20.0 mg/kg de peso húmedo) seguida del bazo (8.92 mg/kg) y del intestino (2.36 mg/kg).

Las alteraciones histopatológicas observadas en el intestino han sido una gran desorganización del epitelio con basal continua, aumento de la capa epitelial, vacuolización manifiesta y corión reducido.

g) Penaeus kerathurus. Cd ($CdCl_2.H_2O$):

Se realizaron 5 experiencias manteniendo en cada una de 5 a 10 ejemplares con 0.8 mg/l de Cd. Los valores medios de la acumulación de Cd en hepatopáncreas y músculo han sido de 319.64 y 7.92 mg/kg de peso húmedo, lo que representa un aumento de la concentración de Cd en el músculo de unas 15 veces mientras que en el hepatopancreas el aumento es de unas 250 veces sobre los valores encontrados en el control. Desde el punto de vista histopatológico se ha observado que los tubos del hepatopáncreas están muy distendidos, adoptando una forma anular, con paredes delgadas y luz circular que afectan a un 65% de los mismos.

Acumulación y efectos histopatológicos.

h) Mugil auratus - Cd ($CdCl_2H_2O$):

Se han realizado experiencias a corto plazo (96 horas) manteniendo ejemplares de M. auratus en agua de mar con 25 ppm de Cd y a largo plazo manteniendo ejemplares de esta especie en agua de mar con 2 ppm de Cd durante 21, 50 y 62 días.

Después de 96 horas con 25 ppm de Cd la máxima acumulación observada ha sido en el hígado (81.70 mg/kg sobre peso húmedo) seguido del

riñón (68 mg/kg) e intestino (61.20 mg/kg). En las experiencias a larga duración las acumulaciones máximas se han observado, al cabo de 62 días de tratamiento en el hígado (349.00 mg/kg sobre peso húmedo) e intestino (124.20 mg/kg).

Desde el punto de vista histológico se ha observado a nivel de hígado de ejemplares tratados con 25 ppm de Cd durante 96 horas, vacuolización en varias áreas del parénquima con buena estructura de los núcleos de los hepatocitos.

Las branquias de los ejemplares mantenidos durante 50 días con 2 ppm de Cd presentaban hiperplasia, sangre y descamación del epitelio con presencia de material fibrilar oxífilo.

En los ejemplares tratados 62 días con 2 ppm de Cd se observó en el intestino epitelio hipercromático en la zona basal, disminución de las células mucosas, aumento de la migración leucocitaria, gran celularidad de la lámina propia y en la luz detritus oxífilos. En el riñón se vió cierto grado de retracción de los glomérulos y núcleos picnóticos en las células tubulares en campos aislados.

i) Sparus aurata Hg ($HgCl_2$):

Se han realizado experiencias manteniendo ejemplares de S. aurata en agua de mar con 0.1 ppm de Hg inorgánico por tiempos de 20, 41 y 54 días con objeto de determinar la acumulación de este metal en diversos órganos y ver al mismo tiempo las alteraciones histológicas producidas por estas acumulaciones.

Se ha observado que en todos los órganos estudiados la máxima acumulación se produce a los 41 días de tratamiento, siendo, no obstante, progresiva la acumulación con el tiempo en el músculo. la máxima acumulación de mercurio se ha encontrado en el hígado con un valor medio de 323.6 mg/kg sobre peso húmedo, seguido del bazo y riñón con concentraciones de 235.8 y 234.3 mg/kg respectivamente.

Las alteraciones histopatológicas más destacadas producidas por el Hg han sido: Engrosamiento de las laminillas branquiales que se hace más manifiesto a medida que aumentan los días de tratamiento con este catión acompañado de descamación del epitelio. En el hígado las alteraciones son progresivas y se observa vacuolización del parénquima y modificaciones de los islotes pancreáticos. En el riñón se observa una disminución de la hematopoyesis que es más manifiesta a los 54 días de tratamiento. Hay vacuolización a nivel de las células de los tubos renales con retracción progresiva de los glomérulos que presentan grandes espacios de Bowman. En el intestino, a nivel de la mucosa, se ha apreciado despolarización de los núcleos con hipercromatismo de la región parabasal-basal y dilatación de las vellosidades que se hacen más patentes a los 54 días de tratamiento.

j) Sparus aurata L. CH_3Hg^+ (CH_3HgCl):

Se han realizado experiencias manteniendo ejemplares de esta especie en agua de mar con 0.008 ppm de Hg (CH_3HgCl) durante 38, 52, 62 y 80 días.

Se ha observado que en todos los órganos y tejidos estudiados la acumulación de mercurio está relacionada directamente con el tiempo de exposición, habiéndose detectado la máxima acumulación, después de 80 días de tratamiento, en el riñón e hígado con valores medios de 23.54 y 21.35 mg/kg de Hg respectivamente. Las alteraciones histopatológicas producidas más destacadas han sido: En las branquias descamación y engrosamiento hiperplasia del epitelio siendo máximas las alteraciones a los 62 y 80 días de tratamiento. En el hígado hay signos degenerativos con vacuolización del parénquima que se manifiesta nítidamente a los 62 y 80 días. En el páncreas hay signos atróficos de los islotes con reducción de la talla de las células específicas. En la vesícula biliar se ve hiperplasia del epitelio siendo máximas las alteraciones a los 62 y 80 días de tratamiento. En el hígado hay signos degenerativos con vacuolización del parénquima que se manifiesta nítidamente a los 62 y 80 días. En el páncreas hay signos atróficos de los islotes con reducción de la talla de las células específicas. En la vesícula biliar se ve hipercromatismo en la región apical de las células de la mucosa. En el riñón se observan signos de glomerulonefritis subaguda de tipo intracapilar presentándose un penacho glomerular denso y compacto con espacios de Bowman muy reducidos y claros, a nivel de los tubos hay una vacuolización con acúmulo de material oxífilo.

En el intestino hay tendencia a la despolarización nuclear, hipercromatismo de la zona parabasal y disminución de las células mucosas, en fases más avanzadas hay zonas discretas de vacuolización en la región apical y en las fases finales de la experimentación hay tendencia a dilatarse las vellosidades.

k) Dicentrachus labrax - Cd ($CdCl_2$):

Se han realizado experiencias manteniendo ejemplares adultos de esta especie (34.1-36.1 cm de longitud total) en agua de mar conteniendo 25 ppm de Cd durante 96 horas. Se ha determinado la acumulación de Cadmio producida por este tratamiento en el músculo, hígado, intestino, bazo, riñón, branquias y sangre, observándose que la máxima acumulación se produce en el intestino seguido del hígado y riñón con valores medios, sobre peso húmedo de 57.53, 35.38 y y 23.12 mg/kg de Cd respectivamente.

Las alteraciones histopatológicas más destacadas producidas por este tratamiento han sido: En las branquias signos muy discretos de descamación e hipertrofia de las laminillas sin que se vean alteraciones en el esqueleto branquial. En el riñón los glomérulos están discretamente retraídos, mientras que los tubos están algo degenerados con vacuolas y cierta desorganización nuclear. En el parénquima hepático hay zonas de vacuolización con núcleos picnóticos. La vesícula biliar presenta un epitelio hipercromático y migración de leucocitos. El páncreas presenta cierto grado de retracción estando rodeado por abundante grasa. En el intestino hay vacuolización del epitelio y aumento de la migración de leucocitos con pocas células mucosas.

l) Sparus aurata. Cd($CdCl_2$):

Se han realizado experiencias de corta duración manteniendo ejemplares de S. aurata en agua de mar con 25 ppm de Cd durante 96

horas y de larga duración manteniendo ejemplares de esta especie 60 días en agua de mar con 3.0 ppm de Cd.

En las doradas tratadas durante 96 horas con 25 ppm de Cd se ha observado que la máxima acumulación se produce, al contrario que en el D. labrax en el hígado seguido del intestino con valores de 74.76 y 46.04 mg/kg respectivamente sobre peso húmedo.

Con este tratamiento las alteraciones histopatológicas más importantes han sido: En las branquias discreta hipertrofia de la porción terminal de las laminillas y células de descamación. En el hígado amplias zonas de vacuolización núcleos picnóticos. Los glomérulos renales están ligeramente retraídos con pigmento melánico y células sanguíneas con disposición normal. Los tubos renales en general presentan en la luz material oxífilo y núcleos situados preferentemente en la porción apical. En el intestino hay gran migración leucocitaria con disminución de las células mucosas y moco en la luz. El páncreas intrahepático tiene un aspecto normal.

En los ejemplares de S. aurata mantenidos 60 días en agua de mar natural y agua de mar con 3.0 ppm de Cd se ha observado que las máximas acumulaciones se producen también en el hígado e intestino con concentraciones de 140.07 y 130.11 mg/kg referido a peso húmedo, respectivamente. Las alteraciones histopatológicas más importantes observadas han sido: En las branquias hipertrofia e hiperplasia con manifiesta descamación de las laminillas, teniendo el esqueleto branquial aspecto normal. En el hígado se observan signos degenerativos con vacuolización del parénquima y picnosis. El páncreas encogimiento de las células de los acinos con aparente desorganización. Los tubos renales presentan detritus oxífilos y núcleos pobres en cromatina, mientras que los glomérulos están vacuolizados y parcialmente retraídos. El intestino tiene despolarización de los núcleos del epitelio, gran migración leucocitaria, células mocosas en número normal o discretamente disminuído y moco en la luz intestinal.

PUBLICACIONES

Las publicaciones efectuadas con los resultados obtenidos en este proyecto han sido las siguientes:

GUTIERREZ, M., ESTABLIER, R. y ARIAS, A. Acumulación y efectos histopatológicos del cadmio y del mercurio en el Sapo (Halobatrachus didactilus). Inv. Pesq. 42 (1), 141-154 (1978).

ESTABLIER, R., GUTIERREZ, M., y RODRIGUEZ, A. Acumulación del cadmio en el músculo y hepatopáncreas del Langostino (Penaeus kerathurus L.) y alteraciones histopatológicas. Inv. pesq. 42(2), 299-304 (1978).

ESTABLIER, R., GUTIERREZ, M., y ARIAS, A. Acumulación y efectos histopatológicos del mercurio inorgánico y orgánico en la Lisa, Mugil auratus. Inv. Pesq. 42(2): 317-324 (1978).

ESTABLIER, R., GUTIERREZ, M. y ARIAS, A. Acumulación del mercurio inorgánico a partir del agua de mar por el róbalo, Dicentrachus labrax, y sus efectos histopatológicos.

ESTABLIER, R. y GUTIERREZ, M. Accumulation and histopathological effects of cadmium to the Lisa, Mugil auratus Risso. International Council for the Exploration of the Sea. C.M. 1979/E: 33. Marine Environmental Committee.

GUTIERREZ, M. y ESTABLIER, R. Acumulación del mercurio inorgánico y orgánico a partir del agua de mar por la Dorada, Sparus aurata L., y sus efectos histopatológicos. Inv. Pesq. 43 (2): 181-191 (1979).

ESTABLIER, R. y GUTIERREZ, M. Acumulación de cadmio a partir del agua de mar por el Róbalo, Dicentrachus labrax y la Dorada, Sparus aurata y sus efectos histopatológicos. Inv. Pesq. 44 (1): 43-54 (1980)

Centro de investigacion: Laboratorio Oceanográfico del Mar Menor
Instituto de Oceanografia
San Pedro del Pinatar (Murcia)
España

Investigador principal: J. ROS

El informe final no fué enviado por el Laboratorio.

Research Centre: Hydrobiological Research Institute
Faculty of Science
University of Instanbul
INSTANBUL
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Principal Investigator: I. ARTÜZ

No final report was received from the Principal Investigator.

Research Centre: Department of Biological Oceanography and Institute of Hydrobiology
Faculty of Science
EGE University
BORNOVA - IZMIR
Turkey

Principal Investigator: H. UYSAL

No final report was received from the Principal Investigator.

Research Centre: Biological Institute
DUBROVNIK
Yugoslavia

Principal Investigator: F. KRSINIC

Period of Reporting: November 1976 - March 1980

INTRODUCTION

Many chlorinated insecticides and industrial aromatic chlorinated hydrocarbons (especially PCBs) are extremely resistant to degradation in the environment. Their toxicological and other harmful effects on aquatic and terrestrial ecosystems are well documented. These factors emphasize the urgency for further investigation on the distribution of these pollutants in the global environment, their primary sources of release, and their effects on the basic food-chain in which, certainly, marine phytoplankton plays an important role.

In our previously published papers, there has been some discussion of experimental problems encountered in investigations related to the extremely low water solubility of DDT and PCBs and their high adsorption affinity towards solid phases (Picer, et al. 1978: Krsinic, et al. 1978). A recently published paper (Picer, et al. 1979) discusses certain difficulties causing the low recovery yields of DDT and Aroclor 1254 added to the system of laboratory-grown phytoplankton culture.

The objective of this work is to shed more light on the fate of DDT, DDE, TDE, dieldrin, and Aroclor 1254 in laboratory-grown phytoplankton culture system.

METHODOLOGICAL CONSIDERATIONS

Selection of species:

The phytoplanktonic species Dunaliella tertiolecta has been selected.

Pollutants analyzed:

Effects of chlorinated hydrocarbons (DDT, DDE, TDE, Dieldrin and Aroclor 1254) have been studied.

METHODOLOGY

Problems linked with solubility of chlorinated hydrocarbons:

300-ml Erlenmeyer flasks, each containing 100 ml of filtered sea-water (1.2 µm Millipore filter), salinity 30°/••, and nutrients for maintenance of marine algae, were used. These culture media were inoculated with the monoculture phytoplanktonic species Dunaliella tertiolecta. The contamination of inoculated phytoplankton media was performed in two ways:

(1) direct addition (chlorinated hydrocarbons solution in ethanol was carefully injected into a solution with inoculated media using a 3 µl glass syringe, and gently swirled); and

(2) indirect addition (chlorinated hydrocarbons were first dispersed in sea-water; aliquots of this polluted water were added into a system and gently swirled with inoculated media).

Flasks were capped with aluminium foil and phytoplankton was cultured at 13°C by the method already described. Flasks which were not inoculated with phytoplankton culture were treated in the same manner. Distribution of organochlorine compounds was investigated after one day (point one) and after six days (point two) of the phytoplankton culture growth. At predetermined times the contents of the flasks were filtered through a 1.2 µm Millipore filter. Pollutants retained on the Millipore filter were determined after washing the filter with hexane-methanol mixture and also in filtrate after the extraction with hexane. Chlorinated hydrocarbons adsorbed on aluminium foil, Erlenmeyer flask walls and vacuum filtration bottle walls were desorbed with methanol-hexane (1:1) mixture and determined after the alumina purification and silica gel separation by means of ECD gas chromatography.

Effects of Cadmium on growth of the unicellular green algae Dunaliella tertiolecta:

MATERIALS AND METHODS

The axenic cultures of unicellular green algae Dunaliella tertiolecta were maintained in a simplified inorganic medium (Guillard, 1962). The composition of the medium was as follows:

1.	$NaNO_3$	0.88 mM
2.	$NaH_2PO_4 \times H_2O$	0.0363 mM
3.	$Na_2SiO_3 \times 9H_2O$	0.06 mM
4.	Thiamin	10 µg/l
5.	B_{12}	1 µg/l
6.	Biotin	1 µg/l
7.	Sea-water	800 ml
8.	Distilled water	200 ml

The cultures were grown in static 500 ml Erlenmeyer flasks containing 200 ml medium and incubated at 18°C ± 1°C.

Illumination was provided on a 12/12 hour/dark cycle by fluorescent cool white light and light intensity was 2000 ± 200 lux from above. Culture flasks and glass used in the preparation of culture were presoaked in concentrated nitric acid to minimize the trace metal contamination.

Dissolved cadmium in the form of $CdSO_4$ was added to the culture medium providing final concentrations of 10^{-6}, 5×10^{-6}, 10^{-5} and 6×10^{-5}MCd^{++} and the media were adjusted to a pH value of 8 to 8.1. The media were inoculated with stock culture which was at the beginning of the stationary growth phase, at an initial cell density of ca 3.10^4 cells ml^{-1}.

Experiments were terminated upon reaching the stationary growth phase as determined by cell density.

The response of algae to the addition of dissolved cadmium to the culture medium was evaluated on the basis of maximum (terminal) yield, which was calculated from changes in the cell number.

RESULTS

The ranges obtained for the investigated pollutants recovered from the experimental system, expressed in percentages of amounts added into a system, are presented in Table I.

It can be seen that the ranges of recovery spread very broadly (for instance for TDE from 3 to 41%) and are relatively low, especially for Aroclor 1254. Fig. 1 shows all recoveries of DDT and Aroclor 1254 from the experimental system.

In the case of DDT it appears that the recovery of pesticide added into the system is slightly higher for the indirect mode than for the direct mode. But such differences, due to the higher variability of results, are not significant. In the case of Aroclor 1254, recoveries are low but the variability of results is not large. It is interesting to note that there is no significant difference in the recoveries of pesticides whether phytoplankton is present or not in the culture. This result is especially important due to the possibilities of the low recovery of adsorbed/absorbed pesticides from phytoplankton organisms.

This means that the procedure used for the recovery of chlorinated hydrocarbons from phytoplankton retained on the Millipore filter is satisfactory at least for the recovery of pollutants retained on the Millipore filter when phytoplankton is not present. There are no significant differences in the distribution of DDT within the experimental system in respect to the phytoplankton growth period (1 day vs. 6 days).

Table I also gives the distribution of pollutants within the system, expressed in percentages of the total recovery from the system.

Fig. 2 shows percentages of the total yield for DDE, TDE and dieldrin retained on the Millipore filter.

It is evident that most of pesticides from the experimental system are recovered on the filter by the adsorption/absorption process. There is no significant difference in recovery with respect to the phytoplankton growth period. It seems that dieldrin is retained to a lesser degree on the Millipore filter in comparision with the other pesticides presented. (Fig. 3). Pollutants of low solubility in water show a great affinity for absorption to solid phases.

The recovered chlorinated hydrocarbons absorbed on walls are presented in Table I. Percentages of recovered DDE, TDE and dieldrin absorbed on the glass walls of the Erlenmeyer flask for all experimental systems are given in Fig. 4. The separation of phytoplankton was carried out by vacuum flask. After each filtration procedure the walls were rinsed down with a mixture of ethanol and hexane to check the amounts of absorbed chlorinated hydrocarbons. From Table I and Fig. 5 it can be seen that certain amounts of pesticides were absorbed on the walls of the vacuum flasks.

An attempt was made to see if a significiant amount of volatized pesticides was retained on the aluminium caps by which the Erlenmeyer flasks were closed during the experimental period. From Table I and Fig. 6 it can be seen that the amounts of pesticides retained on the aluminium caps were not significant. Most of the results obtained fall below 5%. It is highly probable that the aluminium cap retained only pesticides which have a particulate nature in the gas phase over the water phase in the experimental system. But it is obvious that significant amounts of chlorinated hydrocarbons escaped from the experimental system as a real gas phase and certainly that the aluminium cap is not an effective barrier against such volatilized chlorinated hydrocarbons.

It may be concluded that a higher variability of results for the investigated chlorinated hydrocarbons in our experiments is due to some kind of colloid aggregate of the species investigated.

The fate of DDT and other investigated low-soluble organic pollutants in the system of laboratory-grown phytoplankton and other similar laboratory systems is very complex and unpredictable.

Effects of cadmium on D. tertiolecta:

In Table II, due to the concentrations of Cd^{++} (in mols and µg/l respectively), the following results are presented: culture densities after 12 days of incubation (No/ml, t=12); culture density differences according to the control expressed in percentages (%Δ No (t=12); generation time (t_g); growth constant (k), and growth constant differences according to the control expressed in percentages (%Δk).

According to Table II and Fig. 7 one sees that the negative influence of Cd^{++} exists particularly at the concentration of approximately 10^{-5} MC Cl^{++} (1124 µg Cd^{++}/l).

The median effective concentration (EC_{50}) during a 12-day period is equivalent to the concentrations of approximately 5620 µg Cd^{++}/l. At the same concentration the population density of the cells quickly decreases to 41% of the control. The growth constant also decreases at the concentration of 5620 and 11241 µg Cd^{++}/l.

Surfactants in phytoplankton culture medium:

Our previous experimental results have shown that surfactants are produced in culture media by healthy exponentially growing cells. The content and composition of the total surfactants depend on the particular species, age of culture and illumination. The total surfactant content generally increases with cell density, while surfactant content per cell shows an inverse relation to cell density.

Further investigations have been made on the effect of pollutants, particularly cadmium, on the production of surface active substances by different marine and freshwater unicelullar algae grown in batch cultures.

Axenic cultures of phytoplankton were grown in a medium to which cadmium was added in concentrations that cause sub-lethal and lethal effects. The concentration of free metal in the medium was determined at different stages of growth by differential pulse polarography.

Total surfactant content was measured by two electrochemical methods based on absorption of organic molecules at the mercury/solution interface. No pre-treatment such as filtration, pre-concentration or de-aeration was involved.

The electrochemical methods used have different specific sensitivities to various types of surfactants, such as lipids, peptides, polysaccharides, glycoproteins, and humic acids.

A characterization of type and amount of surface active material in phytoplankton culture medium is possible through comparison with model substances.

The following preliminary experiments with cadmium were carried out:

Dunaliella tertiolecta was grown at 18°C and 2000 luxes in a medium containing 5×10^{-5} M of total cadmium.

Pediastrum, freshwater algae, was grown at 25°C and 5000 luxes in a medium containing 5×10^{-7} to 5×10^{-5}M of total cadmium (free metal concentrations ranged from 3.6×10^{-8}h to 2.3×10^{-6}M).

Scenedesmus, freshwater algae, was grown at 25°C and 5000 luxes in a medium containing total cadmium 10^{-5}M and free cadmium ion 1.5×10^{-6}M.

Generally, a higher sensitivity to cadmium for freshwater algae was observed than for marine phytoplankton, which was presented in our experiment by Dunaliella tertiolecta.

The surfactant production is decreased in the presence of cadmium although the values of the surfactant content per cell do not show greater discrepancies from parallel measurements in a medium without cadmium.

PUBLICATIONS

PICER, M., PICER, N., KRSINIC, F. and SIPOS, V. (1979) - Investigation on the distribution of DDT and Aroclor 1254 in laboratory-grown marine phytoplankton. Bull - Environm. Contam. Toxicol. 21, 743-748.

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KRSINIC, F., VILICIC, D., PICER, M. and PICER, N. (1978) - Noxious effects of Diesel Oil D-2 and synergistic effect of polychlorinated biphenyls (Aroclor 1242) on zooplankton species Eurydice truncata. IVes Journées Etud. Pollutions, pp. 307-312, Antalya, CIESM.

Table I

Ranges of recovered investigated pollutants and their distribution within the experimental system

		DDT	DDE	TDE	Dieldrin	Aroclor
Total recovery (%)	from	3	9	3	4	2
	to	34	32	41	42	18
Recovery distribution of pollutants within a system (%)						
Millipore filter	from	0	13	0	0	28
	to	99	90	100	93	93
Filtrate	from	0	0	0	0	0
	to	5	03	01	66	9
Erlenmeyer flask	from	0	3	0	0	7
	to	86	75	72	59	36
Vacuum flask walls	from	0	0	0	0	0
	to	15	15	27	26	10
Aluminium cap	from	0	0	0	0	0
	to	26	15	4	4	20

Table II

Conc.Cd^{++}(M/l)	Conc.Cd^{++}(µg/l)	No/ml (t=0)	No/ml (t=12)	%△No (t=12)	t_G	k	△%k
Control	0		7.9×10^5	100	1.3	0.53	100
10^{-6}	112.4		8.8×10^5	110	1.1	0.63	118
5×10^{-6}	562		9.2×10^5	115	1.1	0.63	118
10^{-5}	1124.1	3×10^4	8.3×10^5	104	0.9	0.77	145
5×10^{-5}	5620.5		3.8×10^5	41	2.1	0.33	62
10^{-4}	11241		1.5×10^5	18	1.9	0.36	67

No/ml (t=0) Culture density after inoculation

No/ml (t=12) Culture density after 12 days incubation

%△No (t=12) Culture density differences according to the control expressed in percentages

t_G Generation time

k Growth constant

△%k Growth constant differences according to the control expressed in percentages

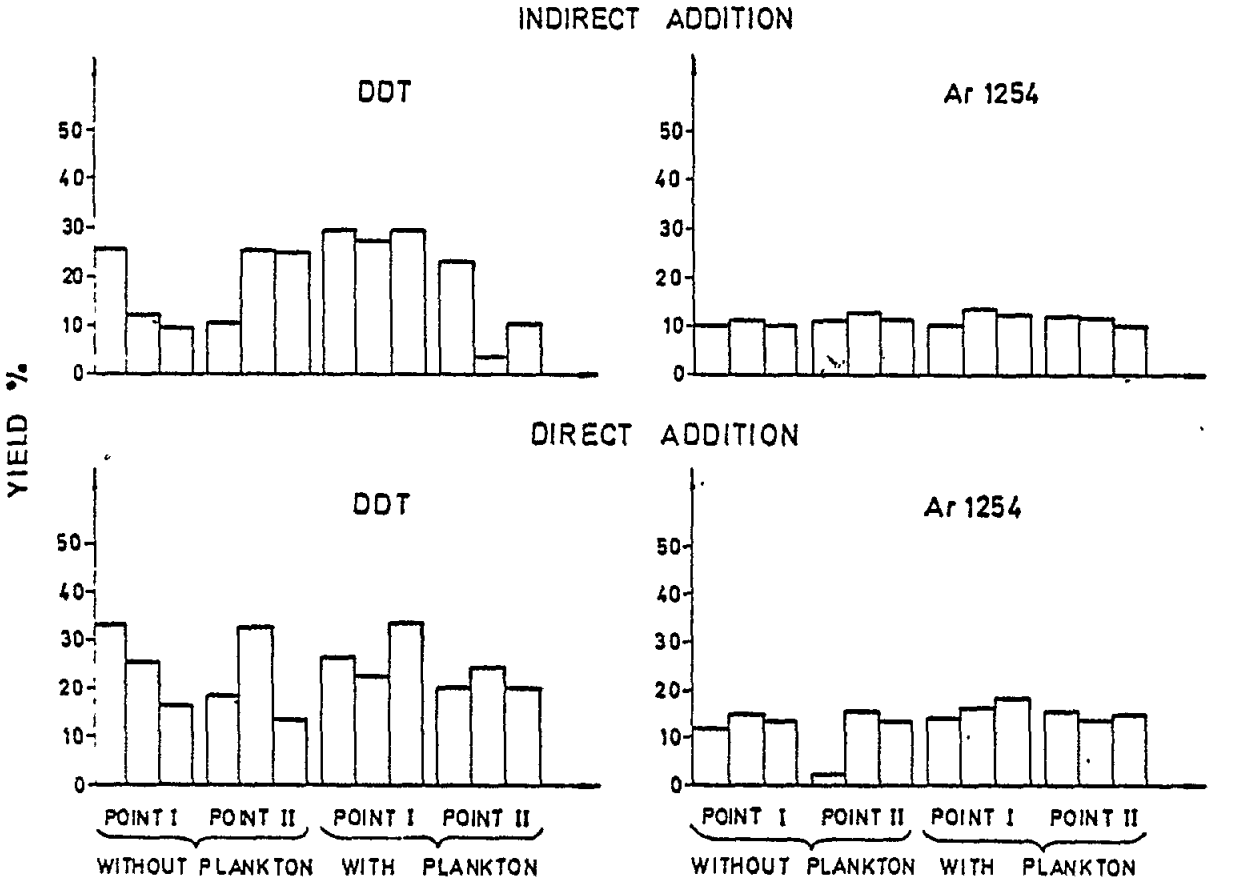

Fig. 1. Percentages of total yields of chlorinated hydrocarbons recovered from investigated phytoplankton growing systems

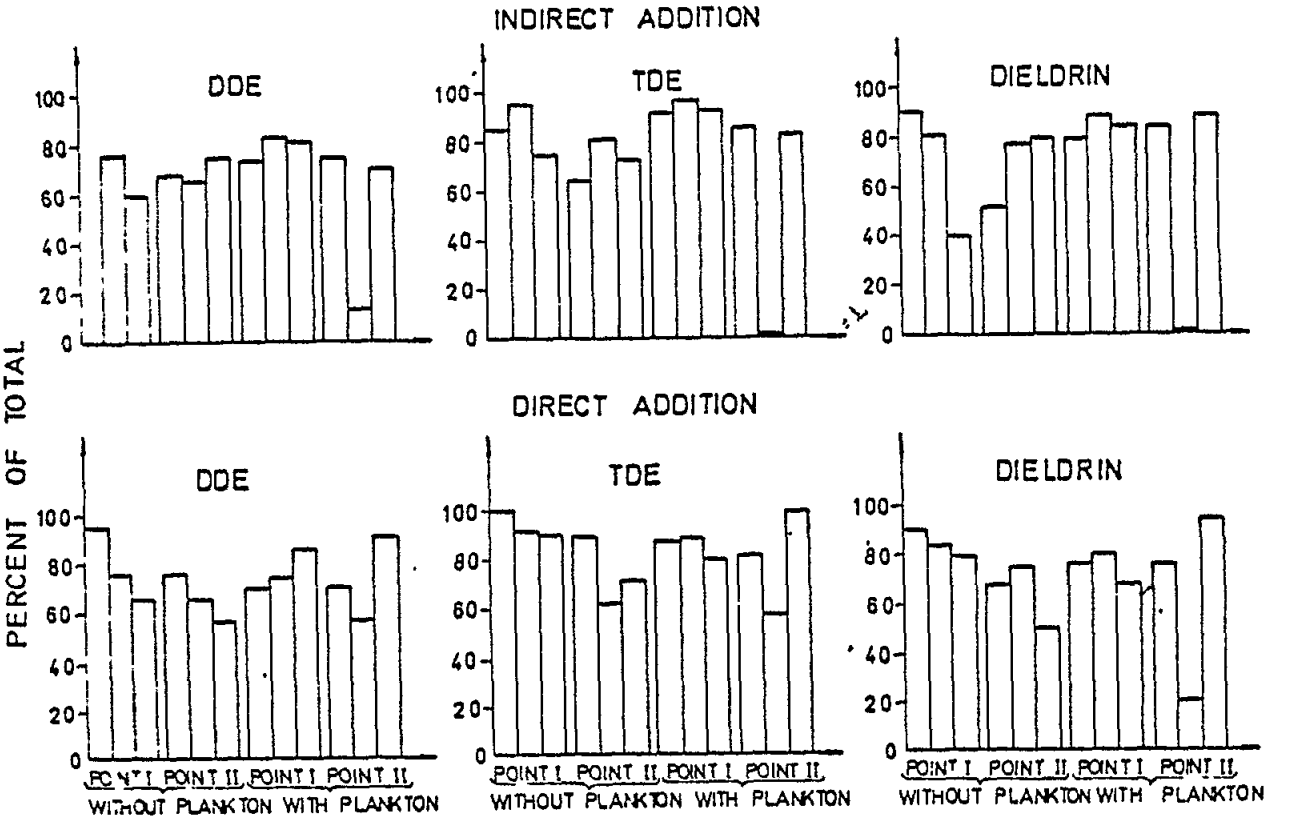

Fig. 2. Percentages of the amounts of chlorinated hydrocarbons retained on Millipore filter

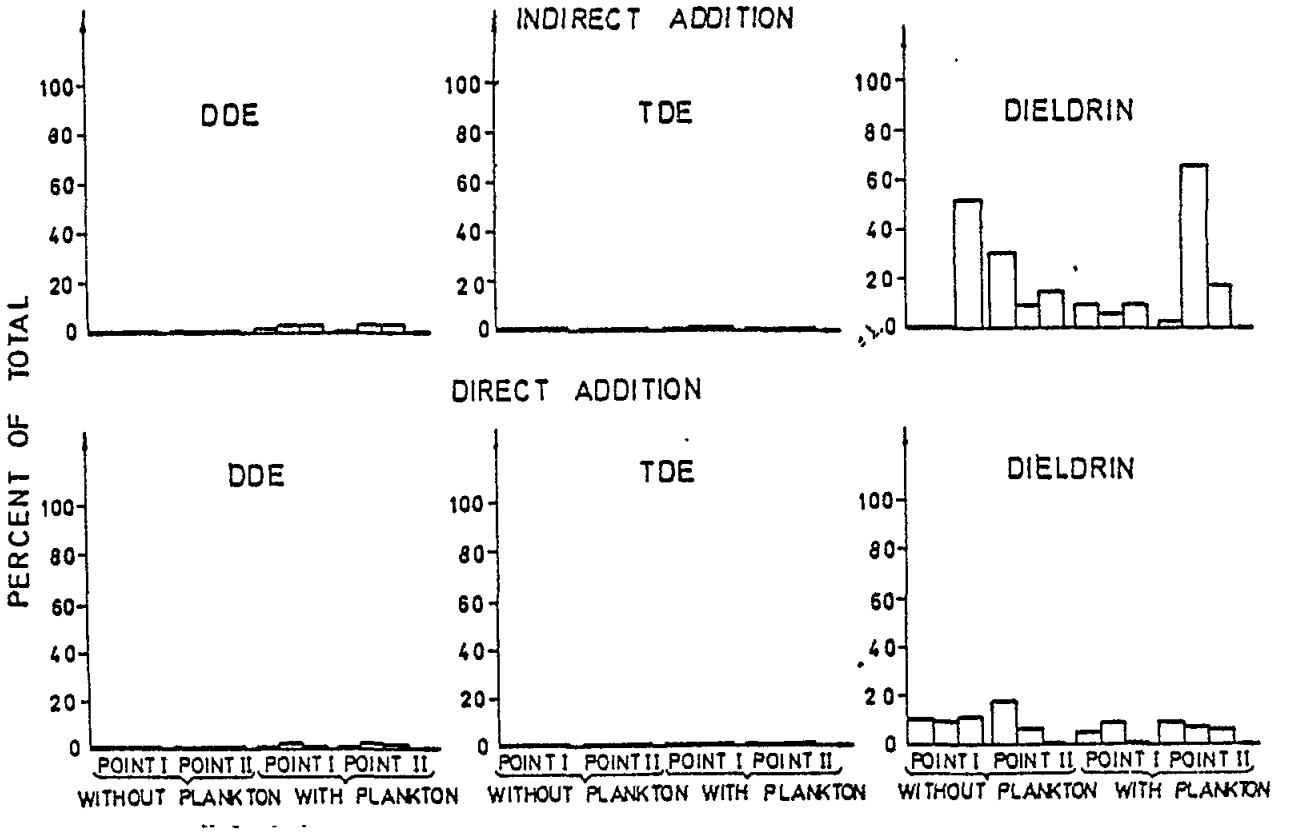

Fig. 3. Percentages of the amounts of chlorinated hydrocarbons in filtrate

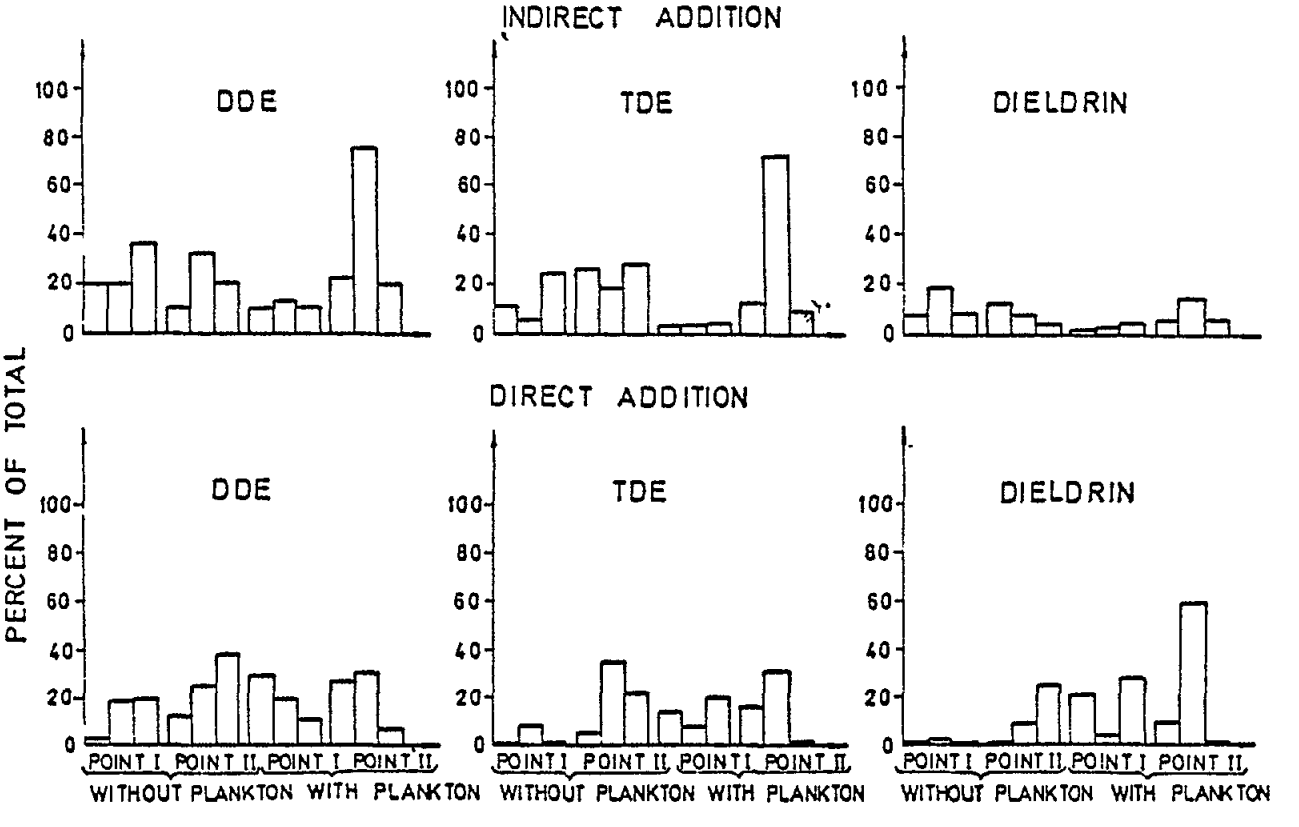

Fig. 4. Percentages of the amounts of chlorinated hydrocarbons adsorbed on flask walls

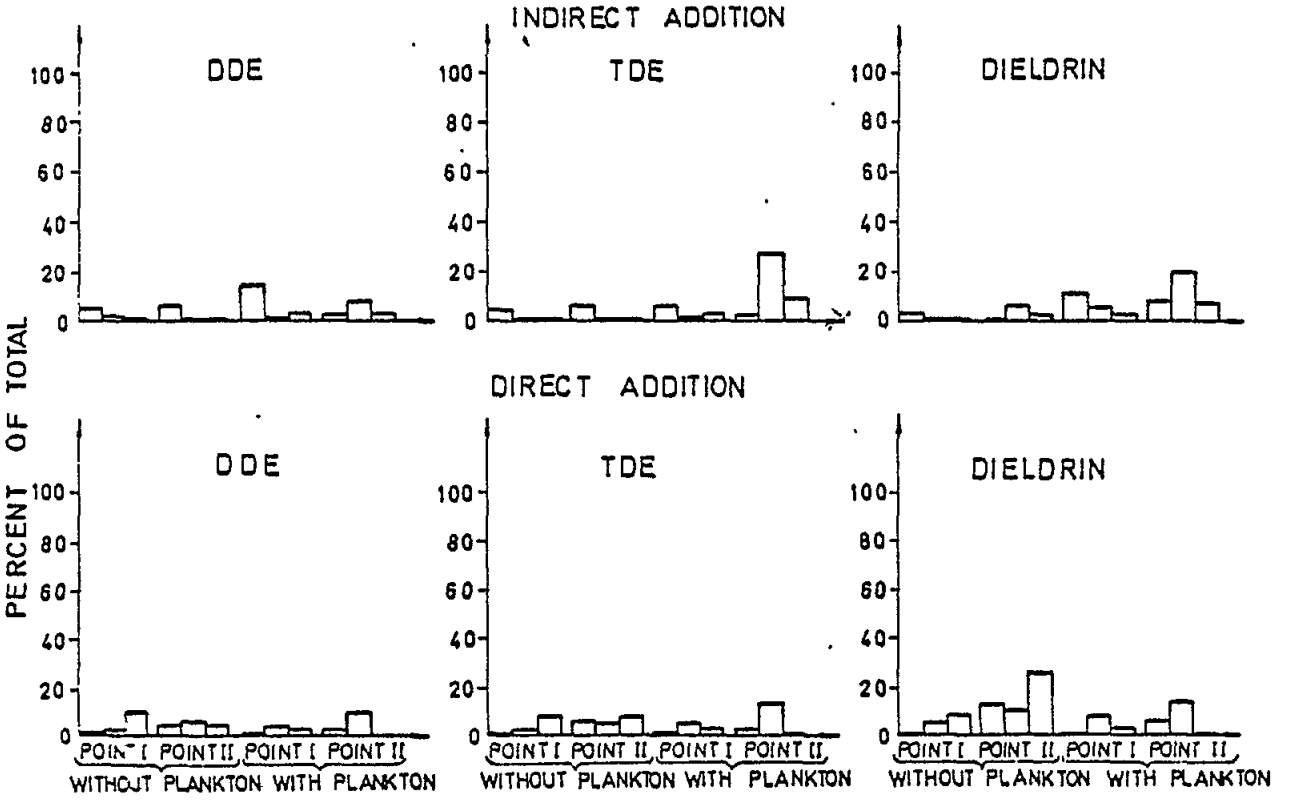

Fig. 5. Percentages of the amounts of chlorinated hydrocarbons adsorbed on vacuum flasks

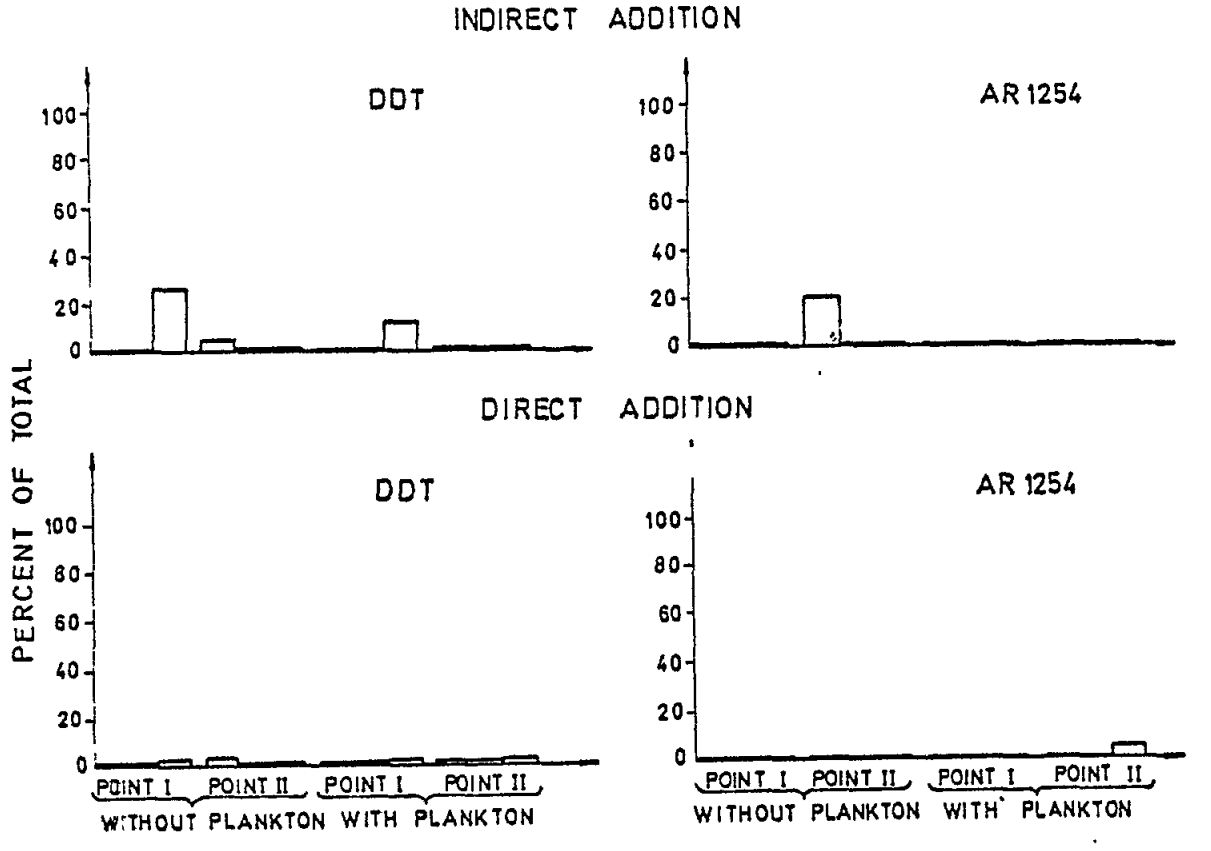

Fig. 6. Percentages of the amounts of chlorinated hydrocarbons adsorbed on aluminium caps

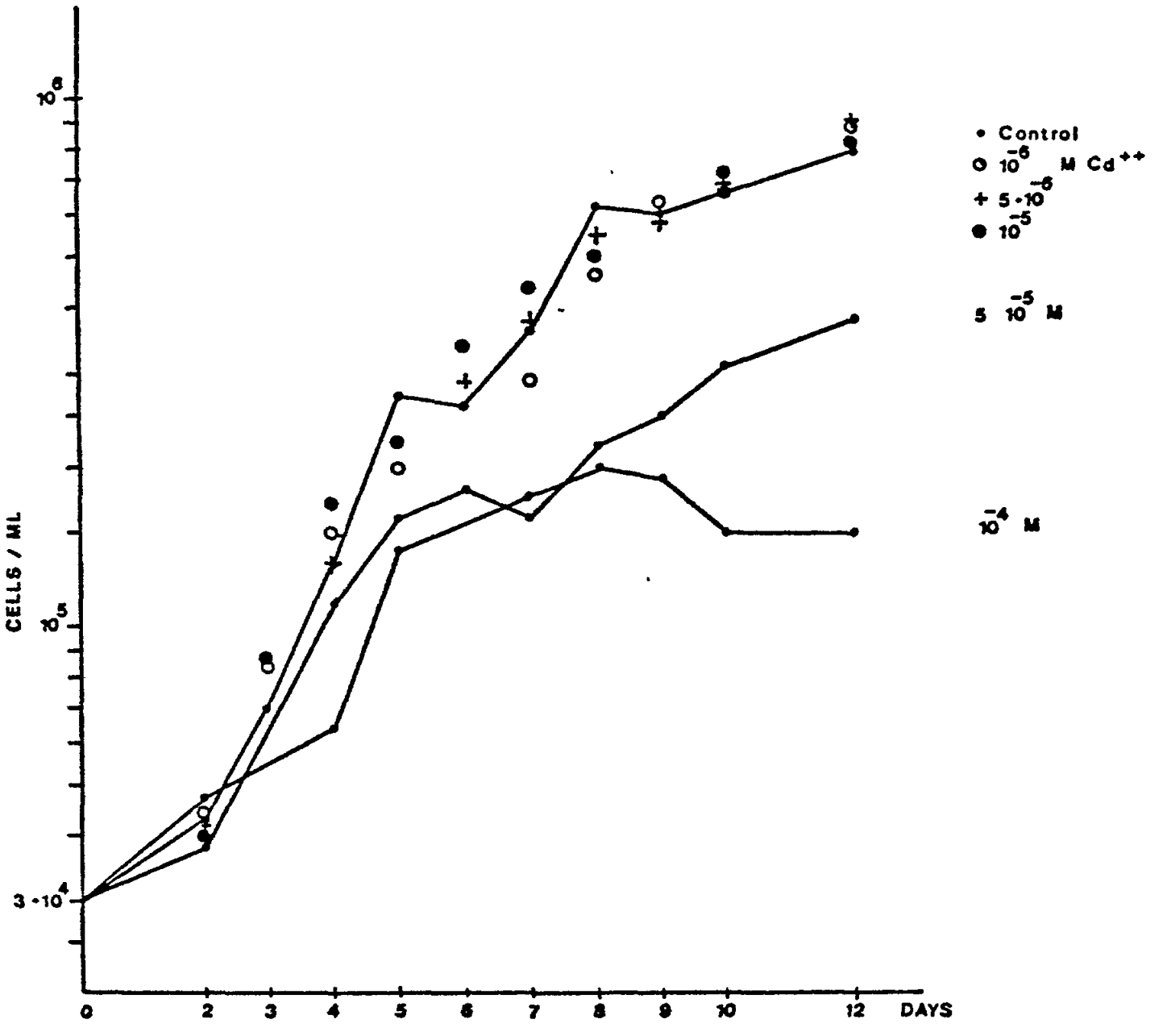

Fig. 7. Effects of cadmium on growth of the unicellular green algae Dunaliella tertiolecta

Research Centre: Center for Marine Research
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Yugoslavia

Principal Investigator: B. KURELEC

Period of Reporting: September 1976 - March 1981

INTRODUCTION

The Centre carried out previous activities relevant to MED POL IV. Since three groups of collaborators are actually active within this Programme the report will be divided into three separate summaries: Group A, Group B and Group C.

GROUP A

METHODOLOGICAL CONSIDERATIONS

Selection of the species:

Mugil auratus, Sardina pilchardus, Blennius pavo, Microcosmus sulcatus, Geodia cydonium, Phytoplankton.

Pollutants tested:

Crude oil (Lybian "Sara"), Diesel 2, Detergents (commercially available: Faks, Radion and sodium dodecyl sulphate), Benzo (a) pyrene, ^{3}H-B(a)P, ^{14}C-B(a)P, 20-Methylcholanthrene, 9,10 Dimethy 1-2 benzanthracene, Phenobarbital, actual polluted sea-water (mixed type of domestic/industrial pollution).

METHODOLOGY

Test condition: natural exposure (field experiment), exposure in tanks (flow system), exposure in specially constructed incubators.

The procedures for evaluation of effects comprise the measurement of the Benzo (a) pyrene monooxygenase (BPMO) activity in the postmitochondrial fractions of the homogenates of organs; the induction of BPMO after exposure to pollutants; the inhibition of BPMO by benzoflavone; the measurement of binding of labelled B(a)P on the DNA, RNA and proteins; the measurement of the biomass content of sea-water, and the measurement of free amino acid content of sea-water.

RESULTS

Blennius pavo

Oil: population exposed to a crude-oil accident induces its BPMO level by an 8-fold increase. Similar induction was observed in experimental exposure to sea-water artifically polluted with 170 ppb Diesel 2.

Mugil auratus

Organic waste: natural population, living in the mixing zone of the waste of a fish cannery is highly induced with respect to BPMO.

Sardina pilchardus

Field study: schools of Sardina pilchardus caught in the northern Adriatic differ in their BPMO activity. The members of the same school have the same BPMO activity. Thus, measuring of the BPMO activity in the livers of local fish offers a good indication of the presence of pollutants in the corresponding area. It could and/or should be used as a tool for monitoring the xenobiotica level in sea-water in wholly non-migrant fish species.

Geodia cydonium

Field study in polluted areas: a model of a regenerating sponge has been developed. In field experiments actual pollutional situations such as occur in bays or harbours of industrial and living areas have been studied by positioning regenerating sponge cubes in nets hanging from anchored buoys for defined times. The changes in the macromolecular precipitation pattern, measured with a specially designed technique and by radio-precursor studies have been evaluated.

Anionic detergents: it is clearly demonstrated that the sponge model is by far the most sensitive one for the study of the biology of marine detergent pollution.

Phytoplankton

Attempts have been made to correlate the concentration of the most attractive part of dissolved organic material (free amino acids concentration) in sea-water with other water quality criteria. The northern Adriatic level of free amino acids has been shown to be within "physiological" limits, with the exception of local phytoplankton blooms caused by localized sources of pollution.

PUBLICATIONS

KURELEC, B., BRITVIC, S., RIJAVEC, M., MULLER, W.E.G., and ZAHN, R.K. (1977). Benzo (a) pyrene monooxigenase induction in marine fish - molecular response to oil pollution, Marine biology-4, 211-216.

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GROUP B

METHODOLOGICAL CONSIDERATIONS

Selection of the species:

Mugil auratus, Sardina pilchardus, Boops salpa, Xanto hydrophilus, Palaemon serratus.

Pollutants analysed:

Lead, Mercury, Copper, Cadmium, Zinc, Aluminium, water soluble fraction of crude Iranian oil, phenol, organophosphate and carbamate pesticides: Dichlorvos, Bromphos, Phosalone, Paraoxon, Malathion, Gusathion, Carbaryl, Baygon.

METHODOLOGY

Lead ($Pb(NO_3)_2$, 50 to 500 ppb): continous flow system, aerated and thermoregulated water conditions (15 or 18°C ± 0.2). Water soluble fraction (WSF) of crude Iranian oil (100, 200 and 500 ppb) static system, aerated and thermoregulated. Phenols (500 to 2500 ppb), experimental conditions the same as for lead. Organophosphate and carbamate pesticides were used in vitro in concentrations of 10^{-3} to 10^{-6}M at increments of 10^{-1}M. Metal ions Zn^{2+}, Al^{3+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Pb^{2+} were used in vitro in concentration of 10^{-2}M to 10^{-7} at increments of 10^{-1}M. Haematological, biochemical and whole blood, plasma and tissue enzymes activity were tested: ALA-D, LDH, AP and AC, GOT, CPT. Liver esterases were analysed by electrophoretic and histochemical methods.

The lead concentration in whole blood was measured by the ASV method.

Intercalibration exercise:

Lead concentration in blood was measured by the Laboratory for Physico-Chemical Separations, Center for Marine Research, Rovinj - Zagreb.

RESULTS

Mugil auratus

Heavy metals: Lead effect was measured by means of ALA-D activity inhibition. The highest inhibition was reached after a period of two weeks and it was proportional to the lead concentrations. At the same time a significant haemoglobin concentration decrease occurred.

The ALA-D activity response in normal blood haemolysate, loaded in vitro with several concentrations of cadmium, mercury, copper, aluminium, zinc and lead itself were measured. The restoring effect of zinc and aluminium ions added in vitro to blood samples of lead-exposed mullets was studied and it was found that zinc is very effective in restoring activity of ALA-D.

Boops salpa

Crude oil: The tested concentration of the crude oil WSF influenced the activity of the AP and AC in the blood plasma of the fish Boops salpa. Regarding the enzymes tested the cell membrane structure might have been affected and some disfunctions at the subcellular level occurred.

Phenol: Higher concentrations of phenols induced the appearance of neurotoxic symptoms, excitability, intensive breathing rate, equilibrium disbalance and others. Haemorrhage, oedema and blood infiltration were observed in most major tissues. The increased activity of LDH, GOT and GPT in the blood plasma indicates that cellular damage had occurred in the liver. Phenol also affects blood protein levels.

Sardina pilchardus

Organophosphate and carbamate pesticides had differential inhibiting effects. All liver esterases were completely inhibited by 10^{-3} to 10^{-6} para-oxon, but the inhibition was non-specific. For example, Phosalone had no inhibition effect on sardine liver esterases. Other tested pesticides had specific inhibition effects, depending on concentration.

Xanto hydrophilus

Crude Oil: WSF influences the activity of the AP and AC in the blood plasma.

Palaemon serratus

Crude Oil: WSF influences the activity of the AP and AC in the blood plasma.

DISCUSSION OF RESULTS

The ALA-D activity test is satisfactory for assessing lead contamination in fish: it is more sensitive and easier to perform than direct determinations of lead concentrations in tissues.

The accumulation of lead in mullet produces anaemia, probably as a result of injury to the haematopoietic system. Our results concur with those obtained on freshwater fish. Cadmium, copper and mercury added in vitro produce significant inhibition of ALA-D. High concentration of zinc reactivate the inhibited ALA-D of the Mugil previously exposed to lead.

Effects of organophosphate and carbamate pesticides on sardines was studied through inhibition of esterase isozymes. The role of esterases isozymes in organophosphate toxication are explained in detail in a paper by M. Krajnovic-Ozretic and W. de Ligny (Marine organism 1978). Comparison of our results on sardines with results of other authors on centrarhid fish disclosed some differences: they found that none of the carbamates inhibit the esterases from fish, which differs from our results.

We should also note that OP dichlorvos and paroxon behave differently in the inhibition process in centrarhid and clupeid fish.

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GROUP C

METHODOLOGICAL CONSIDERATIONS

Selection of the species:

Palaemon elegans, Leptomysis mediterranea, Mugil capito.

Pollutants analysed:

Mercury, Selenium, Cyanides, Thalium.

METHODOLOGY

Mercuric chloride ($HgCl_2$) and SeO_2 in static renewal toxicological tests, at various concentrations. Sodium cyanide (NaCN) concentration 10 µg/l to 300 µg/l, continuous flowing system.

Thalium nitrate, concentration 1 mM/l, continuous flowing system. The effect of mercury and selenium interactions on toxicity, as well as radioactive $^{203}HgCl_2$ and ^{75}Se outfluxes, was investigated.

Intercalibration exercise:

Mercury and selenium concentrations in the shrimps were measured by Neutron Activation Analyses at the Institute "Jozef Stefan" - Ljubljana.

RESULTS

Palaemon serratus

Heavy metals: The release of $^{203}HgCl_2$ (5.0 µg/Hg/b body weight) from the shrimp pre-treated with selenium in the SeO_2 form - nominal injected dose 1.97, 3.95 and 7.90 µg Se/g fresh weight, had significantly decreased compared to the control group to which 5.0 µg Hg/g fresh weight had been administered. In the presence of $HgCl_2$ (5.0 µg Hg/g fresh weight) the release of ^{75}Se had also diminished significantly with the higher stable Se pre-treated dose (7.90 µg Se/g), while at a lower selenium concentration the difference was not statistically confirmed.

A decrease in rate of mercury loss in the presence of selenium, demonstrated by radioisotope tracer technique, was also confirmed by the stable Hg and Se analyses.

A dose of 7.9 µg Se/g fresh weight injected 12 hours before exposure of the shrimp to the various mercuric chloride solutions had not produced a significant difference in 24 h LC_{50} compared to the group which was not pre-treated with selenium. However, when the mercury concentration was 4.0 mg/l sea-water, the median lethal time (LT_{50}) for the shrimps pre-treated during a 4 day period by sublethal selenium (6.8 and 10.5 m M Se/l) concentration, was delayed (19.2 and 33.2 hours) compared to the group which was not pre-treated by selenium.

Leptomysis mediterranea

Cyanides: Very low CN concentration in the sea-water continuous flowing system induced lethal toxicity 96 h LC_{50} = 45 µg CN^-/l (confidence limits 20 to 71 µg CN^-/l). Results indicate a high degree of sensitivity of myzid to cyanide toxicity.

Mugil capito

Heavy metals: Thalium at a concentration (1mM) 10 times less than K does not produce any change in the gill potential, but produces a significant increase in the Na and Cl effluxes as shown below: Effects of K^+ and Tl^+ added to fresh water (FW) on Na^+ and Cl^- effluxes and transgill P.D. of Mugil capito adopted to sea-water (SW).

	S.W.	F.W.	+ Tl	(F.W. Tl-E.W.)	n
Jout Na	6349 ± 1047	1795 ± 400	2400 ± 491	+605 ± 185*	5
Jout Cl	5356 ± 1490	2089 ± 704	2416 ± 642	+327 ± 119*	5
Ve - Vi	-14.3 ± 3.4	+14.5 ± 5.9	+12.5 ± 4.9	-2.0 ± 1.0	4

Jout Na: Na or Cl effluxes in u equiv h^{-1} x 100 g^{-1}, Ve - Vi: transgill P.D. in mV. All data are expressed as mean ± S.E., n: number of experimental animals. Level of significance: *$p < 0.05$.

DISCUSSION OF RESULTS

Palaemon elegans

The experiments strongly support the hypothesis that the interaction of mercury and selenium produces slower release of these metals from shrimp. We believe that the main route of the selenium-mercury release is through permeable membrane barriers in the gills and to a lesser degree through the urine and faecal pellets. By coupling with proteins, both compounds may separately compete for the same carrier protein at a transport site. It was found that after the simultaneous administration of selenate and Hg, Hg-Se aggregations or bound formations were present in 1:1 ratios in mammal tissues which protect the animals from Hg toxicity. Mercury binds very strongly in vitro to albumin and haemoglobin and weakly to gamma globulin in mammals, including humans. In spite of selenium-mercury dose variations in rates, the ratio of selenium to mercury in blood plasma proteins remains close to 1.

The data confirmed the fact that selenium was bound to a sulphydryl group and mercury attached to the selenium. Selenium was attached to a sulphydryl group of proteins forming seleno proteins. Seleno-free amino acids, which have a higher affinity for mercury, established stronger bounds than in the case of the single mercury or selenium treatment. Protective effects of selenium against the toxicity of inorganic mercury may be affected by interaction of both compounds. This interaction might be an important way of preventing a large portion of the mercury from reaching a target in the functional systems which are most sensitive to mercury toxicity.

Mugil capito

Although Tl^+ ions can substitute for K^+ with a ten-fold higher affinity in activating the sodium pump in mullet, they cannot mimic the effects of K^+ in stimulating Na^+ and Cl^- efflux across the gill. This is interpreted in the light of an additional K^+ -sensitive transport component being involved.

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Research Centre: Institute of Oceanography and Fisheries, SPLIT, Yugoslavia

Principal Investigators: R. MUZINIC - G. SINOVCIC - M. TUDOR

Period of Reporting: February 1977 to March 1980

INTRODUCTION

No previous research dealing with the effects of pollutants on marine organisms in experimental conditions has been done except some initial observations on the effects of lead on the enzyme activity in erythrocytes of Scyliorhinus canicula L.

The importance of the research on the effects of pollutants on marine organisms is increasing. The coastal waters have become more polluted and they include nursery grounds of some commercially very important fish.

METHODOLOGICAL CONSIDERATIONS

Selection of the species:

Scyliorhinus canicula , Halobatrachus didactylus Schneider, Liza aurata (Risso) (=Mugil auratus), Mugil cephalus cephalus L. (=Mugil cephalus), and Liza saliens (Risso) (= Mugil saliens).

Pollutants analyzed:

Mercury, Cadmium, Lead, DDT, Oil dispersants (M-2, MXG-2, BP 1100 WD, BP 1100 X).

METHODOLOGY

Scyliorhinus canicula (Lead):

The ALA-D activity was determined with the little modified Berlin and Shaller method. The blood was taken by a cardiac puncture.

Halobatrachus didactylus (Mercury):

8 specimens and 2 controls were used. Two thirds of the 300 l sea water was renewed every 48 hours. The blood was taken by a cardiac puncture.

Liza aurata (Cadmium):

9 specimens were kept at 18 mg/l and 10 specimens at other concentrations (32, 56 and 100 mg/l). Mortality was recorded within 144 hours. The fish were not fed.

Mugil cephalus cephalus (DDT)

1 and 5 ppm in vitro, 0.5 and 1 ppb in vivo. 5 specimens were used for

tissue homogenates. 10% homogenates were prepared in a phosphate buffer at 7.4 pH. Supernatants were obtained at 10.000 rpm during 30 minutes. Analyses were carried out through a series of substrate concentrations by a spectrophotometer. Then the maximum velocity (V max) of enzyme activities was determined. Enzyme activities were expressed as mU/mg protein.

Liza saliens (Oil dispersants):

10 specimens were used at each concentration and in two control groups. A day-night rhythm was artificially maintained. Mortality was recorded during 48 hours.

RESULTS

Scyliorhinus canicula

Heavy metals (Lead): Effects on 5-aminolevulinic acid dehydratase in erythrocytes. In in vitro test 0.1 ml lead acetate water solution was added to 1 ml of blood.

The ALA-D activity in erythrocytes of Scyliorhinus canicula in vitro at various concentrations of Pb^{++} after a 30 minute preincubation at 37°C (Units = µM PBG/min/l erythrocytes)

Pb^{++} concentration (mg/ml blood)	Activity (Units)	Activity (%)
0.0000	20.89	100.0
0.0002	24.44	117.0
0.0010	22.68	108.6
0.0045	19.38	92.8
0.0090	18.46	88.4
0.0180	17.87	85.5
0.0315	16.37	78.4
0.0450	15.99	76.5
0.0630	15.43	73.9
0.0810	16.37	78.3
0.1080	14.67	70.2
0.1350	13.36	64.0

In in vivo test the lead was applied intraperitoneally.

The ALA-D activity in erythrocytes of Scyliorhinus canicula in vivo before and after the 48 hour incubation with various lead doses (Units = µM PBG/min/l erythrocytes)

Weight of fish (g)	Activity before (Units)	Pb^{++} dose (mg/kg B.w.)	Activity after (Units)	Activity (%)
230	32.20	1.0	36.06	111.0
192	33.65	1.1	34.66	103.0
238	99.94	2.7	92.26	92.3
221	32.73	3.0	31.34	95.8
204	35.16	3.1	33.97	96.6
224	49.57	5.0	43.48	87.7
221	25.56	8.0	20.84	81.5
234	27.72	8.6	22.95	82.8
206	32.77	12.2	22.97	70.0
200	23.01	12.6	17.00	73.9
166	41.95	22.5	28.17	67.1
170	57.76	43.0	35.72	61.8

The ALA - D activity in erythrocytes Scyliorhinus canicula before and after 48 hour exposure to lead in sea-water (Units = µM PBG/min/l erythrocytes).

Weight of fish (g)	Activity before (Units)	Pb^{++} concentration (mg/l)	Activity after (Units)	Activity (%)
188	80.51	0.25	32.85	40.8
255	47.09	0.25	20.81	44.2
292	78.93	0.50	27.78	35.2
226	61.86	1.00	6.57	10.6
203	61.21	1.00	15.51	25.3

Halobatrachus didactylus

Heavy metals (Mercury): Accumulation, cytohaematologic and histopathologic effects.

A progressive accumulation of mercury was recorded in the blood, liver, kidney and spleen. It reached the maximum value in the liver (if referred to dry weight in the kidney).

The data on haematologic characteristics did not seem consistent.

Cytohaematologic alterations included slight erythroanisocytosis, isolated evrythrohypocromia and tendency to fragmentation of erythrocytes with formation of erythroplastids.

In the liver a tumefaction, vacuolization and increase in the number of nuclei with an irregular disposition of these were recorded. In the kidney a gradual vacuolization with depolarization of nuclei and accumulation of eosinophilous detritus obstructing the renal tubes lumen took place.

Liza aurata

Heavy metals (Cadmium): Effect on survival of juveniles

Mortality of juvenile Liza aurata at various cadmium concentrations during 144 hours.

Hours	Mortality (%)			
	18 mg/l	32 mg/l	56 mg/l	100 mg/l
8	-	-	-	1
14	-	-		
24	-	10	100	100
33	11.1	-		
48	-	20		
72	-	30		
96	33.3	40		
120	22.2			
144	-			

No deaths occurred in the group of controls. Some damage to the caudal fin was recorded at the end of the 2nd day. In 22 individuals dead within 24 hours no damage was recorded.

Mugil cephalus cephalus

DDT: Effects on enzyme activities in muscles and liver of young fish.

White muscle	In vitro		In vivo	
	Control	1 ppm	5 ppm	0.5 ppb	1.0 ppb
LDH	27730 ± 3750	17290 ± 2650	26330 ± 940	8360 ± 1340*	7030 ± 600*
SDH	56.6 ± 6.6	46.3 ± 7.0	68.4 ± 23.3	7.2 ± 0.8*	6.4 ± 0.2*
FUM	169 ± 7	70 ± 2*	115 ± 5*	118 ± 3*	101 ± 3*
MDH	7900 ± 730	8750 ± 3130	10160 ± 1390	7780 ± 740	14160 ± 3170
Red muscle					
LDH	9880 ± 1690	11470 ± 3850	11480 ± 770	6400 ± 960*	5670 ± 1840*
SDH	6.4 ± 1.3	6.7 ± 1.6	6.7 ± 0.2	1.4 ± 0.7*	4.8 ± 0.5
FUM	796 ± 19	864 ± 21*	876 ± 20*	319 ± 13*	257 ± 7*
MDH	23840 ± 1750	21970 ± 2380	24580 ± 2120	28650 ± 1850	26550 ± 2490
Liver					
LDH	52.4 ± 4.4	208.3 ± 27.5*	609.9 ± 40.5*	81.7 ± 20.8	99.8 ± 13.3
SDH	33.3 ± 8.9	28.7 ± 2.5	21.0 ± 2.3	7.9 ± 0.5*	3.9 ± 0.3*
FUM	245 ± 7	306 ± 8*	339 ± 3*	109 ± 2*	166 ± 7*
MDH	15460 ± 3530	17320 ± 2860	15510 ± 2000	15160 ± 1080	13250 ± 1700
B-HBDH	82.8 ± 6.5	43.0 ± 4.2*	200.0 ± 12.3*	18.6 ± 0.5*	16.9 ± 1.6*
CYT OX	12.8 ± 2.2	17.5 ± 3.5	20.8 ± 3.3	-	-

The in vitro and in vivo DDT effects on activities of enzymes from white and red muscles, and from liver in young Mugil cephalus cephalus (the maximum velocity is expressed in mU/mg protein with standard errors) (statistically significant effects at $P < 0.05$ are marked by an asterisk).

In vitro DDT had a statistically significant ($P < 0.05$) stimulating effect on lactate dehydrogenase from liver and fumarase from red muscles and liver. Such an inhibiting effect was recorded on fumarase from white muscles and B-hydroxybutyrate dehydrogenase from liver (1 ppm). The DDT in vivo effects were quite different. A statistically significant ($P < 0.05$) inhibiting effect was found in the activity of lactate dehydrogenase from white and red muscles, succinate dehydrogenase and fumarase from all the tissues, and B-hydroxybutyrate dehydrogenase from the liver. A statistically significant stimulating influence of DDT in vivo was recorded in the liver lactate dehydrogenase (1 ppb). No statistically significant change was detected in the malate dehydrogenase. It follows, therefore, that DDT has strong effects on the respiratory chain and citric acid cycle. Both of these processes were slowed. A difference was found between the in vitro and in vivo DDT effects on the enzyme activities analyzed.

Slowing DDT in vivo effects on the respiratory chain and citric acid cycle, as well as catabolism of fatty acids and glycolysis were recorded.

Liza saliens

Oil dispersants: Effect on survival of juveniles.

Mortality of juvenile Liza saliens at various concentrations of four oil dispersants during 48 hours.

Oil dispersant	Mortality (%)				
	10 ppm	100 ppm	1000 ppm	5000 ppm	10000 ppm
M - 2	-	-	-	100	100
MXG-2	-	-	100	100	100
BP 1100 WD	-	-	100	100	100
BP 1100 X	-	70	100	100	100

No deaths occurred in two groups of controls.

Median survival time (LT_{50}) in juvenile Liza saliens at various concentrations of four oil dispersants.

Oil dispersant	LT_{50} (h)				
	10 ppm	100 ppm	1000 ppm	5000 ppm	10000 ppm
M - 2	-	-	-	13	7
MXG-2	-	-	5.5	2.1	1.8
BP 1100 WD	-	-	7.2	3.5	1.8
BP 1100 X	-	17	7.2	7	6

DISCUSSION OF RESULTS

A test on Scyliorhinus canicula blood showed that the lead in vitro effect was rather small.

In the in vivo test the regression of the ALA-D activity on the lead quantity had a higher slope than that found in the in vitro test. Even low lead concentration will, therefore, influence the enzyme activity in vivo. The lead had the highest effect on the ALA-D activity in Scyliorhinus canicula when added to sea-water. At 0.013 mg/l the reduction in this activity was statistically significant ($P < 0.05$) and amounted to 13.5% as shown by an extrapolation to our data.

A progressive accumulation of mercury was also recorded in Liza aurata. In this species similar histopathologic effects were also recorded; i.e. in the liver, vacuolization, tumefaction and an increase in the number of nuclei with their irregular disposition; in the kidney, a progressive vacuolization with degeneration of tubuli and disorganization of the cell. (Establier et al 1978)

In Dicentrarchus labrax cytohaematologic alterations (erythroanisocytosis, erythrohypocromia, erythroplastids) were found after a 48-day exposure to 0.1 mg/l of mercury. (Gutiérrez et al 1978 a) A 96-hour exposure to 50 mg/l of cadmium caused similar cytohaematologic and histopathologic changes in liver and kidney of Halobatrachus didactylus. (Gutiérrez et al 1978 b)

The cadmium lethal effects were studied in only one preliminary experiment on Liza aurata. There seems to be no data for a comparison, judging from the available literature.

On Mugil cephalus cephalus, the DDT in vivo effects show a strong inhibition of enzyme activities. The inhibition is due to the fact that DDT easily links to proteins, lecitin and cell organels. It was shown that DDT inhibits the activities of enzymes participating to the oxidative phosphorilation and, therefrom, to ATP-ase (Cutkomp et al, 1971). Such an effect will cause a change in the activity of allosteric enzymes and processes which they regulate. (Engel et al, 1972). It is quite possible that the inhibition of metabolic pathways in vivo takes place through an allosteric disactivation. This is why a slowing of the citrate cycle and respiratory chain occurs in the in vivo test.

On Liza saliens, the oil dispersant M-2 was found to be the most appropriate of four dispersants used and BP 1100 X the least appropriate, while MXG-2 and BP 1100 WD were intermediary as to their toxicity degree and similar to each other.

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Publié et imprimé par:

Plan d'action pour la Méditerranée
Programme des Nations Unies pour l'Environnement

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