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MEETING ON THE TOXICITY AND BIOACCUMULATION OF SELECTED SUBSTANCES IN MARINE ORGANISMS

Rovinj, Yugoslavia, 5-9 November 1984

prepared as part of the

Long-term Programme for Pollution Monitoring and Research in the Mediterranean (MED POL Phase II)

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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the World Meteorological Organization, and
the International Atomic Energy Agency
as cooperating agencies.

DEFINITION OF MARINE POLLUTION

Pollution of the marine environment means: "The introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) which results in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of sea water and reduction of amenities".

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PREFACE

The Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL - Phase II), which is the scientific/technical component of the Mediterranean Action Plan, is basically divided into two groups of activities, namely Monitoring and Research. The Research component is divided into twelve topics one of which is concerned with the toxicity and bioaccumulation of selected substances in marine organisms (research activity 'G').

The Meeting on the Toxicity and Bioaccumulation of Selected Substances in Marine Organisms (Rovinj, Yugoslavia, 5 - 9 November 1984) was jointly convened by FAO and UNEP in the framework of research activity 'G'. One of the objectives of the meeting was to provide a forum for Mediterranean scientits to present their work on the subject. Many of the authors of the papers are principal investigators of research projects carried out in the framework of the above activity. The papers were not reviewed before presentation. Furthermore, no attempt was made to group the papers by subject and they appear here in alphabetical order of the senior author's name.

The views expressed in the papers are those of the authors and do not necessarily represent the views of either FAO or UNEP.

Final editing and compilation of this volume was done by the staff of the FAO Fishery Resources and Environment Division, particularly Mr. G.P. Gabrielides. Ms Linda Kiakides was responsible for the typing and Ms Gloria Soave for correcting the references.

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EFFECTS OF TOXIC POLLUTANTS ON THE FILTRATION RATE OF MEDITERRANEAN BIVALVE MOLLUSCS

by

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1. INTRODUCTION

Effective control measures for toxic pollutants require the development of a range of standard procedures for the measurement of toxicity to aquatic organisms. Such procedures vary from complex and time-consuming protocols designed for research purposes and for the establishment of water quality standards, to fairly simple and rapid procedures designed solely for routine screening and monitoring of pollutants and effluents. Examples of the latter type include the APHA/AWWA/WPCF (1980) Standard Methods and the residual oxygen bioassay (Carter, 1962; Ballard and Oliff, 1969; Vigers and Maynard, 1977). In some cases, such simple tests may form part of an administrative or legislative pollution control scheme, such that permission to discharge an effluent or pollutant is conditional upon the result of a toxicity test. Examples of this include the toxicity test prescribed by the former Ministry of Housing and Local Government (1969) U.K., and the various tests prescribed in different countries for the assessment of oil dispersant toxicity, which have been recently reviewed by Moldan and Chapman (1983). Although it is important to recognise the limitations of such simple procedures, it appears that many Mediterranean countries lack the physical or manpower resources required to undertake the rigorous assessment of the many toxic pollutants already being discharged into the sea. Therefore the establishment of simple and rapid protocols for toxicity testing could make a contribution in the short term to the control of pollution in the Mediterranean.

This paper describes some preliminary experiments designed to assess the usefulness and accuracy of a simple toxicity test using the common bivalve mollusc Mytilus galloprovincialis. Bivalve molluscs feed and respire by drawing a current of water into their bodies under the influence of the ctenidial cilia, organic particulate matter being filtered from the water current. It has been shown (Abel, 1976) that in the temperate water species Mytilus edulis the rate of filtration of water is reduced by the presence of toxic pollutants. For a range of poisons, the concentrations required to reduce the filtration rate (FR) to half its normal value were smaller than the concentrations required to kill half the animals in 96 hours. Since measurement of FR can be achieved in less than one hour, it appears that the procedure offers the opportunity for the rapid screening of toxicity of a range of pollutants, and that the toxicity of pollutants can be rapidly ranked in order of magnitude, or compared to a standard, such as is required in a test whose results may have legal or administrative significance. Therefore we have investigated the effect of some heavy metals on the filtration of M. galloprovincialis, paying particular attention to the accuracy and reproducibility of the results obtained.

2. MATERIALS AND METHODS

The method for measuring FR is based on that described by Abel (1976), and depends upon the fact that mussels will remove from solution by their filtering activity the vital stain Neutral Red. Mussels are washed in sea water to remove silt, placed in vessels containing an appropriate quantity of sea water (100-200 ml per animal), and allowed to recover from handling for about 30 min. At this stage, a quantity of neutral Red stock solution is added, sufficient to give a concentration of dye in the test vessel of between 1 and 2 mg l^{-1}. The solution is mixed thoroughly, taking care not to

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disturb the animals. A sample of water is immediately removed from each vessel. The animals are left for 10-20 minutes, during which time the dye is removed from solution and absorbed onto the gills of the mussels. The vessels are gently stirred periodically to ensure thorough mixture of the dye. At the end of the experiment a second water sample is removed.

Concentrations of dye in the sea water are measured spectrophotometrically, in this case using a Perkin-Elmer/Hitachi Model 200 at a wavelength of 460 nm, in a cuvette of 10 cm light path. The filtration rate is given by the formula:

$$FR = \frac{M}{nt} \cdot \log_e \left(\frac{Co}{Ct} \right)$$ (Coughlan, 1969)

where M = the volume of the sea water in the test vessel
n = the number of animals
t = the time interval between the two water samples
C_o = the dye concentration in the initial sample
C_t = the dye concentration in the final sample

A full discussion of the method and its assumptions is given by Abel (1976).

Mytilus galloprovincialis were obtained from a commercial supplier and acclimated to laboratory conditions at 20±2°C for several days before use. The effects of three metals - mercury, copper and zinc - on FR were studied. Replicate groups of 10 animals were placed in vessels containing sea water to which appropriate quantities of stock solutions of $HgCl_2$, $CuSO_4$ and $ZnCl_2$ had been added. Some preliminary experimentation was required to establish the correct range. Prior to the experiments with heavy metals, some preliminary experiments on the FR of animals in clean water were carried out.

3. RESULTS

Initially, determinations of FR were made on individual specimens in clean water. Individual FR's were very variable. Among a sample of 30 specimens ranging in size from 50-80 mm, FR's varied from 1.15 ml animal^{-1} min^{-1} to 26.17 ml animal^{-1} min^{-1}, with a mean value of 10.72 ml min^{-1} and a standard deviation of 7.57 ml min^{-1}.

Size is an obvious potential determinant of FR, and in the original method (Abel, 1976) animals were graded into groups of similar size, ±5mm. To investigate the effects of size on FR, after the FR determination each mussel was measured (shell length to the nearest mm), cut open, blotted dry and weighed (wet weight including shell to nearest 0.1 g). There was a good correlation between length and weight (Weight = 0.7 length - 28.17, $r = 0.94$, $n = 50$, $p < 0.0001$). There was also a significant correlation between FR and weight (FR = 0.47 weight + 1.75, $r = 0.43$, $n = 30$, $p < 0.02$), but not between FR and length (FR = 0.33 length - 11.25, $r = 0.36$, $n = 30$, $p > 0.05$). However, expressing the results in weight specific terms (i.e. ml g^{-1} min^{-1} instead of ml animal^{-1} min^{-1}) did not appreciably reduce their variability. Mean weight-specific filtration rate was 0.57 ml g^{-1} min^{-1}, with a standard deviation of 0.37 ml g^{-1} min^{-1}. Thus expressing the results in weight-specific terms gave a s.d. of approximately 64% of the mean value, compared with an s.d. of approximately 71% of the mean value when FR is expressed as ml animal^{-1} min^{-1}. Thus it appears that in practice there is little advantage in expressing results in weight-specific terms.

In a second series of filtration rate measurements, groups of ten animals were placed in vessels containing 2 litres of sea water. Using groups of 10 gave a mean FR of 10.39 ml animal^{-1} min^{-1} with a standard deviation of 3.67 ml animal^{-1} min^{-1}. To investigate the effects of heavy metals on FR, animals were therefore exposed in groups of 10 to each of a range of concentrations of the chosen metal, and their FR determined. For mercury each experiment was replicated 10 times and for zinc and copper, five. The results are shown in Figs 1, 2 and 3. In the latter two figures, FR values are expressed as percentages of the control value, for reasons explained later. Concentrations required to reduce the FR to 50% of its control value, calculated in different ways (see below) are shown in Table I.

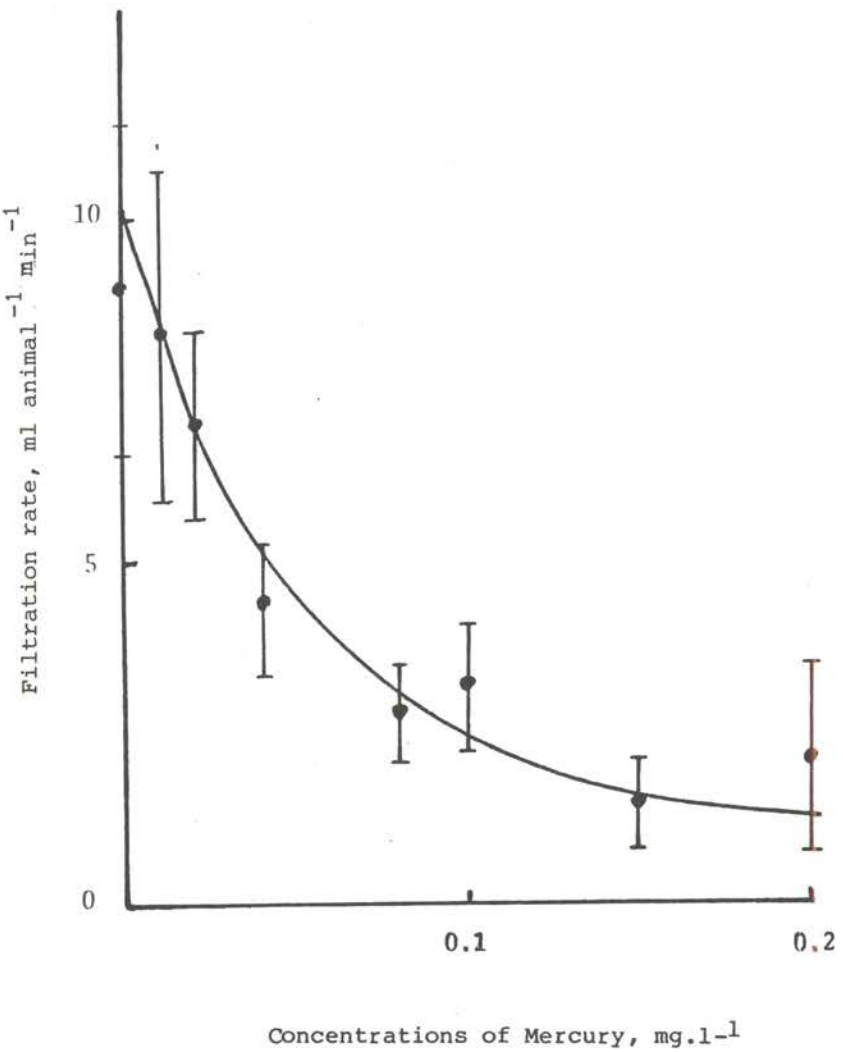

Figure 1. Effect of mercury on the filtration of M. galloprovincialis. Each point is the mean of 10 determinations; vertical lines are 95% confidence limits.

4. DISCUSSION

It is clear that the presence of toxic pollutants reduces the filtration rate of M. galloprovincialis, which appears to be about equally as sensitive to the three metals tested as M. edulis (Abel, 1976). Watling and Watling (1982) reported results for the Southern-hemisphere species Perna pernà exposed to several metals, and this species also appears to be about equal in sensitivity to Mytilus. There are few data available on

Table I

Concentrations of metals required to reduce the filtration rate of M. galloprovincialis to half its normal value, estimated from the experimental data by three methods. (See text for description of methods)

	Mercury	Zinc	Copper
Method (a)	0.055 ± 0.018	1.15 ± 0.72	0.065 ± 0.017
Method (b)	0.04	1.10	0.15
Method (c)	0.064	1.0	0.07

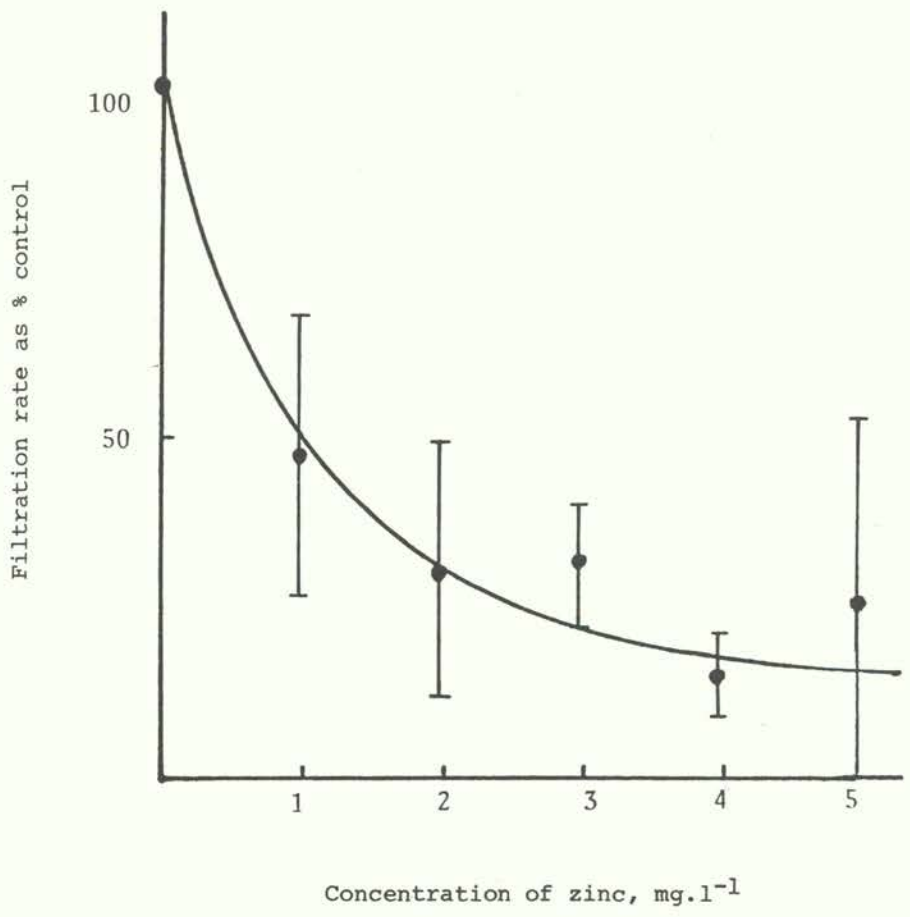

Figure 2. Effect of zinc on the filtration rate of M. galloprovincialis. Each point is the mean of five determinations, all filtration rates being expressed as a percentage of the control value recorded in the experiment in which the determination took place. Vertical lines are 95% confidence limits.

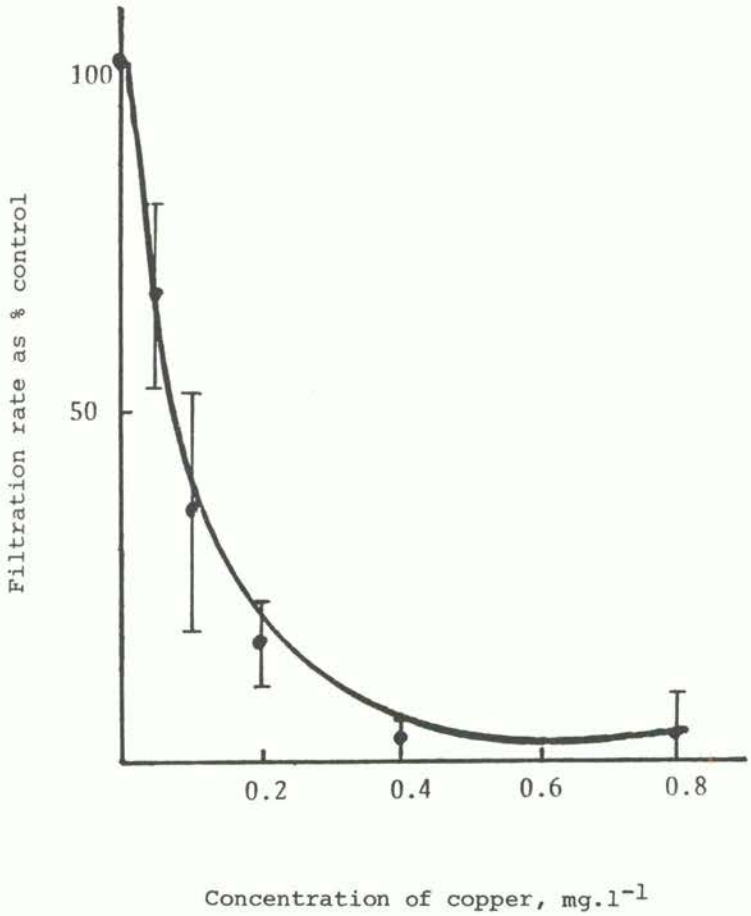

Figure 3. Effect of copper on the filtration rate of M. galloprovincialis. Each point is the mean of five determinations, all filtration rates being expressed as a percentage of the control value recorded in the experiment in which the determination took place. Vertical lines are 95% confidence limits.

the lethal toxicity of pollutants to bivalves, but it appears that the filtration rate is a more sensitive indicator of toxic stress than mortality (Abel, 1976). D'Silva and Kureisky (1978) reported 48h LC50 values for Mytilus viridis exposed to copper and zinc of 0.14 and 2.31 mg l^{-1} respectively, both figures rather higher than those in Table I. The sensitivity of Mytilus galloprovincialis filtration rate to copper is similar to that of more elaborate indicators of toxic effect in the same species, Viarengo et al. (1980) reported that exposure of M. galloprovincialis to 0.08 mg l^{-1} copper for several days produced marked reductions in the rates of protein synthesis and amino acid absorption, and in the levels of ATP in the tissues. Thus it appears that measurement of mussel FR could form the basis for a rapid assessment of the toxicity of pollutants. Its advantages include the ready availability at low cost of animals, from the field or commercial supplies; rapidity and relatively small scale; and the ease with which the animals may be maintained in the laboratory for reasonable periods.

A disadvantage however is some lack of precision in the result. A 50% reduction of FR is chosen as the criterion of toxic effect since smaller reductions are generally not statistically significant (Fig. 1). The limitation on the precision of the result is due to the high variability between recorded FR's under similar conditions. No systematic study of the causes of this variability was undertaken, and possibly such investigation would reduce variability by allowing the optimum experimental conditions to be more precisely defined. However, a large number of factors may influence FR and not all of these could be readily standardised. For example, FR may be influenced by

the physiological status of the animals, the environmental conditions, or by circadian rhythms. It was noticed during these experiments that FR values tended to decline after animals had been maintained in the laboratory for more than 14 days, and that on certain days all experiments produced consistently lower FR values than on other days. A major source of variability in the result is likely to be disturbance of the animals prior to or during the experiment. M. galloprovincialis has a conspicuously lighter shell and finer byssal threads than M. edulis and is clearly adapted to very calm conditions. Its response to disturbance is to adduct its valves and cease filtering. Further there is no reason to assume that all mussels are always filtering at their maximum rate.

The variability of the measured FR's creates two difficulties which influence the precision of the result. First, relatively small alterations in the curve fitted to the points on the graph can produce large differences in the estimate of the effective concentration of poison, even when reasonably large numbers of replicates are available (e.g. Fig.1). Secondly, the consistently high variability of control values creates difficulties in nominating a control value for FR as a basis for determining the effective poison concentration. The latter difficulty can be surmounted in one of two ways: expressing all FR values as a percentage of the control value of the relevant experiment, as in Figs. 2 and 3; or by taking as control value the point at which the fitted curve intersects the filtration rate axis, as in Fig.1.

Table I summarises the results obtained from the same data by three different methods:

(a) by taking the mean value of the effective concentrations estimated from individual plots of FR against concentration for each replicate experiment. This method allows the confidence limits of the mean to be displayed.

(b) from a plot such as Fig.1, of the mean FR and its confidence limits in all replicates plotted against poison concentration.

(c) from plots such as Figs. 2 and 3, of mean FR and its confidence limits from all replicates, expressed as a percentage of the corresponding control value, plotted against poison concentration.

It can be seen from Table I that as may be expected, mercury is most toxic and zinc least, with copper intermediate in toxicity. However the results for copper have a wide range, from 0.065 mg l^{-1} by method (a) to 0.15 mg l^{-1} by method (b); and two of the three results for copper do not differ much from the values for mercury. Values for mercury and zinc are reasonably consistent regardless of the method used to estimate the effective concentration.

Thus it appears that the measurement of filtration rate will allow the toxicities of pollutants to be compared or ranked, but that differences in effective concentration values which lie within about 1 order of magnitude should not be considered significantly different. Further experiments are continuing with a wider range of poisons and species.

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TOXICITY AND BIOACCUMULATION OF PCBs IN MARINE ORGANISMS

by

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In this paper the accumulation of some polychlorinated compounds in marine organisms and their harmful affects on marine organisms are described.

Food transfer in the ecosystem takes place through the food chain among the organisms. The links of the food chain which make up the ecosystem in the marine environment consist of phytoplankton, zooplankton and small and large fishes. Large fishes are also consumed by human beings. A gradually increasing accumulation of toxic materials in the consumer animals in consecutive links of the food chain, is called bioaccumulation.

In a marine ecosystem DDT is accumulated by the sea plants which is the first link of the chain, then passes to the herbivorous fishes (e.g. atherines) which make up the second link and then to the carnivorous fishes (e.g. needle fishes) which make up the third link of the chain, and finally to the fish-eating birds.

Investigations have shown that the accumulation increases at each trophic level and accumulation of DDT in consumer animals which make up the highest level of the food chain reaches up to ten million times its concentration in the marine environment. Generally, the longer the food chain the higher the concentration of pesticides accumulated in the consumer tissues.

It has been observed that in different organisms the effects of biological accumulation occur in different ways. It is found that the marine fishes and shrimps which live in shallow waters are very sensitive even to low DDT concentrations. It is known that DDT at levels between 0.6 and 6 mg l^{-1} can kill many kinds of shrimps.

In Fig. 1 below the range of concentration of some organic pollutants which exhibit acute toxicity is shown.

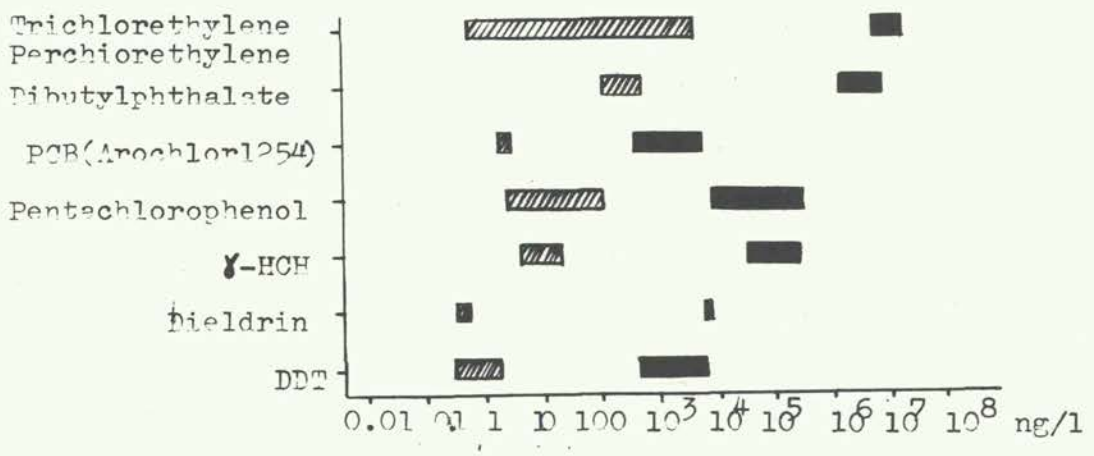

Figure 1. Range of concentrations of organic pollutants in seawater (hatched areas) in comparison to range of concentrations which exhibit acute toxicity effects in experiments with fish and shrimps (black bars) (Ernst, 1980)

As seen in Fig. 1 , some pesticides may be very toxic to fishes even at very low concentrations, and they may affect different organisms, in different ways. Toxicity can kill an organism because of acute effects or it can cause chronic harm. These compounds can have an effect on the activity, feeding and reproduction of the organism in different degrees.

There is a correlation between solubility of the pesticides in water and their bioaccumulation. As the solubility increases, the accumulation decreases. Those pesticides which have greater solubility are less resistant as they can be diluted more easily. However, insoluble pesticides do not go into the water rapidly, and they are adsorbed more by the living or dead organic materials, or by the bottom sediment fractions, immediately.

Salinity, organic materials, pH and temperature affect the solubility of pesticides in water.

Bioaccumulation depends upon the physico-chemical properties of pesticides, environmental conditions and the nature of the organism. Organochlorine compounds are very hydrophobic and they accumulate more easily than the other pesticides. The accumulation of chlorinated hydrocarbons take place not only from the surrounding sea water, but through food as well.

If a fish has been fed with 17 µg of DDT during a 4-week period, a DDT content of 0.5-0.8 µg shows up in its muscles, while its brain and liver show a higher concentration. In this experiment it has been shown that fish accumulated approximately 60 % of the total amount of DDT fed to them. (Fig.2)

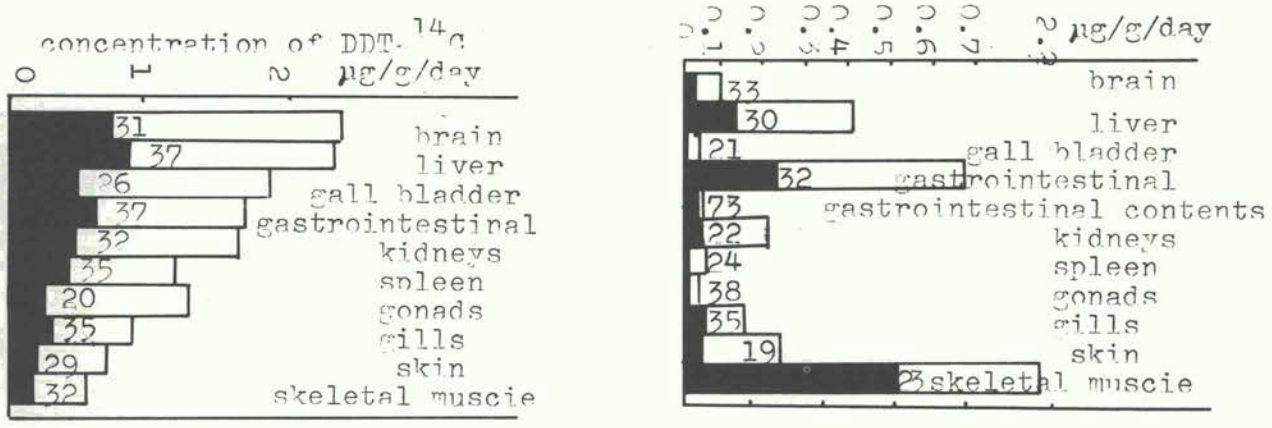

Figure 2. DDT is stored in different quantities in the various organs of a fish. The diagram shows the result of experiments with sole (Solea solea) which had been fed 17 µg of radioactively-marked DDT. The DDT concentrations (on wet weight basis) in the various organs are indicated in (a). The absolute DDT amounts in fish that were analyzed after being fed DDT for 3 days are indicated in the white bars of (b). The black bars indicate how much DDT was still in tissues after the fish were kept in a DDT-free environment for 2 months and were able to eliminate DDT into the aquarium water. The figures indicate the percentage of DDT that was not eliminated in this period (Goerke and Ernst, 1977).

Likewise, 58 % - 93 % of the PCB's fed to them through food was found in the body tissue of the polychaete Nereis virens after a 3 week period of digestion (Goerke and Ernst, 1977). Approximately 60 % of the DDT contained in their body is given off when the affected soles are kept in water that is free of DDT for two months. Accumulation and elimination of DDT and other chlorinated hydrocarbons is, then, a complex interrelationship between harmful substances in water, in food and in the organisms. This explains why animals living close together in the same region may have different spectra of chlorinated hydrocarbon concentrations in their tissues. They all have different habits of feeding and they all come in contact with the chlorinated hydrocarbons dissolved in seawater, or adsorbed to suspended particles in the seawater, or adsorbed to suspended particles in the seawater, or incorporated in their food. (Fig. 3)

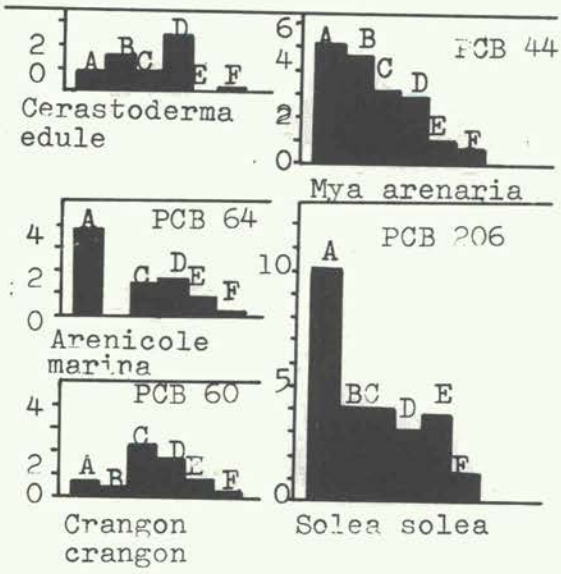

Figure 3. Even in one and the same marine locality different organisms have different patterns of chlorinated hydrocarbons (per wet weight) in tissues. Different sources of food and different metabolic pathways are reflected. The graph shows patterns for cockle, lugworm, shrimp, clam and sole from a subtidal area in the Weser Estuary, North Sea, several miles away from the coast and away from the main shipping route, and not by a point source polluted. PCB's are represented by figures, the other compounds by bars with the meaning: A:DDD, B:dieldrin, C:alpha-HCH, D:lindane, E:DDE, F:alpha-endosulfan. (Goerke et al., 1979)

In general predators show higher concentrations of DDT and PCB's as they go higher in the feeding hierarchy.

But even for very well-studied organisms, for example for mussels, no-one has been able to establish with sufficient clarity the differing rates of accumulation under laboratory conditions or in the natural environment which is much more complicated.

It is confirmed that 260 mg kg^{-1} DDT accumulated in the brain of a sole which is fed with artificial food and 80 mg kg^{-1} DDT accumulates in its liver. In the same way, it is determined that 5% DDT accumulates in 14 days in the brain, kidneys and intestines of a plaice which is fed with foods containing 15 mg kg^{-1} DDT, 13% DDT accumulates in its muscle tissues and the rest is eliminated. According to investigations like this, although in phytoplankton DDT exists in trace amounts, in zooplankton, it reaches measurable amounts (0.1 oil mg kg^{-1}) and in fishes and in sea birds fed with the fishes and in sea mammals, it is found at higher levels.

As seen in Fig. 2, DDT is stored in different quantities in the various organs of a fish.

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OIL SPILL DISPERSANT TOXICITY TO FISH AND MOLLUSCS

by

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1. INTRODUCTION

When oil spill occurs, the major concern to prevent its impact on any shoreline is to use an oil spill dispersant, which can greatly aid in the handling of floating oil at sea. This oil spill dispersant can be of extremely high toxicity to marine life when used in underisable concentrations. This study deals with the toxicity test of an oil dispersant (Dispolene 32S) using defferent test organisms: three kinds of fishes (Mugil ramada, Atherina hepsetus, Aphanius fasciatus), and two molluscs (Mytilus galloprovincialis, Tapes decussatus).

2. MATERIALS

Oil spill dispersant, Dispolene 32 S (SEPPIC, MONTANOIR)

Test organisms: Mugil ramada (mean length, 4.6 cm), Aphanius fasciatus (mean length, 3.0 cm), Atherina hespetus (mean length, 4.8 cm), Mytilus galloprovincialis (mean length, 3.8 cm) and Tapes decussatus (mean length, 3.5 cm).

Dilution sea water: After aeration and sterilisation for 24 hours, the dilution water has a pH of 7.9, a salinity of 37.6°/oo and a temperature of 23°C.

3. PROCEDURE

All stages of the procedure were carried out in an atmospere free of dust and toxic vapors. The test is carried out in graduated Pyrex cylinders (2000 ml capacity) at a temperature of 23°C and gently aerated. A series of dilutions of the toxicant were prepared with filtered sea water as follows:

100 ppm (o.1 ml/l), 1000 ppm (1 ml l^{-1}), 2000 ppm (2ml l^{-1}), 5000 ppm (5 ml l^{-1}),
10 000 ppm (10ml l^{-1}) and control.
LC10, LC50 and LC90 values after 24 and 48 hours of exposure were determined by plotting percentage mortality at 24 and 48 hours (probit scale) against dispersant concentration (log scale) (Doudoroff et al, 1951; Stora, 1972; Vanhaecke et al., 1981).

4. RESULTS

Results and observations are reported in Tables I and II and in Fig. 1. They show that Dispolene 32 S is very toxic even at low concentrations. Even the most resistant species shows a complete mortality in a few minutes when Dispolene is used at concentrations between 5 000 and 10 000 ppm, and can only survive for a few hours at concentrations between 1000 and 2000 ppm. The effect of Dispolene 32 S is almost the same on all species tested. At concentrations between 2000 and 10 000 ppm Mugil ramada is the most sensitive and bivalves are less resistant than Atherina and Aphanius; but at Dispolene concentrations between 50 and 100 ppm, bivalves are more resistant than fishes. It seems that in the second case, the molluscs can tolerate very high concentrations of Dispolene, by adducting their valves. In the first case, the molluscs keep their valves open and show a normal biological activity, for a short period, after which they die. It seems that Dispolene, when used at small concentrations, inhibits some physiological mechanism and causes the death of animals.

Table I

Percentage mortality related to different concentrations of Dispolene 32 S, of the following organisms:

a) Mugil ramada

Time / concentrations: (hours) (ppm)	0	100	1000	2000	5000	10 000
000.00	0	0	0	0	0	0
0.08	0	0	27	100	100	100
0.19	0	0	73			
0.30	0	0	100			
20.40	0	60				
47.20	0	80				
143.50	0	100				

b) Atherina hepsetus

000.00	0	0	0	0	0	0
.10	0	0	0	0	100	100
20.40	0	0	100	100		
143.50	0	0				

c) Aphanius fasciatus

000.00	0	0	0	0	0	0
0.20	0	0	0	0	33	100
0.30	0	0	0	0	67	
0.40	0	0	0	0	100	
20.40	0	0	100	100		
47.50	0	10	100			
119.50	0	20				
143.50	0	25				

d) Mytilus galloprovincialis

000.00	0	0	0	0	0	0
24.00	0	0	9	45	68	86
44.30	0	5	48	85	100	100
72.30	0	11	100	100		
144.40	0	76				
168.00	0	100				

e) Tapes decussatus

000.00	0	0	0	0	0	0
24.00	0	0	10	47	70	80
44.30	0	5	60	84	100	100
72.30	0	5	100	100		
144.40	0	50				
168.00	0	100				

The reaction time of molluscs to the Dispolene is higher than that of fish; Dispolene 32 S can kill 50% of fish and molluscs in less than 24 hours, when used at concentrations between 100 and 3000 ml l^{-1}. This LC-50, for 24 hours, is one to four times bigger, when compared to LC-50 for 48 hours (Fig.1). Mussel is a little more sensitive than clam; The LC-10, LC-50 and LC-90, for 24 hours, are two to five times higher than those killing the same bivalves in 48 hours.

On account of its high density, when mixed with sea water, Dispolene 32 S, by sinking from the surface to the bottom, can kill all marine organisms within the water column. For this reason, such oil spill dispersant is dangerous to both plankton and benthos.

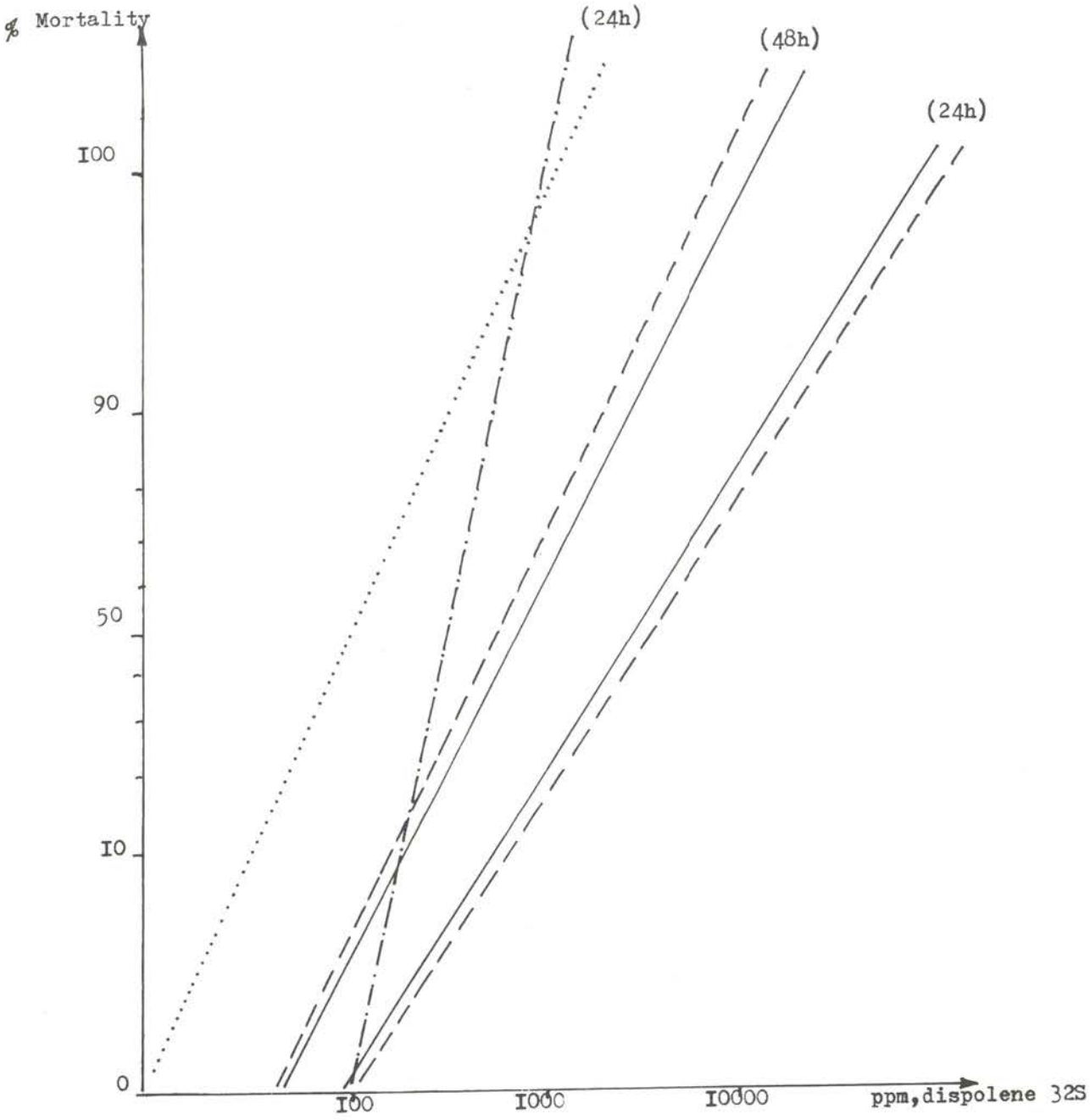

Figure 1. Determination of lethal concentration values which kill 10%, 50% and 90% of Mugil ramada (....), Aphanius and Atherina (__.__.__.), of Mytilus (_____) and Tapes (_ _ _) during a period of 24 and 48 hours.

Table II

Lethal concentrations of Dispolene 32 S using, as test organisms, fishes and molluscs, during 24 and 48 hours.

Test organisms	24 hours			48 hours		
	LC-10	LC-50	LC-90	LC-10	LC-50	LC-90
Mugil ramada	40	100	300	20	60	200
Atherina hepsetus	200	300	600	LC is between 100 and 1000		
Aphanius fasciatus	200	300	600	90	200	400
Mytilus galloprovincialis	600	3000	10000	200	700	3000
Tapes decussatus	600	3000	10000	200	600	2000

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THE ROLE OF LOG P IN PREDICTING BIOCONCENTRATION AND TOXIC POTENTIAL OF ORGANIC CHEMICALS FOR AQUATIC ORGANISMS

by

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1. INTRODUCTION

Schematically, one can say that the 50's were the years of the perception of the pollution problems; during the 60's the activities were directed mainly toward protection, regulating the discharges of effluents or the use of molecules; and the 70's were the years of prevention. This was based on the assumption that toxicological and ecotoxicological characteristics of molecules must be known before the chemical is marketed. This is the philosophy of the Toxic Substances Control Act (U.S.EPA, 1978) in the USA and the 6th Amendment of the Directive 831/79 on Dangerous Substances in the European Economic Communities (EEC, 1979). Similar approaches have been used in many countries. The problem of prediction is now a key point in scientific and regulatory activities. Most ecotoxicological studies are therefore performed in order to enhance the capacity of prediction.

About 50,000 chemical substances are, at the moment, marketed in large amounts and ecotoxicological data are scarce or absent. The two major undesirable effects of several organic substances in the aquatic environment are bioaccumulation and toxicity. In the last 10 years much effort has been devoted to predicting bioaccumulation and toxic potential from physico-chemical characteristics of the molecules. In 1974 Neely et al. proposed the n-octanol/water partition coefficient (P) as a parameter to measure bioconcentration potential of organic chemicals in fish. Examining various chemicals they found a linear relationship between the logarithm of P and the logarithm of bioconcentration factor (BCF). In a two-compartment model (water and fish) BCF is defined as Cf/Cw at the steady state where Cf and Cw are the concentrations in fish and water respectively.

Referring to the problem of the toxic potential of a molecule, the study of the relationship between chemical structure and biological activity has had a wide development in recent years, in particular through the contribution of Hansch and co-workers (Hansch, 1969; 1973). This type of approach has been successively employed in aquatic toxicology (Veith and Konasewich, 1975).

This paper reviews the experimental work and the experience of the authors on the role of log P in predicting the bioaccumulation and toxicity of organic chemicals on aquatic organisms.

2. BIOACCUMULATION

As stated in the introduction, bioaccumulation of organic substances in fish can be predicted on the basis of their physical and chemical characteristics (Neely et al., 1974). This has been verified in a number of cases and the margin of error is relatively limited (Veith et al., 1979). These last authors found that the log BCF and n-octanol/water partition coefficient as log P were linearly correlated. The correlation for 55 tests were expressed by the following equation:

$$\log BCF = 0.85 \log P - 0.70$$

which had a correlation coefficient $r^2 = 0.897$.

The relevance of investigations into developmental and young stages of fish to the evaluation of the deleterious consequences of pollution has been recognised and the importance of these studies in establishing water quality criteria for aquatic life has been stressed by several authors (McKim, 1977; Calamari and Marchetti, 1978). Nevertheless studies on bioaccumulation of organic substances have been carried out mainly on adult fish, with few exceptions (Korn and Stanley, 1981).

During an extensive investigation on paradichlorobenzene (1,4DCB) studies on bioaccumulation were made on early life stages of rainbow trout (Salmo gairdneri) (Calamari et al., 1982). Bioconcentration tests were performed twice, on alevins, in a 7-day treatment, plus a 1-day release, and in a 60-day test from egg to alevin. The BCF (bioconcentration factors) at the steady state were 112, 40, and 85 for 0.003, 0.015, and 0.073 mg l^{-1}, respectively, and a complete release was obtained in less than 1 day in the first test. The levels of accumulation were remarkably higher in the second test, particularly in certain developmental stages, (more than 1000 as BCF at hatching), but for the fed alevins this returned to around 100 at the end of the experiment (Fig.1). Observed BCFs approximately correspond to the theoretical values, which are 90 or 105 according to the mode of calculation. Barrows et al. (1980) found a BCF in Lepomis machrochirus with a half-life in the tissues of less than 1 day. The $t_{1/2}$ of 15 hr theoretically calculated by Neely (1980) corresponds exactly to those found in the release experiment. Könemann (1979) found the patterns of uptake and release in Poecilia reticulata to be as rapid as those found for rainbow trout in the present research. However, a BCF as high as 1400 was observed in hatching embryos, and this phenomenon, although not leading to dangerous consequences in this particular case, has to be taken into account from a methodological point of view. It is therefore necessary not to be too confident in the calculated low levels for adult fish, but to also take into account the high potential of bioconcentration that fish exhibit in certain developmental stages.

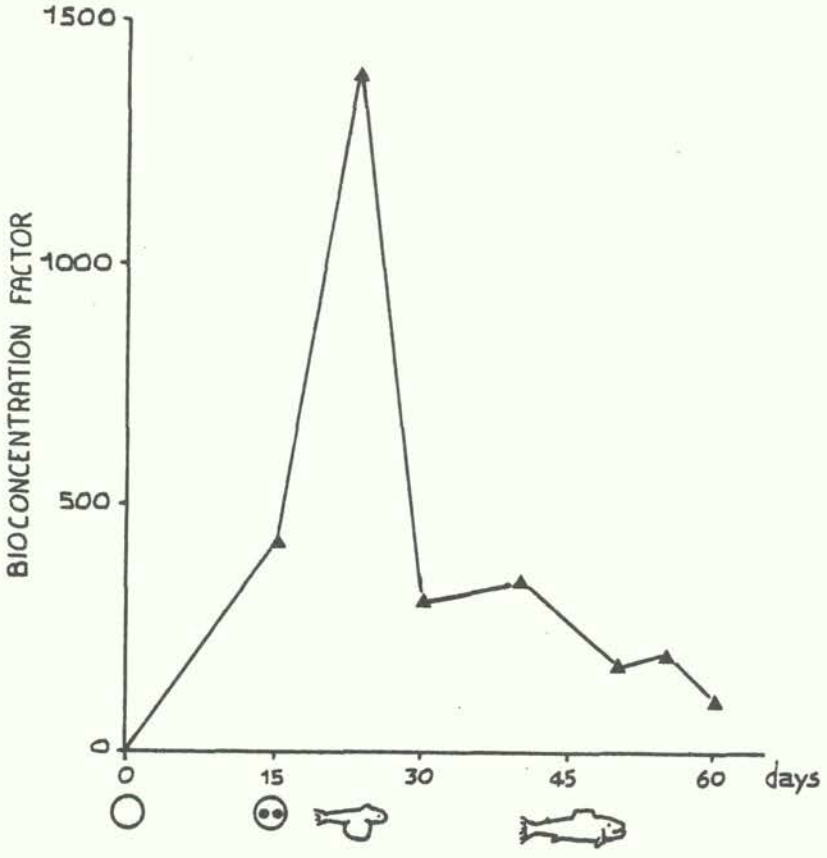

Figure 1. Bioconcentration factors (BCF) of 1,4DCB in different developmental stages of Salmo gairdneri during long-term treatment with 3 µg l^{-1}.

Organic substances are stored mainly in the deposited fat of the organisms and significant correlations between pesticide residues and lipid content were found in some species of fish (Earnest and Benville, 1971; Keck and Raffenot, 1979). As it is well known that lipid content in developmental stages of fish is higher than in alevins or adult fish it can be expected that early life stages accumulate higher quantities of chemicals with a potential for chronic effects. In order to confirm previous data and explore the possible differences in the manner of uptake and release during various stages of development a series of tests was set up to study accumulation of 1,4DCB in Salmo gairdneri early life stages (Galassi et al., 1982). The relationship between developmental stages and 1,4DCB content in total body weight for continuous exposure is shown in Fig.2. The highest concentration was tested only once. A peak of uptake is evident during the hatching phase; the maximum was clearly identified in the first experiment. The accumulation pathway is very similar for comparable concentrations.

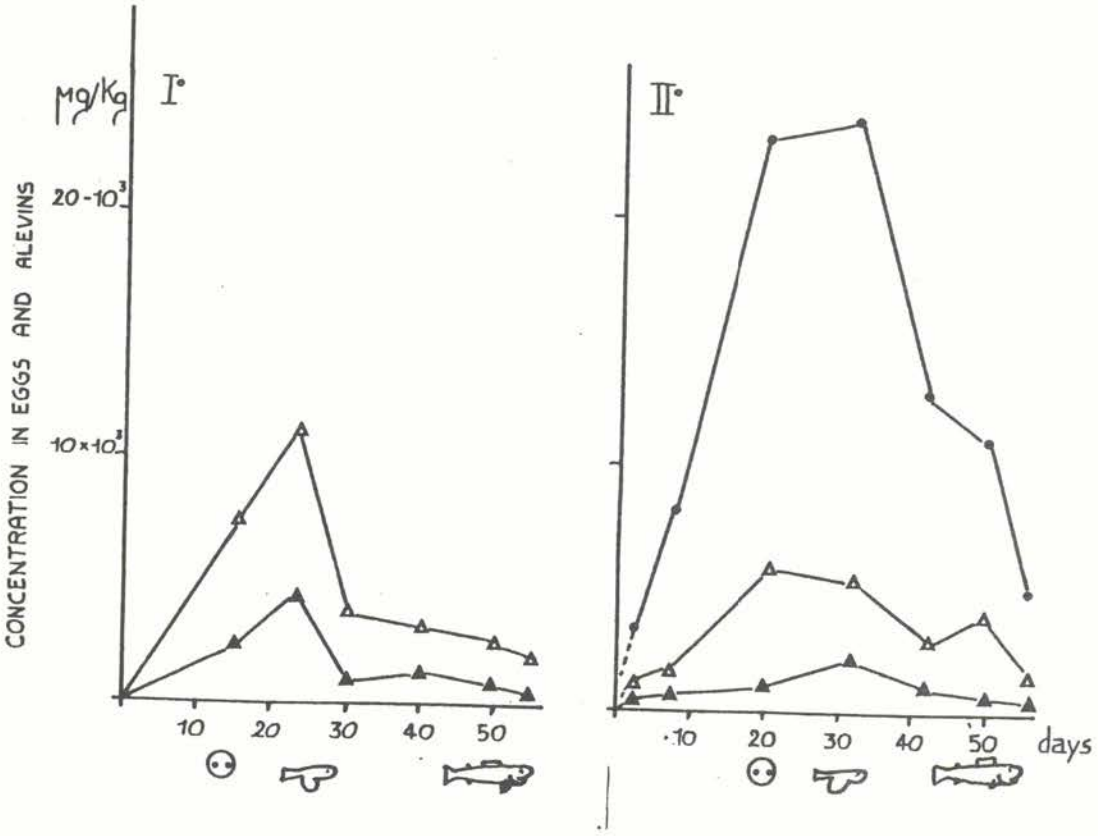

Figure 2. (I) First series of long-term tests of 1,4DCB from eggs to alevins at two concentrations: (▲) 12.4 and (▲) 3.2 µg l^{-1} at 12°C. (II) Second series of long-term uptake tests at three concentrations: (.) 79.5, (△) 16.4, and (▲) 3.6 µg l^{-1} at 10°C.

Feeding stages accumulated the compound at very low levels in comparison to eggs. Uptake and release of 1,4DCB in alevins 2-3 cm long are shown in Fig. 3. It is to be noted that the release curves are particularly sharp and that the levels of uptake are not high. Eggs, until the hatching stage, have a particular inability to release the 1,4DCB. Immediately after the hatching, uptake and release are notably more rapid than in the previous stages. The potential of bioaccumulation is quite high, particularly at the hatching stage. In one case a CF as high as 1000 was observed. For most of the cases the ratios of accumulation in critical developmental stages in regard to alevins range from 6 to 20.

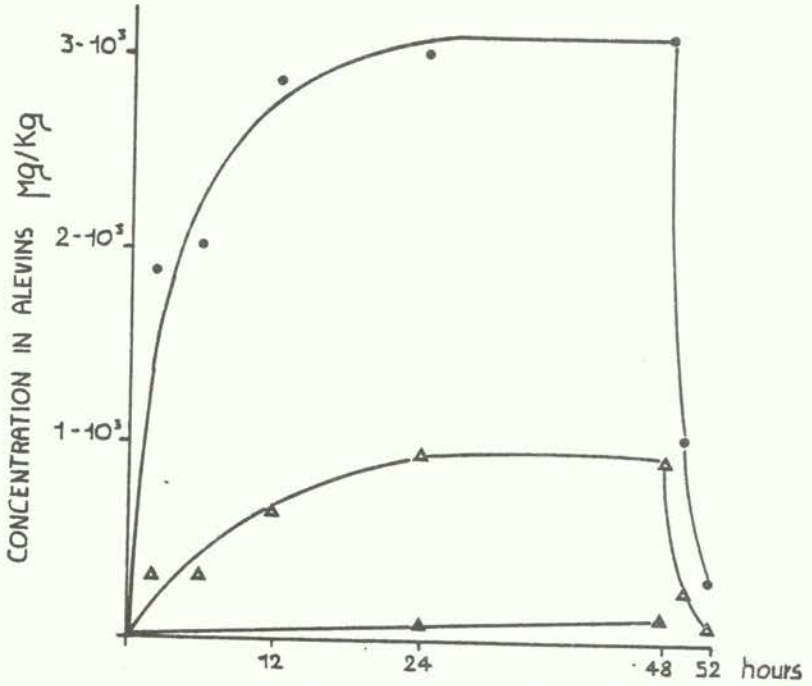

Figure 3. Short-term uptake and release tests on alevins at three different concentrations of 1,4 DCB : (.) 73.2, (△) 14.6 and (▲) 3.0 μg l^{-1}.

Kinetic parameters were calculated assuming a first order uptake model. A comparison between toxicokinetic parameters is given in Table I for alevins and eggs. Wide differences were evident for all the parameters considered C_s, K_1, K_2 and $t_{1/2}$. However, the referred theoretical asymptotic concentration can never be reached by eggs as the time required is too long. At hatching only 50% of the possible maximum was attained. A greater potential for accumulation in eggs in regard to posthatching stages is still evident even if the concentrations of 1,4DCB are expressed on the basis of lipid content. A theoretical BCF of 17000 can be calculated for eggs, compared to 300 for alevins.

Considering the result of the tests from a methodological point of view several considerations can be drawn:

(a) Developmental stages of fish are very useful for bioaccumulation studies as well as for mortality and teratogenicity.

(b) Certain stages accumulate more than others having a low release capacity.

(c) The particular substance tested here reached CF up to 1000 in developmental stages when theoretical BCF is around 100; BCFs calculated from the n-octanol/water partition coefficient, reliable for adult fish, are therefore not applicable to early life stages.

(d) Even if data refer to lipid content, a higher bioaccumulation potential has been demonstrated in eggs by means of a toxicokinetic model.

(e) There is a need to explore the possibility of prediction of accumulation also for developmental stages of fish; on the basis of the results obtained in the present study it seems more advantageous to test a limited number of key stages (eyed eggs, hatching) with short-term exposures and frequent sampling; kinetic constants and steady-state concentrations can be calculated applying the toxicokinetic models.

(f) It is necessary in this kind of study to separate the thermodynamic point of view (maximum bioaccumulation potential at the steady state) from the kinetic aspects because of the deep biological modification involved in the developmental stages.

Table I

Kinetic constants and asymptotic concentrations for 1,4DCB uptake and release in eggs and alevins of Salmo gairdneri, from continuous-exposure tests at 10°C.

Eggs	c_w ($\mu g\ l^{-1}$)	c_s ($\mu g\ kg^{-1}$ lipid)	K_1 (hr^{-1})	K_2 (hr^{-1})	$t_{1/2}$ (hr)
	79.5	1355×10^3	22	0.0013	531
	16.4	303×10^3	26	0.0014	493
Alevins	c_w ($\mu g\ l^{-1}$)	c_s ($\mu g\ kg^{-1}$ lipid)	K_1 (hr^{-1})	K_2 (hr^{-1})	$t_{1/2}$ (hr)
	73.2	200×10^3	460	0.17	4
	14.6	52×10^3	390	0.11	6

Linear inverse relationships between log K_{oct} and K_2 were found for homologous series of organic molecules (Zitko, 1980; Könemann and Van Leeuwen, 1980), indicating a very slow accumulation rate for molecules with very high BCF. These kinds of molecules are expected to accumulate to a limited extent in early life stages owing to the limited exposure time. Considering the possibility of extending these studies to other organic molecules, and assuming that compounds with BCF around 500 are potentially suspect in relation to bioaccumulation problems, attention should be given to molecules with medium BCF due to their quick uptake. An investigation was therefore planned in order to confirm the potential danger of molecules with medium BCF, to see if by the application of toxicokinetics models it is sufficient to test a limited number of key stages after short term exposure and finally to explore the possibilities to predict bioaccumulation in certain early life stages.

Two trichlorobenzenes were tested (1, 2, 3 and 1, 2, 4 TCB) (Galassi and Calamari, 1983). Uptake and release tests were performed for each compound on three different developmental stages (eyed egg, alevin after hatching, alevin). A variable number of specimens, according to the size of the stage, were exposed in continuous flow for 48 hours and then left in clean water for a release phase of at most 96 hours. Samples of different numbers of individuals were taken approximately at 8,24,32,48 hours during the uptake phase and at 8,24,32,48,72,96 hours during release phase for analysis of 1,2,3 and 1,2,4 TCB content.

The kinetic model utilized for the uptake phase was the one currently described in the literature (Rescigno and Segre, 1966) that is based on the assumption that the uptake and release of a substance in fish from and to water can be described by a two-compartment model:

$$c_w \underset{K_2}{\overset{K_1}{\rightleftharpoons}} c_f$$

in which c_w = concentration of the toxicant in water; c_f = concentration in fish; K_1 = uptake constant; K_2 = release constant during the uptake phase.

Assuming first order kinetics, the closed form of the initial differential equation system is:

$$c_f = \frac{K_1}{K_2} c_w (1 - e^{-K_2 t}) \quad (1)$$

in which $K_1/K_2 \cdot c_w = c_s$ is the asymptotic concentration in fish. If the model satisfactorily describes the phenomenon, then

$$\frac{c_s}{c_w} = \frac{K_1}{K_2} = BCF \quad (2)$$

and K_2 can be calculated by expressing (1) in the logarithmic form:

$$\log (c_s - c_f) = \log c_s - \frac{K_2}{2.303} t \quad (3)$$

First order kinetics do not seem to satisfactorily describe the release phase for chlorobenzenes (CBs) and the experimental data fit better into an equation corresponding to second order kinetics:

$$c_f = c_0/(1 + c_0 K_2' t) \quad (4)$$

in which c_o is the toxicant concentration in fish at the beginning of the release phase and K_2 is the release constant. K_2 can be calculated by expressing the release time as a function of the reciprocal of c_f

$$\frac{1}{c_f} = \frac{1}{c_o} + K_2' t \quad (5)$$

The data were used in equations (1), (3) and (5) to calculate the kinetic constants and the asymptotic concentrations. The rate constants K_2 and K_2 and the bioconcentration factors, calculated by dividing the asymptotic concentrations in tissues by the concentrations in water, are shown in Table II. When there are no data for K_2 and K_2 the uptake and release kinetics were very fast. In those cases tissues were already saturated with the substances at the first sampling (8 hours) during the uptake or were completely eliminated during the release phase. BCF_1 is the bioconcentration calculated on lipid contents.

Table II

Asymptotic concentrations and kinetic constants for uptake and release of TCBs in developmental stages of Salmo gairdneri

		Eyed-egg	Hatching	Alevin	Alevin (mixture)	
BCF	1,2,3 TCB	108	710	52	55
	1,2,4 TCB	85	349	39	49
BCF_1	1,2,3 TCB	5118	22050	7761	-
	1,2,4 TCB	4028	10838	5821	-
$K_2(hr^{-1})$	1,2,3 TCB	0.0129	0.0143	-	-
	1,2,4 TCB	0.0313	0.0356	-	-
$K_2(hr^{-1})$	1,2,3 TCB	0.00001	0.00004	0.00166	-
	1,2,4 TCB	0.00003	0.00003	0.00188	-

BCFs at hatching were about ten times higher than in alevins. These differences can be reduced, but not suppressed, by expressing the data on the basis of lipid weight. It is also evident that alevins with yolk-sac have the highest bioaccumulation potential and that hatching is the most critical stage. Moreover, it has been shown that the toxicokinetic approach is advantageous, since short-term exposure were enough, in most cases, for the calculation of the asymptotic concentration. Theoretical accumulation in alevins nearly corresponded to those calculated by the kinetic constants. A linear relationship was found for each developmental stage between log BCF_l (BCF on the basis of lipid weight) and log P for CB_s, when data on 1,4DCB were added to those for the TCBs (Fig. 4). The equation of the straight line for the hatching stage is:

$$\log BCF_l = - 1.21 + 1.48 \log P \qquad (6)$$

Similar relationships were not found when BCF_s based on the total body weight were considered, due to the remarkable differences in lipid content in the fish of the two experiments. If these correlations are confirmed with a larger number of compounds, the prediction of theoretical BCF_s for early life stages of fish could become possible. However, from data in the literature one can expect that chemical substances with very high BCF will follow different toxicological pathways and probably will not fit exactly into these relationships.

3. QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS

Among the methods to predict toxic effects on the basis of physico-chemical parameters of a molecule one of the most widely utilised is the Quantitative Structure-Activity Relationships (QSAR) approach, mainly in the form described by Hansch and coworkers (Hansch, 1969; 1973). The aim of a QSAR system is to describe by means of a mathematical equation the effect of different molecules on a biological system. The basic Hansch equation relating a biological effect (i.e. toxicity) with hydrophobic properties, electronic and steric characteristics has been variously modified, for example:

$$\log 1/EC50 = a \log P + b\ pKa + c\ Es + d \qquad (7)$$

where log P is the hydrophobicity parameter, n-octanol/water partition coefficient, which indicates the ability of certain molecules to pass through the biological membranes.

The electronic parameter is the Hammett constant or pKa from which Hammett constant is deduced. The use of the electronic parameter is desirable when chemicals have strong polar groups, such as phenols or amines. The degree of ionization is dependent on their pKa and the environmental pH and it is well known that the ionization affects the uptake of the molecules. Es, the steric parameter, represents the intramolecular effects of substituents on nearby reaction centre and, according to the enzyme-substrate or "lock and key" theory, should be the most important characteristic. However, at least in aquatic toxicology, the use of this parameter is rare and most of the biological activities of the molecules studied have been explained simply on the basis of the two properties, log P and pKa.

In theory it is then possible to predict a defined biological activity of a molecule knowing an appropriate equation and the physico-chemical and structural characteristics of the substance. Although the results of the use of QSAR systems in aquatic toxicology are extremely promising, the number of cases, related to different groups of organic chemicals, are not yet sufficient to allow a general application. The experience of the authors in this field has been made on three groups of organic substances with different physico-chemical characteristics: chlorobenzenes, amines and organotin compounds.

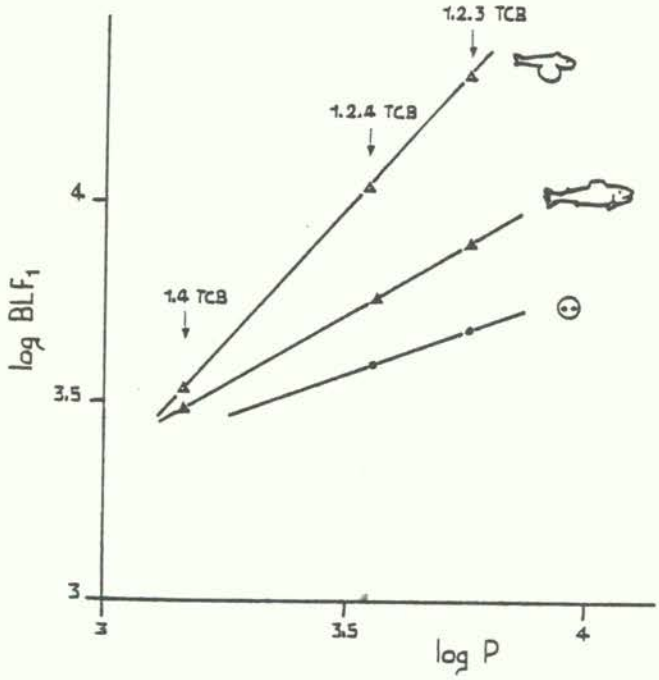

Figure 4. Relation between log BCF_1 and log P for (.) eyed-egg; (▲) hatching; (▲) alevin of S. gairdneri.

Acute toxicity tests with six chlorobenzenes (MCB; 1,2DCB; 1,4DCB; 1,2,3TCB; 1,2,4TCB; HCB) were performed on several aquatic organisms at different trophic levels: primary producers (Selenastrum capricornutum), primary consumers (Daphnia magna), and secondary consumers (Salmo gairdneri and Brachydanio rerio). Fertility impairment in Daphnia and photosynthesis inhibition in Selenastrum were also carried out (Calamari et al., 1983). Chlorobenzenes being non-ionizable molecules, the Hansch approach was applied in its simplified form:

$$\log 1/EC50 = a \log P + b \qquad (8)$$

In Table III the results of correlation between log $1/EC50$ (m mol l^{-1}) and log P for the different species and type of test, are reported. Correlation coefficients were always statistically significant and in a number of cases highly significant. Only for Selenastrum photosynthesis inhibition and Daphnia fertility test correlation was made for all six chlorobenzenes. In all other cases hexachlorobenzene was not toxic at water solubility, in agreement with the prediction in function of log P based on the results obtained on other five molecules. In Fig. 5, as an example, the relationship log 1/EC50 versus log P for Daphnia fertility is drawn. In order to improve the description of the system an attempt was made to apply the curvilinear equation with the quadratic term:

$$\log 1/EC50 = a \log P - b (\log P)^2 + c \qquad (9)$$

Veith et al. (1983) found that with the highest values of log P the relation with toxicity assumes a parabolic trend. Hansch (1969) previously hypothesized that there would be an optimal value for the partition coefficient of a chemical for finding its site of action. Above this value the biological activity of the molecules tend to decrease. In the original equation a quadratic term was therefore included. Applying this hypothesis to the case of chlorobenzenes, it was found that although in several correlations the data fitted better for five compounds, the extrapolation for the HCB were inconsistent with the few experimental data obtained (Fig. 6).

Table III

Correlations between toxic effects of chlorobenzenes and octanol-water partition coefficient (P) for the different organisms and tests

Selenastrum growth inhibition	$\log \frac{1}{96hEC50} = 0.92 \log P - 1.4$	r = 0.97 n = 5
Selenastrum Photosynthesis inhibition	$\log \frac{1}{3hEC50} = 0.99 \log P - 1.8$	r = 0.997 n = 6
Daphnia acute toxicity	$\log \frac{1}{24hIC50} = 0.78 \log P - 0.7$	r = 0.889 n = 5
Daphnia fertility test	$\log \frac{1}{14dEC50} = 0.73 \log P - 0.04$	r = 0.980 n = 6
Salmo	$\log \frac{1}{48hLC50} = 0.66 \log P - 0.2$	r = 0.914 n = 5
Brachidanio	$\log \frac{1}{48hLC50} = 0.51 \log P - 0.2$	r = 0.925 n = 5

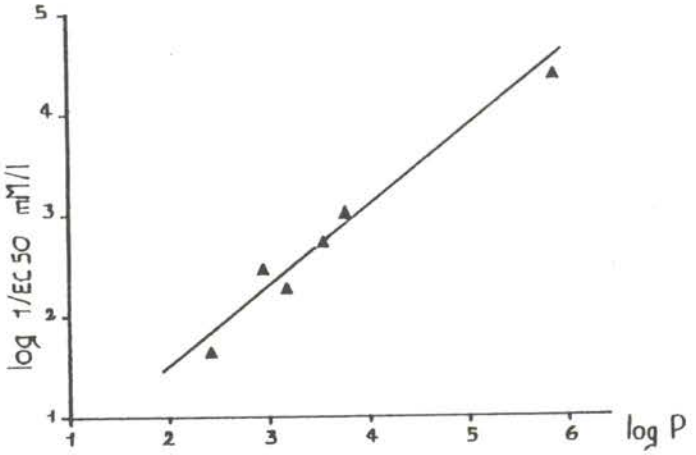

Figure 5. Linear correlation between toxicity of chlorobenzenes and octanol-water partition coefficient (P) for the fertility test on Daphnia magna.

The toxicity of several amines was also tested by means of acute toxicity tests on different aquatic organisms (Calamari et al., 1980). For Salmo gairdneri the following relation between 96 h LC50 (m moles l^{-1}) and pKa was found for aliphatic amines (Fig.7).

$$96\ h\ LC50 = -5.59\ pKa + 63.28 \quad (r = 0.95,\ n = 5) \qquad (10)$$

No correlation was found between toxicity and log P. The results indicate that for this group of highly ionizable and highly soluble substances the octanol/water partition coefficient plays a negligible role in determining toxicity, whereas pKa is a relevant parameter.

Recent unpublished data on the toxicity to Daphnia magna of several organotin compounds demonstrated the importance of both the hydrophobic and electronic characteristics. Tested compounds were three di-substituted (dimethyl tin dichloride: Me_2SnCl_2; dibutyl tin dichloride Bu_2SnCl_2; diphenyl tin dichloride: Ph_2SnCl_2)

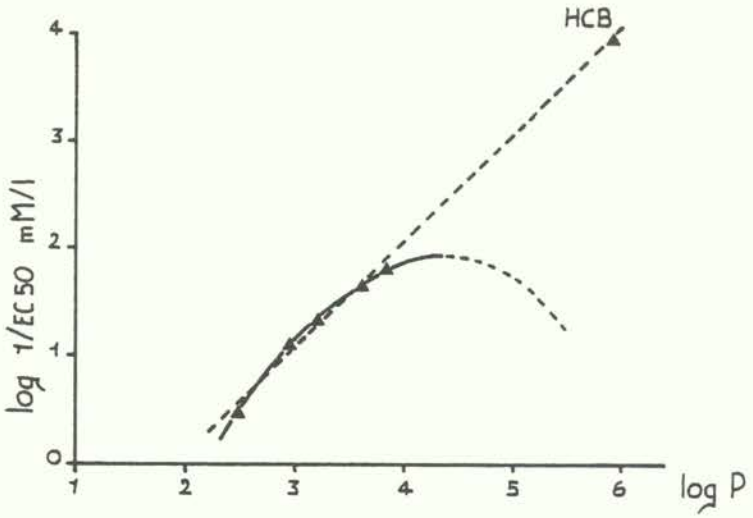

Figure 6. Parabolic correlation between toxicity of chlorobenzenes and octanol-water partition coefficient (P) for the photosynthesis inhibition tests on Selenastrum capricornutum.

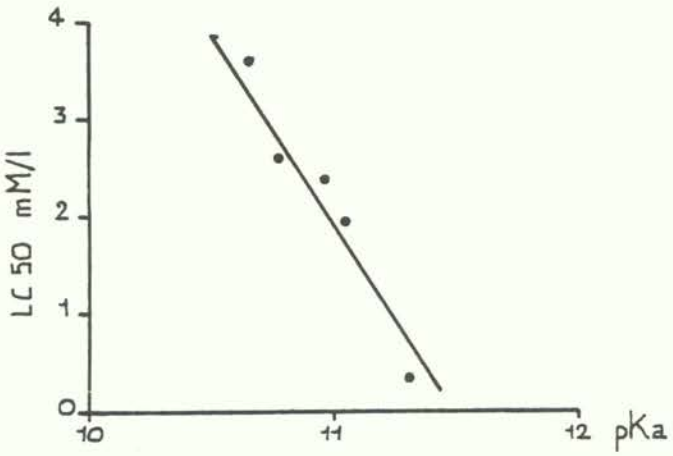

Figure 7. Correlation between pKa of the aliphatic amines and toxicity on Salmo gairdneri (96 h LC50 expressed as m moles l^{-1}.

and five tri-substituted (trimethyl tin chloride: Me_3SnCl; triethyl tin bromide: Et_3SnBr, tripropyl tin chloride: Pr_3SnCl; tributhyl tin oxide: Bu_3SnOH). Values of log P were taken from the literature (Wong et al., 1982) or calculated according to the method of Leo et al. (1971). Values of pKa, if not available from the literature, were calculated both experimentally, by measuring the pH of solutions at different molarity, and by calculation according to the method of Barlin and Perrin (1972). The correlation between log P and 1/EC50 for all compounds was scarcely significant ($P < 0.05$). However, by considering di-substituted and tri-substituted compounds separately, highly significant correlations ($P < 0.01$) were found (Fig. 8). The relationships between toxicity and log P are represented by the following equations respectively for di-substituted and tri-substituted compounds:

$$\log 1/EC50 = 0.46 \log P + 1.83 \qquad (r = 0.99, n = 3) \qquad (11)$$

$$\log 1/EC50 = 0.34 \log P + 3.51 \qquad (r = 0.98, n = 5) \qquad (12)$$

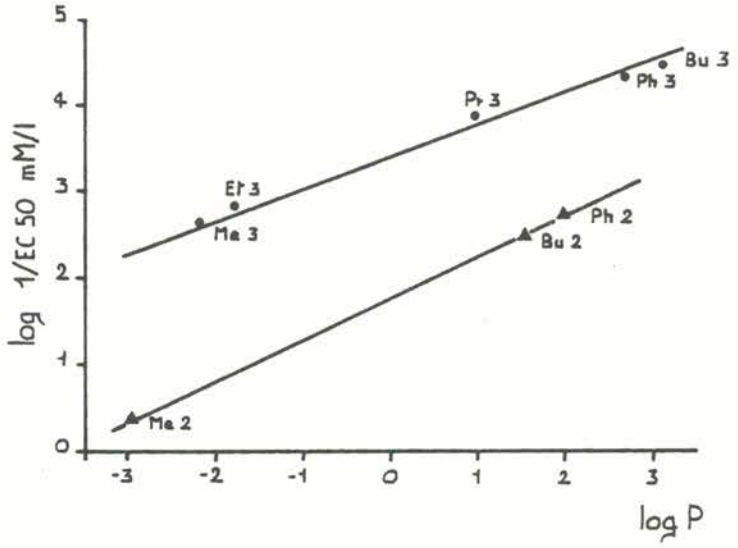

Figure 8. Correlations between log P and toxicity on Daphnia magna (log $\frac{1}{EC50}$ expressed as μ moles l^{-1}) for diorganotin and triorganotin compounds.

The two regression lines are roughly parallel, indicating the possibility of a similar mode of action. No significant correlations were found between toxicity and pKa, neither considering all compounds together nor examining di-substituted and tri-substituted separately. A multiple correlation performed by introducing simultaneously as independent variables log P and pKa and considering all compounds together gave a highly significant correlation ($P < 0.001$). Therefore the toxicity of organotin compounds could be represented by the following equation:

$$\log 1/EC50 = 0.44 \log P + 0.48\ pKa + 0.32 \quad (r = 0.93,\ n = 8) \qquad (13)$$

As a consequence the toxicity of this group of chemicals could be explained by both lipophilic and electronic characteristics.

4. CONCLUSIONS

Through a short review of literature and a discussion of the practical experience of the authors, the relevance of log P in predicting potential bioaccumulation and toxicity in aquatic organisms has been shown. In particular from the experimental data referred to on bioaccumulation the following conclusions can be drawn:

(a) theoretical calculations of BCF are actually acceptable and reliable within less than one order of magnitude. However, this method is not applicable to early life stages of fish which have been demonstrated to be able to accumulate to an extent over 10 times greater than adults. Some stages have been found with higher bioaccumulation capacity than others;

(b) due to the rapid metabolic changes of the development stages of fish, time and kinetic parameters are critical factors. Molecules with medium BCF seem in practice to be more cumulative than others with higher BCF because of their faster uptake;

(c) notwithstanding the limited number of cases available, an equation is proposed for the prediction of the accumulation in hatching alevins, which are the stages of highest bioaccumulation potential. Obviously the tentative equation should be verified on a higher number of substances.

Regarding the QSAR, the few cases shown demonstrate the interest and the complexity of the Hansch approach in aquatic toxicology. No general equation is then applicable to all chemicals, and each group of compounds should be studied by itself; moreover confidence limits of the predicted concentration of effect have to be defined case by case. Notwithstanding these limitations we can refer to and share the opinion contained in a document prepared by Könemann and Calamari (1983) in the framework of the OECD activities of existing chemicals:

(a) QSARs have been developed for several classes of chemicals and make it possible, within these classes, to make good estimates of the toxicity of chemicals on the sole basis of their physico-chemical and structural characteristics;

(b) most QSARs have been calculated for a small number of chemicals. Expert judgement is necessary to set the limits to the validity of QSARs as well as reliable confidential limits to the predicted effect values;

(c) when used in a proper way, QSARs can be very helpful in selecting "priority chemicals" in case no experimental data is available;

(d) toxicity data calculated from QSARs is a general estimate and should not be used permanently as surrogate for experimental data.

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ACUTE TOXICITY OF AN OIL DISPERSANT AND A DISPERSANT /OIL MIXTURE TO *Artemia salina*

by

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1. INTRODUCTION

Pollution of the sea with hydrocarbons occurs almost continuously without apparent harmful effects. However, massive pollution due to accidents taking place in semi-closed seas as the Mediterranean could upset the ecological balance. According to a GESAMP report (IMO/FAO/UNESCO/WMO/WHO/IAEA/UNEP, 1977) the biological effects of hydrocarbon pollution depend on several factors such as geomorphology, water temperature, salinity, and the characteristics of the organisms (planktonic or benthic). Various dispersants are used against oil pollution but their inocuity is not demonstrated. The destruction of flora and fauna due to hydrocarbon pollution is much more extensive in semi-closed seas, especially in coastal areas where the variety of species and the density of population are high. Larval stages are often more sensitive to pollutants than adults. Greece, having a lengthy coastline, (the 3rd largest in the world, after Japan and Norway, in relation to land surface area) is extremely sensitive to this pollution.

Several publications on hydrocarbon and/or dispersant pollution -some of them using *Artemia* as test animal - have appeared recently in the literature(Portmann and Connor, 1968; Maggi, 1972; Maggi and Cossa, 1973; Anderson *et al.*, 1974; Blackman *et al.*, 1978; Castritsi-Catharios *et al.*, 1980, 1983; Bellan, 1981; Vanhaecke *et al.*,1980; Thompson and Wu, 1981; Ozelsel, 1983; Papineau and Le Gal, 1983; Power, 1983; Verriopoulos and Moraitou-Apostolopoulou, 1983).

2. MATERIALS AND METHODS

The following substances were used in the toxicity tests:
- Mixture of Finasol OSR_5 dispersant with gas oil in 1:10 portions.
- Dispersant OSR_5 alone.

Artemia nauplii, stage I, were raised from cysts collected at Messolonghi saltworks.

The experiments were carried out at two temperatures ($25\pm0.5^{o}C$ and $27\pm0.5^{o}C$) and lasted for 24 hours. We used natural sea water (S $^{o}/_{oo}$:38.0), double filtered, pH 8.10-8.11. One hundred animals were tested at each concentration in 5 dishes containing 10 animals each, the tests being carried out in duplicate.

The reason we used *Artemia* as test organism was its higher sensitivity than other Crustaceans, although the Messolonghi parthenogenetic strain was more resistant to pollution than others.

3. RESULTS AND DISCUSSION

For the analysis of the results the Bliss (1937) method was transferred to a computer program specially prepared for the purpose. Results are summarized in Table I and in Figs. 1-3. From these results we can conclude that the larvae are less sensitive at lower temperatures, the other parameters remaining constant.

LD_{50}^{24} ($25^{o}C$ - LD_{50}^{24} ($27^{o}C$) = 70 ppm

The difference is probably due to an increase of the metabolic activity of nauplii in higher temperatures. This result is in accordance with observations in the field made by many scientists working with other types of organisms.

From the results it is quite clear that the dispersant alone is more toxic than the mixture. On the other hand, other dispersants (OSR_2) which belong to older generations are significantly more toxic than Finasol OSR_5. The OSR_5 composition is based on biodegradable emulsifiers.

Table I

24 h LC50 values for FINASOL OSR_5/Gas-oil mixture, FINASOL OSR_5 and Gas-oil alone.

Mixture FINASOL OSR_5/0 Gas-oil (1:10)	FINASOL OSR_5	Gas-oil
25±0.5°C	27±0.5°C	25±0.5°C
726 ppm	656 ppm	259 ppm

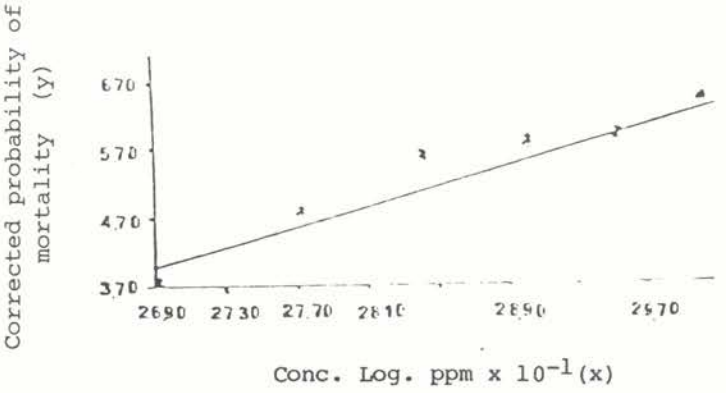

Figure 1. Correlation between corrected probability of mortality (y) and log. concentration of the mixture, dispersant/gas-oil (x). Animal tested: Artemia nauplii, strain from Missolonghi, stad. I. temperature 27°C.

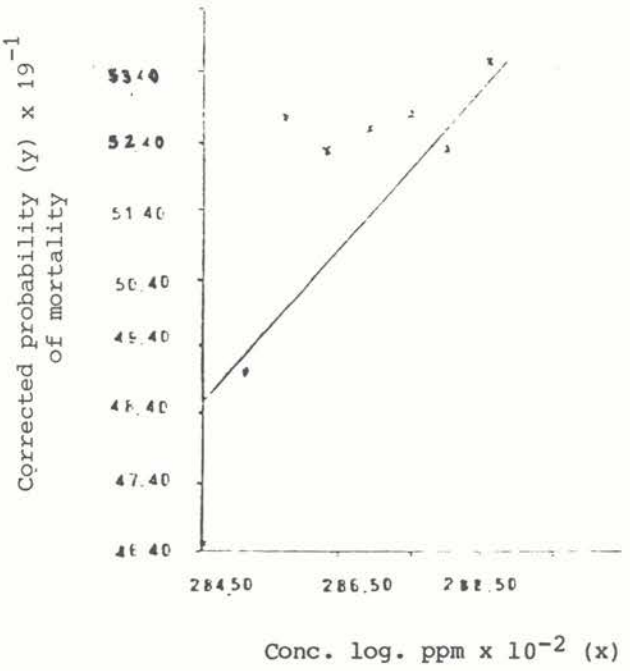

Figure 2. Correlation between corrected probability of mortality (y) and log. of dispersant (Finasol OSR_5) concentration (ppm) (x). Animal tested: Artemia nauplii, strain from Messolonghi, stad I. temperature 25° C.

Acute toxicity tests in the laboratory, like the ones we report here are not sufficient enough to give an overall idea of the action of dispersants used against pollution.

Tests in the field are certainly more valuable, since one can observe the effects on the food chain as well. In that case, however, costly and time-consuming tests are needed, which should be based on a previous deeper knowledge of the ecosystem.

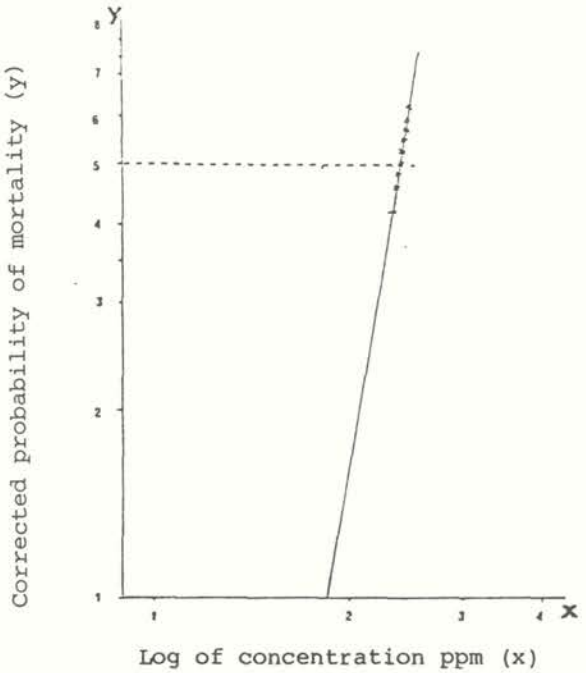

Figure 3. Correlation between corrected probability of mortality (y) and log. concentration of the mixture dispersant/gas oil (x). Animal tested: Artemia nauplii, strain from Messolonghi, stad. I. temperature: 25^0 C.

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THE VARIABILITY IN LEVELS OF COPPER AND ZINC IN THE TISSUES OF SELECTED SPECIES FROM THE BERRE LAGOON (FRANCE)

by

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1. INTRODUCTION

A 17-month survey was made to study the bioaccumulation of copper and zinc ions by 3 species of molluscs in the Berre lagoon (France). This lagoon, on the Mediterranean side, is subject to urban and industrial pollution (Arnoux, 1976). It is located near Marseille and has the peculiarity of receiving both freshwater from the Durance channel to the North and sea water that passes through the Caronte channel from the South. Its salinity varies from 4 to 30°/oo (Minas, 1973) and is governed by the freshwater supply from the Durance channel.

In the Berre lagoon wide fluctuations occur in the quality of both the water and the sediment. This paper investigates the interaction between water and sediment quality and the levels of two pollutants, copper and zinc, in the tissues of molluscs.

2. MATERIALS AND METHODS

The three species of molluscs were collected from three sites in the lagoon, which were located in the Caronte channel and its extension into the lagoon (Fig. 1). The species chosen included two filter feeders, Mytilus galloprovincialis and Tapes aureus; and the gastropod Murex trunculus which is a carnivore and scavenger.

Sampling was carried out by dredge and was limited to the Southern part of the lagoon since the Northern part has anoxic conditions (Stora, 1976). The specimens were kept in the deep freeze (-30°C) from two to five days, after which they were lyophilised and homogenised.

The tissues were weighed and 0.5 g of tissue was digested in 5 ml of nitric acid in sealed Teflon bombs for 2h at 150°C. After digestion, the determination of copper and zinc in the tissues was made using a flame (air-acetylene) Philips (IL 251) Atomic Absorption Spectrophotometer (UNEP/FAO/IAEA, 1982).

3. RESULTS AND DISCUSSION

The bioaccumulation of copper and zinc ions varied with the species and the stations of study. A wide change was also observed in relation to time.

The results that were obtained during this study are presented in Figs.2 and 3.

It can be seen that the fluctuations in the concentration of both metal ions follow the same pattern for the 3 species which are:

(a) Decrease of copper and zinc concentrations in July 1978 specimens, which continues for T. aureus up to October.

(b) Decrease in the concentrations of the metal ions by M. trunculus and T. aureus collected during November 1978 (Station A).

(c) Increase in the concentrations of copper and zinc in specimens collected during May and June 1979. This increase could be attributed to different metabolism that these species have during this period (Romeril, 1974).

Measured concentrations of copper and zinc in the tissues of the tested species from all three stations follow the same pattern, although much higher levels of zinc were present.

At station B there is an almost constant gradual decrease in the concentration of copper ions in T. aureus from February to November (Fig.2); on the other hand a sharp decrease of the zinc concentration was observed. At station A, although the rate of decrease of zinc is similar to that of copper, the elimination rate of copper in T. aureus is much slower.

For mussels (M. galloprovincialis) the sharp decrease in the concentration of copper and zinc ions between February and July is followed by a slow increase during the second phase of study (July - November 1978).

The metal concentrations in M. trunculus were much higher, but the corresponding decrease was not as intense. The reason for this may be the food source of M. trunculus. It has been shown that many predators accumulate metal ions to greater concentrations than species which belong to lower trophic levels (Aubert et al., 1976).

The results show that the rate of change of metal content represents the sensitivity of the species to environmental conditions or the intensity of the variations of the environment.

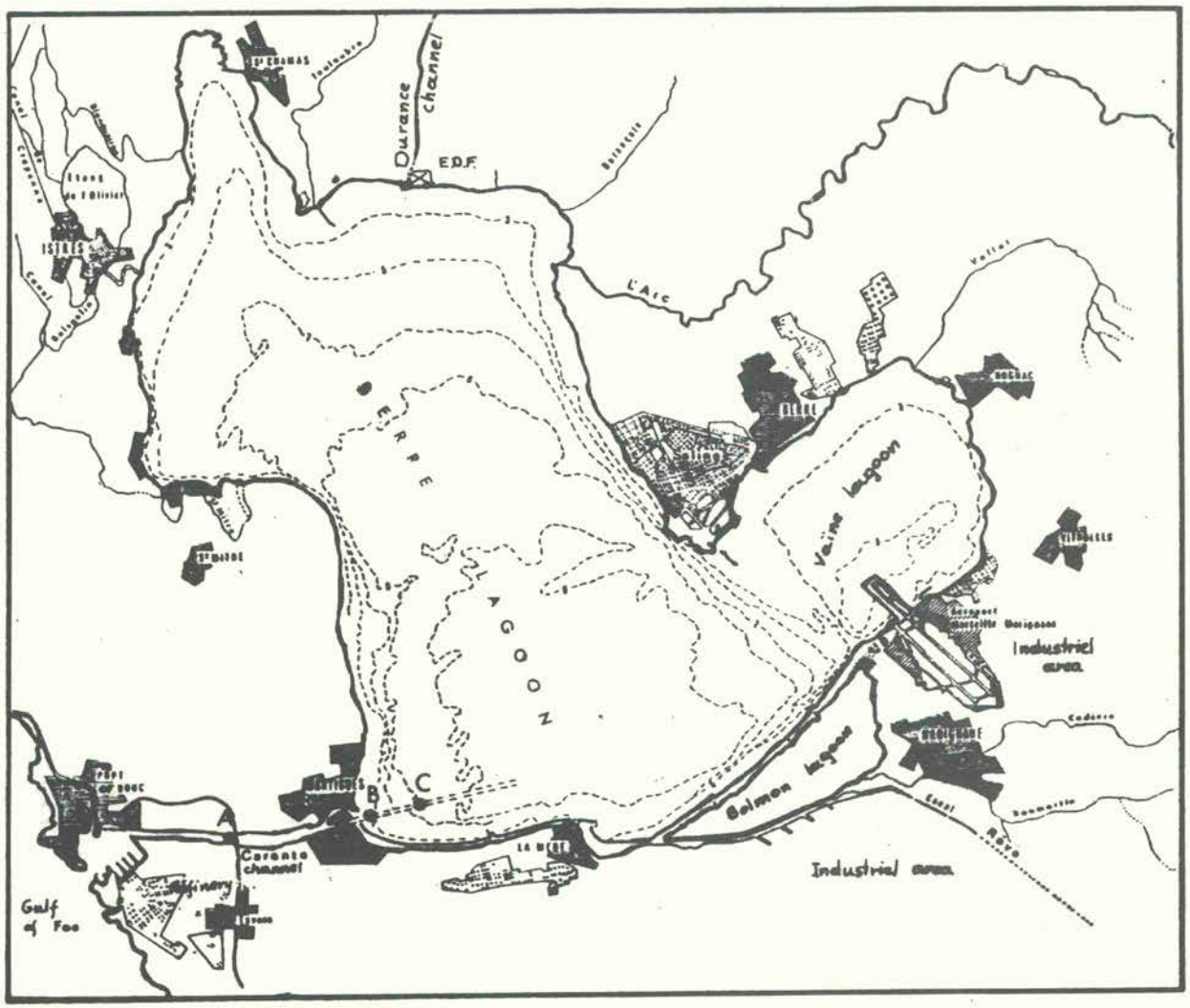

Figure 1. Sampling area

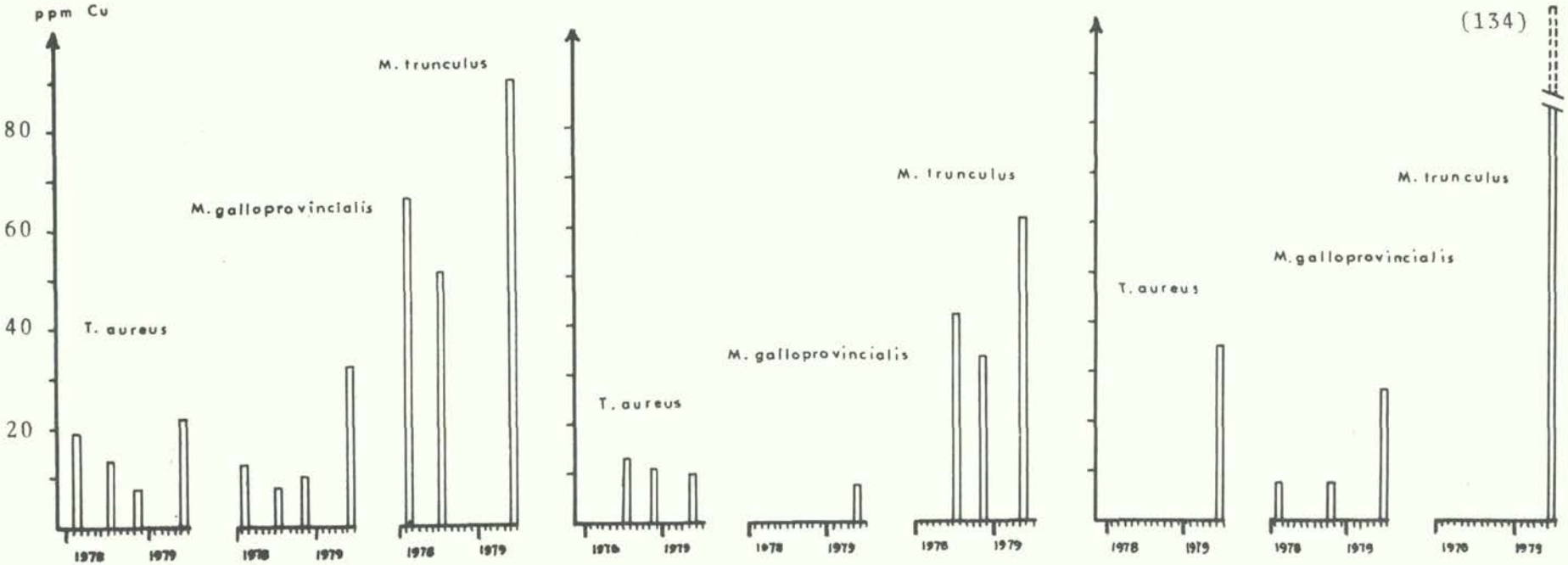

Figure 2. Cu levels in the tissues of Berre lagoon molluscs

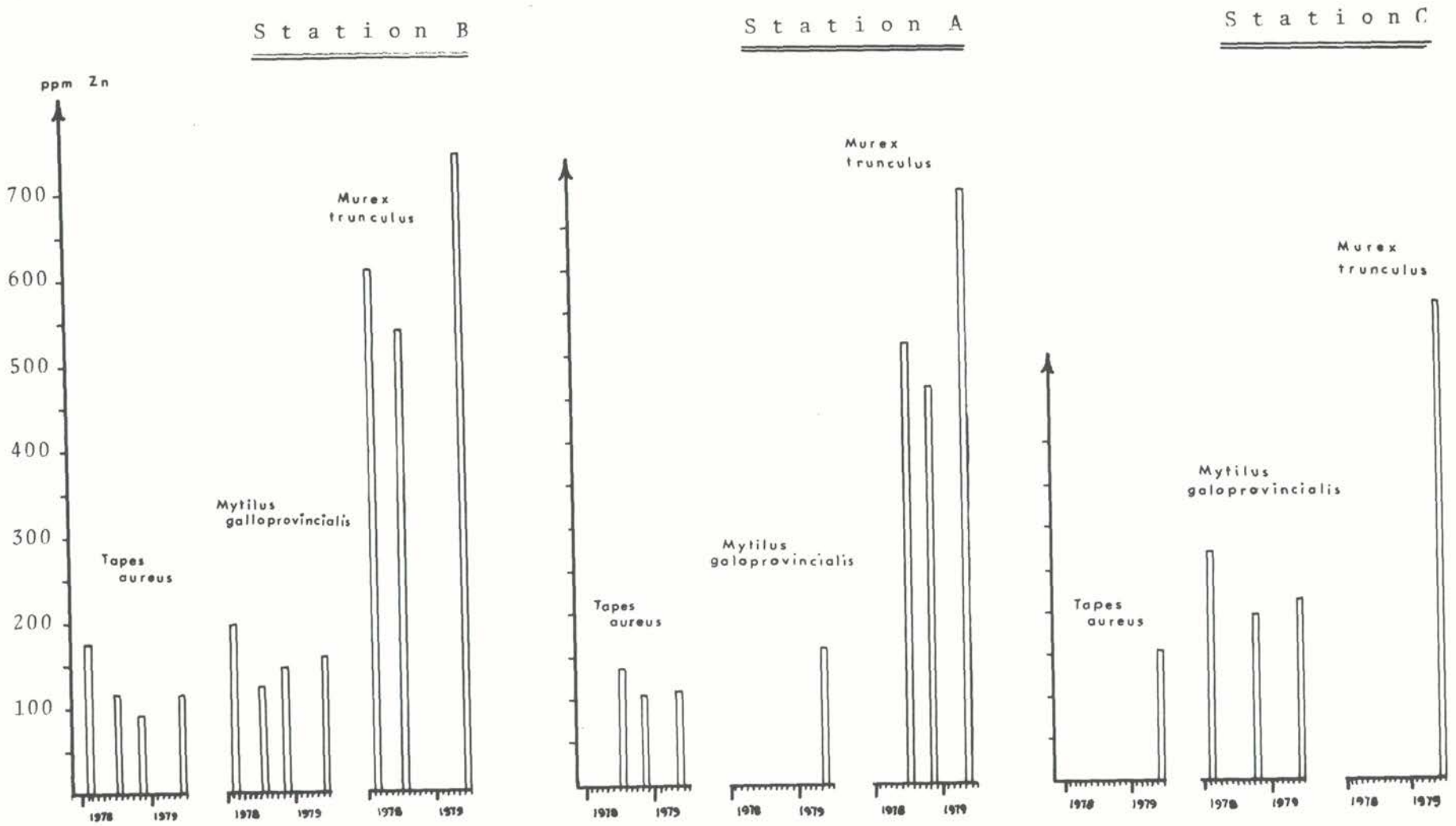

Figure 3. Zn levels in the tissues of Berre lagoon molluscs

Many authors that have studied coastal populations reported variability of the assimilation of metal ions depending on age, season of sampling and location of sampling sites, although no definite systematic relation was observed with these factors (Fowler and Oregioni, 1976; Capelli et al., 1978; Majori et al., 1978).

The water supply and suspended solids of the Durance channel play a very important role in the water quality of the Berre lagoon, not only because they are abundant but also due to their ability to fix and transport pollutants dissolved in the water.

The large inputs of water and mud, and their variability in quantity, may be responsible for the variations that are observed in the concentration of metal ions in the molluscs. In an effort to find a relation between the above mentioned phenomena a correlation between the input of water and suspended solids of Durance and the concentrations in T. aureus was made (Figs.4 and 5).

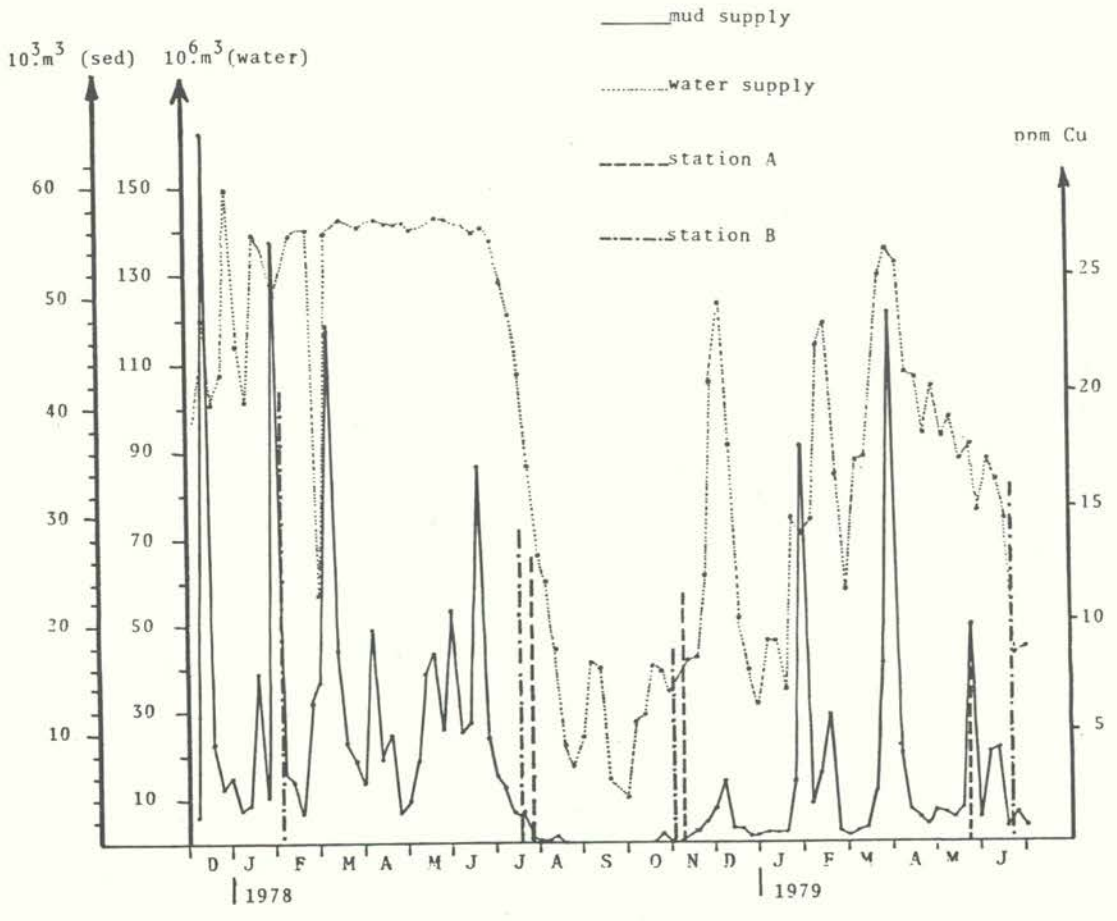

Figure 4. Copper levels in T. aureus tissues and water and mud supply of Durance channel during February-June 1979

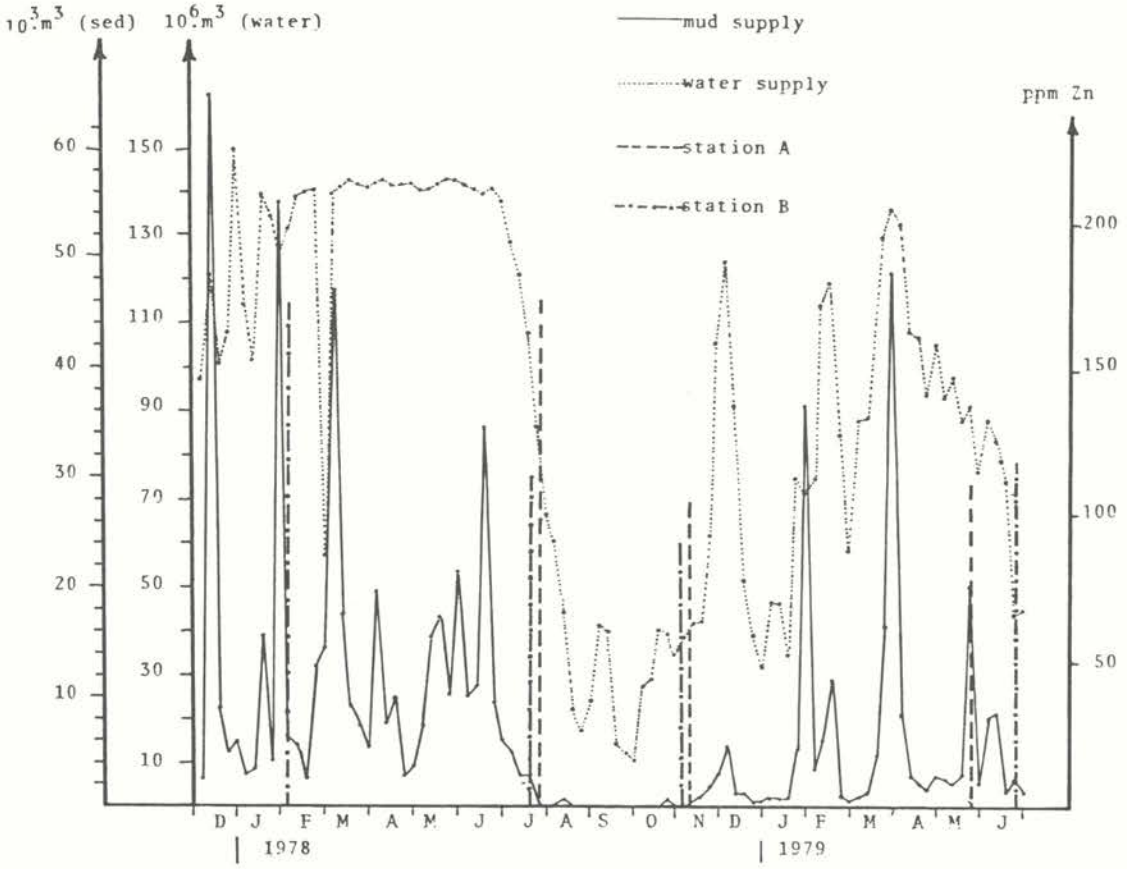

Figure 5. Zinc levels in T. aureus tissues and water and mud supply Durance channel during February 1978-June 1979

T. aureus were collected at stations A and B at 9m depths in the Caronte channel and its extension into the lagoon. The decrease in concentrations of copper and zinc ions that was found in their tissues seems to be related to the decrease of suspended solids of the channel : the peak of copper bioaccumulation of T. aureus follows the peak of mud input. The minimum recorded copper level follows a period in which there is no mud input.

A gradient of metal ions accumulated by the molluscs in stations A, B and C was frequently observed; the relation of which was st A st B st C. This could be explained by the difference of the hydrodynamics at those stations (Catsiki, 1980).

4. CONCLUSIONS

The results suggest that the concentrations of copper and zinc ions in the tissues of the selected molluscs are dependent on the species and the reaction of these organisms, during the period of study, to the environmental conditions. The ability of T. aureus and M. galloprovincialis to assimilate or to eliminate metal ions follows the same pattern for each metal; this could be because fluctuations in levels of copper and zinc in the environment follow a similar pattern, and because the species react similarly. The gastropod M. trunculus shows the same pattern, although much higher values of copper and zinc concentrations were observed. Variations in mud input to the Durance channel may also affect the bioaccumulation by the molluscs of copper and zinc ions.

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EFFECTS OF PCBs ON Leander adspersus: TOXICITY, BIOACCUMULATION, OXYGEN CONSUMPTION, OSMOREGULATION

by

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1. INTRODUCTION

The short- and long-term effects of PCB Aroclor 1254 on marine organisms have represented our principal research and study topic in the last years. We studied the copepod Tisbe bulbisetosa (Dalla Venezia et al., 1981) and the bivalve Mytilus galloprovincialis (Dalla Venezia et al., 1983) under polluted conditions, in regard to different parameters, such as survival, fecundity and coupling, for the first species; survival, behaviour, bioaccumulation and oxygen consumption, for the second one. Both species were found to be very tolerant to concentrations of 100 $\mu g\ l^{-1}$ (T. bulbisetosa) or even 1000 $\mu g\ l^{-1}$ (M. galloprovincialis) for a period of at least a week, at normal salinity level (34 $\pm$ 2‰).

For the present research we chose the crustacean decapod Leander (syn. Palaemon) adspersus, firstly since it was previously used in similar experiments with good results (Pihl Baden 1982, 1982a), and secondly because it is frequent in the lagoon of Venice. On the basis of our previous work, we carried out cross-experiments, which consisted in the exposure of test animals to nine PCB - salinity combinations, in order to test the toxicity of pollutant also in extreme environmental conditions. In a second phase, we have investigated whether a long exposure to low concentrations of PCBs produces any significant alteration on oxygen consumption and osmoregulation of the shrimp.

2. MATERIALS AND METHODS

Shrimps, Leander adspersus, were collected by a special trawl net from the shallow waters of an area of the lagoon of Venice, far from pollution sources. After collection, the shrimps were transferred into laboratory aquaria, kept at a temperature as close as possible to the external one and at a salinity of 34 $\pm$ 1‰. The temperature was raised, or lowered, by 1°C per day to reach the maintenance temperature of 18°C, by the use of a cryo-thermostat. During the acclimation period, of at least one week, water was continuously filtered and aerated, and shrimps were fed on soft parts of mussels. All experiments were carried out in a constant temperature room.

PCB suspensions were prepared by adding to sea water an ethanolic solution of 10 g l^{-1} Aroclor 1254. In the toxicity experiments the emulsifier Corexit 7664 was added, as stabilizing agent (see Dalla Venezia et al., 1981).

2.1 Toxicity

Groups of adult Leander adspersus were transferred from the acclimatization aquarium into nine jars containing sea water at nine pollution-salinity combinations, in the range 10-1000 $\mu g\ l^{-1}$ PCB and 10‰-50‰ salinity, plus controls. The temperature was 18°C. Aroclor 1254 was mixed with 0.2 ml l^{-1} Corexit 7664. Every second day the water was changed in all the jars, because it was not advisable to use any filter, working with a suspension. Dead animals were counted and removed daily.

2.2 Oxygen consumption

Shrimps were kept in two aquaria, the first containing unpolluted sea water as control, the second sea water plus 1 $\mu g\ l^{-1}$ PCB (both aquaria at 34 $\pm$ 1‰ and 18°C). The control shrimps were fed on soft parts of wild mussels (PCB content: 0.041 to 0.055 $\mu g\ g^{-1}$ wet weight); the PCB-treated shrimps were moreover fed on PCB-contaminated mussels (PCB content: 3.2 $\mu g\ g^{-1}$ww). These mussels had been

maintained for one month at 10 $\mu g\ l^{-1}$ PCB for this purpose, and analysed in our laboratory. For incubation no emulsifier (Corexit) was added to PCB, because the shrimps have to be used also in the following osmoregulation experiments. It is possible in fact that an emulsifier may interfere with osmoregulation (Butler et al., 1982). In both aquaria, water was renewed twice a week. No filter was used, for the reasons mentioned above.

After a period ranging from 22 to 29 days, groups of adult Leander adspersus were distributed individually in flasks containing oxygen-saturated clean sea water at three salinities (10°/oo, 30°/oo, 50°/oo). Flasks were stoppered and after two hours the residual oxygen was determined by the Winkler method (Ansell, 1973). The difference between the oxygen content in the experimental and the control (without shrimps) flasks were taken as the oxygen consumed during the period. The measurements were repeated at three different temperatures (10°C, 18°C, 25°C).

At the same time, other shrimps were kept in 1 $\mu g\ l^{-1}$ PCB and fed on wild mussels, like the controls. After a period ranging from 17 to 38 days, shrimps were chosen, two at a time, one from PCB-contaminated aquarium, another from control and put into two flasks, in which oxygen probes were inserted. In this way it was possible to record the oxygen consumption of each single individual, first at 35°/oo, then at 50°/oo salinity. Measurements, done at 18°C, were repeated in six contaminated individuals and in six controls. At the end of each experiment the shrimps were dried at 110°C for 48 hours. All results were converted to $\mu g\ g^{-1}$ dry weight h^{-1}.

2.3 Osmoregulation

Adult Leander adspersus, used in this experiment, were maintained as described in 2.2 above for a period ranging from 21 to 30 days. They were distributed in three jars containing sea water at different salinities (10°/oo, 30°/oo, 50°/oo). After two hours, a sample of haemolymph was withdrawn from each animal. The samples were kept in a refrigerator. This procedure was repeated at three different temperatures (10°C, 18°C, 25°C). Before making the measurements by a Vogel Osmometer (based on freezing point depression), each sample was centrifuged at 15,000 rpm for 2-3 min., to separate the serum from the clot.

2.4 Bioaccumulation

At the end of two of the three experiments described in 2.3, that is after 21 and 28 days of exposure to PCB suspension and PCB-contaminated feeding, the shrimps were shucked. The flesh was freeze-dried, then extracted for eight hours with n-hexane in a Soxhlet apparatus. The extract was concentrated, cleaned by shaking with sulphuric acid and subjected to gas-chromatographic analysis. The results were calculated on the basis of wet weight.

3.1 Toxicity

The graphs in Fig. 1 show the percentage mortality at three PCB-concentrations and three different salinities, plus controls over a period of nine days. The 96-hour LC50 may be calculated by graphic interpolation, and ranges from 10 to 100 $\mu g\ l^{-1}$ PCB at 50°/oo, and from 100 to 1000 $\mu g l^{-1}$ PCB at 30°/oo and at 10°/oo.

3.2 Oxygen consumption

Mean values and standard deviations of oxygen consumption data, determined by the Winkler method, are reported in Table I. A large individual variability (high standard deviations) is evidenced, and that advises against the comparison of the means. Mean values reported in Fig.2 show a significant difference in oxygen consumption with the temperature, while the differences with salinity are questionable.

Table II shows the results of measurements, repeated at two different salinities, on each single individual, numbered from 1 to 12. In this case also, the comparison of means does not improve the information, while the comparison of the value at 35°/oo with the value of 50°/oo for each shrimp, using the "sign test" (Sokal and Rohlf,

1969), shows that the difference between oxygen consumption rates at the two salinities is significant ($P < 0.05$). The pre-exposure to PCBs does not influence the consumption.

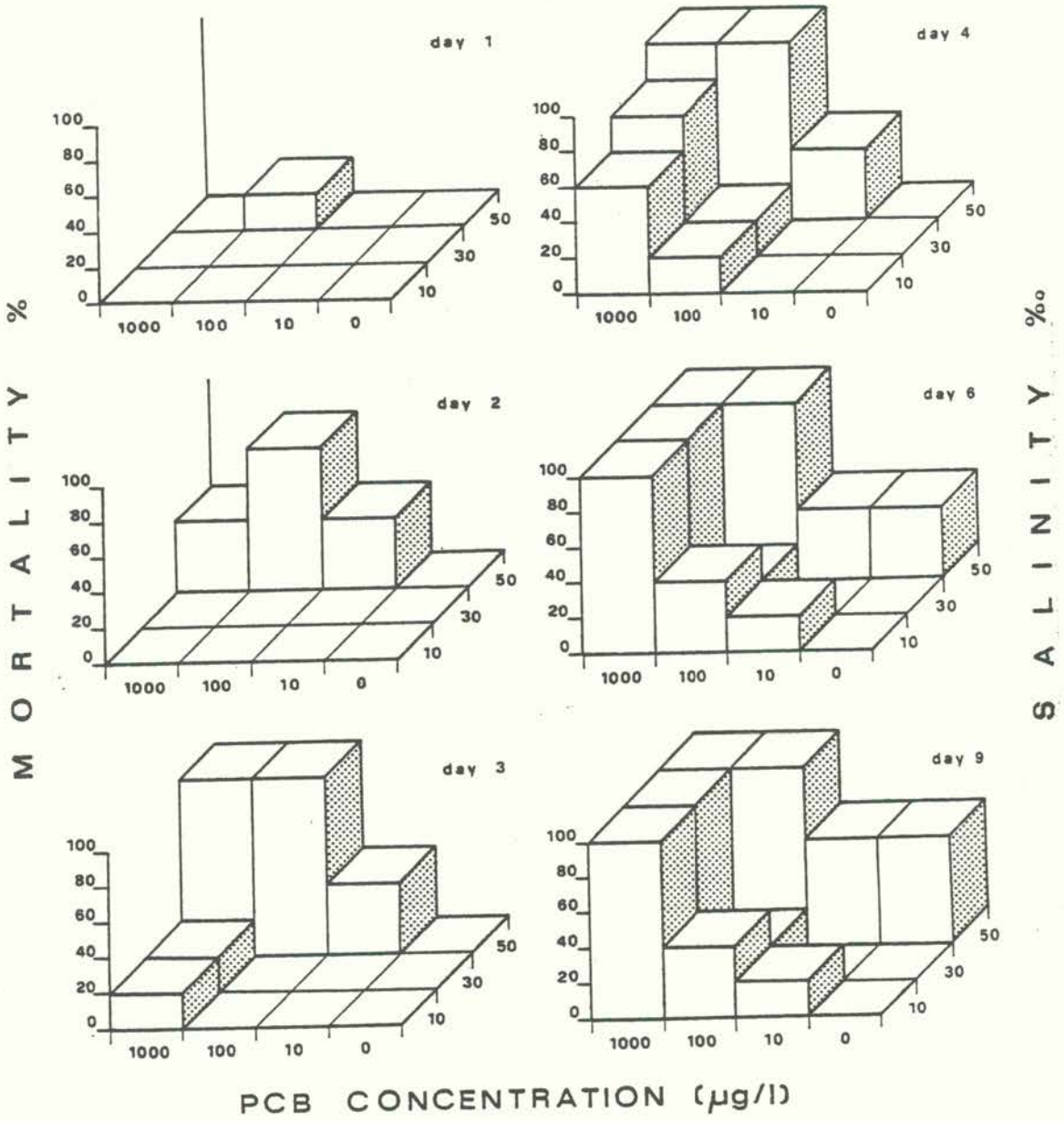

Figure 1. Leander adspersus. Tri-dimensional graphs showing the toxicityof Aroclor 1254 at different salinities and 18°C. The length of exposure (days) is reported on the right of each graph.

3.3 Osmoregulation

The graphs in Fig.3 show that Leander adspersus is an exceptional osmoregulator. Its osmoregulatory ability is almost independent of both temperature and PCB pollution. Table III shows that individual variability of osmotic concentration of serum is rather small, and that the difference between PCB-treated and controls is not significant.

3.4 Bioaccumulation

Table IV shows the PCB content of flesh of shrimps after different treatments, and of mussels, which served as food. The ability of shrimps to concentrate the PCBs

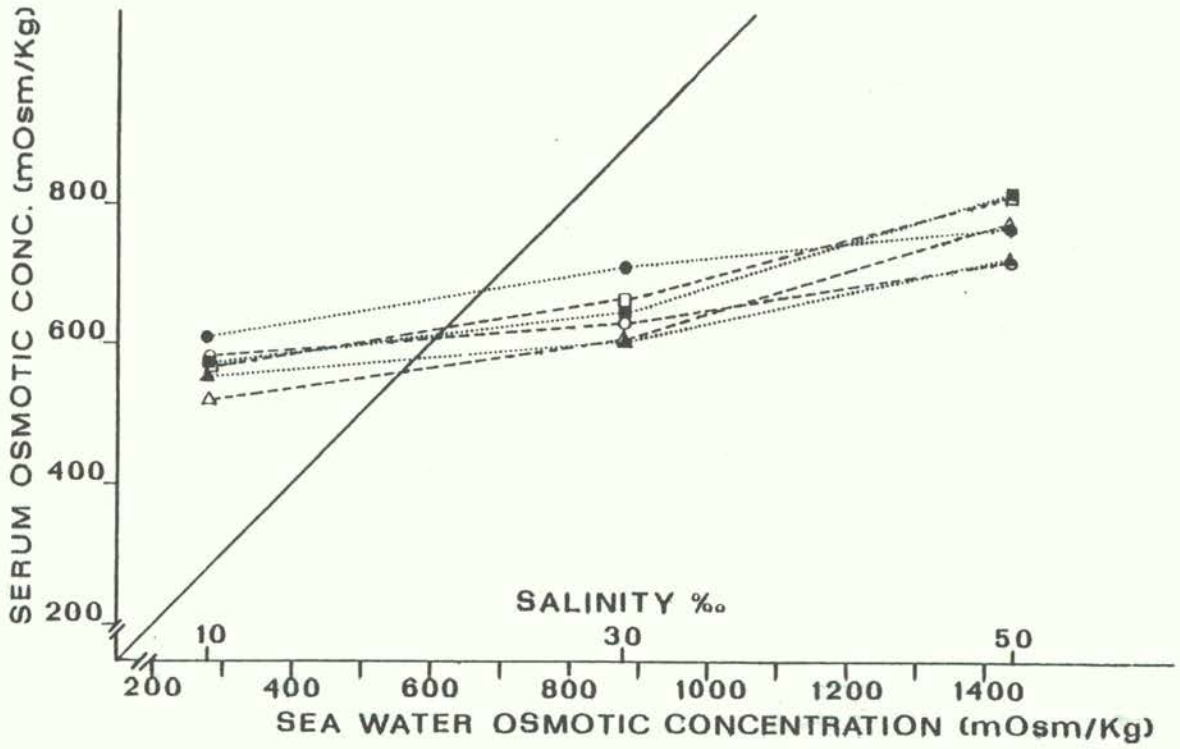

Figure 2. Leander adspersus. Mean oxygen consumption measured on groups of five individuals by Winkler method in relation to salinity and temperature. Open symbols: controls; filled symbols: PCB-treated animals (see text). Circles: 10°C; triangles: 18°C; squares: 25°C.

from food is confirmed, when we work at levels similar to those of the natural environment. Instead, when we use as food mussels with high levels of PCBs, the content in tissues of shrimps increases, but not proportionally to that of food.

4. DISCUSSION

Before drawing any conclusion from the results obtained in the present work, or comparing these results with those of other authors, it is right to recall the levels of PCBs in open and coastal waters of the Mediterranean (Table V). Some examples of acute toxicity data of PCBs on different species are presented in Table VI. By comparing Table VI with Table V, one can see that the 96-hour LC50's for Leander adspersus, Tisbe bulbisetosa, Mytilus galloprovincialis, as well as those calculated by other authors for Penaeus duorarum and Palaemonetes pugio, are three to six orders of magnitude higher than PCB concentration in sea water. Even though lower values of lethal PCB concentrations (0.94 to 19.0 $\mu g\ l^{-1}$) have been observed by Nimmo et al. (1971) by exposing Penaeus duorarum for periods ranging from 15 to 53 days in flowing water, one must conclude that the mortality does not represent a parameter sensitive enough to be used in biological monitoring of marine pollution.

Table I.

Oxygen consumption ($\mu g\ g^{-1}$ dry weight h^{-1}) measured by Winkler method at different temperature-salinity combinations on groups of five individuals. Means and standard deviations.

S°/oo	Controls	n	PCB-treated	n	t°C
10	868.3±87.5	5	825.9±257.3	5	10
30	1180.3±299.6	5	1290.8±395.4	5	10
50	909.3±183.7	5	875.7±174.3	5	10
10	1630.6±218.6	5	-		18
30	1779.4±612.4	5	-		18
50	1681.1±199.7	5	-		18
10	2308.9±240.3	5	2118.6±490.9	5	25
30	2711.6±466.5	5	1907.2±955.8	5	25
50	2487.1±603.9	5	1968.9±350.4	5	25

Table II.

Oxygen consumption ($\mu g\ g^{-1}$ dry weight h^{-1}) measured by polarographic electrodes at 18°C and two salinities, in consecutive days on individual shrimps, coming either from clean or from PCB-treated water.

EXPOSURE days	CONTROLS			PCB-TREATED		
	no	35°/ooS	50°/ooS	no	35°/ooS	50°/ooS
17	1	2175.3	1977.5	7	1106.1	663.6
24	2	1977.3	1258.3	8	1468.8	856.8
29	3	1360.6	1107.1	9	2429.2	1670.1
31	4	1522.1	1343.1	10	3742.8	1981.5
36	5	2813.7	2110.3	11	1740.4	1160.3
38	6	2950.1	1659.4	12	1601.5	800.7
Mean		2182.7	1576.0		2014.8	1188.8
St.dev.		589.0	407.1		951.6	528.7

Several authors have utilized the oxygen consumption rate as a parameter for investigating the physiology of different organisms in polluted and unpolluted conditions. Hagerman (1970) has studied oxygen consumption in clean sea water in relation with salinity and temperature, working on Crangon vulgaris and using an experimental model similar to ours. The results on Crangon and Leander agree as regards temperature: the higher the temperature, the higher oxygen consumption rate; as for salinity, however, the response of Leander seems to be different from that of Crangon. However, the large individual variability does not allow comparisons to be made on the basis of mean values unless the difference between PCB-treated animals and controls is wide enough.

Table III.

Osmotic concentration (mOsm kg^{-1}) of serum in relation to salinity and temperature. Means and standard deviations.

S^{o}/oo	Medium mOsm kg^{-1}	Controls	Serum n	PCB-Treated	n	$t^{0}c$
10	282	579.2 ± 52.0	9	607.1 ± 47.7	8	10
30	878	631.0 ± 51.3	9	710.4 ± 93.3	9	10
50	1444	721.4 ± 73.9	9	769.6 ± 23.9	9	10
10	282	520.6 ± 52.9	8	555.6 ± 21.8	5	18
30	878	611.3 ± 59.7	7	606.9 ± 61.2	7	18
50	1444	777.3 ± 93.5	9	723.8 ± 84.6	8	18
10	282	567.4 ± 32.1	9	571.2 ± 21.5	10	25
30	878	663.7 ± 33.3	11	643.6 ± 30.5	9	25
50	1444	816.6 ± 44.9	11	824.7 ± 46.1	10	25

Table IV.

Bioaccumulation of PCBs in soft parts of Leander adspersus and Mytilus galloprovincialis ($\mu g\ g^{-1}$ wet weight).

Wild mussels	0.041 to 0.055
Control shrimps (fed on wild mussels for about one month)	0.085 to 0.150
PCB-treated mussels (exposed to 10 $\mu g\ l^{-1}$ PCB for about one month)	3.200
PCB-treated shrimps (exposed to 1 $\mu g\ l^{-1}$ PCB and fed on PCB-mussels for 21 days)	0.791
PCB-treated shrimps (as above for 28 days)	0.689

Vernberg et al. (1978), in order to overcome this variability, experimented on single organisms, which served as their own controls. These authors, working on Uca pugilator, found with Aroclor 1254 a significant increase in oxygen consumption rate when animals were transferred from clean sea water to a suspension of 50 $\mu g\ l^{-1}$ PCB (see also Dalla Venezia et al. 1983).

However, when animals were maintained for a long period at low concentrations of PCBs (as in this work), no difference was observed in oxygen consumption rate with the controls. Probably acclimation, rather than bioaccumulation, plays an important role in determining their response.

On the basis of the work of Bursey (1978) on the crab Emerita talpoida and Hagerman and Uglow (1983) on Palaemonetes varians, we expected to find some significant difference on haemolymph osmotic concentration at least following an abrupt change of both temperature and salinity. Furthermore, Roesijadi et al. (1976), keeping Palaemonetes pugio for 96 hours at constant temperature in a concentration of about 30 $\mu g\ l^{-1}$ PCB, found some alteration in this parameter, after exposure to different salinities. Considering all these results, it was reasonable to suppose that a

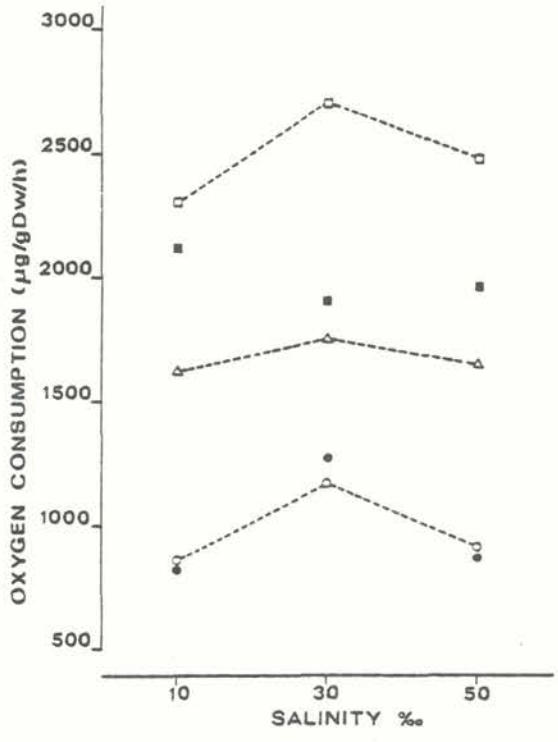

Figure 3. Leander adspersus. Mean osmotic concentration of serum in relation to salinity and temperature. Symbols as in Figure 2.

prolonged treatment with PCB plus PCB-contaminated food, combined with changes in temperature and salinity, should give a more evident alteration in the osmoregulation capability. This result was not obtained in our experiments.

As for bioaccumulation, Table VII shows the values of PCB concentration in flesh of crustacean decapods in natural and in various experimental conditions. It was demonstrated that concentrations up to 9.6 µg g^{-1} wet weight, which are 10^2 times higher than those of wild shrimps, do not significantly affect osmoregulation (Nimmo and Bahner, 1974). Unfortunately, in most cases it is not possible to find a threshold concentration of PCBs in water and in animal tissues, above which osmoregulation and oxygen consumption rate are disturbed. This instead represents the aim of our future researches. On the basis of the limited number of papers concerning the sublethal effects of PCBs, it seems advisable to adopt a few standardized experimental models. In this way it would be possible to improve the comparability of results on toxicity, bioaccumulation and physiological indexes of stress.

Table V.

PCBs in sea water. Data expressed in $\mu g\ l^{-1}$

Date	Location	Average	Range	References
1975	N.W.Mediterranean Coast	13×10^{-3}	$(1.5 - 38) \times 10^{-3}$	Elder, 1976
1975	Open Mediterranean Sea	2×10^{-3}	$(0.2 - 8.6) \times 10^{-3}$	Elder and Villeneuve, 1977
1976	Tiber Estuary	297×10^{-3}	$(9 - 1000) \times 10^{-3}$	Puccetti and Leoni, 1980
1977	Tiber Estuary	135×10^{-3}	$(n.d. -380) \times 10^{-3}$	Puccetti and Leoni, 1980
1977-78	Rijeka Bay	-	$(0.1 - 0.9) \times 10^{-3}$	Picer and Picer, 1979
1979	Po River Delta	7.9×10^{-3}	$(3.7 - 13.7) \times 10^{-3}$	Fossato et al., 1982
1980	Po River Delta	2.6×10^{-3}	$(1.2 - 5.5) \times 10^{-3}$	Fossato et al., 1982

Table VI.

Toxicity of PCBs to aquatic animals. Data expressed in $\mu g\ l^{-1}$.

Species	96 hour LC_{50}	References
Penaeus duorarum	10-100	Duke et al., 1970
Palaemonetes pugio	60	Roesijadi et al., 1976
Tisbe bulbisetosa	500	Dalla Venezia et al., 1981
Mytilus galloprovincialis	1000	Dalla Venezia et al., 1983
Leander adspersus	100-1000	Present work

Table VII.

Concentrations of PCBs in some species of Crustacean decapods from differently polluted areas or after experimental treatments. Concentrations are expressed in $\mu g\ g^{-1}$ wet weight.

Species	Location	Exp. Conditions	PCB	References
Leander sp.	Lagoon of Venice	-	0.019-0.098	Fossato, 1983
Crangon crangon	"	-	0.018-0.082	"
C. mediterraneus	"	-	0.015-0.092	"
Crangon crangon	Weser Estuary	-	0.056-0.064	Goerke *et al.*, 1979
Penaeus aztecus	Gulf Shores (Alabama)	-	0.100	Nimmo and Bahner, 1974
Penaeus duorarum	Tampa (Florida)	-	0.010	Nimmo *et al.*, 1971
Penaeus aztecus	Gulf Shores (Alabama)	PCB 3 ug l^{1} - 7 days (flowing water)	7.1-9.6	Nimmo and Bahner, 1974
Penaeus duorarum	Tampa (Florida)	PCB 2.5 ug l^{1} - 15 days (flowing water)	ca. 40	Nimmo *et al.*, 1971
Leander adspersus	Lagoon of Venice (Venice)	PCB 1 ug l^{-1} - 21/28 days + PCB-contaminated food (static water)	0.69-0.79	present work

5. ACKNOWLEDGMENTS

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BIOACCUMULATION OF CADMIUM IN PLANKTON, BIVALVES, CRUSTACEA AND IN DIFFERENT ORGANS OF SIX FISH SPECIES FROM MEX BAY, WEST OF ALEXANDRIA

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1. INTRODUCTION

Cadmium being in the middle of the periodic sub-group consisting of zinc, cadmium and mercury reveals intermediate properties. All three elements display a profound capacity for combining with -SH (and amidazol-containing ligands); the stability of such complexes increases in the order of Zn $>$ Cd $>$ Hg. Cadmium and mercury compete with and displace zinc in a number of zinc containing metalloenzymes by irreversibly binding to active sites, thereby destroying normal metabolism.

Cadmium is regarded as one of the most toxic metals after mercury (Bryan, 1971). Only since mercury and cadmium accidental poisoning in Japan has the emphasis been shifted towards investigations dealing with the ability of the aquatic organisms to accumulate heavy metals, especially those non-essential metals such as mercury and cadmium.

In Mex Bay, west of Alexandria, mercury in the different trophic levels has been investigated and reported elsewhere by Aboul Dahab et al.(1986). The present work deals with cadmium concentrations in the different trophic levels in this Bay. Some hydrochemical characteristics of the Bay can be found in El-Rayis et al.(1986).

2. MATERIALS AND METHODS

The location of Mex Bay relative to Alexandria and the sampling stations are shown in Fig. 1.

Bivalves, Mactra corallina and Donax trunculus, a crab (Neptunus pelagicus), a shrimp species (Penaeus kerathurus) and six fish species (5 teleostei and one elasmobranch, Table I), were collected from Mex Bay, during February 1983. Mixed plankton samples were collected from the upper half meter at 16 stations (Fig. 1) by means of a nylon phytoplankton net. The plankton samples were filtered through 0.4 um Nuclepore filters. The bottom sessile animals, Donax and Mactra were collected by a bottom trawler. Crab, shrimps and fishes were collected by a commercial trawler net.

All biological samples were identified and prepared for analysis as described by Bernhard (1976). Determination of cadmium in the samples and in the filtered plankton was carried out according to Harms (1980). Measurements of cadmium concentrations were done with a Perkin-Elmer graphite furnace-atomic absorption spectrophotometer. All manipulations were carried out in a laminar flow clean-bench in a dust-free room. Ten replicate analyses of standard 1577 "Bovine liver" from the National Bureau of Standards, USA containing 0.27 ± 0.04 µg Cd g^{-1}, yielded a mean value of 0.25 ± 0.05 µg g^{-1}. The detection limit for cadmium was 1 ng, the standard deviation 5%. Recovery of cadmium after standard addition to homogenized tissue was 93.2 ± 15.6%.

3. RESULTS

The range and mean concentrations of cadmium in the different marine organisms studied in Mex Bay are shown in Table I. The table also shows range, mean and standard deviation of weight and cadmium concentration in different organs of the fish species.

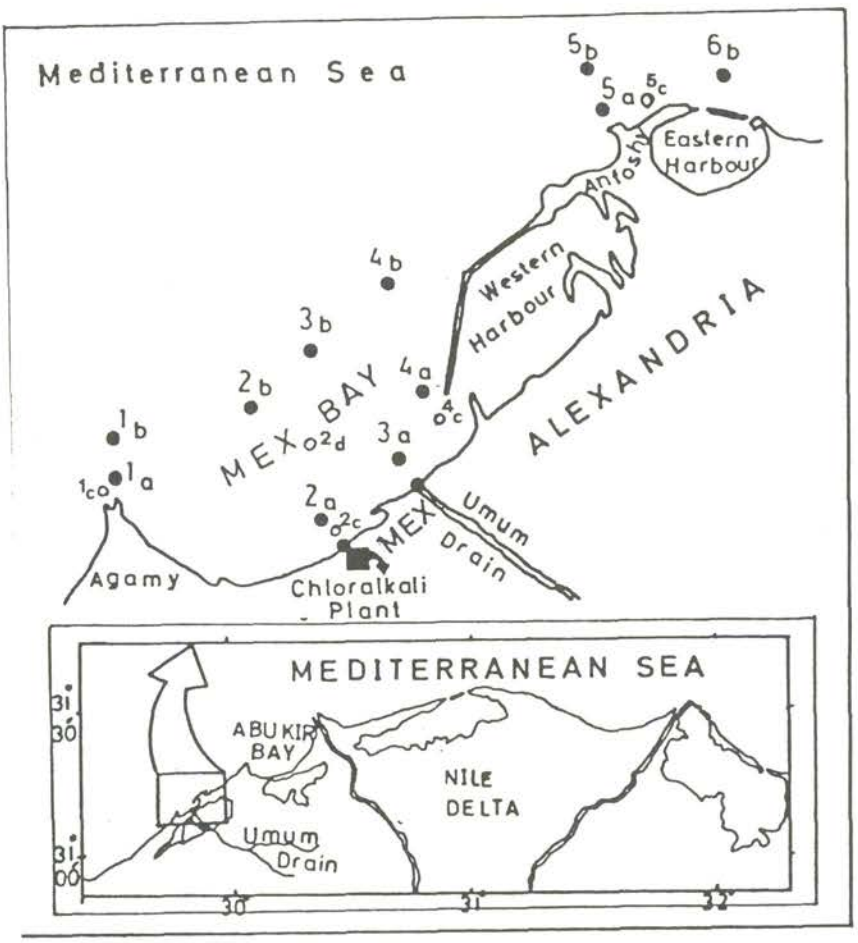

Figure 1. Area of study and sampling stations

Plankton

The distribution of cadmium in the mixed plankton samples is shown in Fig. 2 and presented in Table II. Cadmium concentrations in the mixed plankton collected from Mex Bay ranged between 88 and 258 µg kg^{-1} (fresh weight). Distribution of cadmium concentrations in the surface mixed plankton is shown in Fig. 2. The highest concentrations (248 and 213 µg kg^{-1} fresh weight) were observed in the samples collected from the inshore waters off the two land based-sources, off the agricultural "Umum" Drain and the chlor-alkali effluent, i.e. at stations 3a and 2c respectively (Fig.1). There is a seaward decrease in the cadmium concentration in the plankton.

Bivalves

Both concentration ranges and average values of cadmium in the soft parts of the two bivalves, Donax and Mactra , were close to each other (Table I). The concentrations of cadmium in these organisms ranged between 124 and 875, with an average value of 450 µg kg^{-1} fresh weight. Both are filter feeders, feeding on plankton and suspended organic matter either settling from the above layer or resuspended from the bottom sediments. From Table I, one can see that bivalves are good accumulators of cadmium relative to all the other organisms studied from different trophic levels. However, it must be emphasized that the analysis also included the gut content for each individual. This could be the reason for the observed range of concentrations (124-875 µg kg^{-1} fresh weight).

Higher Marine Crustacea

The levels of cadmium in the flesh of the crab and shrimp studied seem to be quite similar to each other (Table I). The concentrations (µg kg $^{-1}$ fresh weight) ranged from 89 to 493 and from 145 to 419, with an average of 257 and 266 for the shrimp and crab respectively. The level of cadmium in crustacea, therefore, is lower than that in the bivalves but still higher than in organisms from higher trophic levels.

Table I.

Number of specimens (n), range and mean (with standard deviation in parentheses) of the animal weight and cadmium concentration (µg kg^{-1} fresh weight) in the different marine organisms studied.

Organism	(n)	Weight (g) range	Weight (g) mean	Cd concentration range	Cd concentration mean	S.D.	Note
1. Plankton	16			88-258	139	(45.2)	Phyto and zooplankton
2. Crustacea:							
shrimp	27	32.9-49.3	41.2 (1.8)	89-493	257	(102)	Flesh
Crab	17	113-186	150 (10)	145-419	266	(71.7)	Flesh
3. Bivalves:							
Donax	52	--	-	124-831	439	(179)	All soft parts
Mactra	55	--	-	184-875	477	(195)	All soft parts
4. Fish:							
Mullus barbatus	45	73-136	103 (17.2)	15-73	43.8	(13.9)	Flesh
				31.94	51.6	(14.7)	Gills
				40-132	82,8	(25.8)	Liver
Sardina pilchardus	32	40-88	68.7 (12.0)	29-74	51.5	(12.3)	Flesh
				43-90	63.3	(12.6)	Gills
				54-132	98.4	(21.1)	Liver
Boops boops	20	58-128	88.6 (16.8)	20-113	56.0	(23.9)	Flesh
				32-135	71.6	(31.2)	Gills
				42-178	101.8	(35.9)	Liver
Rhinobatus halavi	15	354-569	473 (71.3)	42-103	64.6	(17.1)	Flesh
				-	-	-	Gills
				75-214	131.5	(42.5)	Liver
Sparus vulgaris	29	72-123	95.8 (12.9)	18-133	67.0	(31.0)	Flesh
				32-156	86.2	(42.5)	Gills
				46.219	122.8	(49.1)	Liver
Euthynnus allette-ratus	16	289-517	401.3 (73.4)	49-134	88.3	(28.4)	Flesh
				59-152	103.1	(29.5)	Gills
				99-225	165.9	(41.5)	Liver

Fish

Cadmium concentration in the flesh of the six fish species ranged between 15 and 134 µg Cd kg $^{-1}$ fresh weight. The average concentration increases in the following order: Mullus < Sardina < Boops < Rhinobatus < Sparus < Euthynnus. Euthynnus accumulates more cadmium than the other five fish species, especially Sardina. This is a predatory fish, feeding on smaller fish like Sardina , which in turn is a phytoplankton feeder.

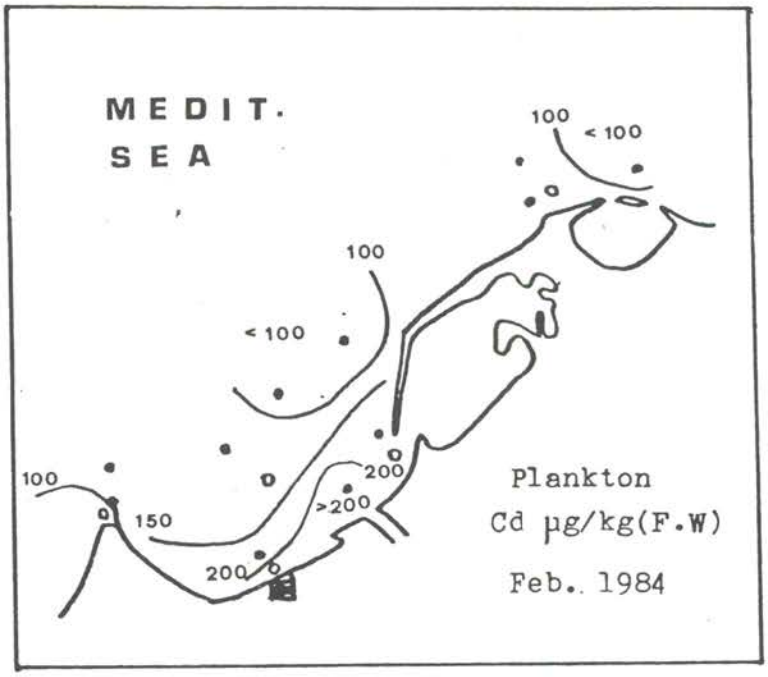

Figure 2. Distribution of cadmium in the surface mixed plankton from the coastal waters of Mex Bay, west of Alexandria

Table II

Cadmium concentration in mixed plankton (µg kg^{-1} fresh weight) from the surface coastal waters, west of Alexandria, Feb. 1984. The identification of the main plankton species is mentioned elsewhere (Halim, 1983).

Inshore station No.	Cd	Offshore station No.	Cd
1-a	127	1-b	153
2-a	151	2-b	131
3-a	248	3-b	94
4-a	158	4-b	91
5-a	111	5-b	102
1-c	95	6-b	90
2-c	213	2-d	146
4-c	177		
5-c	138		
Mean	157.6±48.8	Mean	115.3±27.3
Overall mean		139.±45.2	

Therefore Euthynnus, being at the highest trophic level, accumulates more cadmium than its prey, Sardina. Generally speaking, the levels of cadmium in all the fish species are lower compared to that in the lower trophic levels (mixed plankton). Further study of cadmium concentration in phytoplankton is required.

Examining the cadmium concentration in the different fish organs (flesh, gills and liver) it appears that gills and liver concentrations are about 1.2 and 2.0 times the flesh respectively. The order of accumulation of cadmium in the different organs is: liver $\rangle$ gills $\rangle$ flesh.

4. DISCUSSION AND CONCLUSIONS

Mex Bay receives considerable amounts of cadmium via the waters discharged at rates of 6×10^6 and 30×10^3 m^3 day^{-1} from the agricultural drain and the chlor-alkali plant effluents respectively. The daily input of cadmium in both its dissolved and particulate forms contributed by the sources to the bay is shown in Table III. The agricultural drain contributes 357 times that of the chlor-alkali plant effluent. This has led to increased concetrations of cadmium in the inshore water, to 191 ng l^{-1} relative to the offshore area (depth greater than 10 m) of 106 ng l^{-1}. The distribution of cadmium in water seems to coincide with that in mixed plankton. This suggests that plankton could be used as an indicator for studying cadmium contamination in the present area. This phenomenon was also noticed during the study of Hg distribution in mixed plankton in the same bay (El-Rayis et al., 1986).

In other areas in the world, such as in Sorfjorden in Norway, zoo- and phytoplankton were used as indicator organisms for heavy mercury contamination, where the mercury concentrations in plankton samples gradually diminished with distance from the source, (Skei et al., 1976). However, it seems preferable to select one or more of the dominant phyto- or zooplankton species to study their metal content as indicator species. Skei et al. (1976) and Turekian (1976) showed that the level of cadmium in both phyto- and zoo-plankton are quite similar to each other. The mixed plankton from Mex Bay showed a concentration level of 139 µg kg^{-1} FW, that is 675 times higher than that of the average background concentration level in water.

Bivalves are filter-feeding organisms, thereby reflecting the heavy metal composition of a limited space. They can therefore serve also as heavy metal indicator organisms. Unfortunately, no data for cadmium are available in literature for the two studied bivalves, Donax and Mactra, from other areas in the Mediterranean Sea. A level of 190± 9 µg Cd kg^{-1} FW was reported by Förstner and Wittman (1981) for Mactra clabrata. The level in Mactra corallina from Mex Bay, 457 µg kg^{-1}FW, is significantly higher. However from this it is very difficult to assess if the Mactra from the Bay accumulates more.

Although crustaceans are on a higher trophic level than bivalves, they showed a lower cadmium level than bivalves. Their feeding habits however are different, being phytophagous, carnivorous or both. In addition, their feeding habits change during their life cycle. Uysal (1981) reported a concentration range of 65-764 µg kg^{-1} FW from the Aegean Sea in P. kerathurus, which is slightly higher than that found in Mex Bay (89-393 µg kg^{-1}FW).

The present investigation of cadmium concentration in fish from Mex Bay had two aims, first to compare cadmium levels in Alexandria seafood to that found in other Mediterranean areas and second to locate the sites of greater bioaccumulation in the fish organs.

Table III

Average (6-monthly sampling) cadmium concentration and average daily amount of Cd contributed by the agricultural "Umum" Drain and the chlor alkali plant effluents to the Bay. (After Aboul Dahab et al., 1985).

	Agricultural drain	Chlor alkali plant effleunts
Concentration (ng l^{-1}) Dissolved	307	250
Particulate	109	28
% of particulate/total	26	10
Total daily contribution (g Cd day^{-1})	2496	7

Data about the level of cadmium concentrations in the same six fish species from other parts of the Mediterranean Sea are scarce. Values of 590, 100 and 52 µg Cd kg $^{-1}$ FW were reported for Mullus barbatus from Marseilles (GFCM, 1978); Ankra (Uysal and Tuncer, 1985); and from Greece (Taliadouri-Voutsinou, 1981) respectively. The present mean value (43.8±13.9 µg Cd kg $^{-1}$ FW) is much closer to the lowest level, reported from Greek waters.

On the basis of the varying affinities of the metal for the individual organs, the musculature here proves not to be the most suitable body part for determining the extent of the heavy metal concentration for the entire organism. The level of cadmium in the muscle tissues of the fish is always much lower than in the other organs, as shown in Table I. Interspecific variation in the metal content can not therefore always be determined by analysing the muscle tissue. Table IV, however, shows the correlation coefficients between cadmium concentration in each organ and the fish weight for each individual of the six fish species. The relation was insignificant between flesh content and fish weight.

Earlier work in this laboratory on bioaccumulation of cadmium in the different organs of juvenile Mugil capito, suggested that bioaccumulation of cadmium in the musculature becomes significant only when contamination is extremely high. After 25 days' exposure to 120 µg Cd l^{-1}, the musculature of M. capito reflects a 5-fold increase in cadmium concentration relative to that in the control fish. It was 10-fold for the liver and 43-fold for the gills. Organs with great affinity to heavy metals like liver would therefore appear to be more appropriate for the evaluation of metal contamination in fish, and particularly when the organ shows a good relationship with the total weight of the fish (Table IV). In the present results, the gills also show elevated cadmium levels whereas the flesh, as mentioned, was markedly less enriched with cadmium. This suggests that uptake of cadmium occurs both through food chain and through the gills. However, there is little evidence for biomagnification through the trophic levels. Levels in the predatory fish Euthynnus are not substantially higher than those in fish lower in the food chain, although the level is still higher than that of the background in water but less than in plankton. The sequence for Cd level in Mex Bay is as follows: Water < fish flesh < gills < liver < mixed plankton < crustacea < bivalves < suspended matter.

Table IV

Relationships between concentration of Cd in liver and weight of the fish. (No significant relationship was found between weight of the fish and Cd concentration in either flesh or gills, r < 0.2).

Fish species	No. of individuals	Correlation coefficient		Relationship
M.barbatus	45	0.78	Fish weight	= 61.0 + 0.52 Cd
S.pilchardus	32	0.67	"	= 31.0 + 0.38 Cd
B.boops	20	0.86	"	= 47.4 + 0.40 Cd
R.halavi	15	0.91	"	= 271.5 + 1.53 Cd
S.vulgaris	29	0.73	"	= 72.1 + 0.19 Cd
E.alletteratus	16	0.71	"	= 193.5 + 1.25 Cd

5. ACKNOWLEDGEMENT

Many thanks to Prof. Dr. Y. Halim, for critically reading this manuscript and for all the facilities granted to the laboratories of the Aquatic Pollution Project (UNDP/UNESCO/EGY/73/058). I am also grateful to Mr. O. Aboul Dahab, for his cooperation in all the stages of this work.

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BIOACCUMULATION OF SOME HEAVY METALS IN COASTAL MARINE ANIMALS IN THE VICINITY OF ALEXANDRIA
I. BIOASSAY

by

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1. INTRODUCTION

Some heavy metals like copper and zinc in sea water are considered among essential nutrients for marine organisms, i.e. an organism fails to grow or complete its life cycle in the absence of those metals. However, these same trace metals become toxic when concentration levels exceed those required by factors varying between 40 and 200-fold (Venugopal and Luckey, 1975). Sea water also contains metals like cadmium and lead which are considered non-essential heavy metals (Baccini and Roberts, 1976), but they are harmless as long as they are present at tolerable levels.

In Alexandria coastal waters copper and zinc are present in considerable amounts. According to El-Sayed and El-Sayed (1981) and Abu-El Nagah (1980), concentrations of more than 100 and 500 $\mu g\ l^{-1}$ for copper and zinc respectively can be found in Alexandria coastal waters. These values are about 20-fold higher than those found in open sea water. Open sea water contains 4 and 10 $\mu g\ l^{-1}$ of copper and zinc respectively (Brewer, 1975).

The exposure of our marine environment to such pollutants could represent a hazard to marine life. Toxicity tests and evaluation of median lethal concentrations (LC50) of these metals is therefore necessary. Bioassay tests were done on two of the common marine organisms in Alexandria waters which are easily acclimatized in the laboratory, Mugil capito and Portunus pelagicus. The first is a pelagic fish and the other is a bottom-dwelling crustacean. Bioaccumulation rate and LC50's were determined for the three metals copper, zinc and cadmium. Bioaccumulation of cadmium in the different organs of the fish Mugil was also tested.

2. MATERIALS AND METHODS

Fish used for these tests were brought alive from professional fishermen in Alexandria. Mugil fry of about 3 cm length were used for determination of LC50 and for bioaccumulation of metal in the whole body of the fish. The crabs used in these experiments had a carapace width of about 9 cm. Immature (juvenile) Mugil specimens of length between 8 and 10 cm were used for the determination of the rate of bioaccumulation of cadmium in the different organs, gills, liver, intestine and dorsal and peduncle muscles, of the fish. The water used for the tests was taken from the natural environment, in nearly clean water 5 km away from the coast. Before use the water was filtered through 0.45 um millipore membrane filters. The salinity of the water was adjusted to about 36°/oo, to represent the nearshore waters. Aeration was accomplished by small air pumps and polyethylene tubing. No pumice stones were used, to avoid adsorption of heavy metals on them. All animals used in these experiments were starved. The aquaria used were made entirely of glass.

The bioassay method given in "Standard Methods", (APHA/AWWA/WPCF, 1980) was used. Static bioassay was adopted due to its convenience (Chapman and Stevens, 1978; Holcombe and Andrew, 1978). The LC50 was estimated by interpolation after plotting percentage mortalities against corresponding test concentrations on a logarithmic graph paper (Chrm, 1978).

From the Mugil fry, 8 fishes were put in each aquarium of 10 l capacity. Four crabs were used in each equarium containing 8 l of water. The water temperature was of $22 \pm 1°C$ and the pH was about 8.2. More details about each experiment will be

given in the results. Chemical analysis of the heavy metals in the animal tissues and in water were done according to Riley and Segar (1970) and Riley and Taylor (1968) respectively. Atomic Absorption Spectroscopy (Shimatzu-AA-360-11) was used for the measurements of copper, cadmium and zinc in the samples.

3. RESULTS

3.1 Tests of bioaccumulation and determination of LC50 of cadmium, copper and zinc

3.1.1 Mugil fry

Cadmium

Four aquaria were used, with concentrations of 60, 80, 120 and 180 $\mu g\ l^{-1}$. The graphs of percentage mortalities at the various concentrations of cadmium, and bioaccumulation of cadmium by the fish with time are shown in Figs. 1 and 2 respectively.

Fish started to die after 24 hours of exposure in all aquaria. All fish died on the eleventh day of exposure in the first and second aquaria. In the third and fourth aquaria fish died on the 9th day. The LC50 evaluated from Fig. 1 is 54 μg Cd l^{-1} (at 168 hr). Fig. 2 shows that the rate of bioaccumulation increases both with the initial concentration of cadmium in the water and with time. The rate of bioaccumulation in the first 24 hr is much higher than the following days. It appears here that the bioaccumulation rate also increases by the 6th day of exposure in the aquaria with initial cadmium concentrations more than 60 $\mu g\ l^{-1}$. The concentrations of cadmium in the four aquaria have dropped considerably during the first 24 hrs. and in general the decrease in the concentration in the water follows an inverse pattern to that bioaccumulated by the fishes (Fig. 2).

Copper

Three aquaria were used containing respectively concentations of 1, 2 and 3 mg Cu l^{-1}. Copper was in the form of copper sulphate. The percentage mortalities at the different copper concentrations and the bioaccumulation of copper by fish with time are shown in Figs. 3(a) and 4(a) respectively.

Mortality occurred in all aquaria after 24 hr, where one, two and three fishes were found dead in the 1st, 2nd and 3rd aquarium respectively. All fishes were dead after 12 days at concentration of 1 mg l^{-1}; after 7 days at concentration of 2 mg l^{-1}; and after 5 days at 3 mg l^{-1}. From Fig. 3(a), it appears that the LC50 is 1.3 mg Cu l^{-1} (120 hr). The rate of bioaccumulation of copper in fish tissues (Fig. 4(a)) increases with the increase of copper concentration in the water. The rate of bioaccaumulation in the first 48 hr is much higher than the following days. The decrease in copper concentration in the aquarium water follows an inverse pattern to that of bioaccumulation by fish.

Zinc

LC50 and bioaccumulation of zinc by Mugil fry are shown in Figs. 3(b) and 4(b), respectively. Concentrations used in the three aquaria were 1.5, 2.0 and 3.0 mg Zn l^{-1} (as zinc sulphate). Mortality occurred after 72 hr in the first concentration (1.5 mg l^{-1}), while it occurred after 24 hr at the other two concentrations. All fish died after 9 days in the first aquarium, 7 days in the second and 6 days in the third aquarium. The LC50 of zinc for Mugil fry is 2.05 mg l^{-1} (120 hr). The rate of bioaccumulation of zinc increases as its concentration increases in the water, and this rate in the first 24 hr for the first two aquaria was higher than in the following days. In the low concentration (1.5 mg l^{-1}) aquarium, the bioaccumulation rate is slower. The concentration of zinc in the aquarium water was decreasing in an inverse pattern to that of its bioaccumulation by fish.

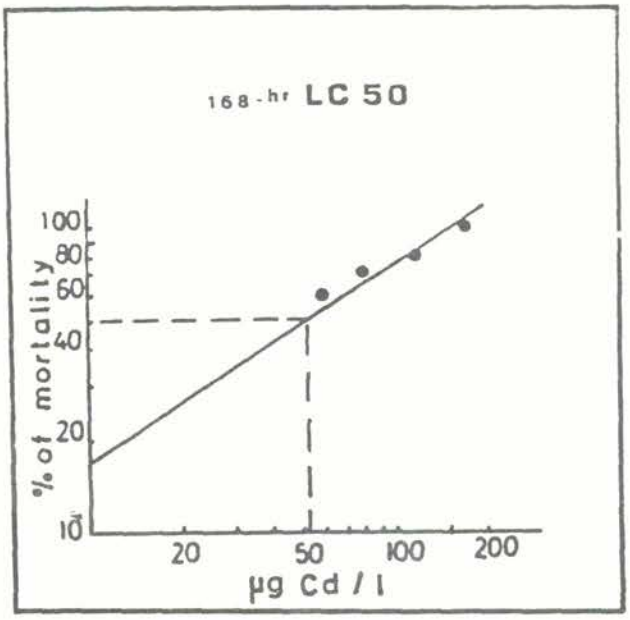

Figure 1. LC50 of Cd in M. capito, fry

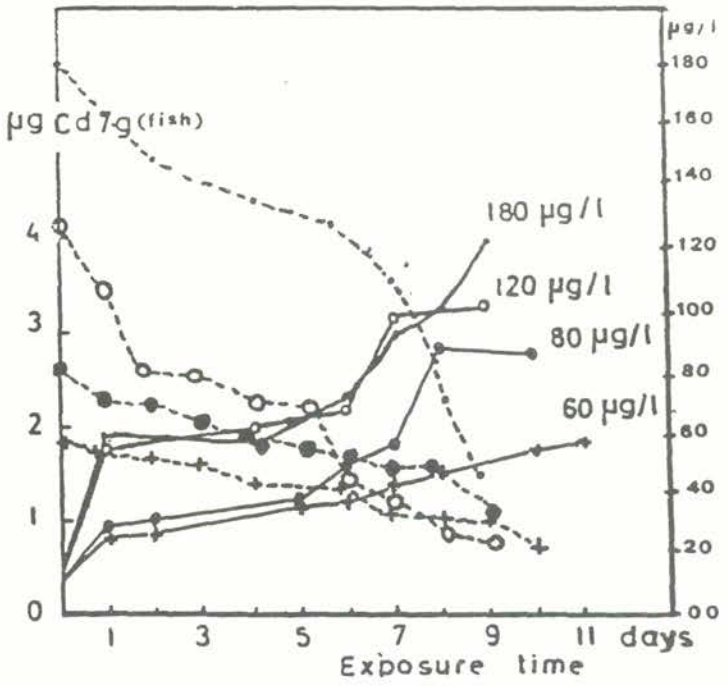

Figure 2. Bioaccumulation of Cd in M. capito, fry, after exposure to different concentrations

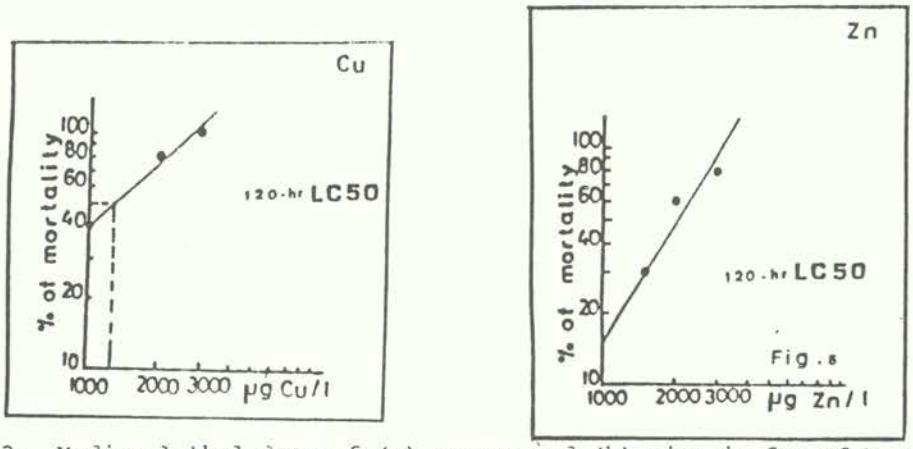

Figure 3. Median lethal dose of (a) copper and (b) zinc in fry of M. capito

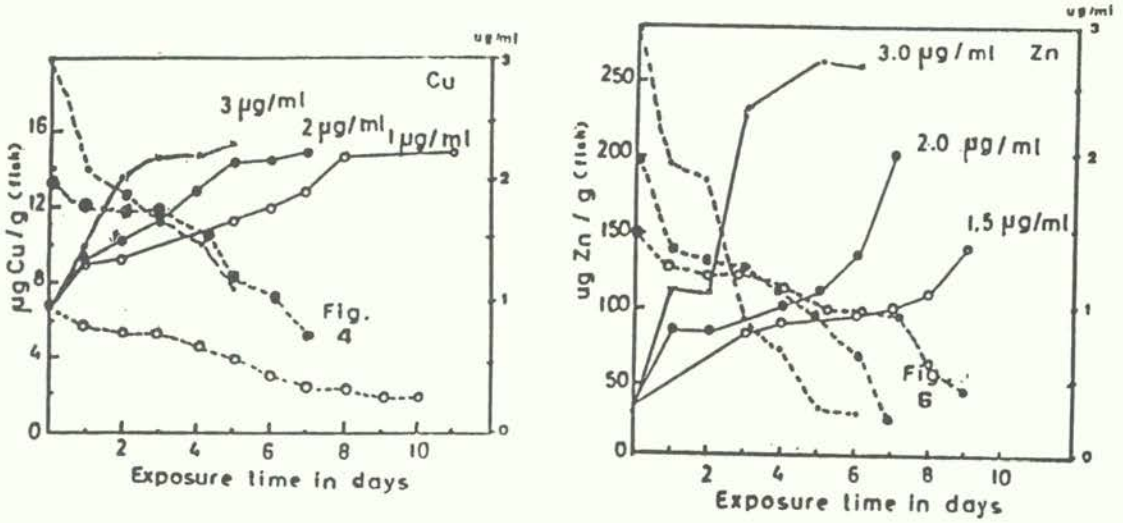

Figure 4. Bioaccumulation of (a) copper and (b) zinc in fry of M. capito after exposure to different concentrations

3.1.2 Portunus pelagicus

Cadmium

Four aquaria of initial cadmium concentrations 0.5, 1.0, 2.0 and 3.0 mg l^{-1} were used for the crab experiment to determine LC50 and the bioaccumulation rate. In the first two aquaria no mortality was observed throughout the time of the experiment (5 days). The results of the bioassay test for the 3rd and 4th aquaria are shown in Table I. After 24 hr. exposure one crab died in each of the 3rd and 4th aquaria. On the 5th day all crabs were dead in these aquaria. From Table I, it appears that LC50 is about 1.7 mg l^{-1} (120 hr). The rate of bioaccumulation was taken from the 3rd and 4th aquaria. The bioaccumulation rate seems to be higher in the 4th than in the 3rd aquarium and that this rate increases with concentration of the pollutant (Fig. 5), especially in the first 24 hr. The cadmium concentration in the water was decreasing at a rate similar to the rate of accumulation by crab.

Table I

Bioaccumulation of cadmium by crabs (P. pelagicus)

Aquarium 3 (2 µg/ml)					Aquarium 4 (3 µg/ml)				
Time in hours	conc. in water µg/l	conc. in crabs µg/g	No. of dead organism	% of mortality	Time in hours	conc. in water µg/g	conc. in crabs µg/g	No.of dead organism	% of mortality
0	2000	0.63	0	0	0	3000	0.63	0	0
24	1800	3.35	1	25	24	2000	3.85	1	25
48	1500	--	1	25	48	1600	4.26	2	50
96	840	3.98	2	50	96	950	--	2	50
120	560	4.15	3	75	120	730	4.39	3	75
192	560	3.88	4	100	144	420	4.15	4	100
Control	0.11	0.63							

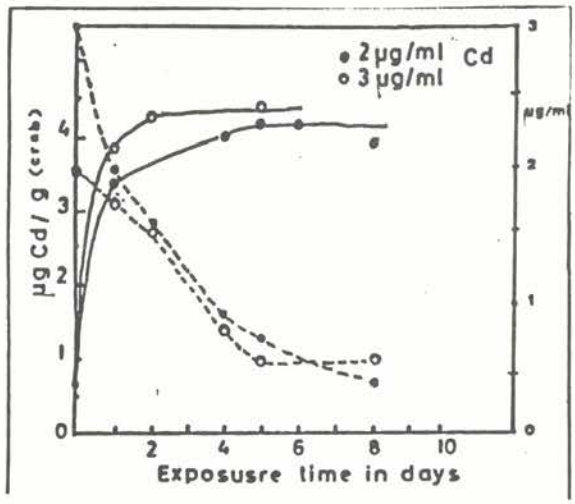

Figure 5. Bioaccumulation of cadmium in muscles of crabs after exposure to different concentrations

Copper

The lowest concentration causing mortality after one day exposure was 10 mg Cu l^{-1}. Two concentrations (10 and 14 mg Cul^{-1}) were used. Mortality occurred after one day in both aquaria. By the end of the 9th day all crabs were dead (Table II). It was difficult to estimate the LC50, although it seems to be around 14 mg Cu l^{-1}, since after two days' exposure half of the crabs were dead. Here also one can see that as the concentration of pollutant increases, the rate of bioaccumulation increases too.

Zinc

The lowest concentration of zinc causing mortality after two days was 80 mg l^{-1} (Table III). Two aquaria were operated, with concentrations of 80 mg l^{-1} and the 2nd was 100 mg l^{-1} respectively. Half of the crabs were dead after 2 days' exposure in the 2nd aquarium. In both aquaria, 50% mortality was observed after 96 hr. This would suggest that the LC50 of zinc on crabs to be around 100 mg l^{-1}. Here, it is noticed that as the initial concentration of zinc in the water increases the rate of bioaccumulation by the organism increases too. From Table III also the concentration of zinc in the water follows a reverse pattern as that for bioaccumulation with time by crab.

3.2. Bioaccumulation of cadmium in the different organs of Mugil capito juvenile

In this experiment, a concentration of 120 µg l^{-1} was used with juvenile fish of lengths varying between 8 and 10 cm. The bioaccumulation of cadmium with time in the different organs (gills, intestine, liver and dorsal and peduncle muscles) is shown in Table IV. The table shows that the rate of accumulation of cadmium in the gills, liver and intestine are much higher than in the other muscle tissues of the fish. The muscle tissues accumulate the least amounts of this metal. Gills, liver and intestine showed an increase in concentration with time. However the intestine shows a sudden decrease in bioaccumulation after the 4th day which lasts to the 6th day. After that a continuous rise in bioaccumulation was obvious. The sudden drop seems to be due to evacuation from the intestine of the food and its residues. The increase in accumulation of cadmium after the 6th day probably comes from the ingestion of the suspended matter from the water which usually concentrate the metal ions on its surface (Sprague, 1969). The suspended matter comes from the faeces given off by the fish.

Table II

Bioaccumulation of copper sulphate by crabs (P. pelagicus)

10 µg Cu/ml					14 µg Cu/ml				
Time in hours	Conc. in water µg/ml	Conc. in crabs µg/g	No. of dead organisms	% of mortality	Time in hours	Conc. in water µg/ml	Conc. in fish µg/g	No.of dead organisms	% of mortality
0	10	30.5	0	0	0	14	30.5	0	0
24	7.3	100	1	25	24	10.5	130	1	25
48	6.4	--	1	25	48	7.31	288	2	50
96	3.2	228	2	50	96	4.18	--	2	50
192	2.15	460	3	75	120	2.29	387	3	75
216	0.83	489	4	100	144	1.13	523	4	100
Control	0.003	30.5							

Table III

Bioaccumulation of zinc sulphate by crabs (P. pelagicus)

80 µg Zn/ml					100 µg Zn/ml				
Time in hours	conc. in water µg/ml	conc. in crabs µg/g	No. of dead Organ-isms	% of mort-ality	Time in hours	conc.in water mg/ml	conc. in crabs µg/g	No. of dead organ-isms	% of mort-ality
0	80	80	0	0	0	100	80	0	0
24	55.6	188	1	25	24	71.5	196	1	25
48	39.5	--	1	25	48	33.6	183	2	50
96	28.8	250	2	50	96	23.4	--	2	50
120	13.5	320	3	75	120	30.8	360	3	75
192	7.6	--	3	75	192	31.8	480	4	100
240	3.2	270	4	100					
Control	0.010	80							

Table IV

Bioaccumulation of cadmium salt by M. capito (10 cm T.L.)

Days	Conc. in water µg/l	Concentration of cadmium in µg/g tissue				
		Gut	Liver	Peduncle	Gills	Muscles
0	120	0.51	0.33	0.30	0.29	0.10
3	43.2	2.81	1.83	0.42	4.63	0.21
5	31.8	1.79	1.69	0.41	6.77	0.22
8	11.2	1.96	1.72	0.51	8.89	0.29
12	10.5	2.30	1.83	0.43	8.98	0.23
15	9.9	3.20	2.23	0.53	9.82	0.43
18	9.3	3.80	2.12	0.49	10.95	0.44
25	3.1	5.83	3.30	0.85	13.13	0.65

4. DISCUSSION AND CONCLUSION

The present results show that there was a certain accumulation of the metals in the examined tissue organs. This is confirmed by the corresponding decrease in cadmium concentration with time in the aquarium water. Gills, liver and intestine show the obvious accumulation of cadmium in them, but not in the flesh of the fish. The first 24 hours show an abrupt increase in accumulation in the three organs, whereas the flesh parts do not. The three organs reacted toward the pollutant differently. The gills accumulate the highest and liver the lowest, while intestine is the intermediate one. This suggests that the accumulation rate depends mainly on whether the organ is in direct contact with the pollutant in the water medium (gills through breathing and intestine through engulfing the water and food). The liver is not in direct contact

with the water and therefore its role seems to come after ingestion of water and food. The food in this case probably consists of the faecal pellets of the fishes in the aquarium. Liver is known as a place of extracting and storage of food, after extraction from intestine and thence to blood circulatory system (Sastry and Cupta, 1979). Some authors (e.g. Holcombe and Andrew, 1978) during their investigation on the metal content in the different organs of fishes from the natural environment found that several tissues like gills, kidney, liver and bones accumulate zinc, cadmium and copper but muscles did so only slightly.

The bioaccumulation of cadmium, copper and zinc in the present work in Mugil fry reach up to 3.9, 15.3 and 263 $\mu g\ g^{-1}$ respectively at the high concentration. In crabs these metals reach to about 4.1, 523 and 480 $\mu g\ g^{-1}$ respectively. From this, one can see that crabs are able to concentrate more metals than fish do. This agrees with observations given in a WHO report (1979) and by the present authors during their survey of metals in marine animals, in the vicinity of Alexandria.

The LC50 of cadmium for Mugil fry and for crabs (P. pelagicus), according to the present results are as follows: 0.054 mg l^{-1} (168 hr) and 1.70 mg l^{-1} (120 hr) respectively. Chapman (1978) found that 408 hr-LC50 was 0.005 mg Cd l^{-1} for Salmo gairdneri and LC 50 (215 hr) was 0.0037 mg Cd l^{-1} for Coho salmon (Oncorhynchus kisutch), which are greatly lower than the present observations. Servizi and Martens (1978) found high doses of LC50 (168 hr) of 1.00 and 4.500 mg Cd l^{-1} in Sockeye salmon (Oncorhynchus nerka) at different stages.

The observed LC50 for Cadmium in Mugil fry and crabs are 1.3 mg l^{-1} (168 hr) and 14.0 mg Cu l^{-1} (96 hr) respectively. These values are higher than those observed by Davis and Shand (1979) who found 96-hr LC50 being 0.220, 0.240 and 0.240 mg l^{-1} for fry, fingerling and smolt of Sockeye salmon respectively. The present LC50 values for copper are less than those given by Richey and Roseboom (1978). Their LC50 values were 2.5 and 1.2 mg Cu l^{-1} (14 days) for juvenile channel catfish and for bluegills respectively. The observed 120 hr LC50, 2.05 mg Zn l^{-1}, for Mugil is comparable to the values observed by Broderius and Smith (1979) and Judy and Davis (1979) who found values of 2.61 and 3.1 mg l^{-1} respectively for the fathead minnow (Pimephales promelas). The 48 hr LC50 of 100 mg l^{-1} zinc in crabs P. pelagicus is much higher than those observed by other authors in fish (Benoit and Holcombe, 1979; Chapman and Stevens 1978; Holcombe and Andrew, 1978).

The safe concentration ranges for the metals under study, according to the present observations, are 0.00054-0.0054, 0.013-0.130 and 0.0205-0.205 mg l^{-1} for cadmium, copper and zinc respectively, for Mugil fry. On the other hand the safe concentrations for crabs seem to range between 0.017-0.170, 0.140-1.400 and 1.00-10.00 mg l^{-1} for cadmium, copper and zinc respectively. Biesinger and Christensen (1972) gave the safe concentration for the three metals to be 0.0002, 0.0046-0.0145 and 0.070 mg l^{-1} respectively in Daphnia magna.

The lower safe concentration values obtained in the present study for cadmium, copper and zinc, however, are still considerably higher than the corresponding values reported recently in three coastal waters off Alexandria (Table V).

Table V

Mean dissolved heavy metal concentrations (µg/l) in three areas of Alexandria coastal waters, during the year 1979 (El-Nady, personal communication)

Area	Copper	Zinc	Cadmium
Abu Kir Bay	4.2	25	0.23
Eastern Harbour	2.7	11	0.30
El Mex Bight	3.2	19	0.13

5. ACKNOWLEDGEMENT

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BIOACCUMULATION OF SOME HEAVY METALS IN COASTAL MARINE ANIMALS IN THE VICINITY OF ALEXANDRIA. II - SURVEYING

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1. INTRODUCTION

Water pollution in the Mediterranean Sea is one of the major problems facing many countries in the area. The present study was done as a part of a survey carried out on three areas of Alexandria waters: (Abu-Kir bay, Eastern harbour and El-Mex bight (Fig. 1.) to detect heavy metal pollution and its effect on marine animals. The three areas studied vary in their degree of exposure to pollution. The Eastern harbour only receives unprocessed domestic sewage at its southern margin, at a rate ranging between 10,000 and 15,000 cubic metres per day. Maximum depth of the harbour is about 12 m. Abu-Kir bay and El-Mex bight receive drainage water and industrial wastes in addition to domestic sewage. In Abu-Kir bay, the industrial wastes are from fertilizers, textile, paper and food processing and canning industries. These wastes are pumped directly to the south west of the bay through El-Tapia pump at a rate of 2 million cubic metres per day (Mitwally, 1982). The drainage water flows through lake Edku and thence through a small channel into the bay at a rate of 146 million m^3 day^{-1} (Sharaf El-Din *et al.*, 1981). Maximum depth of the water in the bay is about 20 m. El-Mex bight receives industrial wastes from the nearby chemical industries, chlor-alkali plant and Alexandria petroleum company. A drainage channel flows into the bight, at a rate of 6 million m^3 day^{-1} through a drain called "Umum drain". This drainage water is usually mixed with surplus water coming from Lake Mariut, an industrially and sewage-polluted lake. The depth in the bight varies between 10 and 20m.

For this, accumulation of copper, zinc, iron, manganese and cadmium were studied in some selected marine animals of different trophic levels: *Sparus auratus*, (pelagic fish), *Raja miraletus* (demersal fish); *Portunus pelagicus* (Crustacean) and *Patella vulgata* (Mollusc).

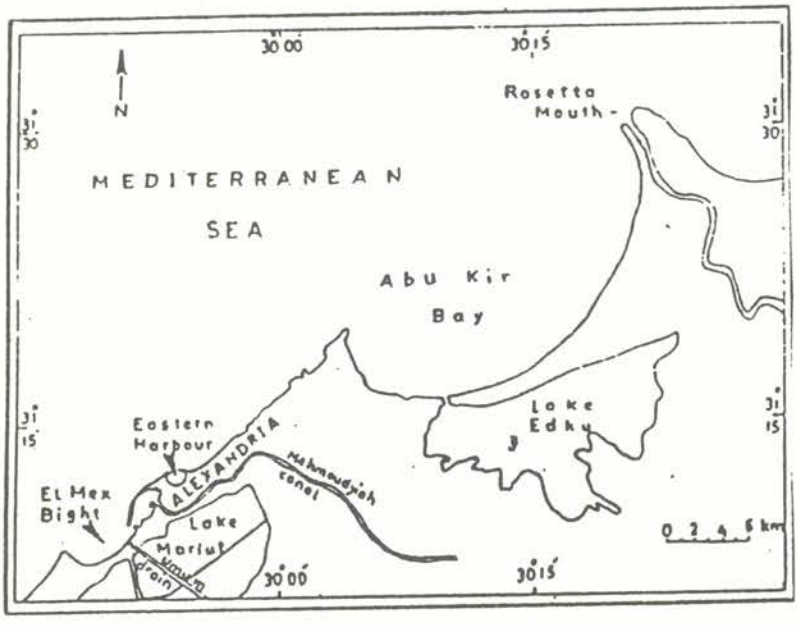

Figure 1. Map showing the position of the three areas of study: Abu Kir Bay, Eastern harbour and El Mex Bight

2. MATERIALS AND METHODS

Monthly samples of the selected marine animals were collected fresh from the three localities off Alexandria during the period from January to December 1979. For fish, composite samples from dorsal fillets and livers of at least 6 specimens of one size class (of 23 and 20 cm total length and disc length for Sparus and Raja respectively) were taken. Four specimens of size 7 x 15 cm of Portunus and 10 specimens of 4 cm shell length of Patella were used for preparation of a composite sample for analysis. Heavy metals were determined in the muscular and hepatic tissues of Sparus and Raja. The muscular tissue was taken from below the pectoral fin on the flank region of the former and on the dorsal area of the disc in the latter. Muscular tissue from Portunus was taken from the tissues underlying its carapace, while all soft parts of the limpets Patella was used. The technique for detection of heavy metals was that of Riley and Segar (1970) using a Shimatzu Atomic Absorption Spectrophotometer (Model-AA-630-11). Concentration of heavy metals in the marine animal studied are usually referred to the dry weight. Concentration or enrichment factor of an heavy metal in the animal body is calculated, which is simply the concentration in organism (mg kg^{-1}) divided by the concentration (mg kg^{-1}) in sea water.

3. RESULTS

The mean concentrations of the five metals in the four animals under investigation from the three areas of Alexandria waters are presented in Table I. It appears from the table that abundance of the metals in the four animals are in the order; Fe > Zn > Cu > Mn > Cd, and copper and zinc are more concentrated in the molluscs and crustaceans than in the fishes. Concentrations of copper and zinc in the fishes vary between 4.0-9.5 and 17-37 µg g^{-1}, and in the invertebrate animals they ranged between 20.7-49.7 and 38-98 µg g^{-1} respectively. Iron is more concentrated in Patella, with a concentration ranging between 450-1239 µg g^{-1}, with a maximum value of 1239 µg/g^{-1} observed in those of Abu-Kir bay. Iron concentrations in the other swimming animals ranged between 16-141 µg g^{-1}.

Manganese follows more or less the same pattern of accumulation in the four animals as that of iron, except that in Portunus from the El-Mex area, where it accumulated high levels (18 µg g^{-1}). The concentrations in Patella ranged between 14 and 17 µg Mn g^{-1} and in the other animals between 2-7 µg g^{-1}.

Table I

Annual mean of heavy metal concentrations in various species according to areas (µg g^{-1} dry-weight basis).

Species	Cu			Zn			Fe			Mn			Cd		
	Areas			Areas			Areas			Areas			Areas		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
S. auratus	4.0	7.1	9.5	19	37	18	24	62	19	4	3	2	0.23	0.54	0.14
R. meraletus	5.9	8.9	-	17	25	-	38	50	-	7	6	-	0.22	0.69	-
P. vulgata	22.9	20.9	20.7	63	38	38	1239	450	504	14	16	17	0.54	0.42	0.88
P. pelagicus	19.3	42.2	41.4	98	66	62	141	16	95	6	4	18	1.98	0.61	2.2

A = Abu Kir Bay, B = Eastern Harbour and C = El Mex Bight
Mean concentration for the twelve monthly sampling times during 1979.

Concerning cadmium, we can see that this metal reaches its highest concentration in the flesh of Portunus, particularly those of El-Mex area. However, it tends to be less accumulated in the tissues of the fish than in the other animals. Patella and Portunus of the Eastern harbour accumulated lower concentrations of cadmium than those in the other areas.

Bioaccumulation of the studied metals in the different organs of the fish species (muscle and liver) in the different areas of study, is shown in Table II. The most striking feature observed from the Table is that regardless of the fish species or samplilng site the liver accumulates more metals than the muscles.

4. DISCUSSION

The present work showed the order of enrichment of metals in the mesopelagic fish as well as bottom animals from Alexandria waters as follows: Fe $>$ Zn $>$ Cu $>$ Mn $>$ Cd. Taliadouri-Voutsinou (1981) and Uysal (1981) gave the same order of accumulation in their study on bioaccumulation of heavy metals in Mullus barbatus in Greek waters and in Mytilus galloprovincialis in Aegean coast respectively.

Bioaccumulation of heavy metals varies from one metal to another in marine organisms. The biological amplification of the metals copper, zinc, iron, manganese and cadmium by the present marine animals is calculated and shown in Table III. It shows that fishes are able to concentrate these 5 metals from 700-3300, 4000-14000, 1000-3300, 1000-2700 and 1000-2300 times respectively, compared to the levels dissolved in the waters of the three areas under study (Table IV). Portunus is a good biological amplifier for the metals copper, zinc and cadmium, while Patella is good for iron and manganese. In general, those crustaceans and molluscs are able to amplilfy the five studied metals (more than in fish species) to the extent of 15600, 5800, 18600, 7700 and 17100 times respectively of those present in Alexandria waters. This is in accordance with the WHO report (1979) which stated that "The invertebrates appear to have particularly high capability of accumulating metals". The accumulation of iron and manganese by Patella to a higher degree than other studied organisms also agrees with the findings of various other authors (Preston et al., 1972; Bryan et al., 1977). Those authors found average values of iron in P. vulgata of about 2255 and 1400 μg Fe g^{-1}, and manganese values in the limpets (soft part) between 18-94 μg g^{-1}. According to Bryan et al. (1977) manganese is an essential constituent of the soft part of the limpets especially that of gonad pigments. These values are relatively higher than those of the present results. According to Abo Nour (1981) iron reaches concentrations ranged between 485-590 μg Fe g^{-1} in the tissues of blue crab (Callinectes sapidus), these values are much higher than the values given in the present study.

Various authors have mentioned the occurrence of cadmium in pelagic fish at very low concentrations, between 0.04-0.09 μg g^{-1} in Mullus barbatus and 0.20 μg g^{-1} in Mugil auratus (Aissi, 1981; Taliadouri-Voutsinou, 1981). Others recorded values in the same species (Mullus and Mugil) between 0.31 and 0.70 μg g^{-1} (Roth and Hornung, 1977; Uysal, 1981). Mullin and Riley (1956), Won (1973) and Karbe et al. (1977) have shown that cadmium might reach high concentrations (up to 16.4 ug g^{-1}) in the bodies of the marine molluscs Mytilus edulis and Patella vulgata. According to the report of U.S.EPA (1978), molluscs can accumulate cadmium up to 2,260,000 times the levels found in the water. George and Coombs (1977) have shown that M. edulis displayed a concentration factor of 165 after 5 days' exposure to 0.7 mg l^{-1} of cadmium. According to Dethlefsen (1977) the shrimp Crangon crangon concentrated cadmium 2,400 times in a 40 days' exposure to 0.005 mg l^{-1}. The present data show that the animals under investigation have high concentration factors for cadmium which can reach 17100 at El Mex area in Portunus pelagicus. This metal is more accumulated in crabs and Patella than by fishes. This observation agrees with that of Fowler and Oregioni (1976) and Ellis et al. (1980), where blue crab accumulated higher values of cadmium (3.5 μg g^{-1}) than the blue mussel (2.4 μg g^{-1}). Comparing these values with the present data, we can see that the cadmium concentrations in the studied animals are relatively low with respect to other areas. However, it is slightly high in Portunus from El Mex bight.

Table II

Annual mean of heavy metal concentrations in liver and muscles of various fish species according to areas ($\mu g\ g^{-1}$ dry-weight basis)

Species	Cu			Zn			Fe			Mn			Cd		
	Areas			Areas			Areas			Areas			Areas		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
S. auratus															
Muscles	4.0	7.1	9.5	19	37	18	24	62	19	4	3	2	0.23	0.54	0.14
Liver	27.6	30.5	22.6	94	105	105	339	495	287	8	10	16	0.58	1.04	0.95
R. meraletus															
Muscles	5.9	9.0	--	17	25	--	38	50	--	7	6	--	0.22	0.69	--
Liver	28.0	29.8	--	24	45	--	136	356	--	7	7	--	0.54	0.75	--

A = Abu Kir Bay, B = Eastern Harbour and C = El Mex Bight
Mean concentration for the twelve monthly sampling times during 1979.

Table III

Means of concentration factor* of heavy metal in various species according to areas (x 10^3) during 1979

Species	Cu			Zn			Fe			Mn			Cd		
	Areas			Areas			Areas			Areas			Areas		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
S. auratus	1.0	2.6	3.0	0.7	3.3	0.9	4	17	4	1.2	1.3	1.0	1.0	1.8	1.1
R. meraletus	1.4	3.3	--	0.7	2.2	--	6	14	--	1.9	2.7	--	1.0	2.3	--
P. vulgata	5.5	7.7	6.5	2.5	3.4	2.0	186	128	101	3.9	7.4	7.7	2.4	1.4	6.8
P. pelagicus	11.7	15.6	12.9	3.9	3.8	3.3	21	4	19	1.5	1.6	8.4	8.6	2.0	17.1

A = Abu Kir Bay, B = Eastern Harbour and C = El Mex Bight
*According to Mullin and Riley (1956) and to the level of the metal in the waters of the three areas off Alexandria (see Table IV.)

Zinc is highly abundant in Alexandria coastal water (Table IV). Its concentration ranges between 11 and 25 $\mu g\ l^{-1}$, and hence it has higher concentration in animal bodies than manganese, copper and cadmium. The present results show that crabs accumulated copper and zinc at higher concentrations than in the other studied organisms. Zinc and copper are essential for enzyme activities. Love (1970) stated that copper in invertebrate animals participates in porphyrin and haemocyanin metabolism. In fact we found more or less similar concentrations of copper or zinc in Raja and Sparus. This is in accordance with the observation of Windom et al. (1973), who stated that in all fish species copper and zinc concentrations appear to be similar.

Concentration factors of copper and zinc in the Eastern harbour for nearly all the studied animals are higher than in the other areas under investigation. This is a semi-closed area, with restricted water exchange with the Mediterranean water outside this harbour. This may show how the area affects the rate of bioaccumulation of heavy metals. El-Sayed and El-Sayed (1981) recorded remarkably high contamination of the harbour by copper ($>100\ \mu g\ l^{-1}$) and zinc (500 $\mu g\ l^{-1}$), Hobden (1967) showed that bioaccumulation of heavy metals in marine organisms are affected by locality. Grimanis et al. (1981) noticed that copper concentrations in the flesh of the fish from three gulfs of Greece showed considerable variations, while zinc and iron showed no significant

difference from those found in the studied fish species. Preston et al. (1972) found great differences in heavy metals in P. vulgata collected from different shore areas. Taliadouri-Voutsinou (1981) found only slight difference in metals concentration of different marine organisms in various areas. Bryan et al. (1977) found that the zinc content of molluscs is not changed with the area. The effect of the area on copper and zinc bioaccumulation is clear from the present study. This leads us to think about the effect of other ecological factors on the physiological state of the animal's body which controls the rate of bioaccumulation of heavy metals in the animal.

5. CONCLUSION

In recent years Alexandria waters, especially El-Mex, Eastern harbour and Abo-Kir bay have been influenced by increased amounts of industrial, urban and domestic wastes. However, significant effects are not apparent in our samples of the fish, molluscs and crustaceans up till now. On the whole our results are within acceptable limits when compared with those of similar investigations carried out in the other parts of the Mediterranean Sea (UNEP, 1980).

However, use of liver and other gut parts of the fish on a big scale for feeding poultry animals in our country needs to be further assessed, since the liver and probably the other gut parts could be a serious source of toxic metals in those birds and thence to man.

Table IV

Mean of dissolved heavy metal concentrations ($\mu g\ l^{-1}$) in the three areas of Alexandria waters under study, during the year 1979 (After El-Nady, 1982)

Area	Cu	Zn	Fe	Mn	Cd
Abu Kir Bay	4.2	25	7	3.7	0.23
Eastern Harbour	2.7	11	4	2.2	0.30
El Mex Bight	3.2	19	5	2.2	0.13

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FACTORS AFFECTING ACUTE AND CHRONIC TOXICITY OF CHLORINATED PESTICIDES AND THEIR BIOMAGNIFICATION IN ALEXANDRIA REGION

by

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1. INTRODUCTION

Hermanutz (1978) evaluated the acute and chronic toxicity of endrin and malathion to flagfish. The 96-hr LC50 for endrin was 0.85 $\mu g\ l^{-1}$ and the lowest concentration having a significant effect in the chronic toxicity was 0.30 $\mu g\ l^{-1}$. Mean accumulation factors for various life stages and exposure periods varied from 7100 to 15000. The 96-hr LC50 for malathion was 349 $\mu g\ l^{-1}$ and the lowest concentration having a significant effect in the chronic exposure was 11 $\mu g\ l^{-1}$. Takeda (1978) studied the effects of DDT, BHC, and PCBs on the growth of fish. Sublethal doses of BHC in food inhibited the growth of carp after two weeks of exposure. Hansen (1980) found that in fish exposed for 9 days, lindane was taken up in proportion to its concentration in water at levels between 10 and 70 $\mu g\ l^{-1}$. Westernhagen et al. (1981) reported that chronic exposures of fish to bioaccumulative substances such as chlorinated hydrocarbons and heavy metals, caused effects on reproduction.

2. MATERIALS AND METHODS

Two strains of Tilapia zilli fry were collected from El-Berdeesey fish farm at Alexandria Lagoon and from some agricultural drains at Etay-El-Baroud, Behera Governorate 60 kilometres to the south of Alexandria. In addition, a field population of Gambusia affinis was also collected from the Behera area. All three fish strains were kept in aquaria at the laboratory under standardized conditions of continuous aeration at a temperature of 25°C. The mean length of Tilapia fry was 3.36 cm and of Gambusia 2.06 while the average body weight was 825 and 222 mg respectively. The fish were acclimatized under laboratory conditions for one week before the bioassay test. Standard imported fish diet was used in the aquaria cultures. Acute toxicity tests were carried out in six replicates for each concentration, in six glass vials, each containing 10 fish in aerated water.

Mortality counts were recorded after 24 and 96 hours. The mortality figures were corrected to Abbott's equation depending upon natural mortality in the control replicates.

Insecticides and chemicals tested

The following chlorinated hydrocarbons were obtained in a pure technical form: p, p'-DDT, endrin, lindane, and Toxaphene. Trichlorophenol was included for comparison as a common industrial waste and one of the expected metabolites of some pesticides. DDT in 25% of E.C. formulation was also used to indicate the role of formulating adjuvents on the acute toxicity of the active ingredient of the insecticide. Pure mercuric chloride was compared with phenyl mercuric acetate regarding their relative acute toxicity and rate of uptake by mosquito fish Gambusia affinis.

Accumulation of the tested compounds

The accumulation experiment was carried out using fry of Tilapia zilli of the Alexandria strain. Thirteen aquaria each containing 20 healthy fry fish were used for each treatment. The fish were exposed to one tenth of the 96-hr LC50 as a sublethal concentration. The exposure was continued for 28 days. Fish samples were taken for analysis for the incorporated insecticides in the tissues. The accumulation experiment procedure was similar to the methodology applied and adopted by Davy and Kleerkoper

(1972) and Jarvinen et al. (1977). The chlorinated insecticides were determined according to Thompson (1974).

3. RESULTS AND DISCUSSIONS

Acute toxicity of chlorinated hydrocarbons to Tilapia zilli and Gambusia affinis

Tables I and II include results of acute toxicity of technical grade endrin, DDT, lindane and toxaphene compared with 25% E.C. of DDT to Tilapia zilli fish from the two locations. There was a clear difference between the fish from the two areas. The Behera population lives in agricultural drains and thus it is directly affected by residues of pesticides and other agricultural chemicals. Such continuous exposure might be responsible for build up of resistance to insecticides in such populations, especially when compared with populations less exposed like that in Mariut lake in the vicinity of Alexandria where most of the effluents are diluted and mixed with organic loads from the municipal sewage and wastes. Table III shows indications of resistance in the Behera population.

It was also observed that DDT in the E.C. form was higher in its acute toxicity to both strains. This indicates the importance of testing the formulated form for regulation of the pesticides.

Table IV presents the acute toxicity of the same chlorinated hydrocarbons to Gambusia affinis from Behera culture. In most cases Gambusia was more susceptible than Tilapia zilli, thus suggesting its use as a sensitive indicator for the hazardous levels of such pollutants.

Table I

Susceptibility of fry of Tilapia zilli (Behera strain) to organochlorine pesticides.

product	24 hour exposure			96 hour exposure		
	LC50 $\mu g\ l^{-1}$	confidence limits	slope function	LC50 $\mu g\ l^{-1}$	confidence limits	slope function
endrin tech.	24.34	16.70-35.47	1.79	10.09	7.56-13.46	2.33
p,p DDT tech.	21.81	17.00-27.98	3.31	15.50	11.69-20.55	2.92
DDT 25% E.C.	12.78	9.56-17.08	2.32	9.52	7.36-12.31	3.20
lindane tech.	680.80	470.5-985.2	2.23	394.90	265.9-586.5	2.08
toxaphene tech.	88.27	59.64-130.6	1.71	68.82	45.95-103.1	1.66

Table II

Susceptibility of fry of Tilapia zilli (Alexandria strain) to organochlorine pesticides.

Product	24 hour exposure			96 hour exposure		
	LC50 $\mu g\ l^{-1}$	confidence limits	slope function	LC50 $\mu g\ l^{-1}$	confidence limits	slope function
endrin tech.	1.20	0.85-1.70	1.49	0.26	0.19- 0.35	2.72
p,p-DDT tech.	63.00	47.40-83.73	2.36	42.00	35.21-50.11	3.81
DDT 25% E.C.	27.50	23.19-32.62	3.42	9.50	8.02-11.25	3.45
lindane tech.	18.00	12.44-26.05	1.18	6.40	4.40-9.32	1.39
toxaphene tech.	19.00	12.63-28.58	1.65	5.00	3.60-6.95	2.05

Table III

Susceptibility of fry of Tilapia zilli (Alexandria and Behera strains) to organochlorine pesticides at 96 hours.

Product	LC50 $\mu g\ l^{-1}$ (96 hours exposure)		resistance ratio level
	Tilapia zilli strains		
	Alexandria	Behera	(R.R.)
endrin technical	0.26	10.09	38.81
p,p'DDT technical	42.00	15.50	00.37
DDT 25% E.C.	9.50	9.52	1.00
lindane technical	6.40	394.90	61.70
toxaphene technical	5.00	68.82	13.76

Table IV

Susceptibility of fry of Gambusia affinis (Behera strain) to organochlorine pesticides.

Product	24 hour exposure			96 hour exposure		
	LC50 $\mu g\ l^{-1}$	confidence limits	slope function	LC50 $\mu g\ l^{-1}$	confidence limits	slope function
endrin tech.	10.91	6.80-17.50	1.10	5.27	3.36-8.27	1.16
p,p'-DDT tech.	22.74	16.61-31.13	2.14	9.87	7.28-13.38	1.71
DDT 25% E.C.	58.58	43.15-79.52	2.69	27.69	21.32-35.96	1.99
lindane tech.	1129.00	811.5-1571	1.76	618.1	465.5-820.8	2.05
toxaphene tech.	71.20	49.16-103.1	1.82	49.48	35.29-69.37	1.99

The bioaccumulation rate of chlorinated hydrocarbon insecticides in Tilapia zilli

Table V and Fig.1 show data from the accumulation study of p,p-DDT, endrin and lindane in Tilapia zilli of the Alexandria strain. It can be observed that biomagnification was higher for DDT than endrin, while it was not clear for lindane.

This might reflect the relative rate of biodegradation in the biological tissues in proportion to the retention affinity and storage of each of the tested compounds. It is well known that DDT has one of the highest partition coefficient values and thus its high lipid solubility will help in intensifying its biomagnification ability.

The present data are generally in agreement with those obtained by El-Bishry (1979) for DDT on Mugil cephalus and Tilapia nilotica. Tilapia species seem to be less susceptible to insecticides than other fish species. Endrin was always more hazardous to fish than other chlorinated hydrocarbons. Similar trends were recorded by Hermanutz (1978) and Anderson and Defoe (1980) for endrin on flag fish.

Table V

Accumulation of organochlorine pesticides in Tilapia zilli (Alexandria strain) after exposure to sublethal concentrations ($\mu g\ l^{-1}$).

Treatment		Time of exposure (days)				
		4	7	14	21	28
p,p-DDT[1]	p,p[1]-DDE	ND	ND	ND	ND	ND
	p,p[1]-DDD	ND	ND	220.78	545.46	1039.00
	p,p-DDT	555.56	611.11	333.33	333.33	777.78
	DDT[4]	555.56	611.11	554.11	878.79	1816.78
endrin[2]		327.44	167.44	297.64	446.50	595.35
lindane[3]		149.78	148.74	105.44	103.45	96.55

ND = Not Detected
1 = Sublethal concentration of p,p-DDT (3.57 $\mu g\ l^{-1}$)
2 = Sublethal concentration of endrin (0.025 $\mu g\ l^{-1}$)
3 = Sublethal concentration of lindane (0.631 $\mu g\ l^{-1}$)
4. = DDT = p,p-DDD + p,p-DDT

Coats and O'Donnell-Jeffery (1979) and Randall (1979) reached the same conclusion that formulated insecticides are more toxic to fish and Daphnia than the corresponding technical insecticides. This implies the requirement of submission of such data for the formulated compounds to assess their relative hazards to fish and non-target organisms in the environment. Similar results were obtained by El-Sebae et al. (1983).

Thus the chemical configuration of toxicant, the presence of adjuvants, the concentration, the time of exposure, the type of tested organism and the ecological environmental factors are all factors determining type and extent of the adverse effect to the non target organisms, particularly the marine biota. The chlorinated hydrocarbons are the most abundant class in the land-based contaminants from the Egyptian coast of the Mediterranean Sea (El-Sebae and Abu-Elamayem, 1979).

Performance of mercuric compounds in aqueous ecosystem and their toxicities to Gambusia and Tilapia.

Table VI presents the comparative data for the toxicity of mercuric chloride and phenyl acetate to Gambusia affinis after different time intervals. The results indicate the increased hazard of the organic mercuric compounds when compared with the inorganic ones. The same trend is shown regarding acute toxicity to Tilapia sp. (Table VII)

Table VI

Toxicity of mercuric compounds to mosquito fish (Gambusia affinis).

time interval (hrs.)	mercuric chloride 24 h LC50 mg l^{-1}	phenyl mercuric acetate 24 h LC50 mg l^{-1}
4	No Kill	0.040
24	1.2	0.026
48	0.95	0.024
72	0.80	0.020

Table VII

Toxicity and accumulation of mercury in Tilapia zilli (Alexandria strain).

compound	24 h LC50 mg l^{-1}	accumulation % of initial heads	after 24 hrs. concentration whole body
$HgCl_2$	4.2	74%	18%
ph-Hg-acetate	0.32	74%	10%

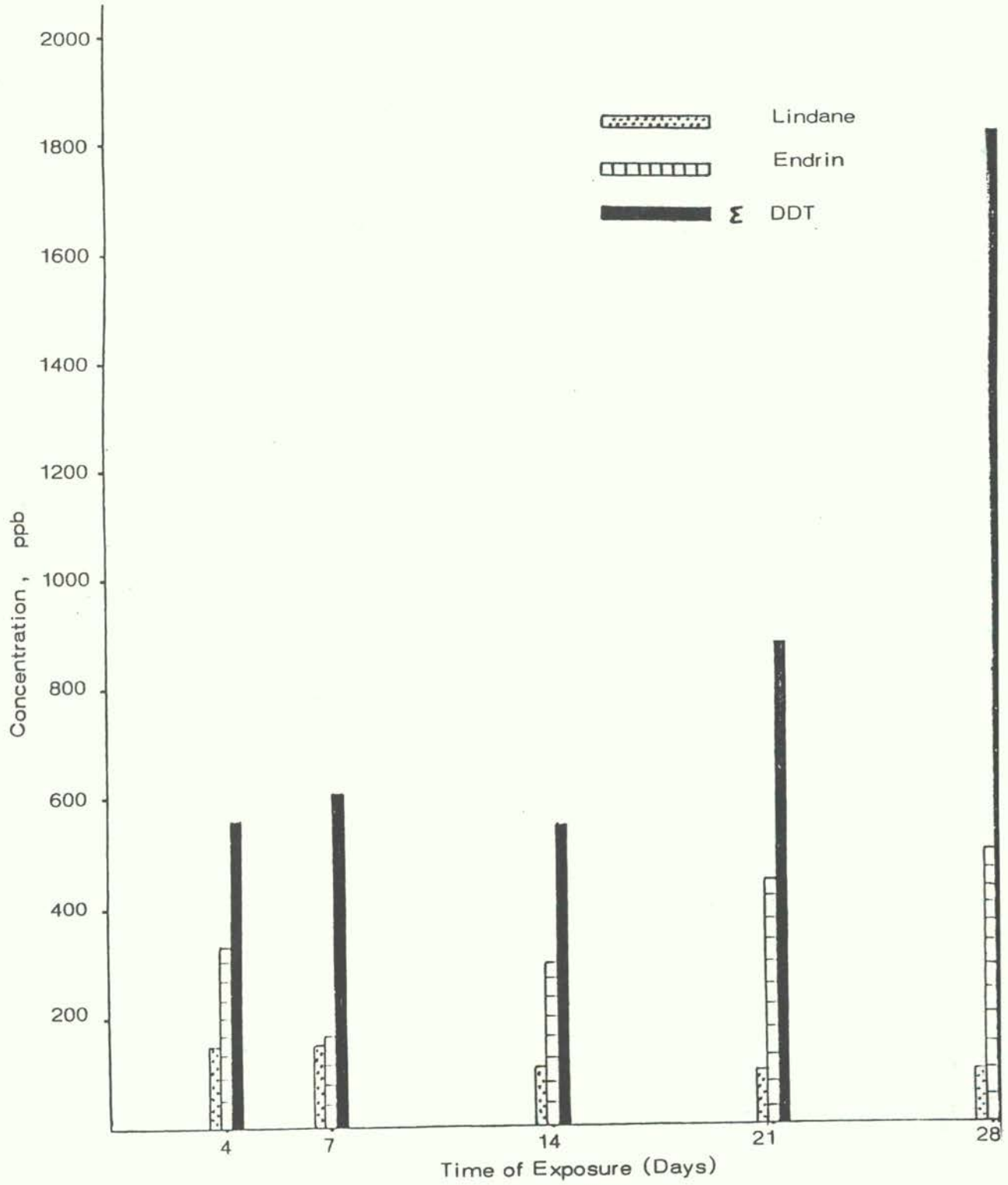

Figure 1. Accumulation of organochlorine pesticides in Tilapia zilli, Alexandria strain, after exposure to sublethal concentrations ($\mu g\ l^{-1}$)

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TOXICITY TESTING IN THE MARINE ENVIRONMENT

by

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1. INTRODUCTION

The earliest aquatic toxicological testing was carried out at the beginning of the 19th century but widespread interest in such work dates from the second half of the 20th century. Modern industrialized societies produce large amounts of chemicals and chemical waste (Ghisalba, 1983). Accidents during production or transportation, and deliberate disposal from industry or domestic uses may cause severe pollution problems in the aquatic environment. Today it is clear that pollution of the hydrosphere represents a major hazard not only to aquatic life but to the health of our whole planet including the human population.

The impairment and progressive deterioration of water systems exposed to pollution was noted from the early times, but the first control measures were taken only after some serious accidents involving human victims occurred. The case of Minamata disease is one of the most illustrative stories. From that time on a proliferation of recommendations, procedures and legal documents were initiated whose purpose was to control and eliminate the introduction of harmful substances into water systems. Various testing procedures have been developed and, since the limitations of acute toxicity tests were recognized, chronic toxicity testing techniques have been developed.

The general approach to toxicity testing is the exposure of an appropriate biological material to the toxic action of a polluting substance under controlled laboratory (or field) conditions. Later, on the basis of the results obtained, the predicted effects of the pollutant(s) are estimated in relation to the environment involved. Such predictions consist of an extrapolative calculation based on the observed behaviour of the test material under experimental conditions. In fact, the toxic effects of the polluting substances are frequently measured at the biochemical, cellular or subcellular level (e.g. including enzyme inhibition and macromolecular disfunction) while the effects that are considered significant in the natural environment occur at higher and more complex (ecological) levels of organisation: populations, communities and ecosystems. The classic, clinical toxicology approach is more simple because it does not necessarily include the extrapolation between different levels of biological organization. However, the ultimate objective in clinical toxicology is the protection of individuals of a single (human) species; while in aquatic toxicology, preservation of whole populations and other higher ecological units is the objective. Actual toxic effects in ecosystems could also be accurately estimated directly in the environment by measuring the changes in population characteristics such as size and density, recruitment, mortality, natality, and other parameters. Unfortunately, in practice that has occurred only in the case of certain uncontrolled or accidental spills. The cases of the Torrey Canyon, Amoco Cadiz and other disasters were very widely studied and documented, but such extensive studies were undertaken because of the wide publicity, and associated political dimensions, of such accidents. (Southward and Southward, 1978; Cabioch et al., 1980). On the other hand, alterations occurring at the level of the ecosystems are not incontrovertible indicators of pollution effects, because population structures change as a consequence of various intrinsic and extrinsic factors not necessarily involving pollution. Recently, there have been attempts to construct large-scale experiments or field enclosures simulating specific ecosystems. Among these the CEPEX (Controlled Ecosystems Pollution Experiment), MERL (Marine Environmental Research Laboratory) are perhaps the best known (Gray, 1982). The results obtained are, without any doubt very useful, but because of the unavoidably high expense these experiments are impracticable for routine investigations. Environmental factors also greatly influence the behaviour of developed pollutants in water, and several methods have been developed for the determination of stability, persistence, transformation and partition of the chemicals in receiving waters. These factors greatly influence the toxic action of pollutants, and also have

implications for the testing procedures. Standards based on the maximal acceptable toxicant concentrations are not completely satisfactory, while in many cases (particularly for organic pollutants) the n-octanol/water partition coefficient appears to be an important indicator of potential toxic effect, regardless of wide variations in the concentration of the chemical in the water. Consequently, in several cases the restrictions imposed on the release of some substances are in practice absurd, because they do not take into consideration the physico-chemical features and the properties of the receiving environment. It is becoming evident that a degree of standardization of procedures is necessary, and the so-called sequential hazard assessment schemes have been proposed. Their main purpose is to systematize and standardize the different methodological approaches, and to avoid irrelevant testing procedures.

Several national and intergovernmental agreements, regulations or codes of practice have been approved, and they particularly consider the premarketing procedure for all substances, their transport, use and release of residuals and end or by-products. For OECD member countries there exists a system of standardized toxicological and ecotoxicological tests that should be carried out before the introduction of new chemicals into the environment, including water bodies (OECD, 1981).

2. TOXICITY TESTING

2.1 SINGLE-SPECIES TOXICITY TESTS

2.1.1 Acute toxicity test

The available literature abounds with data relating to acute, short-term, (LC50) experiments and it is almost impossible to present any information without repeating well-known facts.

Acute toxicity tests are relatively simple, short, reproducible, inexpensive and therefore practical tests. They can be easily adapted to identify a wide variety of important adverse effects. They are very useful in practice, though very often criticized on scientific grounds, but are still in use in aquatic laboratories all over the world. There are two divergent opinions among the aquatic toxicologists. Some advocate that the only good aquatic toxicity tests are "chronic tests", while for others even the routine acute tests are too long and costly and they try to replace them by short tests that last no longer than a few hours. One of the main problems related to acute toxicity tests is the misapplication of the results, particularly through rigid legislative or regulatory decisions. In practice, no single test is capable of establishing or clearly providing all the information necessary to delineate the risk to aquatic organisms of harmful substances; and from the scientific and operational point of view, criticism must be directed towards avoiding time- and money-wasting in extravagant sophistication of experimental designs which can in no way adequately represent the situation in a natural aquatic ecosystem.

A large number of preformulated and tried aquatic toxicity tests, and their respective evaluations, are available in several standard referènce works (Sprague, 1969, 1970, 1971; Brown, 1973, 1981; EIFAC, 1983; FAO, 1977; Lloyd, 1979; Lloyd and Tooby, 1979; Stephan, 1982). In many cases, acute toxicity tests are incorrectly equated to lethality tests. In fact, lethality tests are only one kind of acute test, performed to determine the lethal concentration of a substance (LC50), while in other acute toxicity tests non-lethal effects are observed and the results are generally expressed in terms of the effective concentrations or EC50 (Sprague, 1971). The measured effects may include changed morphological characteristics, impaired physiological responses, or altered biochemical activity of functional macromolecular systems; they may include effects on feeding, breeding, development or growth rate of the organisms, and may be studied at the tissue/cellular or even subcellular level. In this way the usefulness of routine acute tests based on the combination of several measurements may be increased. The result obtained - EC50, or median effective concentration - indicates the concentration of a toxic substance that causes one functional response in 50% of the tested organisms (Stephan, 1982). The acute toxicity test approach can be further promoted by the standardization of methodology and terminology. these improve the quality and the comparability of the results obtained, and also facilitate the study of various aspects

of acute and general toxicology. On the other hand, if applied too rigidly, standardization could decrease the usefulness of the acute testing. For example, if a chemical is partitioned into sediments, it seems clear that it would be more realistic to select test organisms that live in sediments instead of those living in the water column as do most standard laboratory toxicity-testing species. In practice, there are few indications that such arguments are sufficiently widely recognised. The adoption of appropriate terminology is also very important. Thanks to Sprague (1969), for aquatic organisms the LD50 used by medical toxicologists has been definitively replaced by the analogons LC50 value. It is also appropriate to replace the term "bioassay" with "toxicity test", (Brown, 1973). In fact, a toxicity test measures the toxicity of a substance, while bioassays use specific biological effects to estimate the concentration of pollutants in the water. It should also be recommended that we speak about the effects of the tested substances on the organisms involved, instead of the response of the organisms. When the test organisms are exposed to lethal concentrations, they certainly do not respond by dying, they unfortunately fight to survive (Stephan, 1982).

The results of the acute tests are evaluated through 48 or 96 h EC50 and by the shape of concentration-response curve plotted on log scale using EC50 calculated at fixed time intervals (Lloyd, 1979). The shape of the concentration curve gives important information about detoxifying and bioconcentration process, and can be used for the determination of the time necessary for conducting the experiments. The LC50 value cannot be used accurately to predict the minimum concentrations harmful to aquatic life, therefore application factors were used (EIFAC, 1975).

2.1.2 Chronic toxicity tests

Chronic toxicity testing is an expensive and time-consuming way to study the toxic effects of a pollutant. The fish life cycle test was one of the first to be introduced (Mount and Stephan, 1967), and recently there is a tendency to replace it by the fish early life stages test (embryo-larvae test), because they are of equal or even higher sensitivity but significantly shorter and less expensive. These tests take in consideration that not all life stages are equally resistant to pollutants but generally the post egg-hatching-phase is the most sensitive (Eaton, 1974; McKim et al., 1975; Macek and Sleight, 1977; McKim, 1977; Eaton et al., 1978). Very attractive, but very expensive is the most recently proposed multigeneration fish toxicity test, in which the importance of reproductive capacity of fish is emphasized (Newsome, 1980; Bresch, 1982).

The results of chronic tests replace arbitrary application factors with empirical values. The most usual criteria of toxic effect are fertilization capacity, hatchability, survival and particularly the growth rate of juvenile stages. The growth rate and the body condition factor are extremely sensitive and objective parameters because they are the integral expression of the state of health of the tested animals. Under some circumstances, these values should not be readily adopted before the necessary standardization of conditions (e.g. of food and space availability) in the experimental design (Woltering, 1984). These density-dependent components greatly influence the growth rate of fish early life stages, but they have not been included in the routine procedures of the ASTM (1982), OECD (1982) and U.S. Environmental Protection Agency (1982) documents. Another complicating factor is the existence of interspecific differences during growth and consequently of the specific response to toxicants. Thus, as in acute toxicity testing, problems of how to extrapolate results from laboratory conditions to the natural environment still remain.

2.1.3 Sublethal tests

Most minor changes in the natural environment are within the normal range of adaptability of living organisms and they are therefore successfully tolerated. In contrast, major changes ultimately reduce the survival of the organisms involved. Between these two tolerance zones lies a boundary region of sublethal response where survival, potential growth and reproduction are less or more impaired. These changes are the reflection of stress induced by pollutants and they may often occur and easily pass undetected at the level of individuals, but later their significance will be recognised at the population/community levels.

Sublethal effects may be defined in terms of morphological, histological, physiological or ethological changes in the organisms induced at any stage of the life cycle but expressed in later phases in terms of reduced survival potential (Rosenthal and Alderdice, 1976). The identification of small changes occurring in the organisms is the first and essential step in the understanding of sublethal effects, while the extrapolation of these weak responses to a higher level of biological organization and the estimation of their significance at the population and community level is a greatly more complex task. In practice, sublethal effects are generally measured through physiological and biochemical alterations, while histological and histopathological analyses are today relatively rare.

Respiration, osmoregulation, excretion, locomotion, growth, reproduction and other physiological features are mostly considered (Anderson and D'Apollonia, 1978; Bayne et al., 1980). Oxygen consumption has been often used as an indicator of physiological stress, in spite of the fact that various external or intrinsic factors markedly affect the results of the measurements (Davis, 1973; Roberts, 1975; Calabrese et al., 1977; Percy, 1977). Osmoregulatory variables like plasma-ionic concentration, urine excretion rate and activity of ion-transporting enzymes are sensitive systems for the quantitative determination of sublethal effects (Thurberg et al., 1973; Caldvel, 1974; Jones, 1975). Indirect methods for the determination of growth, like the scope for growth values, have some advantages (Bayne, et al., 1979).

Recently, the health of fish and their response to environmental changes have been satisfactorily estimated through the changing values of their haematological and serological characteristics (Buckley et al., 1976; Wedemeyer and Yasutake, 1977).

There are two types of biochemical testing (Uthe et al., 1980). In the first, the concentrations of specific biochemical substances are measured in appropriate tissues and an attempt is made to distinguish normal physiological variation from the reflection of pathological lesions induced by pollutants. The other main area of biochemical investigation involves the study of enzymes and their response to pollutants, but the choice of appropriate enzymes and the correct interpretation of results are two essential aspects. Many enzymes were originally chosen because they have been satisfactorily used in clinical diagnostics for higher animals and humans (Heitz et al., 1974). Very attractive is the study of the enzymes that may be expected to be the specific target of particular pollutants, as in the case of acetylcholinesterase and organophosphate pesticides (Coppage et al., 1975; Gould, 1977). Another approach is the measurement of some branchpoint enzymes important in particular basic metabolic pathways (Livingstone, 1982). However, it is important to appreciate that many aspects of the biochemistry of marine organisms are substantially different from those of the higher vertebrates, and that differences between and within marine phyla are even wider. Changes in the acitivity of mixed function oxidases have also been used in pollution studies, and could be considered as indicators of the presence of specific inducing substances, but not necessarily of pathological changes (Giam, 1978).

Before giving final conclusions, one must take into consideration that observed physiological and biochemical changes are not necessarily deleterious, but could be normal variations within the limits of the normal activity and reactivity of normal organisms. If the limits and variations are not well-known, the toxicological and particularly the ecological significance of the measured variations cannot be precisely estimated. Thus, it becomes very important to choose those parameters that can be objectively evaluated, that do change even at the lowest, sublethal, toxicant concentrations, and which have a clearly detrimental effect on whole animals: on growth, reproduction, development and survival (Livingstone, 1982).

Reliable extrapolation from the sublethal effects observed in individuals to the population or community levels still remains difficult. The opportunity could arise when several species at different ecological levels are studied (Bayne et al., 1980, 1980a), or perhaps by using enzyme inhibition studies in a laboratory model ecosystem. However, the study of sublethal effects should be considered very useful for the understanding of toxicological mechanisms and the modes of action of pollutants.

2.2 MULTISPECIES TOXICITY TESTS - EXPERIMENTAL MICROCOSMS

In order to bridge the gap between laboratory results and the real environment, many authors have recently used complex systems which are considered to approximate field conditions more closely than laboratory experiments (Ringelberg and Kersting, 1978; Dortland, 1980; Oviatt et al., 1980; Giesy, 1980; Grice and Reeve, 1982; Giddings, 1983). Experimental microcosm systems are based on field ecological relationships, where the ecosystem is considered as a fundamental unit. The systems range from a simple Erlenmeyer flask with a mixed population culture, to larger enclosures along coastal waters.

A microcosm usually has several essential features that makes it different from the real environment conditions. Such systems are partially or entirely closed or isolated; their dimensions are limited; and the number of species is drastically reduced and the numbers of the individuals of the species involved are frequently unbalanced. A number of various microcosms have been used in aquatic toxicology (Metcalf et al., 1971; Taub, 1976; Bourguin et al., 1977, 1979; Brockway et al., 1979; Crossland and Stephenson, 1979; Giddings and Eddelmon, 1979; Dortland, 1980; Giddings, 1983).

The mixed-culture microcosm consists of natural or artificially-assembled communities. The main characteristics of these systems are taxonomic and physical simplicity. The best known mixed-culture assembly was prepared in 1959 by Beyers (1962) by innoculating an artificially-prepared medium with microorganisms from a sewage oxidation pond. These systems are generally used to assess the effects of pollutants on the community structure, reproduction and respiration (metabolism). Among other investigators, Taub (1969) was the first to set up an artificial microcosm using selected substrates, media and cultured organisms. The main advantages of these systems are the easier control and possible manipulation of the initial communities, as well as the possibility of replicating the experiments. Artificial systems have been frequently used to evaluate the toxicity, bioaccumulation and fate of several chemical products. The main objections to these systems are the low species diversity and the lack of predators.

The recent use of pond microcosms is based on the old concept of the balanced aquarium, in which the aquatic plants and animals live and develop harmoniously without external inputs other than sunlight and heat. The essential characteristics of these systems are the complexity and constancy of the established structures, and their potential response to pollutants is therefore more accentuated. Pond microcosms are usually obtained by transferring preferably undisturbed sediments, with corresponding biological elements and water, from a natural ecosystem into aquaria where the chosen system is allowed to stabilize and develop. The structure of the microcosm remains constant and equilibrated for months. A pond usually contains about 100 or more species. Because of their sensitivity to physical variations and other laboratory artefacts, experimental ponds in the laboratory are kept under controlled and constant conditions. However, these limitations are not crucial and the results are readily transferable to corresponding natural ecosystems. Soon after the earliest applications of the pond microcosm in the study of the transport, degradation and bioaccumulation of chemicals (Eggert et al., 1979; Giddings et al., 1979; Gledhill and Saeger, 1979) they were also used for the hazard assessment analysis of pollutants (Crossland and Stephenson, 1979; Giddings, 1983).

Outdoor tanks are derived from pond microcosms. They are usually flow-through regulated, larger enclosures that in principle remain under the direct influence of natural, not simulated local hydrometeorological conditions. The effects of low-level, long-term exposure of model drainage ditches to organophosphates were studied by Dortland (1980). The effects of oil pollution on littoral communities were studied in outdoor concrete basins in the Netherlands/MOTIF tidal flat ecosystem (Kuiper et al., 1983), and in Norway on intertidal hard and soft bottom substrates (Gray, 1982).

The largest microcosms simulating natural ecosystems are field enclosures of water columns of various sizes, which have been used both in freshwater lakes (e.g. the MELIMEX experiments, Gaechter, 1979), and in inshore coastal waters, like the Loch Ewe bags in Scotland, the CEPEX experiment in Canada and others (Steele, 1979). Their size ranges from 1.5 to 1300 m^3 (Menzel and Case, 1977; Kuiper, 1981), and the results obtained have provided relevant and detailed information about the fate and effects of several

pesticides, heavy metals and hydrocarbons in the aquatic environment. They are closely analogous to the natural environment, and the reproducibility and comparability between replicated control enclosures is satisfactory (Kuiper, 1977; Davies and Gamble, 1979; Steele, 1979).

3. ENVIRONMENTAL HAZARD ASSESSMENT

The hazard assessment procedure is intended to evaluate the toxicity of polluting substances as well as their fate and behaviour in the environment. Single values are the expected final products, indicating the level of available forms of contaminants to which aquatic organisms may be exposed for chronic, lifetime duration without being harmed. Sequential or tiered testing is the basic approach, and different biological and chemical testing procedures are included (Cairns and Dickson, 1978; Calamari et al., 1979; Dickson et al., 1979; Brown, 1982; Lee et al., 1982). This approach offers the opportunity to estimate, step by step, the degree and acceptability of a hazard in a particular system. In some cases, it is possible without any experimental work to estimate by simple calculations that the hazard associated with the release of a particular chemical is either insignificant or very substantial. In either case, it is often not necessary to make a more precise estimation of the hazard. In the first case, the release is allowed without restrictions; in the second, severe restrictions or even absolute prohibition should be imposed. In the most frequent situations, however, where the expected concentrations in the environment are close to the critical limits, the tiered testing procedure will be necessary.

Biological testing usually starts with simple and rapid screening tests, primarily acute toxicity tests. For those chemicals whose acute toxicity lies above specified limits, further testing includes the determination of chronic and sublethal toxicity thresholds on single, usually standard laboratory species. The purpose of the initial screening phase is the identification and ranking of immediately harmful chemicals, according to their potential for adverse effects and their need for further testing. There are no particular problems if acute toxicity tests consistently overestimate the environmental hazard, because the discrepancies will be resolved later on in the later phases of the hazard assessment process. If, however, a lower risk is estimated for chemicals that are actually hazardous to ecosystem, environmental damage could result.

Chemical testing procedures include biodegradability tests, and the persistence of chemicals is the major determinant of the length of the tests carried out in the laboratory. The next steps are concerned with the determination of the actual concentration in the environment, the n-octanol/water partition coefficient leading to an estimation of the likely extent of any bioconcentration processes.

The prediction phase of the tiered system is also based on chemical data necessary to estimate the probability of their harmful impact in the environment. Predictions must be applicable to general cases rather than to any specific geographical area. Mathematical models and other analytical approaches are used to synthesize and integrate available information, in order to produce either qualitative predictions of effects under specific circumstances or probabilistic statements. In both cases, the conclusion requires experimental confirmation, and the microcosm or experimental ecosystem is generally the best tool for the validation of models and the confirmation of their predictions. Many researchers, however, do not agree with the use of microcosms at the end of the testing procedure, but instead prefer to use it at the early, first stage as an alternative to the present strategy of the tiered testing system.

In the future, microcosm experiments, field studies and mathematical modelling, combined with simpler but sensitive laboratory experiments, will offer a satisfactory basis for the interpretation and prediction of the effects of chemicals in the environment. However at present the majority of research is based in practice on toxicity testing, consisting of "packages (bundles) of standard laboratory tests" prescribed by national legislation or international conventions, while alternative, usually more expensive, elements of the hazard assessment approach remain in the field of science fiction for most of the laboratories collaborating in the MED POL programmes.

4. CONCLUSIONS

To protect the marine environment it is imperative to assess the probable effects of pollutants before they are discharged. In practice, because of the wide gaps between simplified laboratory tests and the innate complexity of natural environments, realistic a priori hazard assessment is difficult. To resolve these problems some essential lines should be followed:

(a) The development and application of sensitive and expeditious laboratory techniques to measure, explain and confirm the biological significance of the exposure of test organisms to actual or potential pollutants in the marine environment.

(b) The study of basic biological, physiological and biochemical characteristics of single species, and of the extent of their natural variability with particular emphasis on the differentiation between pathological effects and normal physiological response to natural and artificial environmental pressure.

(c) Detailed synecological studies to evaluate the relationship between the species involved and to estimate the significance of single elements within the ecosystem to the response of the system as a whole.

(d) The development of a satisfactory hazard assessment scheme, which would include the essential steps and possible alternatives for evaluating potential environmental hazards and prescribing the necessary limitations.

Finally, but not least importantly, public authorities and administrators can make a substantial contribution through the enactment and enforcement of legislature and administrative action designed to manage and protect the marine environment.

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INVESTIGATION OF TRACE ELEMENT DISTRIBUTION IN THE AQUATIC SYSTEM OF THE BOKA KOTORSKA BAY

by

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1. INTRODUCTION

The concentrations of trace elements in sea water and sediment samples originating from the Boka Kotorska Bay region have not been determined earlier by proper analytical techniques. The results presented in this work, with those reported earlier for the content of trace elements in selected marine organisms from the same area (Martic et al., 1980), should contribute to the estimation of the degree of contamination and its effects on this unique environment.

In this work, samples from the two most distant locations in the bay, in the vicinity of Kotor and of Herzeg Novi, were investigated. The two locations are quite different with respect to the distance from the open sea, fresh water and industrial waste water input, anthropogenic influence, etc. Included in this report are the results obtained for the content of trace elements in two marine organisms, Mugil cephalus and Mytilus galloprovincialis, which are important components in the regular diet of the local population.

2. METHODS AND RESULTS

Sea water and sediment samples were collected on locations near Kotor (I) and Herzeg Novi (II) (Fig. 1), where a high degree of contamination is a priori expected. It should be noted that the location in the vicinity of Kotor is exposed to dilution by the continental water from the Skurda stream; the salinity determined on locations (I) and (II) is 24.6 °/oo and 36.3 °/oo, respectively. Samples were collected in July 1983 at depths of 0.5 m (sea water) and 10-16 m (sediment). The details of sample preparation were presented in our earlier work (Martic et al., 1983). As the analytical method, X-ray fluorescence was used on samples irradiated with ^{109}Cd and ^{241}Am. Radiation intensity measurements were carried out with a "Cambera" spectrometer equipped with a Si(Li) detector. The underlined values in Table I represent concentrations determined on the basis of statistically well-defined spectral lines, with an error of max. 10%. The remaining values were estimated by applying statistical criteria and are not discussed.

3. DISCUSSION

By comparing the obtained results (Table I) with those of Goldberg (1972) for the concentration of various elements in sea water, significant differences can be noticed. While good agreement is found for macroconstituents (Ca, Br, Sr, I, Ba), confirming good reproducibility obtained by the analytical method applied, for microconstituents correlation factors in the range of n.10 to n.10^4 are found. However, the deviations from Goldberg's values are of the same order of magnitude for each element on both locations. The fact that the maximal deviations are found for heavy metals and arsenic, which in the soluble state are present in the liquid phase, indicates that these changes in concentrations are due to contamination and not to the difference in natural composition.

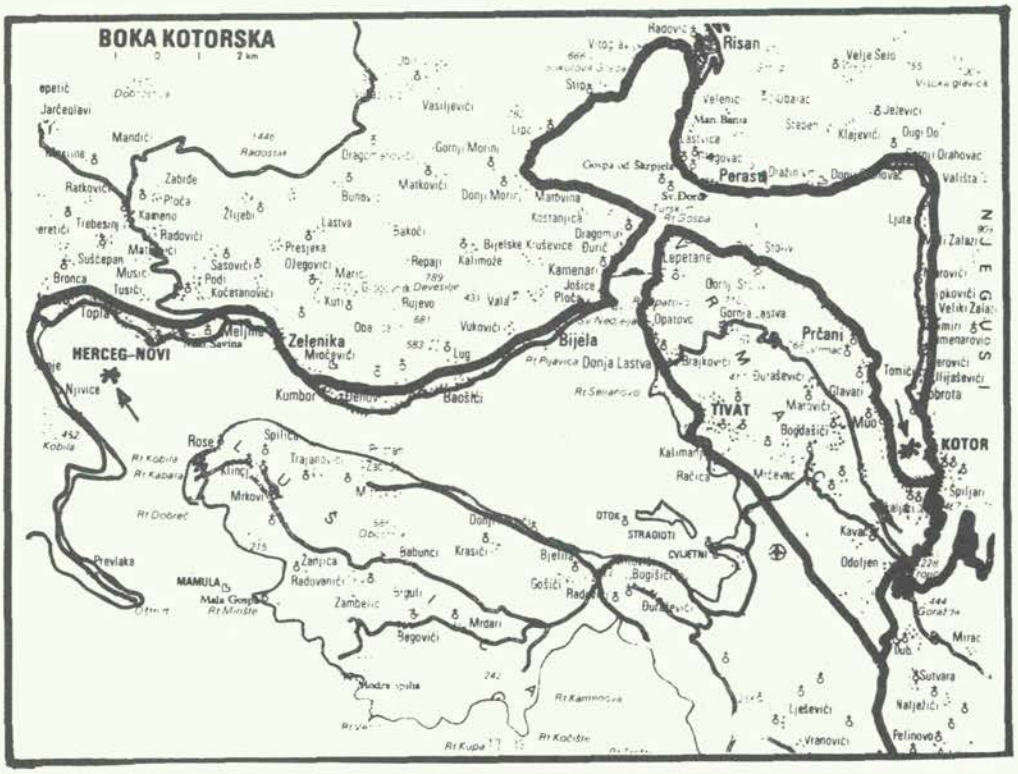

Figure 1. Study area

The comparison of the concentration ratios of various elements in samples from both locations under investigation points to specificities characterizing each location. Although the Kotor Bay was expected to be contaminated to a greater degree, it was found that the concentration of all micro- and macroconstituents, excluding iron, and even taking into account errors in methodology, are higher on the Herzeg Novi location. The highest water contamination was by manganese, zinc, cadmium and arsenic. Whether contamination in the Herzeg Novi area is caused, as appears to be the case at the Kotor location, whose effects are not decreased by the inflow of continental water, could be established only by investigating several locations both in the Bay and in the open sea area.

The analysis of the distribution coefficients (DC) and concentration factors (CF) indicates the selectivity of accumulation of certain microelements in the sediment and in the organisms under investigation. The relatively low DC values indicate that migration of microelements is from the solid to the liquid phase, resulting in a deteriorating effect of pollution on marine life, particularly on the filter feeding organisms. Thus, it was found that the organisms under investigation, especially M. Galloprovincialis, selectively accumulate certain elements (iron, zinc). The resulting visually-recognized deterioration of numerous samples of both species requires thorough biochemical investigation in order to interpret the mechanism of the possible effect of these elements.

Consequently, it can be concluded as follows:

a) In the Boka Kotorska Bay there is an apparent increase in concentration of the majority of the 29 investigated elements as compared to data on average abundance published earlier (Goldberg, 1972), and in particular of the heavy metals, such as chromium, manganese, iron, copper, zinc and the toxic arsenic. However, the obtained results are in good agreement with those found for the concentrations of the above elements in certain Mediterranean industrial and urban regions (Angela et al., 1981, El-Sayed et al., 1981, Taliadouri-Voutsinou, 1981).

Table I.

Concentrations of elements in marine samples collected in the summer of 1982.
with concentration factors (CF) and distribution coefficients (DC)

Element	SEA WATER (mmol m^{-3}) (depth 0.5 m)	Kotor	H/NOVI (16m)	SEDIMENT (mmol kg^{-1} wet weight) ($n.10^3$)	Kotor (10m)	DC ($n.10^3$)	H/NOVI M. cephalus	ORGANISMS FROM KOTOR BAY (mmol/kg wet weight) DC CF	M. gallopro-provincialis	CF
Ca	5.6(3)*	6.4 (3)	1.2 (3)	0.2	1.9 (3)	0.3	255	45.5	2.06	0.4
Sc	187	259	6		13		0.7		0.1	
Ti	99	139	65.9	0.7	82.4	0.6	0.2		7 (-2)	
V	58	80	2		3		0.1		4 (-2)	
Cr	68.3	77.8	4.42	0.06	7.36	0.09	0.1	1.5	2.1 (-2)	0.3
Mn	15	22	10.3	0.7	9	0.4	4.1(-2)	2.7	1.6(-2)	1.1
Fe	51.7	34.3	459	8.9	469	13.6	0.51	9.9	0.27	5.2
Co	7	9.2	0.6		1		1 (-2)		6 (-3)	
Ni	5	7.7	0.2		0.3		8 (-3)		4 (-3)	
Cu	21.9	28.8	0.79	0.04	1.02	0.04	5.3(-2)	2.3	1.6(-2)	0.7
Zn	2.4	4.8	2.27	1.0	0.62	0.1	0.28	120.0	0.64	270.0
Ga	2	3	4 (-2)		7 (-2)		4 (-3)		2 (-3)	
Ge	1	2	3 (-2)		5 (-2)		3 (-3)		2 (-3)	
As	4.0	6.5	0.18	0.01	0.17	0.03	6.0(-3)	1.5	4 (-3)	1.0
Se	0.9	1	2.68(-2)		3 (-2)		1.7(-3)		9 (-4)	
Br	4.3(2)	5.2(2)	0.68	1.6 (-3)	0.47	9.0 (-4)	7.2(-2)	0.2	0.31	0.7
Rb	1	2	0.57	0.6	0.57	0.3	3.3(-3)	3.3(-3)	1 (-3)	1.0(-3)
Sr	69.9	70.9	1.20	0.02	1.77	0.02	0.75	10.7	3.9(-2)	0.6
Y	0.4	0.6	0.15	0.4	0.14	0.2	7 (-4)	1.8	2.6(-2)	65.0
Zr	0.6	0.8	0.60		1.18		2 (-3)		4 (-4)	
Nb	0.4	0.6	2.5(-2)		4.4(-2)		8 (-4)		3 (-4)	
Mo	0.5	0.9	1 (-2)		2 (-2)		8 (-4)		4 (-4)	
Ag	-	0.3	-		-		1.0 (-3)		-	
Cd	0.3	0.5	6 (-3)	0.02	9 (-3)	0.02	5 (-4)	1.7	1.7(-3)	5.7
Sb	-	-	-		-		4.6(-4)		1.1(-4)	
Te	0.3	0.4	6 (-3)		8 (-3)		5 (-4)		2 (-4)	
J	0.3	0.4	0.35		0.10		9 (-4)		6.0 (-4)	
Ba	1.4	1.4	1.03	0.7	0.95	0.7	2.1(-2)	15.0	2.7(-3)	1.9
Pb	-	-	0.19		0.10		4.9(-3)		0.29	

* a (b) = a x 10^b

b) The high concentration factors indicate that dangerous pollutants, such as heavy metals, are in the liquid phase as dissolved species, suitable for the uptake by marine organisms. Our yet unpublished results on the investigation of Crossostrea gigas show an even greater heavy metal concentration.

The results show that parallel investigations on different locations in the Bay area, as well in the open sea, including biochemical studies, are required in order to establish the degree of the effect of contamination on the deterioration of marine life in the Bay.

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LETHAL AND SUBLETHAL EFFECTS OF SOME CONTAMINANTS (HEAVY METALS, OIL, DISPERSANTS) TO MARINE PLANKTONIC ANIMALS

by

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1. INTRODUCTION

The living resources of estuarine, coastal and even oceanic ecosystems are being harmed recently by discharge of waste material and runoff of highly polluted waters. Heavy metals are considered to be among the most harmful aquatic pollutants. These metals are, in general, highly toxic to marine organisms and often accumulate in their tissues. This may, via food chains, contaminate commercially-important food species and through them, humans. In the marine environment oil resulting from natural seeps, oil fields, refinery wastes, normal shipping operation, atmospheric transport and accidental spills, is becoming an increasing threat. Common use of supertankers, development of more offshore oilfields and construction of deep water offshore superports will increase the probabilities of chronic leakage and catastrophic oil spills.

To create standards for environmental management, there is a need for information concerning the effects of pollutants on marine organisms. Attempts to develop regulations based on known effects of metals showed that our knowledge of pollutant toxicity is not yet sufficient. Acute toxicity tests have been utilized in the setting of water quality criteria for protection of the aquatic environment. These studies serve to determine the range of tolerance to pollutants, to set exposure levels for sublethal studies and to evaluate the significance of environmental concentrations. Although survival is considered the best index of a pollutant stress, being the least variable, toxic substances may exert effects on organisms at concentrations much lower than the lethal ones. This may be more important in the long term than acute effects. Sublethal toxicity affects various physiological processes such as reproduction, growth rate, respiration and hatching rate of eggs.

In the framework of the MED POL Programme we have conducted, at the Zoological Laboratory of the University of Athens, several experiments concerning the toxic effects of some marine contaminants (heavy metals, oil, oil dispersants) to various marine organisms. (Moraitou-Apostolopoulou, 1978; Moraitou-Apostolopoulou and Verriopoulos, 1979, 1982, 1982a, Moraitou-Apostolopoulou *et al.*, 1979, 1979a, 1982, 1983, 1985; in press; Verriopoulos and Moraitou-Apostolopoulou, 1981, 1981a, 1982, 1983, Kissa *et al.*, in press).

2. MATERIAL AND METHODS

As test animals we used the copepods *Acartia clausi*, *Tisbe holothuriae* and the anostracan *Artemia salina*. *Acartia* was collected by planktonic hauls (WP2 net) from two areas of the Saronicos gulf, one inside the polluted Elefsis Bay and another at a relatively non-polluted area situated 25 km southwards. *Tisbe* was taken from laboratory cultures and *Artemia* hatched from commercially available cysts. The toxic substances tested were: metals (copper,cadmium,chromium,nickel,cobalt), oil (Tunisian crude oil zarzaitine type) and an oil dispersant (Finasol OSR-2). Two types of toxicity tests were carried out : a) acute lethal toxicity tests and b) sublethal toxicity tests. All experiments (static bioassays) were run in constant temperature rooms and the animals were put, usually, individually in 50 ml containers filled with the toxic solution. Acute lethal toxicity was estimated by calculation of the LC50 48h (concentration of a toxicant which kills 50% of the test animals after 48 hours of exposure) according to the Bliss (1938) method.

In sublethal toxicity tests we estimated the impact of low concentrations of contaminants on various physiological processes of the organisms (feeding as ingestion rate; respiration rate; reproductive capacity as numbers of eggs released by the females; longevity and various parameters regulating the population dynamics of the tested animals). The importance of some parameters influencing the toxicity of toxicants such as environmental factors (temperature, salinity, dissolved oxygen), developmental

stage of the organism and annual generation have also been investigated. Furthermore we have studied the combined effects of heavy metals tested, when acting simultaneously to the organisms.

Finally we have tried to create organisms resistant to pollutants (adaptation). Experiments of oxygen consumption were performed by the polarographic method using an oxygen meter (E5046 pO_2 electrode-radiometer). The principles of this method have been described by Kanwisher (1959). For the feeding experiments we used a mixture of four laboratory cultured phytoplanktonic species: Exuviella baltica, Nitzschia closterium, Skeletonema costatum, Chaetoceros danicus. The experimental solutions of metals were prepared by diluting a stock solution of a metal compound in order to obtain the desired concentration of metal ion. Oil water mixtures for use in bioassays were prepared to form oil-in-water dispersions (OWDs). OWDs were prepared by adding measured volumes of oil (Tunisian crude oil, zarzaitine type) to artificial sea water (prepared by mixing distilled water with Instant Ocean synthetic sea salts) and shaking the mixture vigorously for 15 minutes at approximately 2000 cycles min^{-1} on a shaker.

3. RESULTS AND CONCLUSIONS

a) acute toxicity tests

Table I summarizes the relults of acute toxicity tests. The acute toxicity tests clearly show that there are large differences in the toxicity of the tested metals. Copper was the more toxic of all metals, cadmium was much less toxic while chromium proved about 600 times less toxic than copper. The form of metal compound influences the toxicity of the metal as it was shown for chromium. Temperature seems to be an important factor regulating the toxic effects of pollutants. For all tested toxicants an increase of exprerimental temperature resulted in lower resistance to toxicants. Differences in the tolerance of Acartia to metals have also been observed between the different annual generations of the organism, the summer generation being the more sensitive. Finally significant differences in the tolerance of metals have been observed between the two populations of Acartia: the population of the polluted Elefsis Bay proved more resistant to both copper and cadmium than the population of the same species living at the non-polluted area. The various developmental stages of Tisbe showed differences in the tolerance of copper and cadmium: the younger individuals were more sensitive than the older ones, but also the two reproductive female stages showed increased sensitivity.

Oil proved of low toxicity to Artemia. The "age" of stock solution (time interval between the formation of the toxic stock solution and the dilution and addition of test animals) strongly influences the toxicity of oil, older solutions being much less toxic. The oil dispersant Finasol OSR-2 was much more toxic than oil (about 300 times). The same decrease of toxicity as for oil was noticed with "old" solutions of dispersant.

The toxicity of oil/oil dispersant mixture was lower than that of Finasol (same concentration as that used in the mixture) and much higher than that of oil acting alone. Light conditions influence the toxicity of oil and oil dispersant. For oil the differences in the observed toxicity in the three photic conditions (continuous light, continuous dark, photoperiod: 12 h dark, 12 h light) proved statistically significant. Oil exerted the lowest toxicity to Artemia when acting under continuous dark conditions. A significant increase of its toxicity was noticed under continuous light and especially under photoperiod conditions. Artemia proved very resistant to cadmium, nickel and cobalt but not to chronium.

b) Sublethal toxicity tests

Figures 1-4 show the impact of low concentrations of metals on the longevity of Acartia. All tested concentrations affect the longevity of Acartia. This obviously has adverse consequences on the population density of this organism. Even when longevity of population was taken as an index of the metal stress the population of Elefsis Bay proved more resistant.

Table I.

Results of acute toxicity tests of metals, oil and oil dispersant to various marine organisms

Toxic substances	Organism tested and experimental conditions	48h LC50 mg l^{-1}
	Artemia salina	
Tunisian crude oil (zarzitine type)	"Age" of stock solution	
	a) 0 h.	297.89±2.50
	b) 48 h.	530.00±1.85
	c) 96 h.	407.96
Oil Dispersant (Finasol OSR-2)	"Age" of stock solution	
	a) 0 h.	0.9399±1.05
	b) 48 h.	10.0580±0.55
	c) 96 h.	21.0940±0.50
Oil. (Tunisian crude oil zarzitine type)	a) Continuous dark	
	1) 14°C adults	118.65
	2) 22°C "	677.27
	3) 22°C larvae	260.84
	b) Photoperiod (12h dark - 12h light)	
	1) 14°C adults	7309.8
	2) 22°C "	464
	3) 22°c larvae	201.4
Oil and Dispersant	Continuous light	
	1) 14°C adults	9982.8
	2) 22°C "	616.74
	3) 22°C larvae	250.84
Oil Dispersant (Finasol OSR-2)	a) Continuous dark	
	1) 14°C adults	1.83
	2) 22°C "	0.83
	3) 22°C larvae	2.72
	b) Photoperiod (12h dark - 23h light)	
	1) 14°C	8.001
	2) 22°C	1.190
	3) 22°C larvae	3.427
	c) Continuous light	
	1) 14°C adults	2.42
	2) 22°C "	1.00
	3) 22°C larvae	2.95
Cu as $CuSO_4 \cdot 5H_2O$	Acartia clausi	
	a) from Elefsis bay	0.082±0.0026
	b) from non polluted area	0.034±0.0046
	Tisbe holothuriae	
	a) 1 day old nauplii	0.3142±0.0052
	b) 4 days old nauplii	0.3415±0.0004
	c) 10 days old copepod	0.5289±0.0011
	d) with ovig.bands	0.4473±0.0021
	e) with egg sac	0.4281±0.0027

Table I.
continued

Toxic substances	Organism tested and experimental conditions	48h LC50 mg l^{-1}
Cd as $CdCl_2.2H_2O$	Acartia clausi	
	a) from Elefsis bay	
	1. 14°C	1.50±0.038
	2. 22°C	0.74±0.023
	b) from non polluted area	
	1. 14°C	1.20±0.028
	2. 22°C	0.60±0.043
	Tisbe holothuriae	
	a) 1 day old nauplii	0.5384±0.0062
	b) 4 day old nauplii	0.6450±0.0062
	c) 10 day old nauplii	0.9061±0.0066
	d) with ovig. bands	0.9166±0.0560
	e) with egg sac	0.8727±0.0166
	Artemia salina	159.61
Cr as Na_2CrO_4	Acartia clausi	
	from Elefsis bay	
	a) autumn generation	
	1. 14°C	16.99±2.38
	2. 18°C	11.47±3.87
	3. 22°C	8.83±2.10
	b) winter generation	
	1. 14°C	16.37±0.18
	c) summer generation	
	1. 14°C	12.26±2.62
	d) winter generation	
	1. 14°C	19.27±2.80
	Tisbe holothuriae	
	14°C	17.36
	18°C	15.77
	24°C	16.12
	Artemia salina	7.91
Ni as $Ni(No_3)_2$	Artemia salina	162.99
Co as $Co(No_3)_3$	Artemia salina	171.66

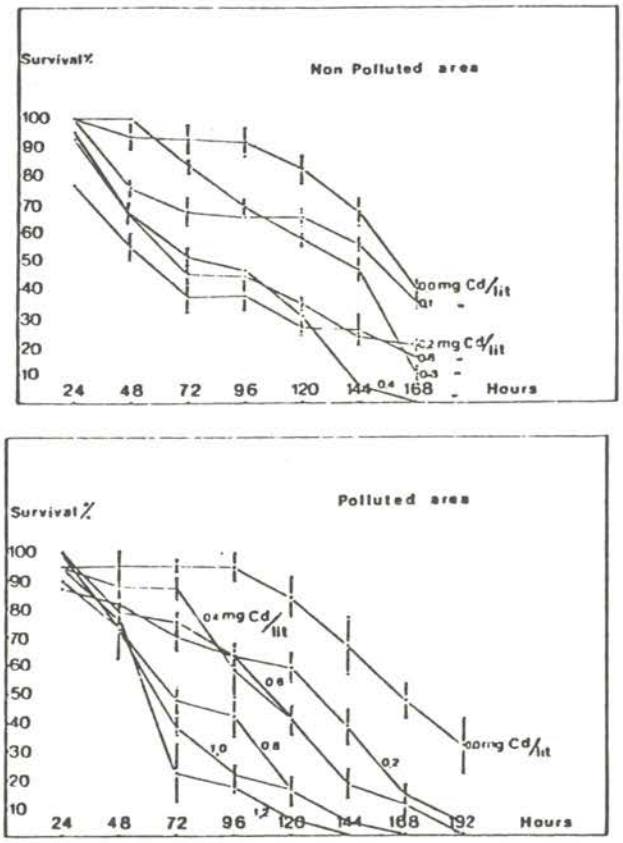

Figure 1. Survivorship curves of the two populations of Acartia exposed to sublethal, concentrations of cadmium.

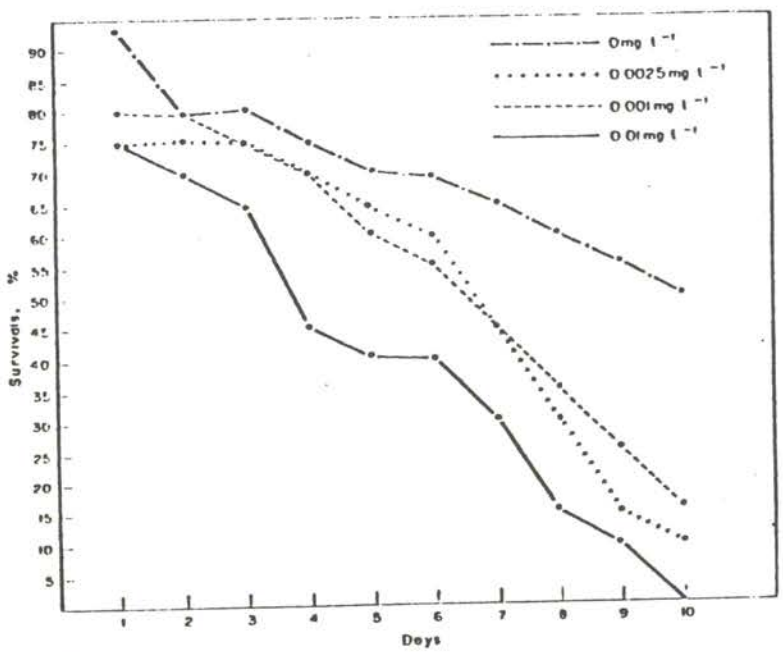

Figure 2. Survival of Acartia clausi in different concentrations of copper. Polluted area.

Table II reports the effects of sublethal concentrations of metals to some physiological processes of Acartia. The general pattern of physiological reaction of Acartia to metal stress was an increase of respiratory rate and a decrease of ingestion rate. The intensity of this reaction is usually proportional to the exposure concentration. This means that, when exposed to low metal concentrations, planktonic animals are subject to serious physiological stress and cannot meet their metabolic requirements.

In all cases, animals from the polluted area are less affected than those from the non-polluted area. The existence of a planktonic population adapted to pollution, observed during the acute toxicity test, is also shown with sublethal tests. The observed increase in the number of eggs released by the females in low concentrations of copper is particularly interesting. It seems that this metal affects some mechanism related to reproduction.

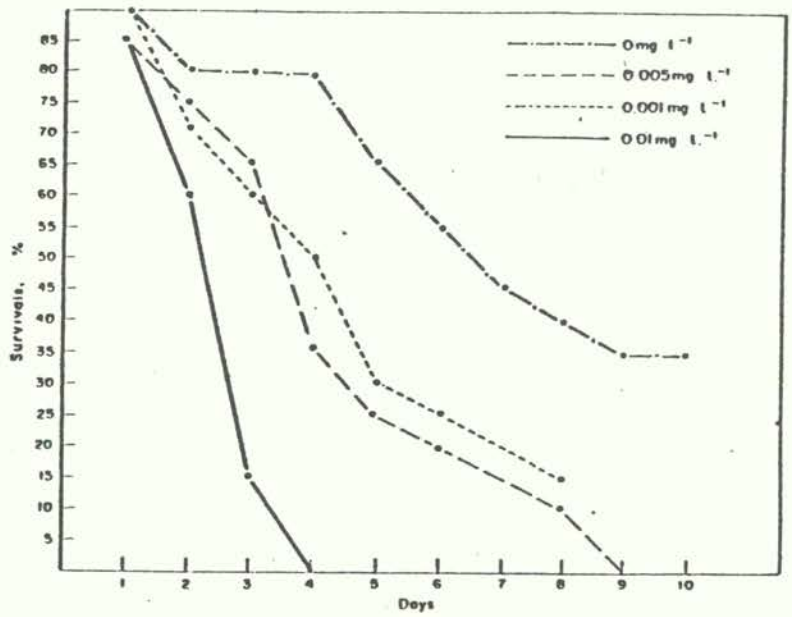

Figure 3. Survival of Acartia clausi in different concentrations of copper. Clean area.

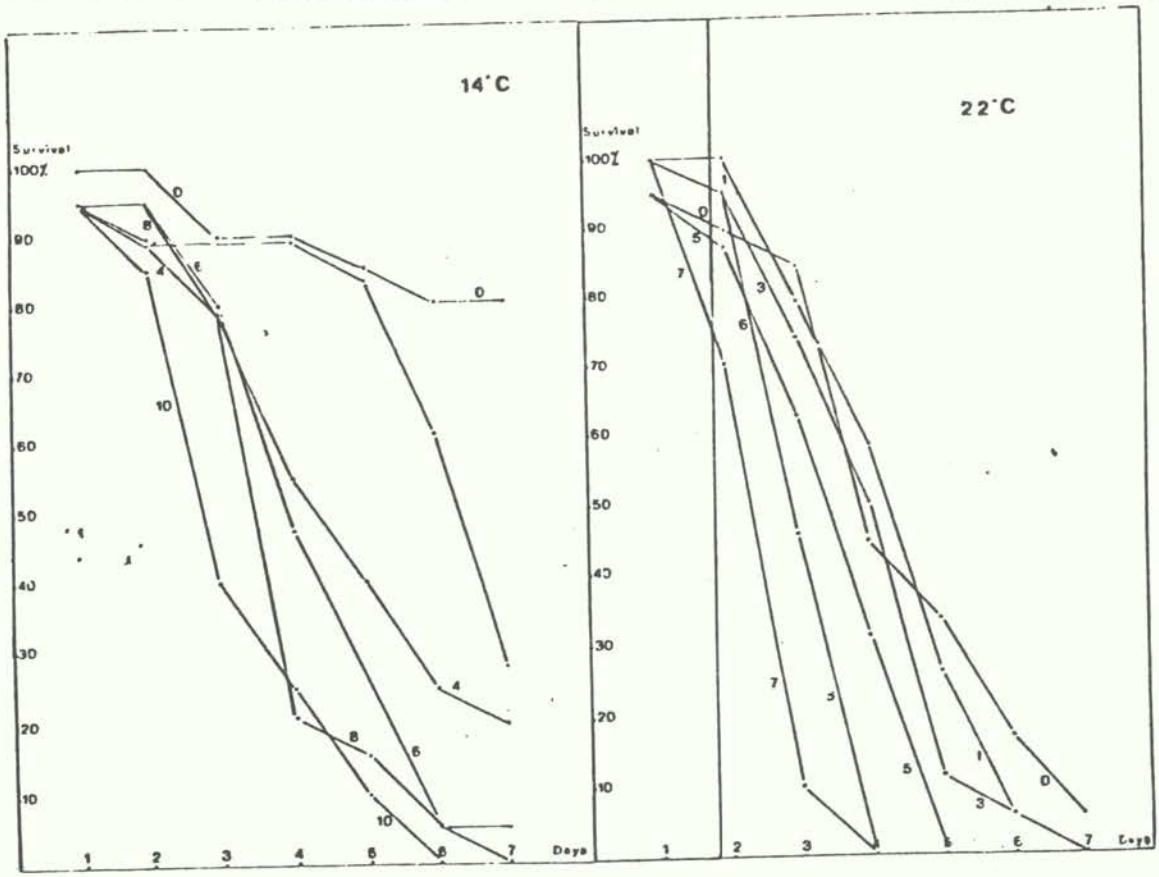

Figure 4. Survivorship curves of Acartia clausi exposed to sublethal concentrations of hexavalent chromium (Na_2CrO_4)

Table II.

Impact of metals to some physiological processes of Acartia

Metal concentration mg l^{-1}		Source of Acartia	Physiological process tested ingestion rate cells/24 h	Egg production (in 3 days)	Oxygen consmp. /animal (µl 02/20h)
Cu	0	polluted	25600	5.25	0.010
"	"	clean	25550	3.12	0.006
"	0.001	polluted	24950	6.0	0.018
"	"	clean	14440	1.0	0.009
"	0.0025	polluted	-	7.06	-
"	"	clean	-	-	-
"	0.005	polluted	12290	-	0.022
"	"	clean	3065	0.28	0.019
"	0.01	polluted	-	5.69	0.030
"	"	clean	-	0	0.024
Cd	0	polluted	11000		0.032
"	"	clean	9500		0.025
"	0.2	polluted	6000		0.037
"	"	clean	7800		0.025
"	0.4	polluted	1800		-
"	2	clean	5800		0.026
"	0.6	polluted	1000		0.038
"	"	clean	5200		0.025
"	0.8	polluted	1000		0.043
"	"	clean	13800		0.044
"	1.0	polluted	19000		0.059
"	"	clean	-		
Cr	0	polluted	11392(14°C)		0.96
"			10162(22°C)		
"	1	"	5715(22°C)		0.99
"	2	"	8218(14°C)		1.10
"	3	"	4646(22°C)		-
"	4	"	5960(14°C)		1.22
"	6	"	5550(14°C)		
			3625(22°C)		1.23
"	8	"	-		1.43

Figs. 5-7 illustrate the importance of some ecological parameters on the toxicity of metals (cadmium) to marine organisms (Tisbe holothuriae). The density of the affected population influences the sensitivity of Tisbe to cadmium, at the higher population densities tested the sensitivity to Tisbe to cadmium was considerably increased.

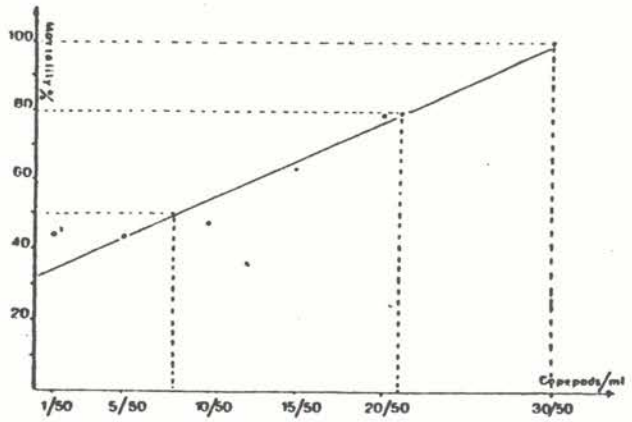

Figure 5. Mortality of Tisbe exposed to cadmium at different experimental population densities

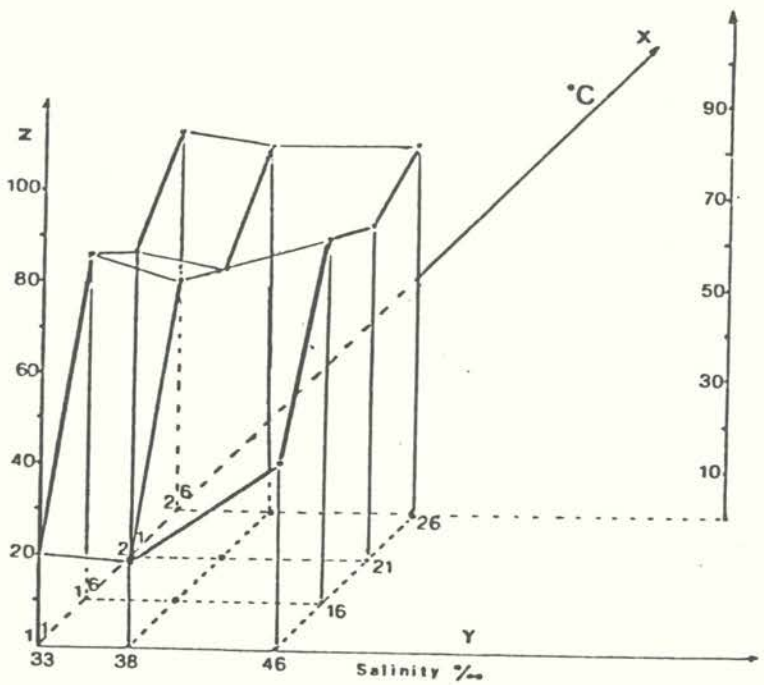

Figure 6. Mortality of Tisbe exposed to cadmium at various temperature and salinity levels.

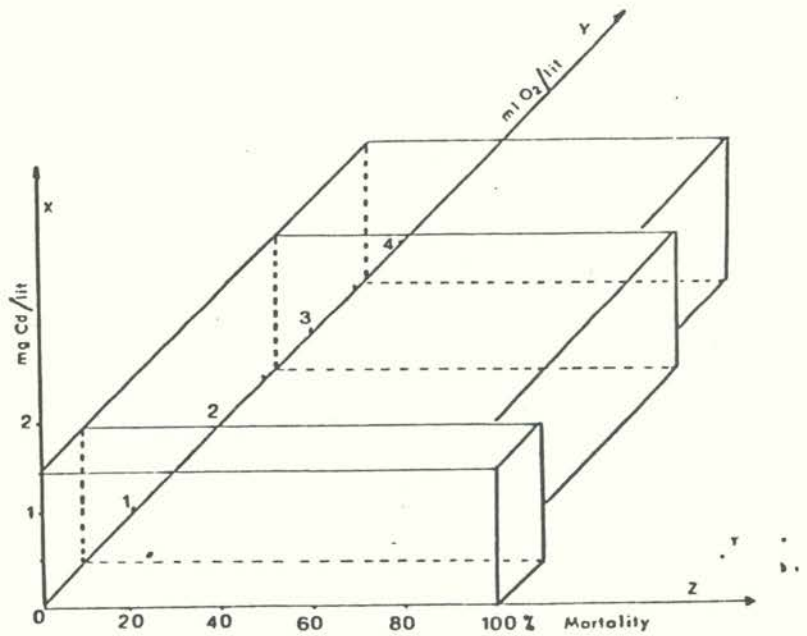

Figure 7. Mortality of Tisbe exposed to cadmium at different dissolved oxygen concentrations.

Table III.

Mean values of the principal studied parameters of population dynamics of Tisbe holothuriae after exposure to chromium.

Concentration of Cr mg/l	Survival of F2 (days)	Number of egg sacs per female	Interval between successive sacs (days)	percentage of abortion of egg	No of F3 offsprings per female sacs	Longevity of F3 (days)	Period of development (egg to adult) (days)
0	22.5 ±7.64	5.86±2.09	4.03±0.92	36.9 ±28.2	110.2±17.2	34.5 ±9.2	12
0.5	18.46±5.71	3.46±1.29	4.46±1.17	40.99±4.02	52.8±13.8	12.95±5.25	20
1	12.09±4.22	2.55±0.76	3.92±1.23	88.8 ±29.9	44.1± 1.1	4.76±2.06	-
2	9.9 ±0.63	1.41±0.63	3.44±.01	96.88±28.18	6.6± 1.2	2	-

Another index of sublethal stress of metals is the hatching rate of eggs. In series of experiments we tested the effects of four metals to the hatching rate of the eggs of Artemia. Artemia eggs have shown high sensitivity compared with adult animals, to metals: concentrations from 1/5 to 1/30 of the 48h LC50 caused a halving of the hatching rate (Tables IV to VII).

Results of regression analysis proved that the toxicity of cadmium was significantly affected by the dissolved oxygen concentration and the interaction between temperature and salinity. Oxygen concentration was negatively related to mortality (r^2=0.789). Mortality was also related to the interaction between temperature and salinity (r^2=0.622).

In another set of experiments we studied the impact of chromium on Tisbe. The parameters studied were the longevity of the F2(parent) generation (Fig.8) and of F3(offspring) (Fig.9); the number of egg sacs produced,the interval between the formation of the successive sacs,the percentage of egg sac abortion and the number of F3 progem (table III). All tested chromium concentration affect the longevity of Tisbe. No inhibition of egg sac formation was noticed. On the contrary the development of egg sacs was strongly influenced. The number of the F3 progeny was decreased with increasing chromium concentrations.

A study was made on the possibility of development of increased tolerance to copper fo Tisbe, due to acclimation under laboratory conditions. When Tisbe was acclimated to copper (exposure to very low doses) a tendency for higher tolerance, due to acclimation, was noticed. The copper induced delay in maturation time observed in the F2 generation became less pronounced from F3 onwards. The 48h LC50 also increased (higher tolerance) due to acclimation (Table IV, Figure 10).

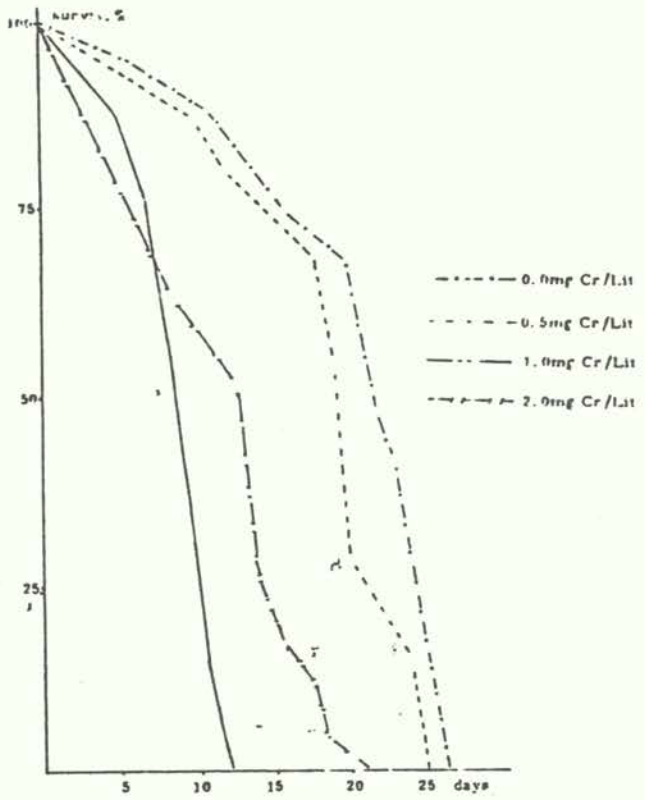

Figure 8. Survivorship curves of the F_2 generation after exposure at different chromium concentrations.

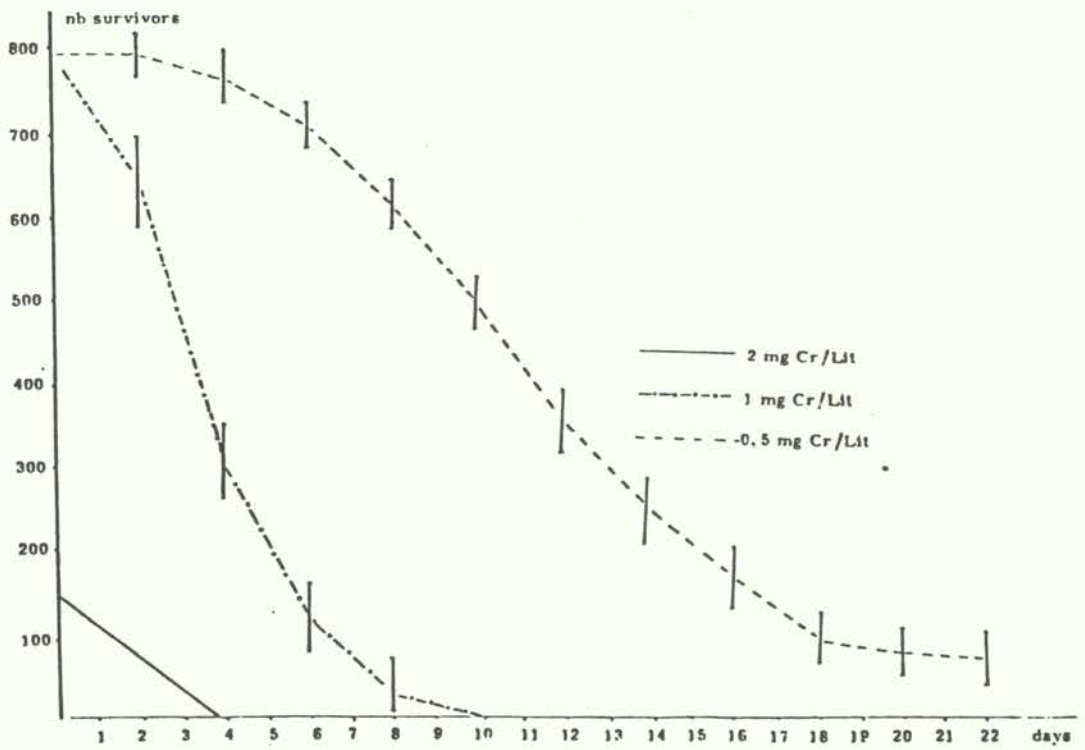

Figure 9. Survival of the F_3 generation after exposure to chromium.

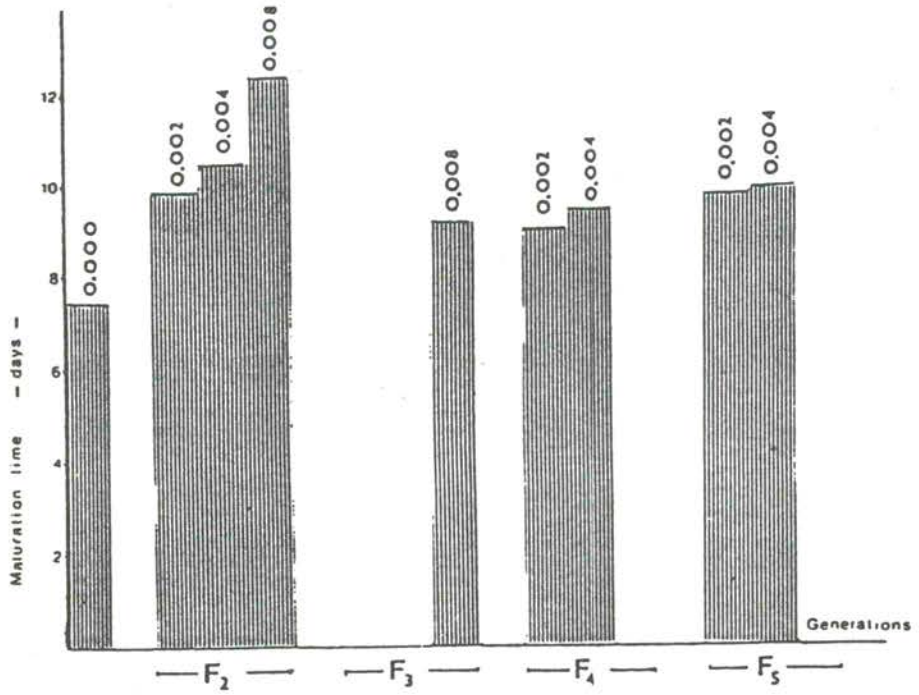

Figure 10. Maturation time of Tisbe exposed to copper. The prolongation of maturation time of F_2 is statistically significant at all concentrations. The animals exposed at 0.008 mg l^{-1} Cu presented at F_3 an important (P=95%)shortening of their maturation time in comparison with F_2. The maturation times of F_4 and F_5 slightly shorter than that of F_2 remain significantly (P=95%) higher than that of F_1.

Table IV.

Effect of cadmium on the hatching rate of eggs of Artemia salina

Concentration mg Cd l^{-1}	No of eggs	Mean hatching per cent	Hatching as fraction of the control
0	260	35.02±3.22	1.0
2	173	22.17±2.62	0.75
5	172	17.26±5.21	0.58
10	252	14.88±3.44	0.44
20	257	13.57±1.85	0.41
30	175	11.44±1.75	0.38
40	270	14.40±4.12	0.43
60	90	3.33±3.02	0.08
80	90	1.11±1.12	0.03
100	90	0	0

Table V.

Effect of chromium on the hatching rate of eggs of Artemia salina

Concentration mg Cd l^{-1}	No of eggs	Mean hatching per cent	Hatching as fraction of the control
0	300	24.26±4.22	1.00
0.5	90	34.44±5.09	1.09
1	90	30.00±3.33	0.95
2	90	31.11±5.08	0.98
3	90	28.89±5.06	0.91
4	90	33.33±3.30	1.05
6	180	27.77±6.23	1.035
8	90	20.00±3.36	0.90
10	90	18.89±1.92	0.85
15	180	22.22±6.94	1.22
20	90	18.89±6.12	0.85
30	180	18.33±4.32	0.89
40	90	55.56±5.05	0.82
60	90	5.56±1.93	0.29
80	90	7.78±1.92	0.41
100	90	6.67±3.38	0.35

Table VI.

Effect of nickel on the hatching rate of eggs of Artemia salina

Concentration mg Cd l^{-1}	No of eggs	Mean hatching per cent	Hatching as fraction of the control
0	270	21.48±5.09	1.00
2	180	16.11±2.86	0.70
5	90	13.33±3.85	0.63
7	90	11.11±3.84	0.53
10	270	16.30±4.22	0.36
15	90	6.67±3.33	0.31
20	180	11.11±2.65	0.56
30	90	4.44±1.97	0.23
40	90	2.23±1.83	0.12
50	90	2.22±1.94	0.12
70	90	0	0

Table VII.

Effect of cobalt on the hatching rate of eggs of Artemia salina

Concentration mg Co l^{-1}	No of eggs	Mean hatching percent	Hatching as fraction of the control
0	180	23.88±4.21	1.00
5	90	21.11±5.92	0.86
10	180	18.3 ±6.66	0.76
20	90	14.44+5.09	0.62
40	90	13.33+3.33	0.57
60	90	8.89±1.92	0.38
80	90	6.67±2.76	0.28
100	180	7.22±1.72	0.20
120	90	4.44±3.85	0.18

Table VIII.

LC50 48 h of untreated and copper acclimated Tisbe

Cu (mg l^{1})	LC50 48 h(in mg l^{1} Cu) F_3 generation	F_5 generation
control	0.428±0.0029	0.4281±0.0029
control+0.002		0.4187±0.0778
control+0.004		0.4451±0.0257
control+0.008		0.5177±0.0987

. The respiratory rate of copper-acclimated Tisbe was also less affected when the animals were exposed to copper. (Fig.11)

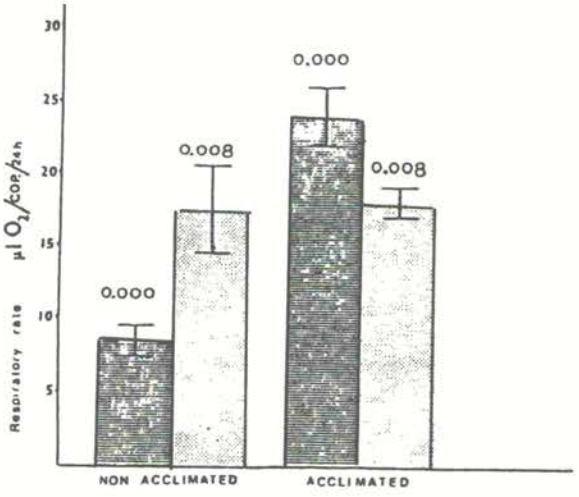

Figure 11. Respiratory rate (in µl O_2 cop. 24h) and its standard deviation of acclimated and non-acclimated Tisbe exposed to copper. The increase of respiratory rate of non-acclimated animals is statistically significant (P=99.99%). The decrease of respiratory rate of acclimated animals is not significant.

Finally we have studied the combined effects of three metals (Cu,Cd.Cr) when acting simultaneously to marine organisms. This is very important because in the field animals are exposed, usually, to more than one toxic substances. In mixtures of the two metals an obvious synergism of the effects was observed in all cases. In all three combinations with the two metals (Cu+Cd, Cu+Cr, and Cd+Cr) the mortality was higher than that expected in a purely additive basis. The mixture of the three metals presented a higher toxicity than that of the individual metals acting separately, but lower than that of all two metals mixture (Fig. 12).

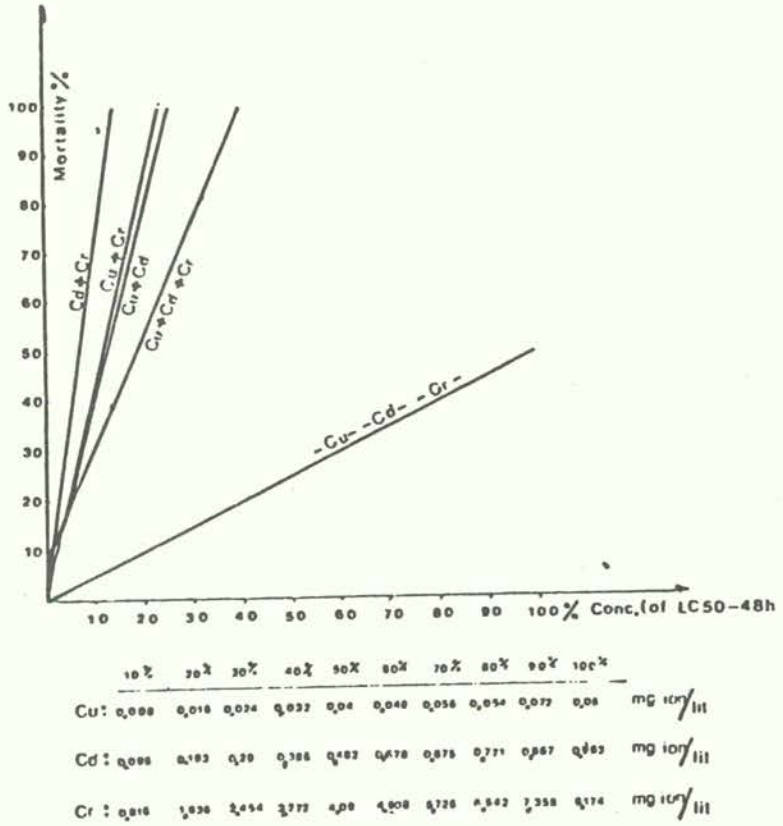

Figure 12. Combined effects of Cu, Cd and Cr to the survival of Tisbe holothuriae.

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EFFECTS OF CADMIUM ON SPERMATOZOA AND FERTILIZED EGGS OF SEA URCHINS*

by

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ABSTRACT

The effects of $CdCl_2$ on sperm motility, fertilization, membrane formation and some other aspects of the early developmental stages of the sea urchins Paracentrotus lividus and Sphaerechinus granularis Lam. were examined.

The swimming speed of spermatozoa incubated for 10 min. in concentrations up to 10 ug Cd l^{-1} of sea water was depressed in comparison to the control group. A slight delay in the formation of the fertilization membrane was induced by short-term exposures of sperms to cadmium prior to fertilization.

Fertilized and non-fertilized eggs had accumulated relatively low amounts of cadmium 15 hours after fertilization. A significant increase in cadmium in the fertilized eggs was evident 15 to 40 hours after fertilization.

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SEA URCHIN GAMETES AND THEIR DEVELOPING EMBRYOS IN MARINE TOXICOLOGY STUDIES

by

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1. INTRODUCTION

If something in the environment is going wrong, today it is very convenient to try to explain these adverse situations with reference to the action of polluting substances. In most cases it is very simple to speculate, but more difficult to identify and explain the primary effects and their eventual environmental consequences. Further work is required both in the laboratory and through field experiments, using advanced and reliable methods, including toxicity tests and bioassay procedures to explain the biological mechanisms involved; and to estimate, by extrapolation, the harmful action of pollutants. Because of the complexity and integrity of the marine environment, ecotoxicological testing should be carried out using representative organisms from various levels of biological and ecological organization, but which at the same time are easy to handle and which have well known and possibly standardized characteristics.

During the last hundred years, as reported by Hyman (1955), Harvey (1956) and Czihak (1971, 1975), sea urchin gametes and their early embryonic development have been amply studied as classic models for experimental embryology as well as in cytology, molecular biology, and physiological and biochemical investigations. In recent decades, the use of sea urchin eggs in ecotoxicological studies has attracted widespread interest. Although problems relating to marine pollution and water quality bioassays are of relatively recent interest, there have been many attempts to introduce these versatile biological objects for marine toxicological testing purposes. Before Kobayashi (1971, 1974, 1977, 1980) proposed echinoid eggs as a convenient bioassay test organism, the earlier works of Wilson (1951) and Wilson and Armstrong (1952, 1954, 1958, 1961) gave the first evidence about the possibility of using sea urchin eggs to test sea water quality. In the meantime the works of Hägström and Lönning (1973), Lönning and Hägström (1975, 1975a, 1976, 1978) and Falk-Petersen (1979), all based on their own very extensive experience in echinoid embryology, have amply illustrated the response of developing sea urchin eggs to various drugs, crude oil fractions and dispersants, with particular attention to impaired morphogenesis and cellular ultrastructure alteration. The depressive action on fertilization induced by various organic and inorganic compounds and the resulting alterations and mortality have been widely studied by many other authors (Allen, 1971; Renzoni, 1974; Nicol et al., 1977; Bougis et al., 1979; Oshida and Goochey, 1980; Bougis, 1981; Castagna et al., 1981; Davis et al., 1981; Kinae et al., 1981; Oshida et al., 1981; Pagano et al., 1982, 1983; Vlasova and Khristoforova, 1982; Wells, 1982; Adams, 1983; Hose and Puffer, 1983; Hose et al., 1983). In general, it was realised that the developing eggs of sea urchins fulfil most of the requirements of bioassay practice. Of particular interest is their high sensitivity: 5-10 times higher than some fish species (Oshida et al., 1981). Some other authors (Dinnel et al., 1981, 1982; Dunham et al., 1982; Greenwood, 1983) elaborated the sperm toxicity test that, in relation to the developing embryos test is more sensitive, quicker and less subjective in the evaluation of the results. All the papers mentioned above mostly considered the morphological alterations occurring during fertilization, cleavage and embryonic differentiation, while the physiological and biochemical consequences were only marginally discussed. Recently, in ecotoxicological studies in general, a selection of relatively simple and convenient biochemical tests is being promoted (Bayne, 1976; Livingstone, 1982). Related to this approach the results of Bay et al. (1983) clearly demonstrate the opportunity to measure the rate of echinochrome synthesis as a corresponding marker of the developmental rate of the sea urchin embryos.

In the investigations described in this paper, the influence of PCP (pentachlorophenol) on developing sea urchin eggs was tested by measuring the change in activity of some basic metabolic enzymes (G6P-DH, GOT, GPT)+ that are normally activated after fertilization or during other specific events of the early embryonal development. At the same time echinochrome production was also measured, and all were compared to the morphological changes recorded by visual estimations. PCP was applied as a model toxicant. It is a frequently-used, broad-spectrum biocide which often generates pollution problems in the aquatic environment. PCP acts as a characteristic uncoupling agent of oxydative phosphorylation (Bevenue and Beckman, 1978) and in several cases it has been shown to affect a variety of other metabolic enzymes (Boström and Johanson, 1972; Holmberg et al., 1972; Desaiah, 1978; Rao et al., 1979).

2. MATERIALS AND METHODS

The sea urchins, Paracentrotus lividus Lam., were collected along the shallow rocky coast near Rovinj (Northern Adriatic). The gonads were separated and the eggs were transferred to sea water while the male gonads were preserved dry. The maturity of the eggs from every female was tested separately. Samples with more than 5% oocytes, and samples in which more than 5% eggs lacked the post-insemination fertilization membrane were excluded. After testing, the mature eggs from several females were pooled and rinsed twice with filtered sea water. Selected aliquots were then distributed into glass beakers to obtain a final density of about 2500-3000 suspended eggs ml^{-1}. The eggs were slowly shaken with an oscillatory stirrer, aerated and maintained at $20\pm0.5^{o}C$. Various quantities of pentachlorophenol (PCP) dissolved in acetone (2 mg PCP ml^{-1} acetone) were added to the beakers to obtain final concentrations of 0.10, 0.20, 0.40, 0.60 and 0.80 mg PCP l^{-1}. Fertilization was initiated 30 min later with a few drops of freshly diluted sperm. In the control beakers aliquots of 0.8 ml acetone l^{-1} were added but, previously, no adverse effects have been observed. The percentage of the eggs with raised fertilization membranes was checked shortly after. Inspections, counting and photorecords of the developmental phases were all made with a conventional light microscope using live or 4% buffered formol fixed samples. At selected time intervals duplicate samples of 35 ml developing egg suspension were taken. The eggs, centrifuged from sea water, were homogenized in 0.5 ml of appropriate buffer: 0.66 mM EDIA in physiological saline (1.3% NaCl) for G6P-DH or in 0.2 M Na-phosphate buffer (pH 7.4) for GOT and GPT testing. After centrifugation (12.000 rpm), the enzyme acitivity was measured in the supernatant phase adopting standard spectrophotometric methods in relation to NADPH formation for G6P-DH (Kornberg and Horecker, 1955), or in relation to the NADH oxidation rate for GOT and GPT respectively (Bergmeyer and Bernt, 1974, 1974a). The assay conditions were previously optimized (pH, substrate concentration, incubation temperature) in relation to the developing sea urchin eggs extracts (Krajnovic-Ozretic and Ozretic, unpublished data). The enzyme activity (U g^{-1}) was calculated on the basis of records obtained with a 300 N Gilford spectrophotometer supplied with a thermoregulated cuvette. The relative echinochrome concentration has been calculated from the absorbence at 475 nm after its extraction into acidified ethanol (25% HCl into 95% ethanol) as described by Bay et al., (1983).

3. RESULTS

3.1 Morphological measurements

The morphological evaluation of fertilization and the time schedule of the ensuing early embryonic development is presented in Table I, following the protocols elaborated by Kobayashi (1971, 1980). At appropriate time intervals, samples with more than 150 developing eggs were counted and the number of units that approached the expected

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+ glucose-6-phosphate dehydrogenase, G6P-DH/EC 1.1.1.49;

glutamate-oxaloacetate transaminase, GOT/EC 2.6.1.1.;

glutamage-pyruvate transaminase, GPT/EC 2.6.1.2.

developing phases (fertilization, first cleavage, swimming blastulae or early and long arm plutei) were expressed as percents and compared to the control groups. It was noticed that PCP, even at the highest concentration (0.80 mg l^{-1}), does not prevent the formation of the fertilization membrane. Later, approximately at the time of first cleavage, it was ascertained that besides an increasing number of non-cleaving eggs a greater number of multipolar divisions occurred in the groups where the PCP concentration was higher (0.60 and 0.80 mg PCP l^{-1}). However, the total number of cleaving eggs was reduced in proportion to the PCP concentration. The most obvious adverse effects occurred after hatching. The number and the size of the swimming blastulae, gastrulae and early plutei were reduced even at the lower (0.20 mg PCP) concentration, while at the higher PCP levels (0.60 and 0.80 mg) the embryonic development was delayed or arrested from the very early cleaving phases.

Table I.

Paracentrotus lividus Lam. Developmental rate of cleaving eggs and early embryonic stages, exposed 30 minutes before fertilization to various PCP (0.10-0.80 mg l^{-1}) concentrations. The results are expressed as % of the expected and observed developmental stages at the indicated time intervals.

Time	5'	60'		12 h	16 h	24 h	40 h	
PCP ml l^{-1}	Fert. membrane	First cleavage 2 cells	multi-polar	Swimming blastulae	Gastrulae	Early plutei	Long arm plutei	General remarks
Control	96	94	-	92	93	62	76	normal
0.10	96	94	-	94	93	80	78	normal
0.20	97	95	2	87	88	84	65	delay, reduced size
0.40	93	92	-	68	16	36	-+	delay, reduced size abnormalit
0.60	96	58	23	35	13	-	-++	arrested embryon development
0.80	95	19	30	-	-	-	-	arrested clevage

+. a certain number of early plutei; ++ swimming blastulae or even gastrulae were present and still alive.

3.2 Biochemical measurements

Because of their fundamental metabolic roles the acitivity of G6P-DH, GOT and GPT have been analyzed and, complementary to the enzymes, the relative concentration of echinochrome as an indicator of the sea urchin development rate was also measured. The measurements were carried out at time intervals corresponding to gastrulation (16 hrs), appearance of early plutei (24 hrs) and long arm plutei (40 hrs from fertilization) in the control groups. The summarized results from three experiments are illustrated in Fig. 1.

The G6P-DH activity (range 15-22 U g^{-1}) was usually low, and in general was slightly depressed by PCP but the results are not statistically significant ($r_{G6P-DH} = -0.386$; $p \gg 0.10$). However, after gastrulation, the activity of both GOT and GPT in the control groups increased (range 110-196 U g^{-1} for GOT and 40-76 U g^{-1} for GPT), while in the PCP treated groups it was progressively and significantly depressed ($r_{GOT} = -0.831$; $p < 0.01$, and $r_{GPT} = -0.890$; $p < 0.01$). The concentration of the synthesized echinochrome was also reduced, and a very high negative correlation was observed in relation to the PCP concentration ($r_{Ech} = -0.933$; $p < 0.01$).

On the basis of the calculated regression equations the effective concentrations (EC_{50}) have been estimated: 0.60 in relation to GOT, 0.42 for GPT, and 0.41 mg PCP l^{-1} for echinochrome respectively. The summarized mean correlation calculated from the GOT, GPT and echinochrome results in relation to PCP concentration is also very significant ($r = -0.877$; $p < 0.01$) and the resulting EC_{50} is about 0.42 mg PCP l^{-1}.

It is also pertinent to mention that the exposure of the eggs to the lowest concentration (0.10 mg PCP l^{-1}) resulted in a slight but evident stimulative effect. The echinochrome concentration in the long arm plutei was about 14% higher and, in the earlier development phases, gastrulae and early plutei, the activity of GOT and GPT was also increased, approximately 7% higher than the control groups.

4. DISCUSSION

The toxic effects of PCP on developing eggs of the sea urchin, Paracentrotus lividus, are very evident and measurable. They can be easily recognised by observing the morphological alterations occurring during the very dynamic and time specific transformations of the synchronously-cleaving eggs and embryos. They can also be identified measuring some specific physiological and biochemical parameters.

The directly observed morphological alterations: cleavage delays and desynchronisation, occurrence of abnormalities, or the definitive failure of the embryonic development, reflect and uncontestably prove the toxic effects of PCP. The previously mentioned results of Hägström and Lönning (1973); Lönning and Hägström (1975, 1975a, 1978) and Falk-Petersen (1979) clearly support this statement. In our case the morphological alterations are in good agreement with the biochemical measurements: the decreased activity of GOT and GPT and the lowered production of echinochrome. The developing phases are very easily identified by microscopical inspections but the accurate evaluation and the routine counting of the morphological alterations is a tedious, time-consuming and an unavoidably subjective practice. As an alternative the measurement of certain enzymes and echinochrome is recommended, with preference to the measurement of the echinochrome production because of the simplicity, sensitivity and objectivity of the method. Echinochrome production is associated with gastrulation and to the differentiation of primary mesenchyme cells (Asashima, 1971). It increases proportionally and linearly to the embryonic development rate and was therefore proposed as an indicator of the sea urchin embryonic development rate (Bay et al., 1983). The rate of echinochrome production is illustrated in Fig. 2, and compared to the photorecords (Fig. 3) it confirms the opportunity to use biochemical methods instead of morphological measurements. In fact, while the lowered echinochrome production as well as the decreased activity of GOT and GPT are easily measurable and significant, the morphological differences due to the increasing PCP concentrations are not always clearly evident, particularly between groups exposed to closely-related PCP concentrations. The microscopic examinations of live or fixed samples should be adopted for a routine but informative checking of the condition of the developing embryos during the experiments, or for appropriate and detailed studies about the effects of drugs, toxicants and other agents on morphogenesis. The decreased activity of GOT and GPT is due to the metabolic effects of PCP while, in the case of the reduced echinochrome, it is an indirect response to the injury produced by PCP to the cellular structures and the slowing of the developmental rate of the embryos. The observed biochemical changes resulted in a corresponding detrimental effect on cleavage (growth) and survival of the developing embryos, thus fulfilling one of the main criteria in sublethal stress indices (Bayne, 1976; Livingstone, 1982).

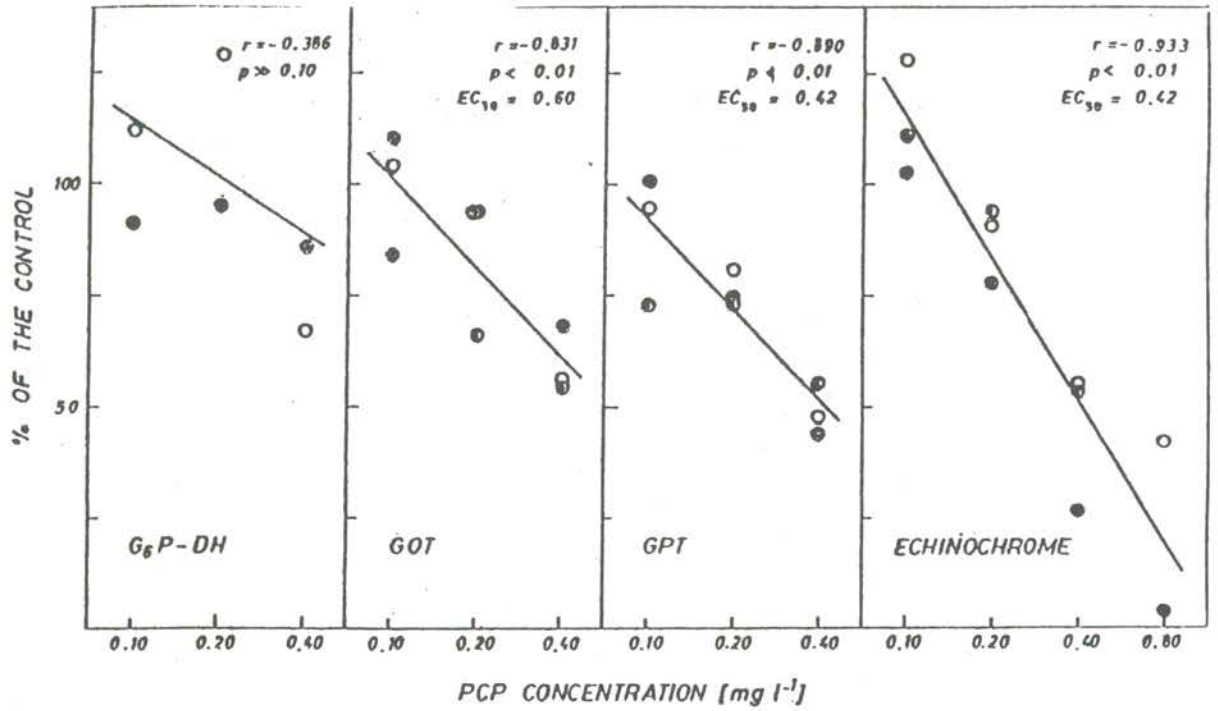

Figure 1. Paracentrotus lividus Lam. Enzyme activity and echinochrome concentration expressed as % of the controls, measured 16 (●), 24 (0) and 40 (O) hours from fertilization of the eggs exposed to various concentrations of PCP.

The metabolically inert sea urchin eggs rapidly activate after fertilization and, among others, the activity of G6P-DH is also highly increased, although it progressively decreases after hatching (Krahl, 1956; Backstrom, 1959). This indicates that during early cleavage the pentose shunt is engaged as an energetically more profitable enzyme pathway, while after hatching carbohydrate metabolism is regulated by the usual glycolytic pathway. The acitivity of G6P-DH after hatching was normally decreased both in control and in the PCP-treated eggs. That indicates that PCP does not induce the expected activation of the pentose phosphate shunt in developing sea urchin eggs as has been observed in fish (Boström and Johanson, 1972) or its significant inhibition as in crustaceans (Rao et al., 1979). In the control groups, the activity of GOT and GPT strongly increases during gastrulation (about 2-3 times in proportion to the early cleaving phases), and it does continue to increase during the pluteus phases. The increasing transamination activity may be related to the accelerated synthetic processes starting with the incipient embryonic tissue differentiation (Czihak, 1971). On the contrary, a substantial depression of GOT and GPT activity was noticed in the PCP-exposed eggs. Since those enzymes are involved in biosynthetic processes, it is speculated that their depressed activity resulted as an evident cleavage and differentiation delay or, with the high PCP concentrations, through a complete failure to develop.

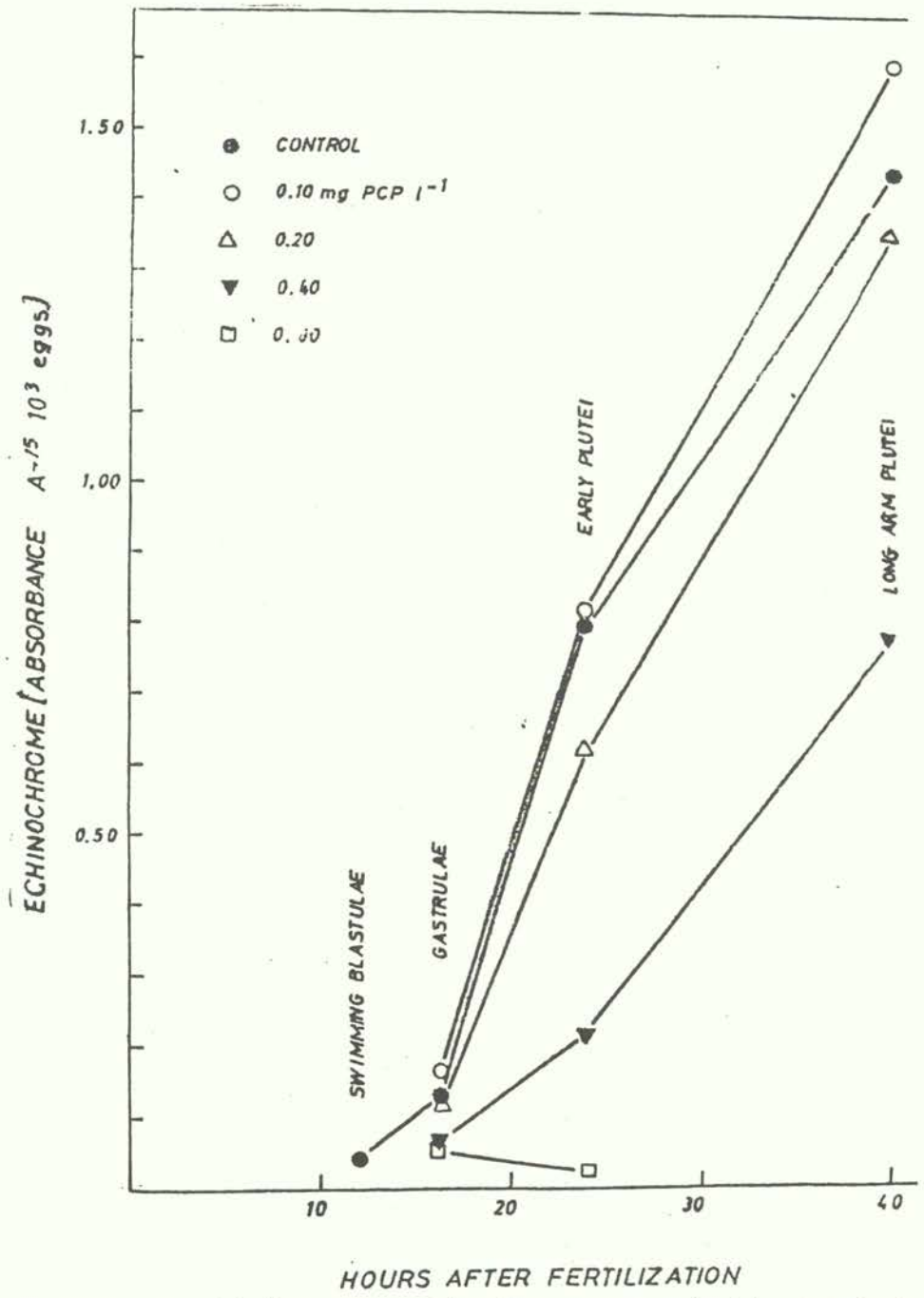

Figure 2. Paracentrotus lividus Lam. Echinochrome production in developing embryos in relation to various exposure of the eggs to PCP.

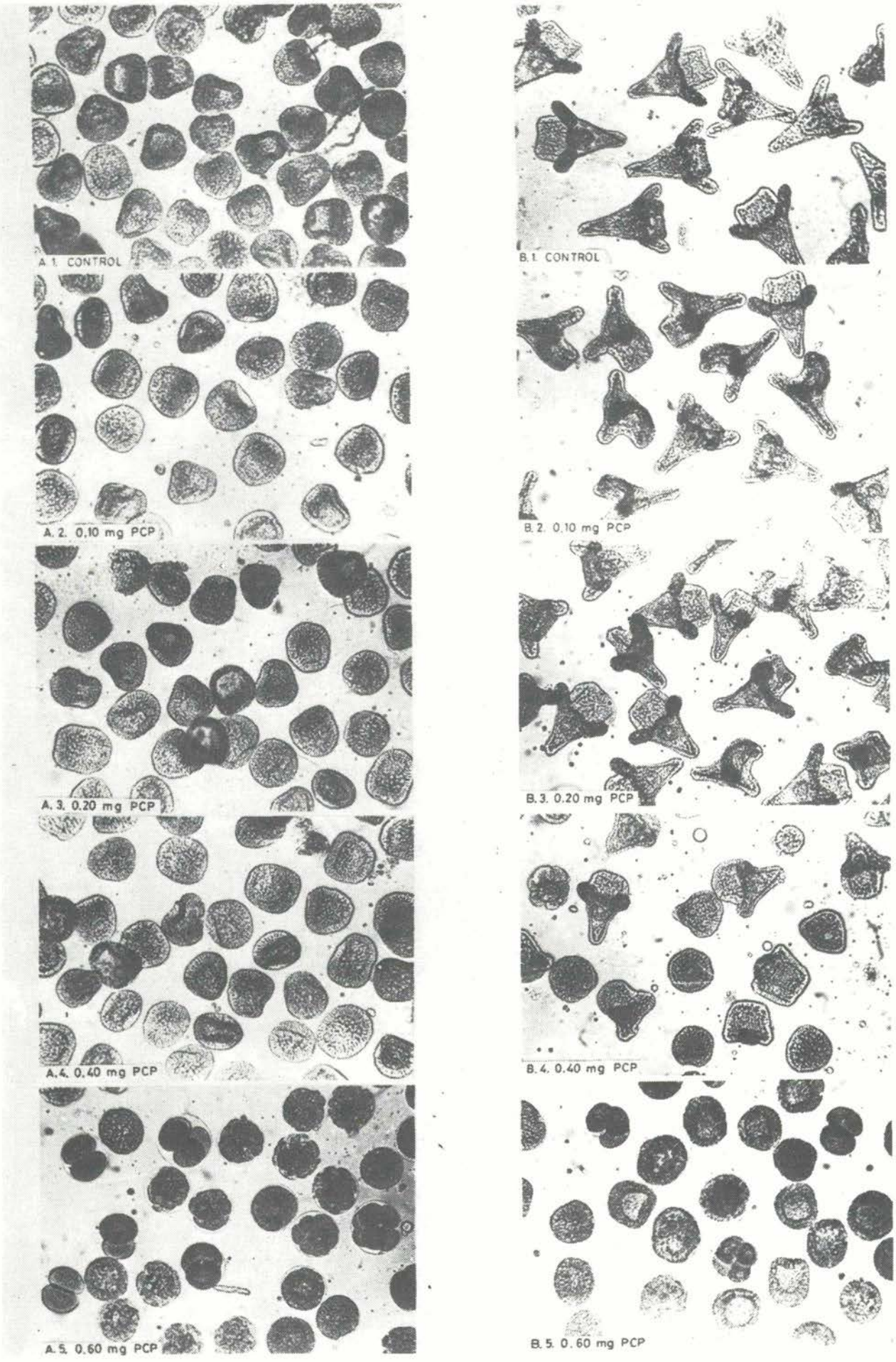

Figure 3. Paracentrotus lividus Lam. Developing embryos exposed to various concentrations of PCP. A. 1-5. gastrulation phase: 16 hours fertilization. B. 1-5. long arm

Some authors, when using sea urchin eggs and/or sperm to test the toxicity of various chemicals, accept the number of eggs with an elevated fertilization membrane as indicative of the degree of fertilization (Dinnel et al., 1981, 1982; Dunham et al., 1982; Greenwood, 1983). This criterion should not be unquestionably adopted. It is a well known fact that in some circumstances fertilized eggs missing the fertilization membrane do cleave and continue to develop to the embryo phases (Harvey, 1956), while in many cases eggs with a normal fertilization membrane never divide. In our case the formation of the fertilization membrane was not disturbed by PCP but, at the higher concentrations an increasing number of non-cleaving eggs was present.

Besides, the frequent appearance of multipolar cleavages indicates that probably the blocking, antipolyspermy mechanism was weakened and some structures of the egg membrane was damaged, but not the cortical granules responsible for the fertilization membrane formation. These are good reasons to suspect the use of the ferlilization membrane as a way to evaluate fertilization. In any case, the fertilization membrane is only one, but not the most essential, element in the articulated fertilization process.

In searching for the most acceptable evaluation of the toxic effects of pollutants in the environment (humans included) two main lines in particular are considered. One related to identifying plurivalent or universal test organisms to be used in standard bioassay procedures. The other is concerned with the highest objective evaluation of physiological and biochemical parameters that change significantly even at lowest/sublethal toxicant concentrations, and which have a clearly detrimental effect on growth, reproduction or survival of the organisms involved (Livingstone, 1982). Sea urchin gamates and developing embryos are likely to satisfy most of the essential criteria for bioassay experiments.

5. ACKNOWLEDGMENTS

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EFFECTS OF CADMIUM IONS IN THE PROCESS OF OOGENESIS OF THE COMMON PRAWN Palaemon serratus (PENNANT)

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1. INTRODUCTION

Many studies have been carried out to assess the origin of protein yolk in oogenesis, which could be extraoocytic, intraoocytic or a combination of both (Nørrevang, 1968; King et al., 1969). It has been described that in the eggs of a number of invertebrates, including those of molluscs (Beams and Sekhon, 1966), polychaetes (Anderson and Huebner, 1968) and echinoderms (Kessel, 1966) the production of yolk is intraoocytic. In contrast to this an extraoocytic yolk production has been reported in the eggs of insects (Anderson, 1974), fish (Shackley and King, 1977) and squids (Selman and Arnold, 1977).

Little work has been done concerning oogenesis in Crustacea. In most of these studies it has been reported that the RER and the Golgi complexes produce the protein yolk, though in later stages there is a small account of extraoocytic contribution. (Beams and Kessel, 1962; Kessel, 1968; Eurenius, 1973).

The productive cycle of Palaemon serratus (Pennant) was studied by Forster (1951) in Plymouth and Cole (1958) in North Wales. Recently Papathanassiou and King (1984) studied the reproduction at the ultrastructural level in P. serratus. The present study describes the effects of heavy metals (in particular cadmium) on this process of oogenesis in P. serratus. This is very important since it will give an indirect indication of how pollutants affect the organs at the ultrastructural level.

2. MATERIALS AND METHODS

Specimens of P. serratus were collected from rocky pools and kept in running sea water of 30±1°/oo salinity at ambient temperature for at least a week before use. After this period, groups of 20 active individuals were placed in crystallizing dishes containing 1 litre of 30°/oo artificial sea water at 15°C. Specimens were placed in three cadmium consentrations, 5, 25 and 50 mg l^{-1} and in clean 30°/oo sea water as control. The sea water was made up by dissolving "TROPICAL MARINE" salts, (Sherley Aquatics Ltd., Solihull, England) in glass-distilled water. After 44 h, which is the lethal time for 50% of the individuals placed in 50 mg l^{-1} cadmium at 15°C (Papathanassiou, 1984), a number of groups of 10 live individuals were removed from each concentration.

For the electron microscope, live treated specimens were placed in 5% glutaraldehyde in buffered sodium cacodylate with sucrose. The ovaries were disssected out and placed in fresh 5% glutaraldehyde solution at pH 7.4 for 2 h. They were then washed in several changes of buffered sodium cacodylate with sucrose added, followed by post fixation in 1% osmium tetroxide solution for 1h at 0-4°C. After dehydration in graded cold acetone the material was embedded in TAAB embedding resin. Sections with gold or silver interference colours were obtained using a Huxley Mark I Ultramicrotome and were mounted on coated copper grids. They were then double stained in 30% uranyl acetate (30 min) followed by lead citrate (10 min) and viewed in a Corinth AEI Electron Microscope.

3. OBSERVATIONS

The paired ovary is pear-shaped with short, wide oviducts. Within the ovary, dividing germ cells are located around the inner margins of the ovarian lobes. The developing oöcytes are surrounded by a layer of follicle cells (Figs. 1 & 2).

Previtellogenic oöcytes occur together with vitellogenic oöcytes in the ovary from March to August (Figs. 3, 4). Mature eggs are released in June and July, and by August the number of vitellogenic oöcytes present in the ovary decreases rapidly, though there is a marked increase in the number of previtellogenic oöcytes present (Fig. 4). From September to January the ovary contains only previtellogenic oöcytes which pass to an early vitellogenic phase in February (Fig. 4).

Oöcytes of specimens exposed to 5 mg l^{-1} and 25 mg l^{-1} cadmium

There were no observable effects on the oöcytes after exposure to cadmium solution of 5 ppm and 25 ppm for 44 hours.

Oöcytes of specimens exposed to 50 mg l^{-1} cadmium

During the process of oögenesis the only change observed in the developing oöcytes of specimens exposed to 50 mg l^{-1} cadmium was in the mitochondria. During vitellogenesis the cristae degenerate and sometimes contain dielectronic material without a limiting membrane (Figs. 5(a)-5(e)). The form of mitochondria varies, being either dumb-bell shaped, cup-shaped, spherical or oval (Fig. 5(d)). The cup-shaped mitochondria eventually enclose either small unaltered portions of cytoplasm or engulf mitochondria (Figs 5(a)-5(e)). During the later stages of vitellogenesis the remaining mitochondrial cristae are swollen, but there is no evidence of mitochondria engulfing cytoplasm (Fig. 5(f)). They are fewer and have a variety of shapes, though at this stage, cup-shaped mitochondria were present.

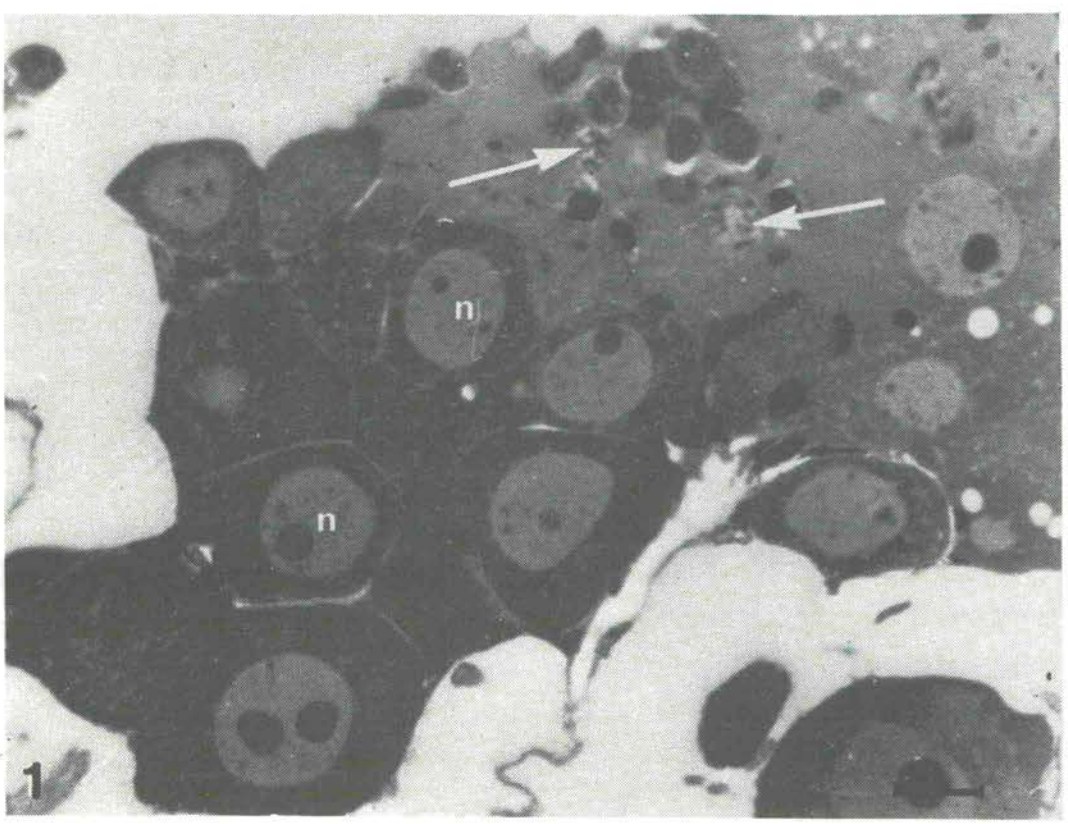

Figure 1. Photo micrograph showing previtellogenic oöcytes. Note the dividing germ cells (arrows). Nucleus (n). Scale bar 10 µm

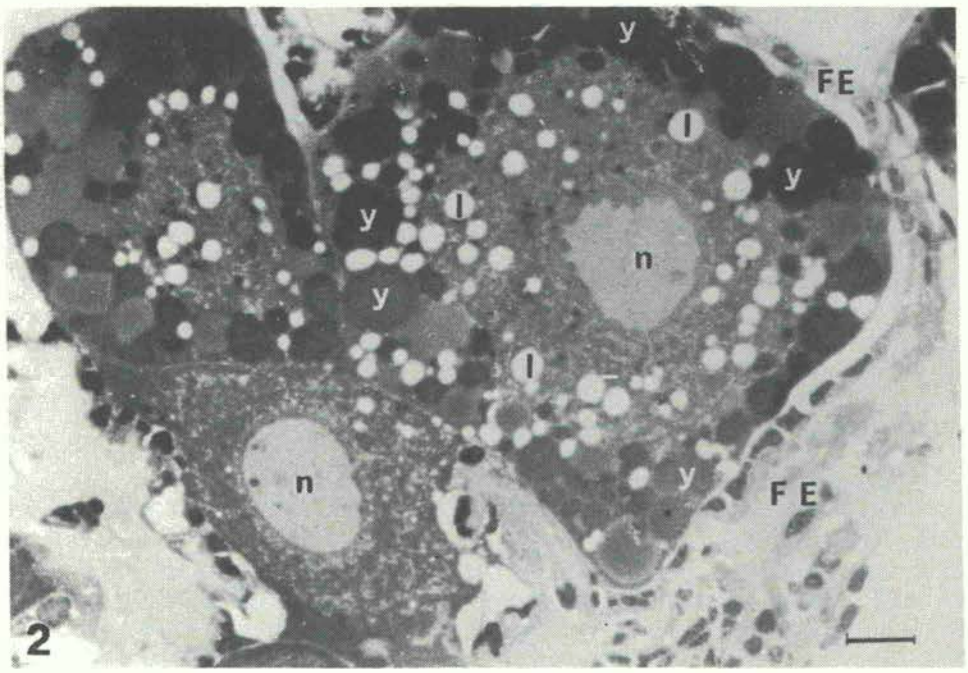

Figure 2. Photo micrograph showing vitellogenic oöcytes in the ovary. Note the presence of yolk bodies (y) and lipid droplets (l). Follicular epithelium (FE), nucleus (n). Scale bar 10 µm.

4. DISCUSSION

When individuals are exposed to 50ppm cadmium, the mitochondria of the oöcytes change shape and the cristae become swollen, especially during the late stages of vitellogenesis. All these features indicate that the mitochondria do not perform their normal function and therefore the energy level declines. Similar studies in the gill cells of crustaceans have shown great differences at the ultrastructural level in specimens treated with heavy metal ions, which were attributed to the effect that these ions have on enzymic and ATP-ase activity, absorption and transportation of salts, active ion uptake and protein synthesis (Bubel, 1976; Papathanassiou and King, 1983; Papathanassiou, 1985).

Cup-shaped mitochondria are observed during vitellogenesis in association with unaltered parts of the oöplasm which contain abundant free ribosomes. Eventually these mitochondria engulf part of the oöplasm. Ratcliffe and King (1969) suggested that similar transformation of mitochondria in the acid gland of starved Nasonia vitripennis (Walker) (Insecta) is an attempt by the mitochondria either to regain their energy level or help them to extract glycolysable substances from both the surrounding cytoplasm and from their own components.

Thus, results show that cadmium ions affect the mitochondria in the developing oöcytes of P. serratus, and therefore, since mitochondria may be involved in the metabolism of the oöcyte, this metabolism may be affected. Further studies, however, must be done to show if the mechanism of yolk product is affected by longer exposure to cadmium ions, in solutions which represent the environmental conditions.

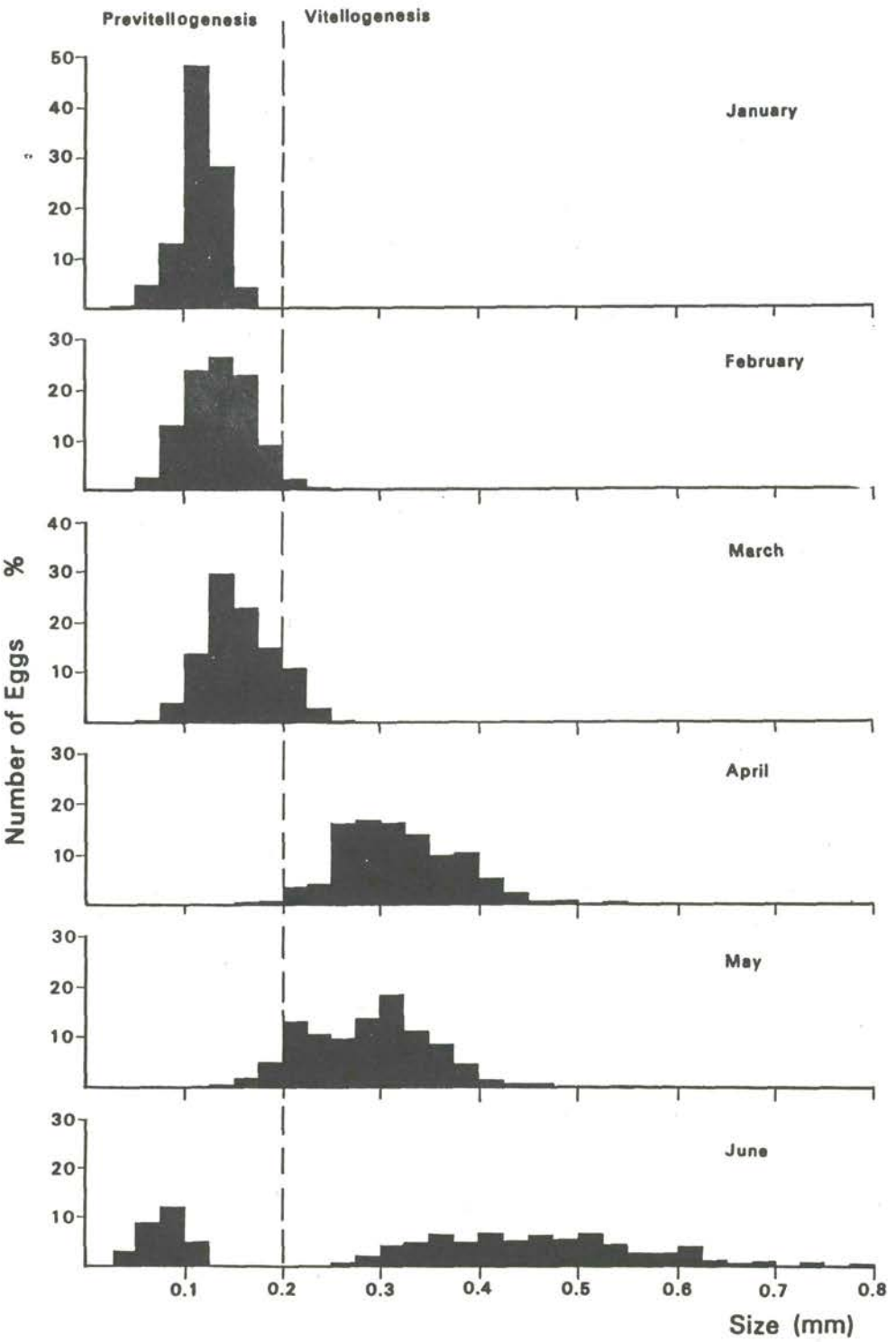

Figure 3. Histograms showing size and number of eggs present in the ovary of P. serratus from January to June.

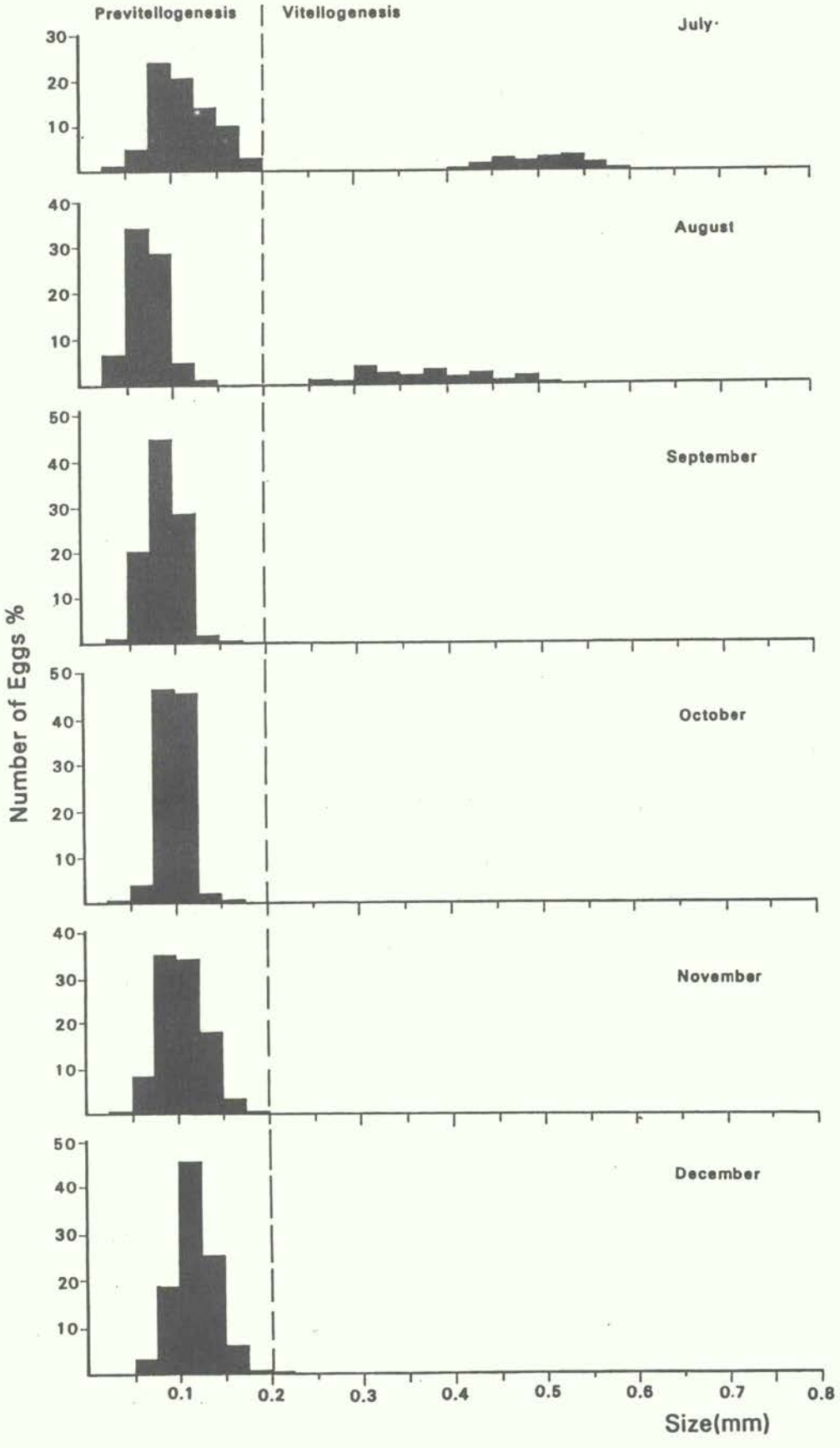

Figure 4. Histograms showing size and number of eggs in the ovary of P. serratus from July to December.

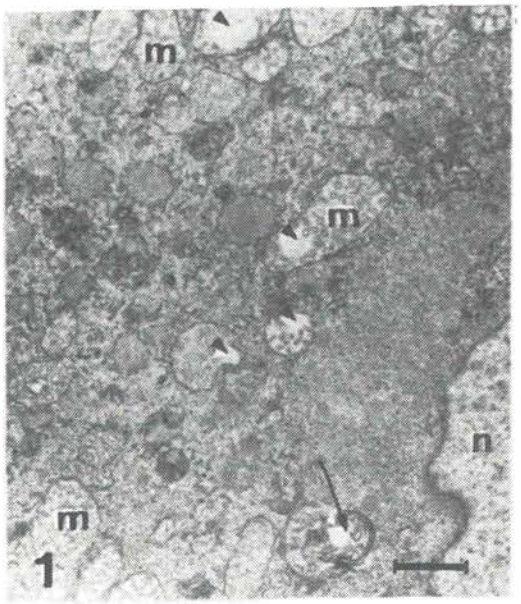

Figure 5(a) Electron micrograph showing a vitellogenic oöcyte after exposure to 50ppm cadmium. Note the mitochondria (m) contaning dielectronic material (arrowheads). Nucleus (n). Scale bar 1 µm.

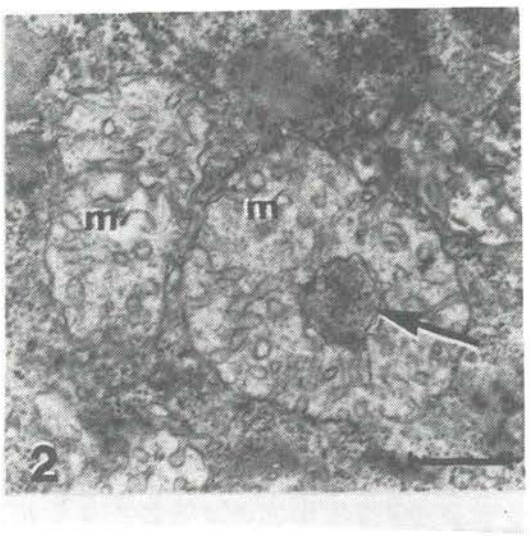

Figure 5(b) Electron micrograph showing mitochondrion (m) enclosing portions of cytoplasm (arrow) in vitellogenic oöcytes after exposure to 50ppm cadmium. Scale bar 0.5 µm.

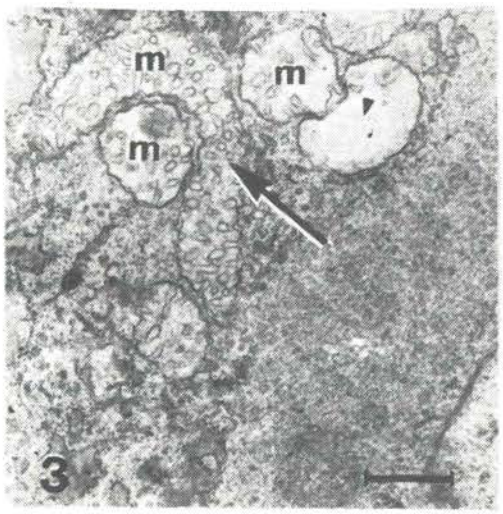

Figure 5(c) Electron micrograph showing a mitochondrion (m) engulfing one of its neighbours (arrow) in a vitellogenic oöcyte, after exposure to 50ppm cadmium. Scale bar 0.7 µm.

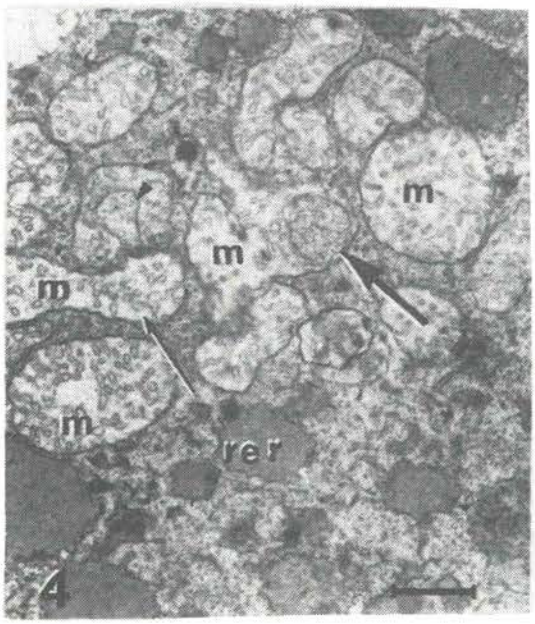

Figure 5(d) Electron micrograph showing the different shapes of mitochondria (m) during vitellogenesis after exposure to 50ppm cadmium. Note the dumb-bell shaped arrow, the cap-shaped (small arrowhead) and the spherical mitochondria. Note that one mitochondrion has portion of the cytoplasm (big arrow). Scale bar 0.8 µm.

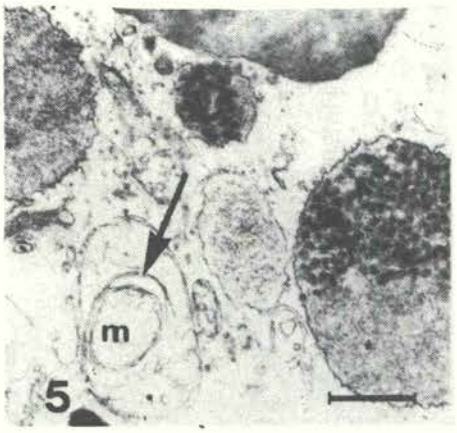

Figure 5(e) Electron micropgraph showing mitochondrion (m) with degenerate cristae (arrow) during late stages of vitellogenesis after exposure to 50ppm cadmium. Scale bar 0.5 µm.

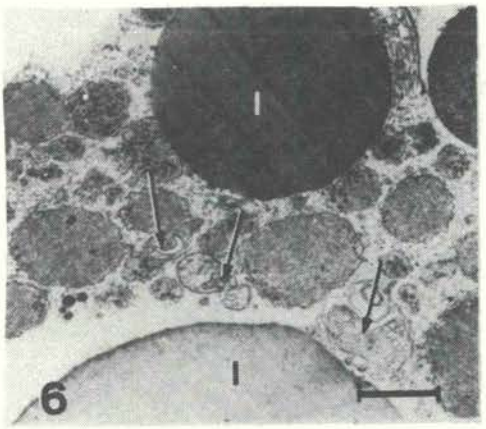

Figure 5(f) Electron micrograph showing mitochondria with swollen cristae (arrows) during late stages of vitellogenesis. Lipid droplets (l). Scale bar 1 µm.

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DETERMINATION OF Cd-BINDING PROTEINS SIMILAR TO METALLOTHIONEIN IN THE DIGESTIVE GLAND OF *Mytilus galloprovincialis* WITH REGARD TO A PRELIMINARY TREATMENT OF THE SAMPLE (+)

by

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EXTENDED ABSTRACT

The formation of inducible proteins similar to metallothioneins has recently been established in various genera of marine invertebrates, especially bivalve molluscs exposed to trace metals under laboratory (Noel-Lambot, 1975) and field conditions (Talbot and Magee, 1978). Several important studies have also been reported concerning the biochemical basis of accumulation and detoxification processes in marine organisms (Coombs and George, 1978).

Metal-binding proteins of marine mussels of the genus *Mytilus* (*M. edulis* and *M. galloprovincialis*) exhibit certain properties not characteristic of mammalian metallothionein and are therefore often referred to as metallothionein-like proteins (MLP) (Roesijadi, 1980). Also, the lack of agreement between certain researchers dealing with characterization of Cd-binding proteins of *M. edulis* may be due to procedural differences (Roesijadi and Hall, 1981).

The present study was mainly focused on methodological problems associated with the preparation of the sample (27000xg supernatant). The main aim was to compare the efficiency of different preliminary treatments of the sample prior to its application to the chromatographic column (Sephadex G-75), in order to obtain the best yield of MLP fraction. Our intention was to minimize the possible breakdown of MLP by application of the best treatment of either crude homogenate or supernatant, and by addition of strong protease inhibitor (PMSF). The addition of 2-mercaptoethanol was designed to prevent a possible aggregation of the native protein under non-reducing conditions. We wanted also to show if the use of ultracentrifugation (140000xg) might contribute significantly to the optimization of the method.

Two separate experiments were conducted on adult mussels (*M. galloprovincialis*) under different experimental conditions, Cd concentrations and duration of exposure (acute exposure, 1,3 μg Cd ml^{-1} for 7 days; chronic exposure, 0,1 μg Cd ml^{-1} for 4 months). The column gel-filtration chromatography, (Sephadex G-75) was performed according to standard techniques (Pharmacia Fine Chemicals). Cadmium content in tissue homogenate, supernatant and in chromatographic fractions was determined using a Varian atomic absorption spectrophotometer (AA-5) by the flame technique. The UV-absorbances of each fraction was determined at 250 and 280 nm by means of a Beckman spectrophotometer (DB-GT).

The inducible proteins isolated and partially characterized from the digestive gland of *M. galloprovincialis* were considered as MLP because of their unique characteristics (strong inducibility and affinity for Cd, thermostability at 70°C for 10 minutes, A 250 : A 280 1 absorbances ratio). The results obtained indicated that each of the pretreatments contributed to the isolation of the higher proportion of Cd bound to MLP in comparison with untreated sample. The application of PMSF (5 mg l^{-1}) resulted only in moderately improved efficiency (2x) in comparison with the untreated sample.

(+) This paper will be published "in extenso" in *Periodicum Biologiorum*.

The sample prepared using heat treatment of the crude homogenate (70°C for 10 minutes) contributed to the highest yield of inducible proteins, containing 3,7 times the amount of Cd associated with MLP (2,7x more than untreated) although it was not as high as the sample treated exclusively by the heating method. Evidently, this method was less efficient than the heat treatment of crude homogenate, possibly because of procedural differences due to the step at which heat-treatment was applied (crude homogenate or supernatant).

The application of 2-mercaptoethanol in order to reduce inter- and intramolecular oxidation, which might result in an apparent reduction of the molecular weight of MLP, was moderately effective because it prevented a part of polymerization. A certain shift in the elution profile was evident corresponding to a change of molecular weight from 28000 Daltons toward 20000 Daltons, although the expected decrease toward 10000-12000 Daltons was not observed. The ultracentrifugation (140000 x g) did not seriously influence the distribution of Cd in the elution profile, because only 5% more Cd was bound to MLP, indicating that the postlysosomal fraction (27000xg) might be satisfactory for routine determination of MLP particularly for biochemical monitoring purposes directed to water quality control.

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STUDY ON THE HEALTH CONDITION OF Tilapia zillii Gerv. LIVING IN THE POLLUTED WATER OF MERGHIM ZONE LAKE MARIUT, ALEXANDRIA, (EGYPT)

by

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1. INTRODUCTION

A number of laboratory experiments with different toxic compounds, abundantly occurring in the aquatic ecosystem, have been performed with fish (for review, see Sprague, 1971). They were shown to induce pathological changes e.g. in pin fish (Lagodon rhomboides) and spot (Leiotomus xanthurus) the PCB Aroclor 1254 caused extreme lipoid vacuolation of the parenchymal cells of the liver, accompanied by sinusoidal congestion, focal necrosis, and inclusion of pigment (Hansen et al., 1971). The coagulative type of rainbow trout liver degeneration could be induced experimentally by exposure to lindane (Cauch, 1975).

This study is a field investigation to study the effect of water pollution in Lake Mariut on the health of Tilapia zillii, which is a popular fish in Egypt and is abundant among the fish caught from the polluted water localities of the lake (Saleh et al., 1983).

2. MATERIALS AND METHODS

Samples of water and Tilapia zillii were collected from some localities of Lake Mariut during the winter season. The fish length and weight were recorded. The livers were removed, weighed, and a small piece preserved in phosphate-buffered 4% formalin to be studied under the electron microscope.

The physicochemical characteristics of the water were measured using the standard methods (APHA/AWWA/WPCF, 1980).

The pollutant concentrations, e.g. heavy metals in the water and in organs of the fish (gills, liver, and flesh) were measured using atomic absorption spectrophotometry.

The condition of the fish flesh (K_f) was estimated using the formula of Graham (1924) where

$$K_f = \frac{\text{gutted weight x100}}{(\text{standard length})^3}$$

The condition of the liver or hepatosomatic index H.S.I. was measured using the equation of Jangaard et al (1967) where

$$\text{H.S.I.} = \frac{\text{weight of the liver x 100}}{\text{gutted weight}}$$

3. RESULTS AND DISCUSSION

Analysis of the water in Merghim zone showed its high content of pollutants e.g. heavy metals (Table I). Moreover, petroleum hydrocarbons were clearly observed covering the coast of Merghim zone due to its connection with some drains carrying the liquid wastes of some factories e.g. the El-Nasr refinery petroleum factory.

Table I.

Physicochemical characteristics of water from two localities of lake Mariut (Merghim zone and El-Berdisi Fish farm) during winter.

Parameters (mg l^{-1}) unless otherwise stated	Merghim zone (Polluted water)	El-Berdisi fish farm
Temperature	15°C	15°C
pH	7.5	8.2
Cl^-	2750	1900
SO_4^{--}	500	270
Alkalinity	300	420
Total hardness	1400	420
Calcium hardness	440	200
Magnesium hardness	960	220
PO_4^{3-}	1.5	5
NO_3^-	0.05	0.05
Total Solids	7093	4599
Suspended solids	1093	425
Volatile solids	1554	1295
Biological oxygen demand	224	280
Chemical oxygen demand	300	320
Dissolved oxygen	5.3	4.4
Fe	14.56	8.16
Hg	0.047	ND
Cd	0.095	0.007
Sn	ND	ND
Cr	0.840	0.159
Pb	0.660	0.02
Cu	1.30	0.1099
Ni	0.51	0.008
Mn	0.025	0.019
Zn	0.351	0.133

The condition of Tilapia zillii living in such polluted water is lower than that living in the comparatively clean water of El-Berdisi fish farm at Lake Mariut (Table II). There is a considerable decrease in the robustness of Tilapia zillii living in Merghim zone-Lake Mariut, which is probably due to water pollution (Sindermann, 1979).

Table II.

Condition of the fish flesh (K_f) for Tilapia zillii living in two localities of lake Mariut (Merghim zone and El-Berdisi fish farm) during winter.

Locality	Condition of the fish flesh
Merghim zone (polluted water)	2.54 ± 0.45
El-Berdisi fish farm (comparatively clean water)	2.94 ± 0.33

Investigation of the liver of Tilapia zillii living in the Merghim zone showed its enlargement (Table III), which is attributed mainly to its high content of pollutants (Table IV). Saleh (1983) explained the high content of pollutants in the liver of Tilapia zillii as due to its fatness. Also the fish liver cleans the blood coming from the intestine, carrying the absorbed matter, from any poisons. This probably means that the high accumulation of pollutants in the liver of Tilapia zillii living in the polluted water of Merghim zone causes a considerable increase in the size of the liver or hepatosomatic index. Moreover, its colour is yellowish white instead of the reddish brown colour of the normal one, i.e. the enlargement of the liver of the polluted fish and its yellowish coloration is probably an indicator of severe liver disease, (Köhler and Halzel, 1980).

Table III.

The liver condition or hepatosomatic index (H.S.I.) for Tilapia zillii living in two localities of lake Mariut (Mergim zone and El-Berdisi fish farm) during winter.

Locality	H.S.I.
Meghim zone	1.47 ± 0.49
El-Berdisi fish farm	0.62 ± 0.24

Table IV.

Heavy metals content (mg kg^{-1}) fresh weight in liver, gills and flesh of Tilapia zillii living in two localities of Lake Mariut. (Merghim zone and El-Berdisi fish farm) during winter.

Element	Tilapia zillii living in Merghim			Tilapia zillii living in Berdisi fish farm		
	liver	gills	flesh	liver	gills	flesh
Fe	1307	487.5	425	281.5	70	108.7
Cd	1.1	0.35	0.92	1.32	0.73	0.91
Cr	3.2	0.78	3.34	0.72	0.34	0.22
Pb	7.2	0.93	1.82	1.55	0.76	1.8
Cu	787	148.7	111.2	420	137.8	121.7
Ni	12.2	5.1	1.18	3.12	8.2	7.3
Zn	240	143.2	190.3	140.5	16.02	13.4
Hg	7.6	0.117	0.112	1.3	0.21	0.99
Sn	0.15	0.112	0.119	ND	ND	ND

The histological studies of the liver structure of Tilapia zillii living in the Merghim zone showed an alteration in its structure, which could be noticed in the generalised swelling of its cells. The blood vessels are extremely congested and the lobular liver structure has largely disappeared. Most of the liver parenchyma are isolated and shrunk (Figs. 1 and 2). It was noticed also that the liver of Tilapia zillii living in the Merghim zone is extremely enriched with intracytoplasmic vacuoles (Fig. 1). However, the cellular structure is largely preserved, the nuclei and the cell boundaries are unaffected, but the cytoplasm is displaced with distinct lining. Some of the vacuoles are even larger than the nuclei (Figs. 3, 4 and 5) Köhler and Halzel (1980) demonstrated by staining such vacuoles with Sudan black that all the vacuoles are composed of lipoid materials, i.e.lipoid vacuolation of the liver cells occurs. Hawkes (1978) showed that the exposure to crude oil led to lipoid vaculation of moderate to heavy extent in sole (Solea solea)liver. It was explained also that the pathological effects were modulated by the presence of lead and cadmium occurring in petroleum (Varanasi, 1978).

Ultrastructural pathological changes were detected in some hepatocytes. The main changes included shrinkage of hepatocytes, affected cells having irregular external outlines and widening of the intercellular space (Fig. 6.). This was associated with nuclear changes in the form of chromatin clumping and margination along the inner membrane of the nuclear envelope (Fig. 7.). The mitochondria of affected cells displayed focal matrical densities whereas the endoplasmic reticulum presented vesiculation (Fig. 8.), and dilation giving the cells cribiform appearance (Fig. 9.). Increase in the number of lysosomes (Fig. 10.) was shown. Moreover, some hepatocytes acquired cytoplasmic vacuoles of various sizes. Small vacuoles appeared almost spherical while larger vacuoles had irregular contours and occupied large areas of the cytoplasm (Fig. 11.). The vacuoles were not membrane-bound and few of them contained flocculent material, suggesting that they were fat vacuoles. These changes denote early injury of the hepatocytes (Trump et al. 1963, 1965).

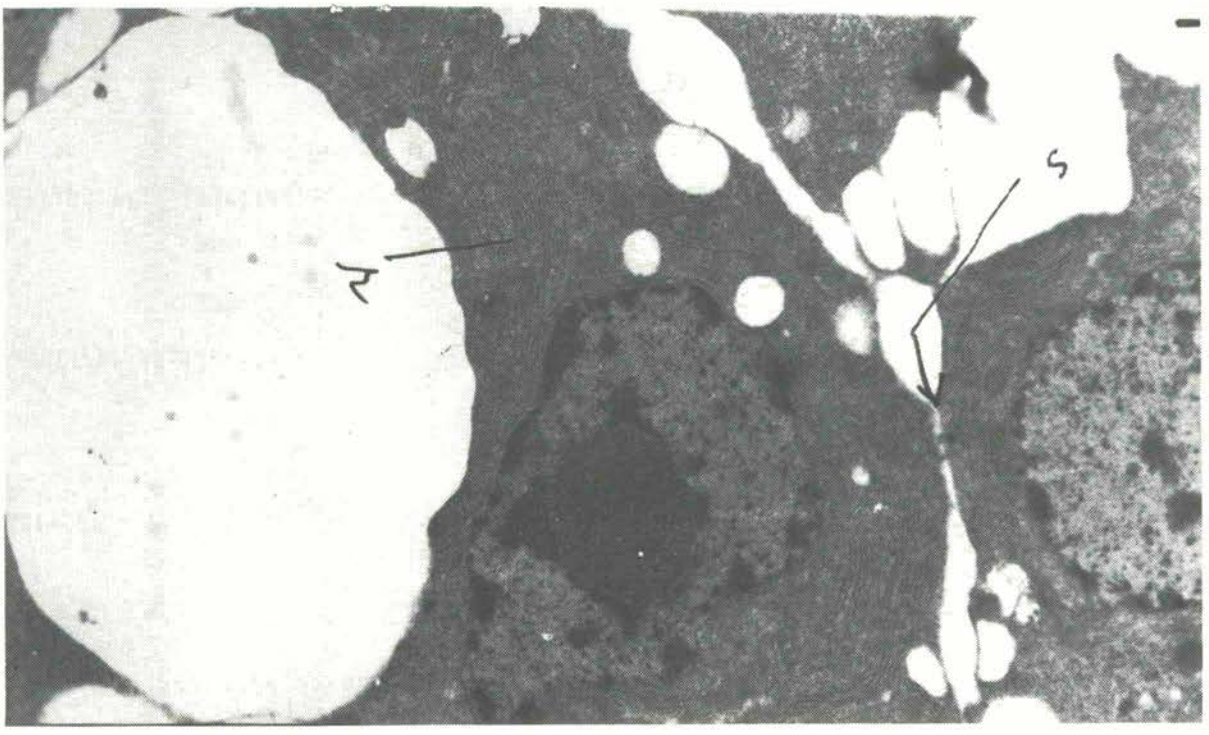

Figure 1. Liver cells rich in rough endoplasmic reticulum (r), and wide intercellular spaces (S).

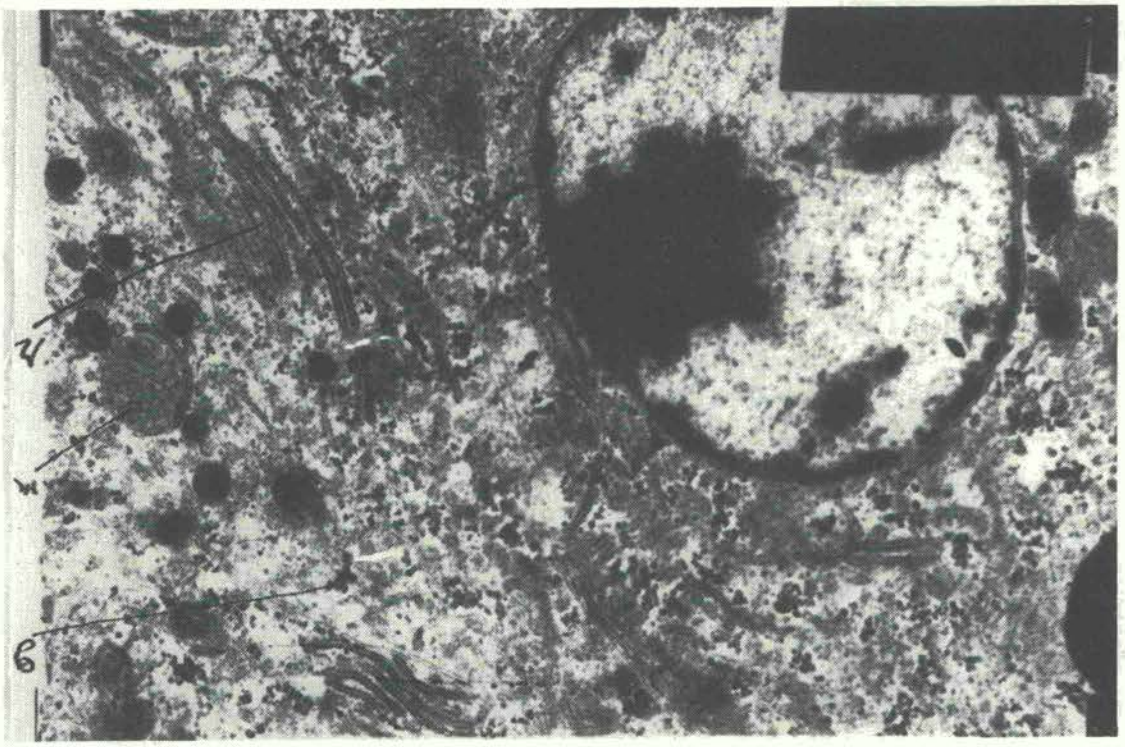

Figure 2. Liver cells with scattered granules (g), few mitochondria (m) and rough endoplasmic reticulum (r).

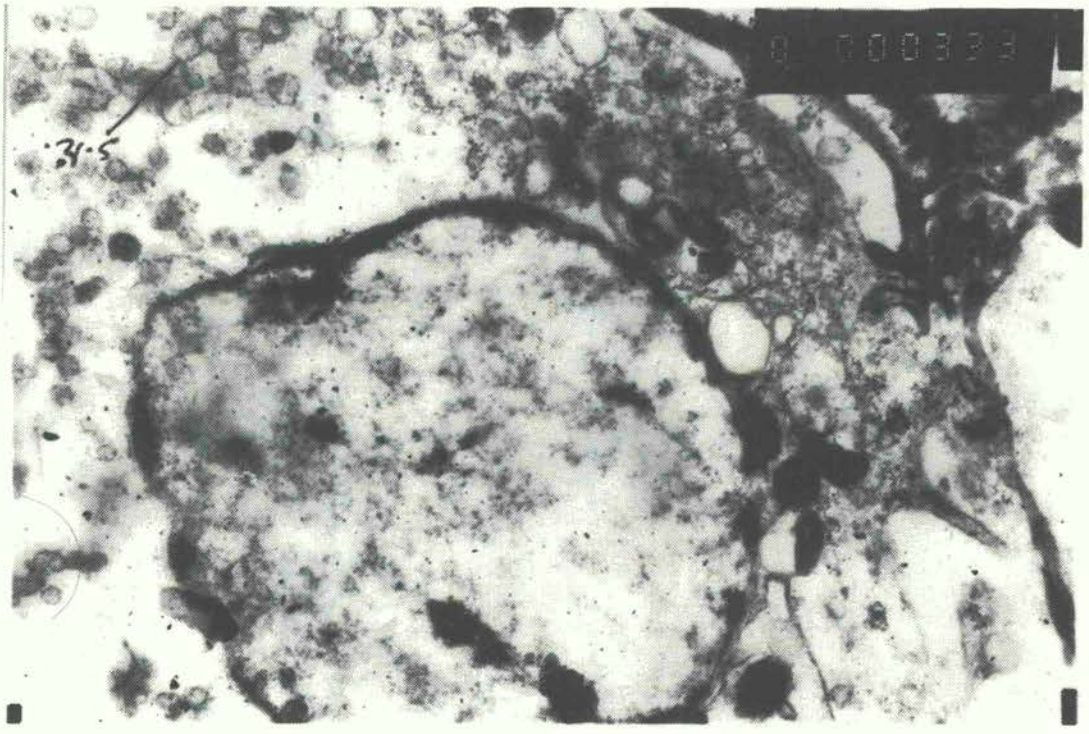

Figure 3. Liver cell with many scattered dense granules and cavities with electron-dense substance, probably lysosomes (I).

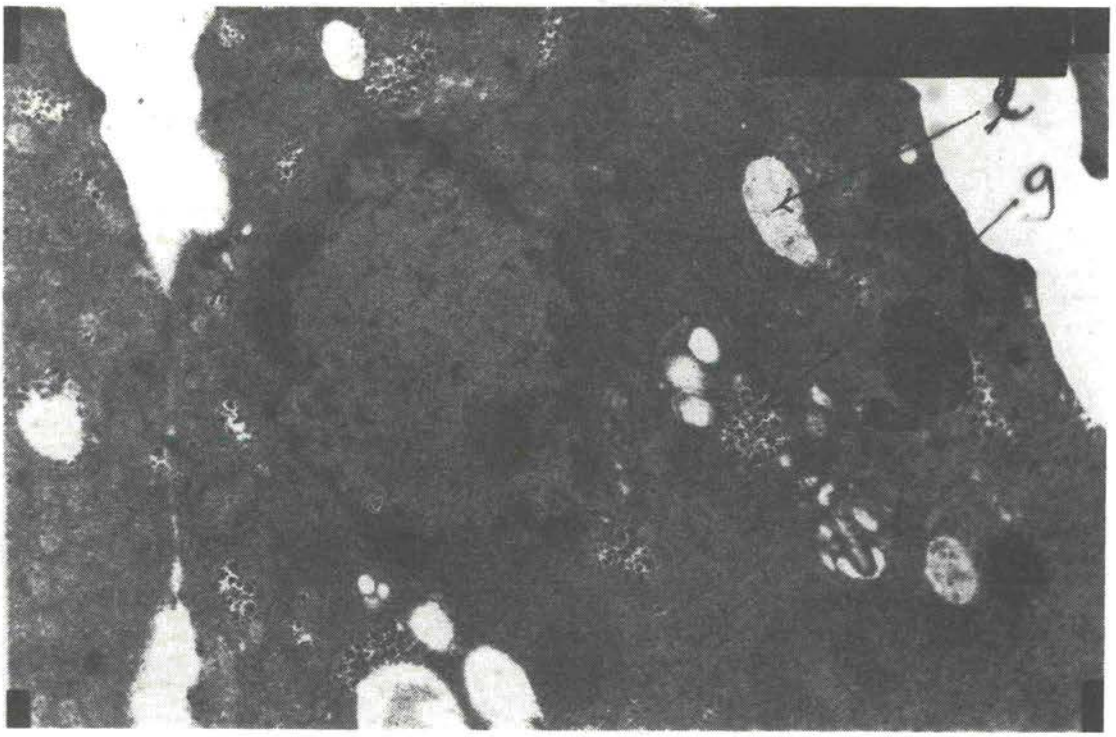

Figure 4. Liver cell with few mitochondria (m) and many vacuolated structures resembling smooth endoplasmic reticulum (r).

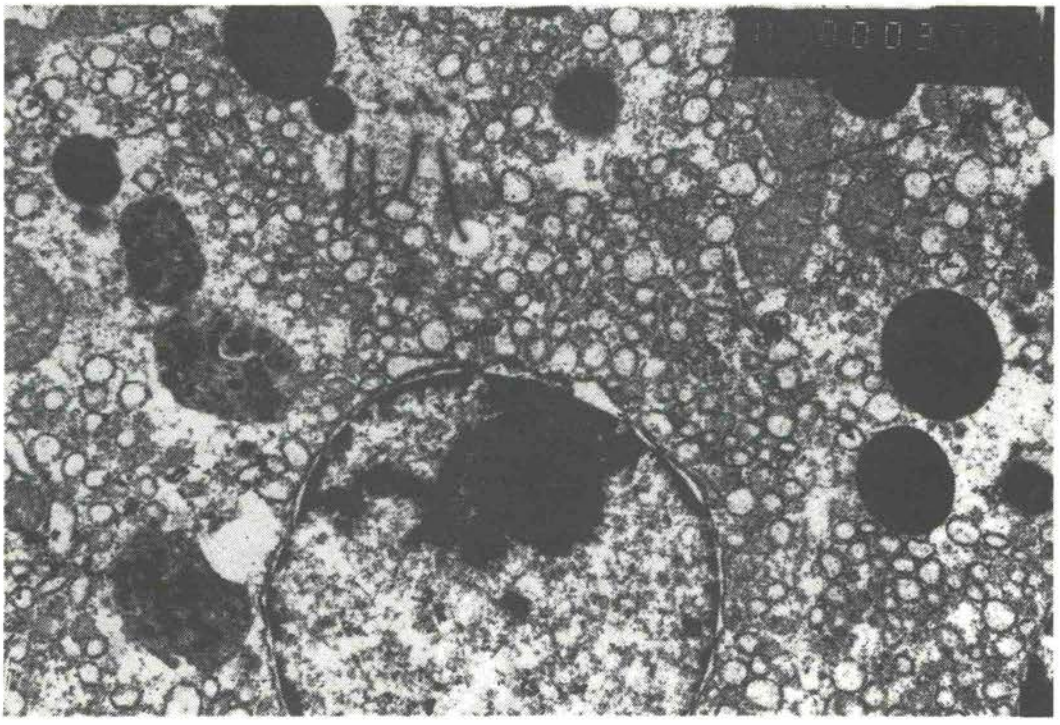

Figure 5. Liver cell with few mitochondria (m), and many vacuolated structures resembling smooth endoplasmic reticulum (S.r).

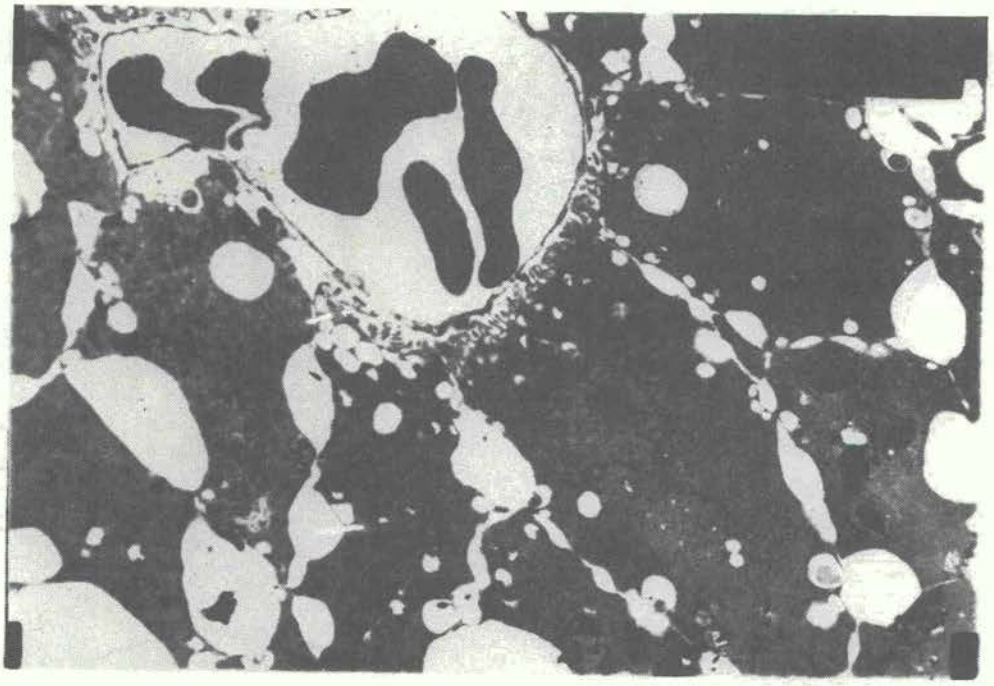

Figure 6. Liver cell showing shrinkage of hepatocytes with irregular external outlines and widening of the intercellular space.

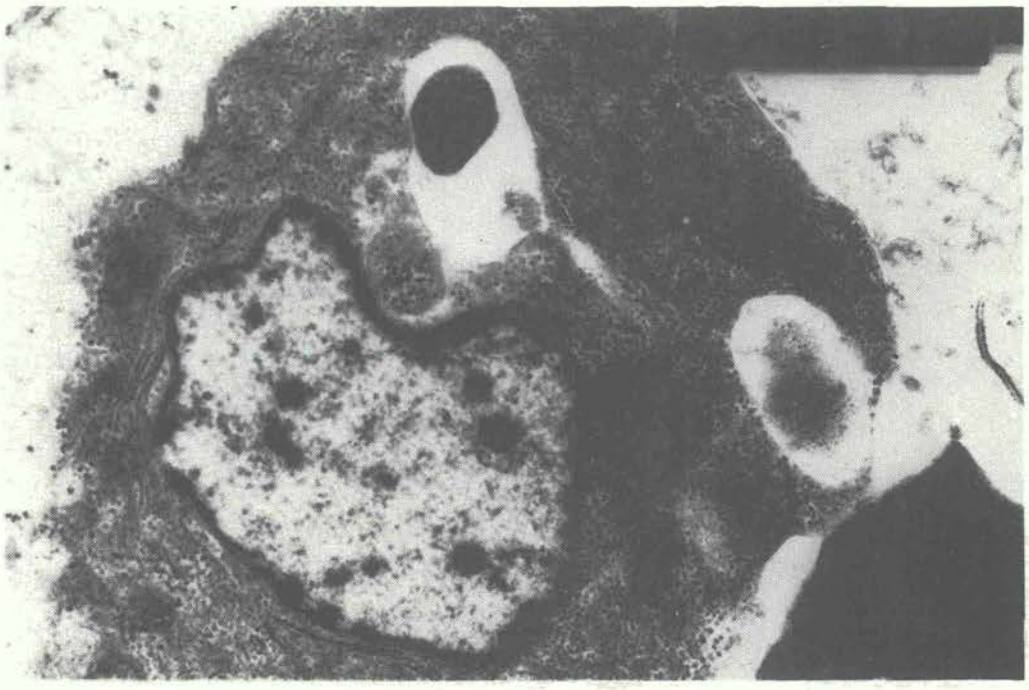

Figure 7. Liver cell associated with nuclear changes in the form of chromatin clumping and margination along the inner membrane of the nuclear envelope.

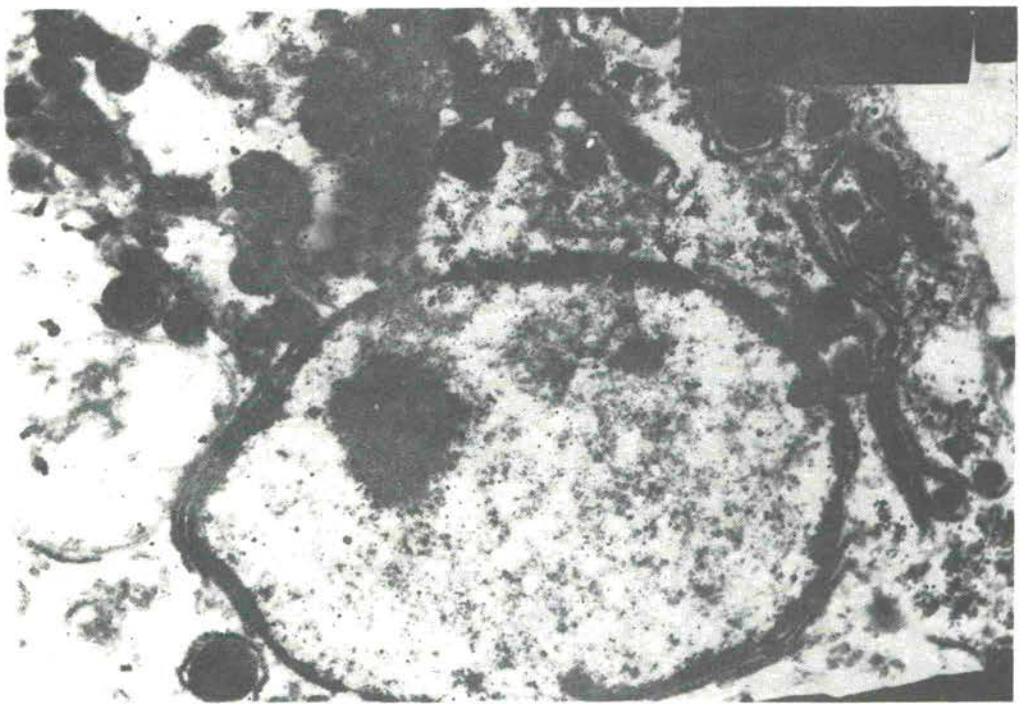

Figure 8. Liver cell with the mitochondria of affected cells displaying focal matrical densities.

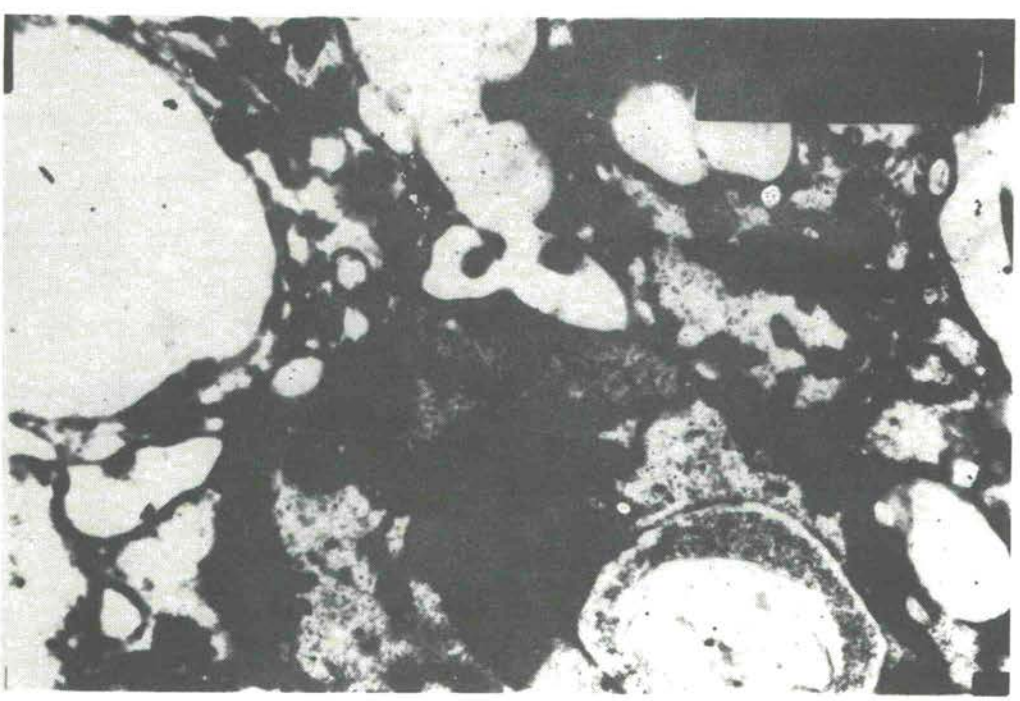

Figure 9. Liver cell showing dilatation giving the cells a cribriform appearance.

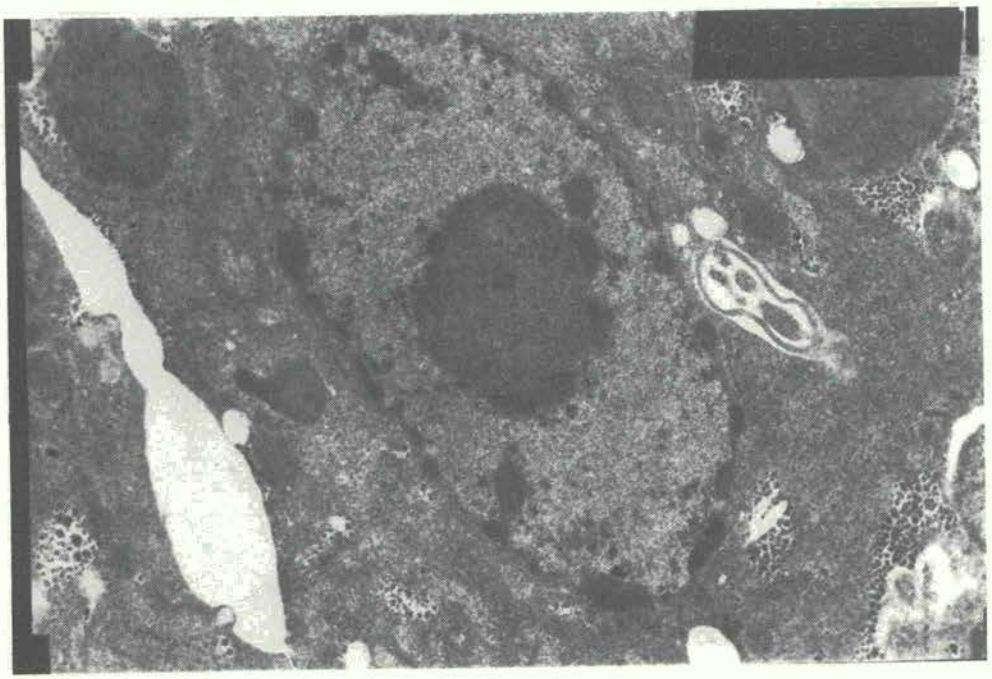

Figure 10. Liver cell with increase in the number of lysosomes.

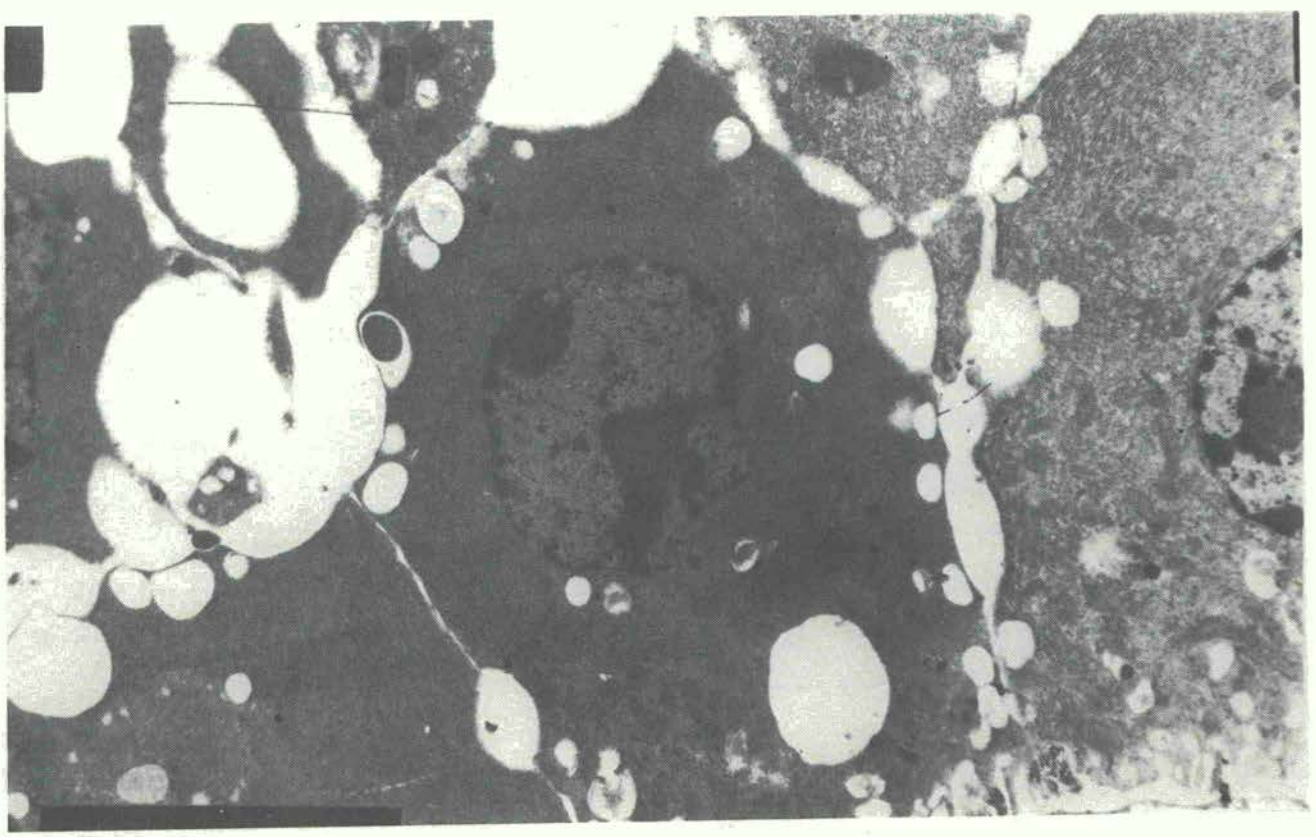

Figure 11. Liver cell with small vacuoles appearing almost spherical, and white larger vacuoles with irregular contours and occupying a large area of the cytoplasm.

4. CONCLUSIONS

The liver of the fish from the polluted lake contains a considerable amount of pollutants and lipids. There is a high attraction between pollutants and lipids, which may give rise to lipoid vacuoles. These lipoid vacuoles may increase in size to be larger than the nuclei. The presence of such vacules in large numbers in the cell probably causes its swelling, and the enlargement of the liver and its yellowish-white coloration. In addition the blood vessels are extremely congested and the liver lobular structure has largely disappeared. The widening of the intercellular spaces and presence of many vacuoles cause the loosening of the liver tissues.

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ACUTE TOXICITY OF AN OIL DISPERSANT ON THE DEVELOPMENTAL STAGES OF THE SEA BASS (Dicentrarchus labrax)

by

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1. INTRODUCTION

Oil dispersants are used for dispersing oil from the surface of the sea and cleaning coastlines after oil spills. Basically they consist of a mixture of a surfactant, a hydrocarbon solvent and sometimes of a stabilizing agent. Their toxicity may depend on the kind of solvent (Portmann and Connor, 1968), surfactant (Swedmark et al., 1971) or on the synergistic effects between the surfactant and the solvent (Nagell et al., 1974; Norton and Franklin, 1980).

It is generally known that younger (earlier) stages of fish are more susceptible to toxic effects (Mironov, 1972; Kühnold, 1977). Thus whereas some organisms are capable of detecting oil dispersants and to avoid them (Portmann, 1972; Gyllenberg and Lundqvist, 1976). Fish eggs are more passive and therefore exposed to strong pollutant effects in the sea water.

Induced spawning and rearing of early stages of sea bass (Katavic, 1984) makes available sufficient quantities of different developmental stages of the same generation for experimental purposes. This paper describes the effects of the oil dispersant BP1100 WD on eggs, larvae, postlarvae and juveniles of sea bass Dicentrarchus labrax.

2. MATERIAL AND METHODS

Measurements of the toxicity of BP1100 WD to eggs, larvae, postlarvae and juveniles of sea bass were carried out under static, aerated conditions. Five different concentrations of oil dispersant, plus controls, were used in 96-hour tests which were duplicated. Eggs, larvae and post larvae were treated in liter volumes, 100 eggs, 100 larvae or 20 postlarvae being placed in each jar. Ten juvenile individuals were placed in each 10-l volume of sea water with dispersant. Newly-fertilised eggs, one day old larvae, 17 day-old postlarvae and five-month old juveniles (average weight 0.89 g) were treated in the dispersant.

BP1100 WD was mixed with the sea water in the ratio 1:10 before use, as recommended by the manufacturer. Test concentrations, therefore, refer to this mixture.

Temperature was kept constant by water thermostats. The temperature range was 15.5 $\pm$ 0.2°C in the experiments with eggs, larvae and postlarvae and 21 $\pm$ 1°C in the experiment with juveniles. No food was supplied during the experiments. The salinity of the sea water ranged from 36.5 to 38.0 °/oo. Oxygen content was observed daily. Oxygen concentration was never lower than 90% saturation in the experiments with eggs, larvae and postlarvae, whereas it reached 75% not earlier that the fourth day in the experiment with juveniles. Experiments were carried under photoperiod conditions of 12 hours light, 12 hours dark.

Mortality was 18% in the controls with eggs, and 10% in those with larvae. Therefore, the mortality correction (Abbot, 1925) was applied for the calculation of LC50. LC50 and 95% confidence limits were determined for each exposure time by the probit method of Bliss (Fisher and Yates, 1949; Stora, 1974).

3. RESULTS

Eggs

Sea bass eggs are relatively very resistant to BP1100 WD. After 24 hours' exposure egg survival gradually decreased with higher dispersant concentrations (Fig. 1). Dead eggs were in the blastulation phase. After 48 hours' exposure egg survival was 70% in

all concentrations, compared with 82% in controls. Embryos begin to develop in eggs in this phase.

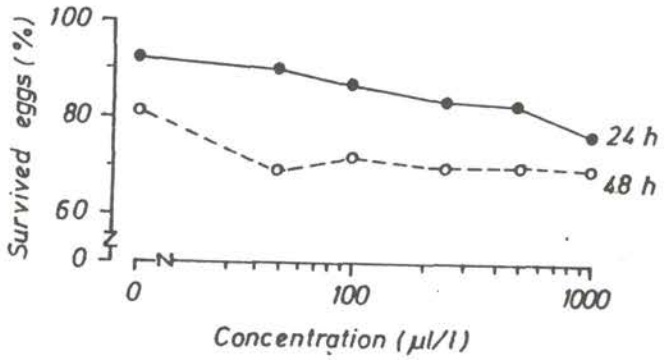

Figure 1. Survival of sea bass eggs after 24 and 48-hour exposure to BP1100WD

Hatching of larvae started during the third day of incubation. Hatching was observed for 5 hours. The delay of hatching compared to the controls was observed. Time to 50% hatching of the population were obtained from the cumulative percentage of hatched larvae and time by means of probit plots (Table I).

Table I.

Differences in the onset hatching between controls and BP1100 WD and the time to 50% hatching of larvae

Concentration ($\mu l\ l^{-1}$)	Delay of hatching (h)	Time to 50% hatch (h)
0	0	7.5
50	±1.4	5.4
100	±1.1	6.0
250	±1.0	4.8
500	±1.0	5.3
1000	±4.5	4.2

Concentrations up to 500 $\mu l\ l^{-1}$ delayed the beginning of hatching by up to an hour compared to the controls. This delay of the beginning of hatching of about 4.5 h in concentrations of 1000 $\mu l\ l^{-1}$ seems to be important with respect to relatively short embryonic development of sea bass at given temperature (Katavic, 1984). The median hatching time was shortened (80-56%) in all BP1100 WD concentrations compared with controls.

After 90 hours' exposure, hatching success showed no significant differences between various BP1100 WD concentrations (Fig. 2). Dead eggs found after this period were in the final phase of embryogenesis.

Some changes in larval behaviour were observed in different dispersant concentrations. Larvae were given a slight stroke with a metal needle to observe their motility. Larval motility was estimated as normal when the stroke evoked immediate reaction and quick escape as far as possible from the source of stimulus, moderate when they escaped 1-3 cm and low when they reacted with a few twitches. Thus mechanically-provoked mobility of larvae was gradually reduced by the increase of BP1100 WD concentrations and the percentage of spinal deformities increased (Table II).

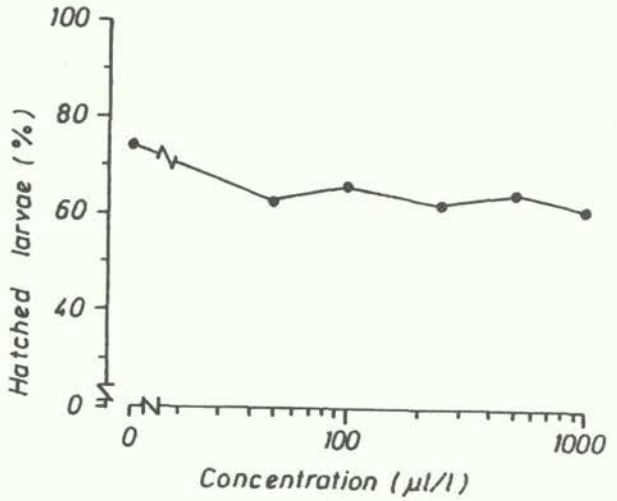

Figure 2. Percentage of hatched larve after 90-hour exposure to BP1100WD

Table II.

Effects of BP1100 WD on the motility and morphological characteristics of hatched larvae

Concentration (µl l^{-1})	Mobility	Spinal deformities (%)
0	normal	0
50	normal	0
100	3-5 cm	27.3
250	1-3 cm	28.6
500	3-4 twitches	50.0
1000	none	26.7

Larvae hatched in 1000 µl l^{-1} concentration showed no mechanically provoked mobility. Average (±SD) beats frequencies (in min^{-1}) in these larvae were 53±0, whereas in normal larvae it was 94±13.

The 96-hour exposure of one day-old larvae to the BP1100 WD concentrations of 5-100 µl l^{-1} caused no significant differences in the mortality between exposed larvae and controls. BP1100 WD increases the rate of yolk sac resorption measured after 48-hour exposure of larvae (Table III).

Toxicity of BP1100 WD to sea bass larvae was tested over the concentration range of 150-1000 µl l^{-1}. BP1100 WD toxicity rapidly increased for 96-h exposure (Fig. 3). At the onset of the test larvae were aged 1 day and at the end they were 5 days old. Sea bass larvae resorb the yolk sac at 15.5°C within five days.

Postlarvae

Sea bass postlarval stages showed considerably greater susceptibility to BP1100 WD than larval stages. The thereshold of lethal concentrations is not reached for post larvae within 96 hours (Fig. 3).

Juvenile

Five-months old juvenile sea bass were more resistant to BP1100 WD than earlier stages (Fig. 3). The relatively slight slope of the toxicity curve is indicative of the

fact that prolonged exposure does not significantly increase mortality at a given concentration. Haemorrhages were observed in dead specimens of juvenile sea bass.

Table III.

Yolk sac resorption in larvae (taken as the surface area of an ellipse) exposed to BP1100 WD for 48 hours

Concentration $\mu l\ l^{-1}$	n	$x \pm SD\ (mm^2)$
0	12	0.900 ± 0.051
5	12	0.899 ± 0.044
10	12	0.789 ± 0.045*
25	12	0.841 ± 0.059*
50	12	0.802 ± 0.054*
100	11	0.800 ± 0.056*

* significant differences to the controls (P 0.05).

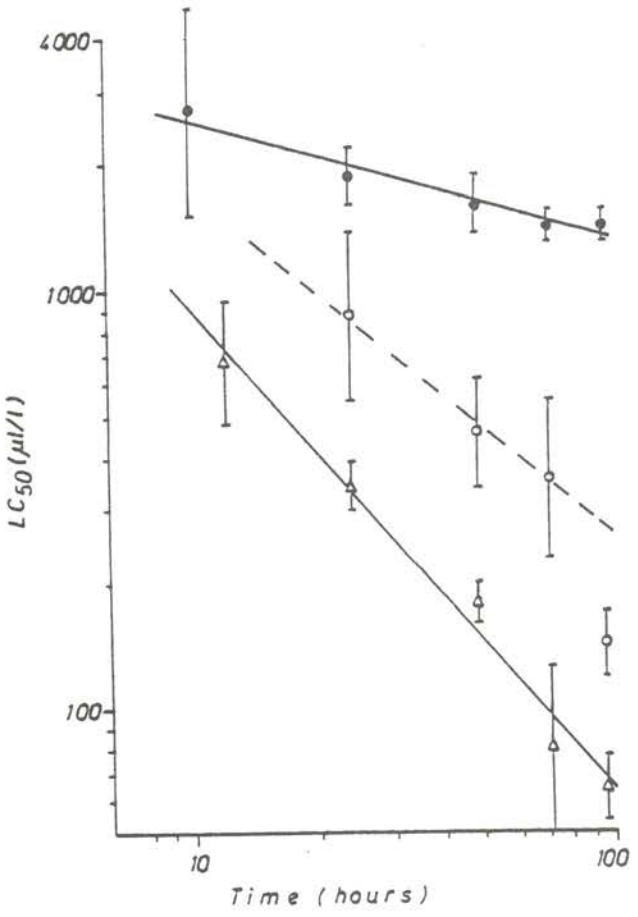

Figure 3. Relationship between median lethal concentrations (LC50) of BP1100 WD and exposure time for larval (o), postlarval (Δ) and juvenile (●) sea bass (Dicentrarchus labrax) stages. Vertical lines mark 95% confidence limits.

4. DISCUSSION

The results have shown that the toxicity of BP1100 WD is highest to postlarvae, somewhat less high to larvae and lowest to juveniles. Generally speaking, sea bass eggs show relatively high resistance to BP1100 WD effects. Mortality is somewhat more marked at the blastula phase and at the time of embryo formation. However, the incubation of sea bass eggs under ambiental conditions shows increased mortality in the morula phase, in the phase of embryo formation after enclosure of blastodermal cap and finally just before hatch (Katavic, 1984). This is indicative of general susceptibility of eggs in these phases of embryonic development. A similar phenomenon was established in herring (Alderdice and Velsen, 1971). High concentrations of BP1002 produce mutagenic effects on the embryos of herring and plaice (Wilson, 1976). Wilson (1976) found that many eggs had died before blastulation occurred.

The onset of hatching as affected by different BP1100 WD concentrations shows no considerable differences compared with controls. The only delay of the beginning of hatching was observed at 1000 $\mu l\ l^{-1}$ concentration. Wilson (1976) found that BP1002 and Finasol ESK delayed the onset of hatching of herring and plaice. BP1100 WD seems to cause a reduction in the time to 50% hatching. Kinne and Rosenthal (1967) considered that stress induced by oxygen deficiency might have some effects on premature hatching. On the contrary, Corexit extends the mean time to hatch in plaice (Wilson, 1976). This is attributed to a bactericidal effect of this dispersant.

BP1100 WD in concentrations exceeding 50 $\mu l\ l^{-1}$ causes distortions in hatched larvae manifested as spinal deformities. Deformities of the larval axis caused by oil dispersants have been recorded earlier (Wilson, 1972; 1976). BP1100 WD in concentrations of 10 $\mu l\ l^{-1}$ inhibits skeleton formation and ectoderm changes in the pluteus phase of the embryonic development of sea urchin (Lönning, 1977). BP1100 WD in higher concentrations shows narcotic effects on sea bass larvae. This was also recorded earlier for oil dispersants (Gyllenberg and Lundqvist, 1976; Wilson, 1976; Kiceniuk *et al.*, 1978).

A statistically significant increased rate of yolk sac resorption compared with the controls was recorded in larvae exposed to dispersant concentrations exceeding 10 $\mu l\ l^{-1}$. This may be attributed to increased energetic requirements of larvae which probably compensate the stress induced by a dispersant. Benzene effects on yolk sac resorption of herring larvae was explained similarly (Struhsaker *et al.*, 1974).

A rapid increase of acute toxicity of BP1100 WD to sea bass larvae between 72 and 96 hours' exposure may be attributed to the transition of larvae to postlarvae. Namely, complete yolk sac resorption in sea bass larvae takes 5.5 days at 15.5°C (Katavic, 1984). Larvae used in our experiment were approximately of the same age. The phenomenon of increased mortality of larvae in the phase of reduced yolk sac content is generally well known (Farris, 1960; Kuo *et al.*, 1973; Sanders, 1975; Katavic, 1984). That is, the dispersant only potentiates larval mortality in this critical phase.

More marked susceptibility to BP1100 WD of postlarvae than that of larvae may be partly explained in terms of the exhaustion of the endogenic mutritive source. In juvenile sea bass the difference in BP1100 WD toxicity between shorter and longer exposures is not drastically marked. This is indicative of the fact that it is the surfactant fraction that is mainly responsible for the toxicity of BP1100 WD. It is well known that surfactants are not easily evaporated and not rapidly biodegradated. Therefore the toxicity of dispersant decreases with the passage of time due to solvent evaporation (Portmann and Connor, 1968). Very marked haemorrhages in dead sea bass are also observed. Nuwayhid *et al.*, (1980) found that 1000 $\mu l\ l^{-1}$ concentration of BP1100 WD caused lesions of the gill epithellium in *Patella vulgata* after 24-hours' exposure.

It seems that marine organisms "in situ" will probably have the ability to avoid oil dispersants (Portmann, 1972; Gyllenberg and Lundqvist, 1976). Thus probably the problem of toxicity in the natural environment is mainly connected with immobile and poorly mobile forms like earlier developmental stages. Sea bass spawn in the wild from December to March (Katavic, 1974) when the sea water temperature is 12-13°C. Nagell *et al.*, (1984) found very high temperature coefficient values (Q_{10}) for oil dispersant toxicity. Accordingly, BP1100 WD toxicity may depend on temperature, which probably is

an additional factor in the reduction of toxicity to eggs and larvae of sea bass in the natural environment. On the other hand it has to be kept in mind that BP1100 WD toxicity was tested in the mixture 1:10 with the sea water, where dispersant concentrations were ten times lower. This means that the recommendations of the manufacturer should be followed when oil dispersant is used.

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MERCURY-BINDING PROTEINS OF THE GILLS AND DIGESTIVE GLAND OF Mytilus galloprovincialis

by

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1. INTRODUCTION

Mussels (Mytilus galloprovincialis, Lam.) are able to concentrate heavy metals without obvious signs of distress. In recent years they have been recognized as suitable organisms for monitoring trace metal pollution of coastal marine waters (Phillips, 1976; Davies and Pirie, 1978). They, like some other marine invertebrates, possess metal-binding proteins which serve to bind intracellular mercury, cadmium, copper, and zinc as a metal storage and detoxification compartment. Synthesis of these metallothionein-like proteins appears to be induced by exposure to the appropriate metal (Noel-Lambot, 1976; Roesijadi, 1980) in both natural conditions and in the laboratory.

The aim of the present work was primarily to study differences in the binding of mercury in the mussel Mytilus galloprovincialis exposed to high level of inorganic mercury in the laboratory and in the marine environment, in comparison with non-exposed control organisms.

2. MATERIALS AND METHODS

The mussles used for analysis, (Mytilus galloprovincialis, Lam., 4-7 cm long) lived in the following conditions:

- a non-contaminated area; mussels were sampled from Lim fjord on the Western coast of the Istrian Peninsula, Northern Adriatic, Jan. 1984,
- a very contaminated area polluted with inorganic mercury; mussels were sampled near the industrial region of Split in Kastela Bay, Central Adriatic, Dec. 1983, June 1984,
- laboratory conditions; mussels were placed in an aquarium with a flowing system for 33 days and the mussels were exposed to 60 ppb of inorganic mercury ($HgCl_2$).

Only two organs were used:

a. the gills; they represent an organ which functions as a site of uptake for mercury and as an important reservoir for the total mercury body burden;

b. the digestive gland, which is known to function in the storage of trace metals.

4-10 g of tissue from 20-30 animals were cut in pieces and homogenized in 3 volumes of ice-cold buffer (20 mM Tris-HCl, pH = 8.6 and 0.1 mM phenyl-methylsulfonyl fluoride). A glass homogenizer with a Teflon pestle was used. The homogenate was immediately centrifuged at 27000 g for 1 hour at 4°C. The supernatant from the digestive gland was filtered (black ribbon filter paper) to remove fats. 3.5 ml of clear supernatant were chromatographed on a 2.5 x 70 cm column of Sephadex G-75 equilibrated with 20mM Tris-HCl, pH =8.6, at a flow rate of 19.2 ml h^{-1} maintained at 4°C. Aliquots of 5-7 ml were collected and monitored for absorbance at 280 nm and 254 nm. In homogenates, supernatants and fractions, total mercury was analysed.

Total mercury was determined by two different methods:

- neutron activation analysis using a pyrolysis technique to separate ^{197}Hg by volatilization and selective trapping (Kosta and Byrne, 1969; Byrne and Kosta, 1974).

- flameless atomic absorption technique using tin(II) chloride reduction followed by volatilization of the mercury and amalgamation on elemental gold (Wittmann, 1981; Konishi and Takahashi, 1983).

In a few selected fractions, both analytical methods were used and good agreement found.

3. RESULTS AND DISCUSSION

All samples of digestive glands and gills were prepared in the same way. The total mercury content and the percentage of extractable mercury for the different sample types are shown in Table 1.

Table I.

Totel mercury content and percentage of extractable mercury in different sample types

S a m p l e	Hg(µg/g)	% of extraction
Control organisms		
gill homogenate	0.08	
supernatant	0.003	3.75
Mussels from natural contaminated area		
gill homogenate	2.07	
supernatant	0.08	3.87
digestive gland homogenate	3.83	
supernatant	0.16	4.12
Laboratory exposed mussels		
gill homogenate	46.53	
supernatant	20.55	44.2
digestive gland homogenate	19.51	
supernatant	6.72	34.4

Although the concentrations of total mercury in the tissue homogenates from mussels from the field (uncontaminated and contaminated) are different, the percentage extracted from the homogenates is similar. The percentage of mercury extracted from tissue homogenates of laboratory-exposed mussels is different, and runs up to 30-44%. In the tissues of these mussels the subcellular localisation of the mercury-binding proteins is thought to be different because of the relatively short exposure time.

Fig. 1 and 2 show the Sephadex G-75 elution profiles of supernatants. The absorbance at 280 nm and 254 nm and the mercury concentrations were determined in each fraction.

Elution profiles on Sephadex G-75 obtained from all supernatants have two separated peaks measured in UV spectrum at 280 and 254 nm. The first one corresponds to high molecular weight proteins (80,000 Daltons), while the second one corresponds to low molecular weight compounds. The second peak contains low levels of total mercury, in

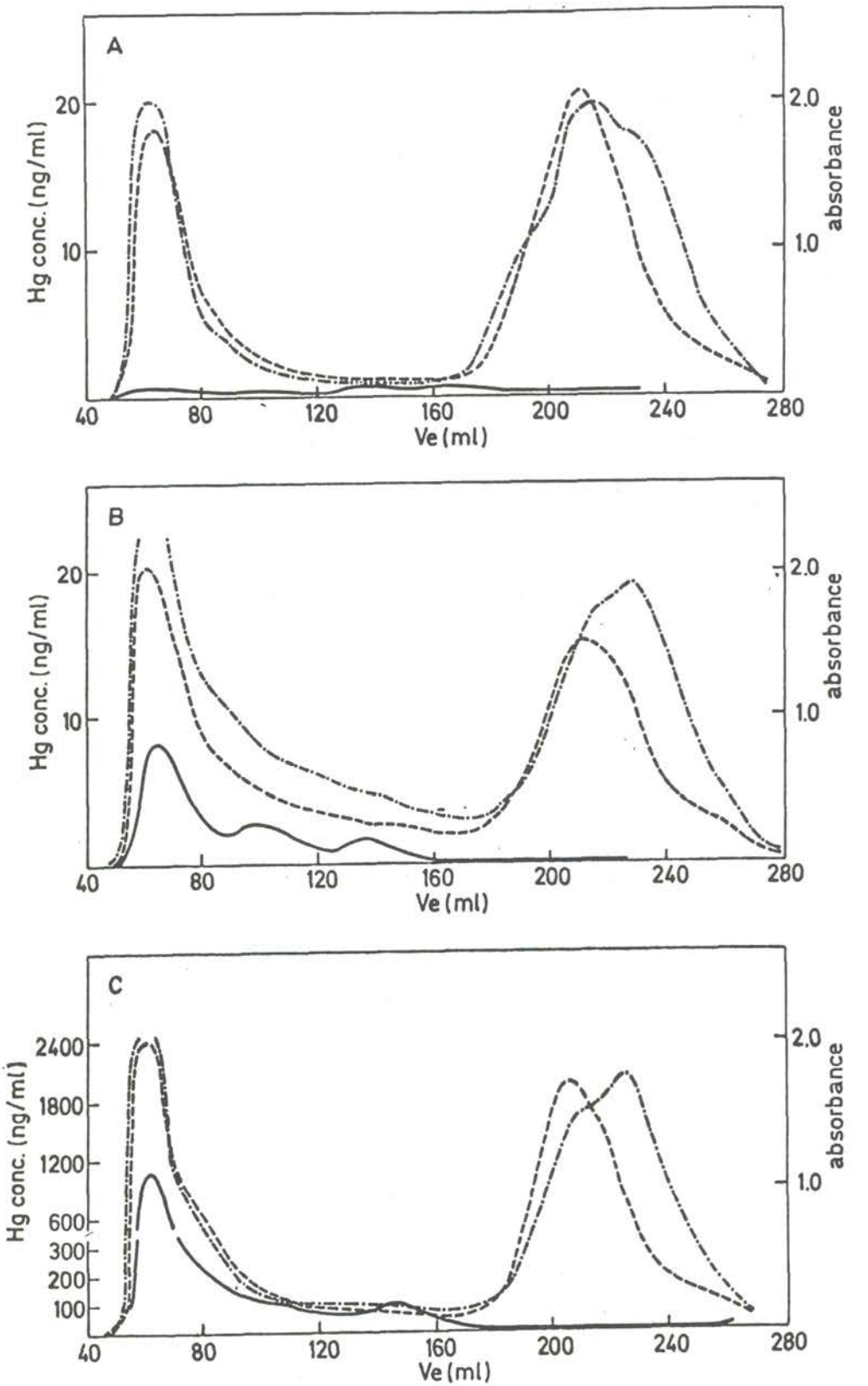

Figure 1. Sephadex G-75 chromatograms of supernatants of mussel digestive glands. In 5-7ml fractions total mercury (——), absorbance at 280nm (----) and 254nm (—·—·) were measured. A: control organisms; B: mussels exposed to high levels of mercury in the marine environment; C: mussels exposed to high levels of mercury in laboratory conditions.

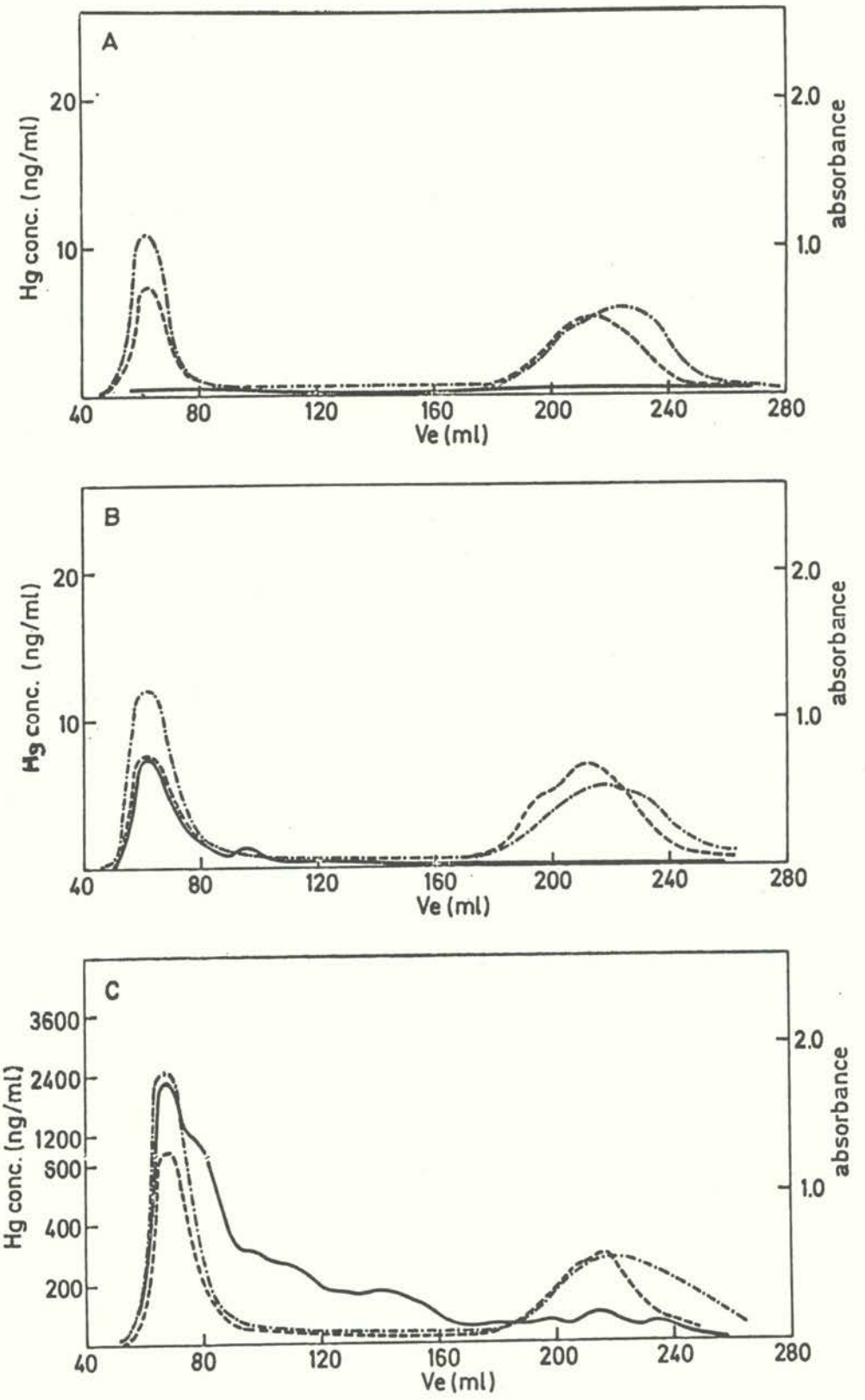

Figure 2. Sephadex G-75 chromatograms of supernatants of mussel gills. In 5-7 ml fractions total mercury (——), absorbance at 280nm (– – –) and 254nm (–.–.–) were measured. A: control organisms; B: mussels exposed to high levels of mercury in the marine environment; C: mussels exposed to high levels of mercury in laboratory conditions.

some cases under the detection limit of the analytical technique. In contrast to other metals (e.g. zinc, copper, cadmium) which can induce the synthesis of metal-binding proteins (George and Pirie, 1979; Viarengo et al., 1981; Köhler and Rüsgärd, 1982; Bray et al., 1983), mercury does not associate with low molecular weight compounds.

Fig. 1 shows the elution patterns on Sephadex G-75 of water-soluble digestive gland extracts. The fractions obtained from the extract of control organisms contain very low mercury concentrations (less than 1 ng ml^{-1}). Fig. 1B demonstrates the separation of the soluble fraction of digestive glands of mussels living in a contaminated area. The first peak at 254 nm which corresponds to high molecular weight proteins has a shoulder. It is possible that the protein fractions in the area of intermediate molecular weight proteins (the position of metallothionein-like proteins) have not separated. The two mercury peaks corresponding to these fractions represent 34% of the total mercury in the extract, and they follow the first mercury peak associated with proteins of high molecular weight. This contains 66% of the total supernatant mercury. In Fig. 2C it can be seen that in the case of the laboratory exposed animals the major part of the supernatant mercury (76%) binds to high molecular weight proteins.

Chromatographic analysis of soluble extracts obtained from the gills of control animals demonstrates that mercury is not found bound to proteins of high molecular weight (Fig. 2A). The mercury concentration in the extract is only 0.003 $\mu g\ ml^{-1}$ and mercury concentrations in fractions from gel filtration are at the detection limit.

In the extracts obtained from the gills of mercury contaminated mussels, mercury is mostly associated with proteins of high molecular weight (70-88% of total supernatant mercury); very little mercury is associated with intermediate molecular weight proteins (corresponding to metallothionein-like proteins) in the case of naturally-polluted mussels. In the laboratory-exposed animals, however, appreciable amounts of mercury are associated with proteins of a wide range of molecular weights.

4. CONCLUSIONS

Mussels, Mytilus galloprovincialis, exposed to high levels of inorganic mercury bind it mostly to high molecular weight proteins (66-88% of total supernatant Hg). This is true for the gills and for the digestive glands. Our results show that in these organs 22-34% of supernatant mercury is bound to proteins in the molecular weight range associated with inducible metallothionein-like proteins found in marine vertebrates exposed to other heavy metals.

It might be instructive to examine more closely the differences found in the intermediate range mercury-binding proteins for naturally-and laboratory-exposed mussels, and to see whether these differences are maintained in experiments of longer duration.

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UPTAKE, ACCUMULATION AND TOXICITY OF VANADIUM AND TIN IN MARINE ORGANISMS

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1. INTRODUCTION

During recent years, chemical contamination of man's environment has caused growing concern. Much of this concern has revolved about the effects of metals on marine organisms, because the salts of various heavy metals and other potentially hazardous materials are discharged in ever-increasing amounts into the marine environment from different sources (Goldberg, 1976) as they find their way into commercial and industrial application.

Since certain heavy metals are essential for life, they are concentrated in marine organisms. The invertebrates appear to have a particularly high capability for concentrating metals, when they filter plankton during feeding (Waldichuk, 1974). Because of the ability of many metals to form complexes with organic substances, there is a tendency for them to be fixed in the tissue and be incorporated in the structure of organic compounds (i.e. proteins and enzymes) and thus, not to be excreted.

Vanadium enters the ocean principally through atmospheric fallout and man's activity and this input in the marine environment has led to the enrichment of this metal in marine waters (Bertine and Goldberg, 1971; Grange, 1974; Duce and Hoffman, 1976). Once in the sea, vanadium may be rapidly solubilized (Walsh and Duce, 1976) or it may sink with the particulates eventually reaching deep-sea sediments. Thus, a number of investigators have reported its existence in sediments (Hopkins et al., 1973; Loring, 1976). Vanadium exists also in petroleum (Zoller et al., 1973) and in industrial effluents. As an example, 710 kg of vanadium per day was discharged to the sea in the acid effluents of a factory producing titanium dioxide (Grange, 1974).

Despite numerous studies on the concentration of vanadium in marine organisms (Nicholls et al., 1959; Fukai and Meinke, 1962; Pesch et al., 1977) with most emphasis being placed on certain species of ascidians which have an ability to concentrate this element to remarkable high levels (Goodbody, 1974; Ladd, 1974; Kustin et al, 1975), a limited number of investigations were carried out on its toxicity in marine organisms (Unsal, 1978; Miramand and Unsal, 1978) and its transfer within the food chain (Unsal 1978, 1978a, 1982, 1983).

Tin was one of the first metals used as a protective coating for steel and other alloys (Hallas et al., 1982). Organotin compounds have been incorporated into such preparations as insecticides, herbicides and fungicides (Zuckerman et al., 1978).

We report here the results of experiments conducted to examine the accumulation of tin by mussels and the accumulation and transfer of vanadium through a neritic and a benthic food chain.

2. MATERIAL AND METHODS

Different experimental designs were used to study the bioaccumulation of vanadium and tin.

Bioaccumulation of tin

Brachidontes variabilis (Krauss) was selected for this study. The organisms were acclimatized to laboratory conditions prior to the initiation of the experiments. Following this acclimatization period the mussels were distributed amongst four glass

aquaria, each containing 8 l of sea water and each being aerated continuously. Experiments started with 30 organisms in each aquarium. In the first aquarium, organisms were maintained in a medium containing 100 µg l^{-1} Sn (as $SnCl_4$) while in the second and third, they were subjected to 250 and 500 µg l^{-1} Sn respectively. The fourth aquarium, without added tin, served as control. Experiments lasted 30 days and during this period, the sea water was changed daily and after each change, tin was added from a stock solution containing 1000 mg l^{-1} $SnCl_4$ in distilled water. Six contaminated and six control mussels sampled at time intervals of 3, 7, 14, 21 and 30 days were deep-frozen pending analyses.

Analyses of samples

Samples were analysed by a Varian Techtron AA6 Atomic Absorption Spectrophotometer. The analytical procedure was described by Tugrul (1982) and by Unsal (1984).

Bioaccumulation of vanadium

The polychaete Nereis diversicolor, the crab Carcinus maenas and the fish Scorpaena porcus were used for these studies.

Prior to experiments, all test organisms were acclimatized to laboratory conditions. Following acclimatization the annelids (N.diversicolor) were placed in three tanks (2000 worms per tank) containing 40 l of sea water each and equipped with a continuous aeration system. The sea water was changed every day and after each change, vanadium (sodium metavanadate) was added to the medium to bring the vanadium concentration up to 500 µg l^{-1}. Experiments were conducted for 7 days, during which time the annelids were not fed. A control tank was prepared separately and maintained under identical conditions.

The same experimental conditions were employed for crabs and fish as for the annelids. The crabs and fish were placed in tanks (15 individuals per tank) containing 40 l of sea water and fed on vanadium-rich Nereis diversicolor (4 Nereis per day per individual). After 15 days' exposure to contaminated food (Nereis diversicolor), all test organisms were removed and deep-frozen for further analyses.

Analyses of samples

Samples were analysed with a Perkin-Elmer model 300 SG Atomic Absorption Spectrophotometer (AAS) equiped with a deuterium background corrector and a HGA-7 heated graphite atomiser. The analytical procedure was previously described in detail (Unsal, 1978, 1978a).

3. RESULTS

Tables I-III show the mean concentrations of tin and vanadium measured in the mussels, in the tissues of the animals used in the two experiments.

Table I.

Metal uptake in soft tissues of Brachidontes variabilis (ng g^{-1} wet wt.) at the outset and after a 30-day accumulation period.

Tin concentration in medium	Tin concentration in soft tissues (ng g^{-1} wet wt.)		
	% of dry wt.	Day 0	Day 30
0 (control)	12.4 ± 1.8	14	19
100 µg l^{-1}		15	641
250 µg l^{-1}		13	298
500 µg		14	382

Table II.

Concentration of vanadium in organisms of the neritic food chain
(μg^{-1} g dry wt.)

	Control (without V added)	Contaminated organisms (exposed to 500 μg V l^{-1})
Annelids after 7 days	4,86	9,90
Crabs after 15 days	2,41	6,64

Table III.

Concentration of vanadium in organisms of the benthic food chain
(μg^{-1} g dry wt.)

	Control (without V added)	Contaminated organisms (exposed to 500 μg V l^{-1})
Annelids after 7 days	4,86	9,90
Fish after 15 days	Gills 9,47 Liver 2,00 Muscle 0,49	Gills 7,63 Liver 1,58 Muscle 0,70

After the 30-day accumulation period, the highest tin concentration was observed in organisms exposed to lowest (100 $\mu g\ l^{-1}$) external concentration. In this group, a slight increase was observed with time (Fig. 1 A). The accumulation pattern was similar in both the 250 and 500 $\mu g\ l^{-1}$ groups (Figs. 1 B and C).

The vanadium concentration in the contamined crabs was calculated to be approximately 3 times the controls (Fig. 2).

In contrast to crabs no accumulation was observed in the tissues of fish, except the muscle, where there was a slight accumulation (Fig. 3).

4. DISCUSSION

The results showed that in all test groups, except controls, tin was accumulated in significant amounts by mussels. We suggest that this accumulation resulted mainly from two sources: the water and the food. The relative importance of these two sources has been previously investigated (Unsal, 1978 a). There are also other mechanisms involved in the accumulation of heavy metals. Delhaye and Cornet (1975), studying the effect of copper on Mytilus edulis during its reproductive period, observed that the spawning period was accompanied by an acceleration of copper uptake. Since, at this time, the animal's metabolism is very high and removal of water caused by ventilation of gills is rapid, so is the copper accumulation. During dissection, we found most mussels, removed in April and in May, to contain ripe eggs, thus proving that Brachidontes variabilis spawns during these months. We therefore suggest that this spawning was a contributory factor to accumulation of tin in mussels.

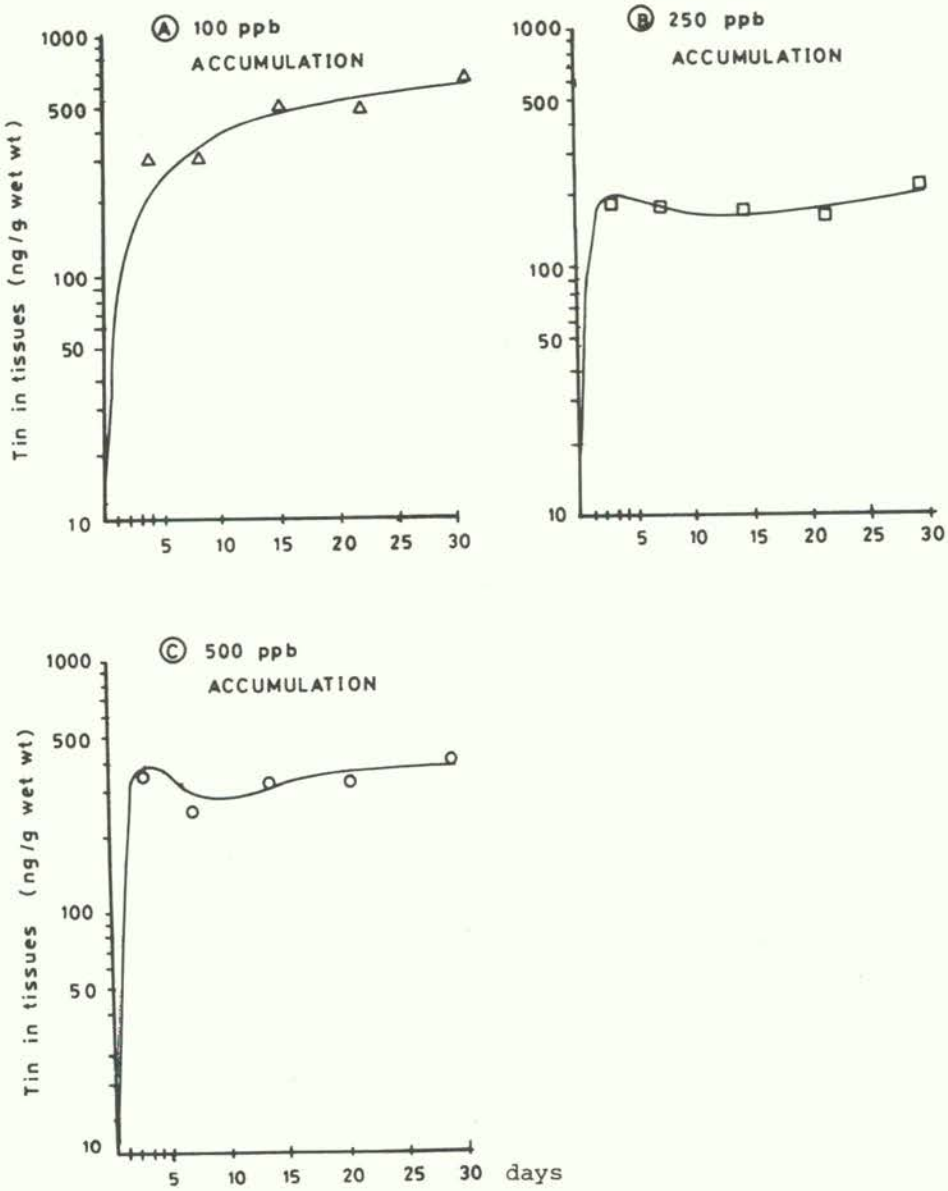

Figure 1. Accumulation of tin in soft tissues of Brachidontes variabilis at different test concentrations and as a function of time.

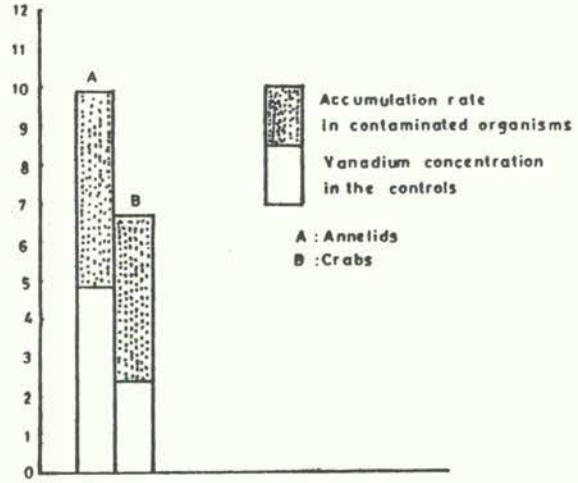

Figure 2. Accumulation of vanadium in different steps of neritic food chain.

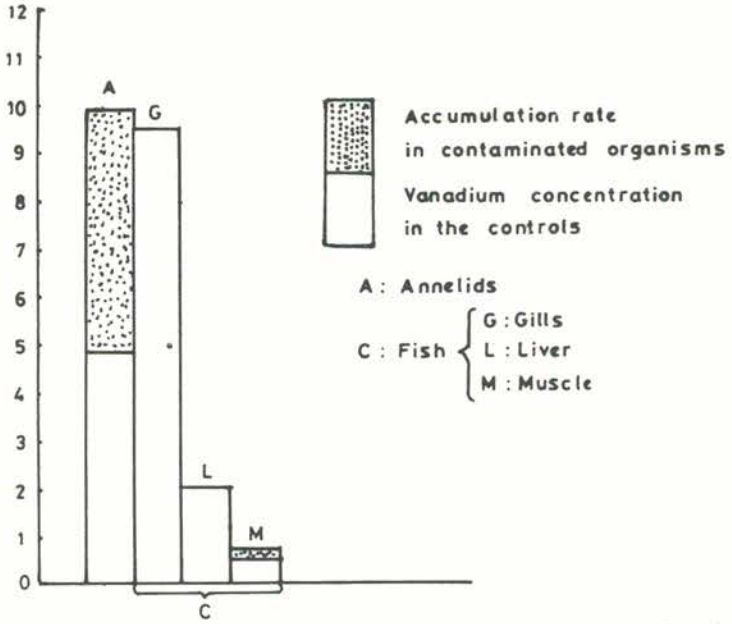

Figure 3. Accumulation of vanadium in different steps of benthic food chain.

The accumulation of tin decreased with increasing tin concentration in the medium as was observed by Bryan (1971) for zinc and by Miramand et al. (1980) for vanadium.

We suggest that the high tin concentrations exert a stress effect and this effect brought about changes in animal's physiology (in filtration rate, in respiration etc.). Therefore the uptake of tin was higher at low concentrations than at high concentrations.

The vanadium concentration in contaminated annelids was double that of the controls. We suggest that an adsorptive process is involved in the bioaccumulation of this element from water. Bryan and Hummerston (1973) have a similar suggestion, that the rate at which Zn was absorbed by Nereis diversicolor is proportional to the degree of adsorption at the surface of the body.

The accumulation of vanadium was significant in crabs. Thus a biomagnification was observed. Because the organisms were fed on vanadium-rich food and maintained in contaminated medium, the uptake took place from two sources. The relative importance of these two sources was demonstrated in a previous study (Unsal, 1983).

Although biomagnification was observed at the second step of the neritic food chain, the transfer of the vanadium was insignificant in the benthic chain. Amongst the tissues analysed, the gills contained the highest vanadium concentration which leads us to the conclusion that most of the metal accumulated in fish was taken up not from food, but from water.

A similar result was reported by Bouquegneau and Noël-Lambot (1977) who studied the accumulation of mercury from water and from food by Merlangius merlangus and by Gadus morrhua. They reported that 85 % of mercury was taken up from water by gills and only 15 % from food.

5. CONCLUSION

Tin and vanadium were taken up in significant amounts from the water by mussels and annelids. Tin uptake decreased with the increase in external concentration. By the end of a 30 days accumulation period, the tin concentrations increased about 35 fold in the 100 $\mu g\ l^{-1}$ group compared to controls. This shows that tin was accumulated in significant amounts by Brachidontes variabilis from its environment, although the tin concentration was 10,000 times greater than that found in unpolluted sea water. This result allows us to suggest that Brachidontes variabilis can be used as an indicator organism of pollution by tin.

We demonstrated also the transfer and biomagnification of vanadium through a neritic food chain. The transfer was less pronounced in the benthic food chain, but it does not mean that vanadium will not be transferred in any other benthic chain. It is worth saying that biological food chains are advisable methods to study the bioaccumulation and specially the biomagnification of substances.

On the other hand, the biochemical studies should be done in order to follow the fate and behaviour of the pollutants in the body of organisms from their uptake to excretion.

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GENOTOXIC RISK ASSESSMENT

by

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When toxic events occur in organisms at the cellular level adverse effects prevail as long as a critical concentration exists. When the event is over, and the damaging agent disappears, if death did not occur, restitution is possible in all cases where action was on molecules other than deoxyribonucleic acid (DNA). When DNA has been altered by a toxic agent - either a physical or a chemical one - the situation is different. In most cases DNA alteration is repaired by enzymatic processes. In about one case out of a hundred million, alterations persist into the DNA synthetic phase - they are genetically fixed into mutations. Most of them are without consequences. Few are such that they interfere with the full fitness of the organism in one way or another. In exremely rare cases, mutations are advantageous. In rare cases they interfere with the "organ-discipline" of cells.

This property of "organ-discipline" keeps cellular balance in tissues and organs well levelled and all cells have to obey signals for either dividing or ceasing to divide; e.g. in the human liver 25000 cells per day are destroyed and have to be resynthesized. If more were synthesized our livers would grow beyond functional size, if less than 25000 per day were produced, the liver size accordingly would decrease. Since however liver size is kept constant "organ-discipline" must be kept by awareness of all cells involved and sticking to the rules.

As a rare mutation, however, organ discipline can be lost, causing DNA synthesis in a cell, leading to unregulated cell division, a genetic marker that is transmitted to the progeny causing a growing clone - a tumour. There may by other ways of tumorigenesis, but most of them, if not all, are rooted in DNA alterations.

Since DNA is a chemical substance whose properties mostly are well known, alterations of it must be discernible and thus measurable. There are several kinds of DNA alterations, including single-strand and double-strand breaks; gaps; coupling of foreign molecules to DNA, (causing distortions or intercalations with minimal distortions); and base changes with changes of hydrogen bonding capacity and extreme bending or twisting, causing changes in hydrogen bonding.

The most frequent alteration is the single-strand break (ssb) or twists in the regular double helix conformation that surpass a critical level of twisting. This will finally lead in most of the measuring procedures to ssb. Another kind of damage is loss of a purine or pyrimidine base, also leading to ssb in the measuring procedure. In addition, most of the other DNA alterations, in the course of repair, lead also to ssbs. We sum all those alterations up under the heading single-strand events (sse).

DNA molecules are double molecules each constisting of an a- and b-strand linked by 2 - 3 hydrogen-bonds per base pair. These hydrogen bonds are weak links, that "melt" in alkaline solution, which does not destroy the rest of the molecular integrity. Thus by this procedure, in intact chromosomes, two thin, very long DNA signle-strands are set free per chromatid in case of undamaged DNA. Yet this setting free takes time, since DNA double molecules are helical, and in the alkali they have to unwind, which may take hours or longer. When, however, there are ssb, which are statistically distributed, unwinding and strand separation is accelerated in a power function. Thus even small sse numbers make very large contributions to the speed of separation.

In one technique we use alkaline filter elution for sse detection. Cells are put on a filter with graded pores (e.g. 2 µm). They are washed with a lysing buffer,

disintegrating the cells, washing proteins, carbohydrates and lipids through the filter. The scaffold structures and the DNA stay on the filter, the DNA hanging to it like a fleece. These purified structures are slowly rinsed for 14 h with a buffer above pH 11.6. The a- and b-strands unwind, the short pieces (DNA with many sse) fall off first, giving rise to fast DNA elution, undamaged DNA staying on the filter to the end. The filter eluate is collected in a fraction collector and the DNA concentrations in the fractions and on the filter are determined.

We may also use ring-shaped DNA molecules with natural supertwists. If such a molecule undergoes a signle break in a strand, the supertwists relax, giving rise to an open circular form. Both molecule types have, for example, very different electrophoretic migration speeds. They can be separated and quantitated. There are many ways of evaluations.

Electron microscopic evaluation gives absolute numbers of strand-breaks. It is too expensive and time consuming for routine use, but it allows for calibration of the other methods.

It is well known that the most dangerous aspect of environmental pollution is genotoxicity. Genotoxic agents are DNA damaging agents. Thus it must be possible to measure the DNA altering effects of genotoxins. This has been proven to be true. We have shown that fish and other animals injected with known pollutants or with extracts from polluted waters have damaged DNA. We are using the DNA damage test in a river survey and we have shown that a kerosene spill even 3 months after sanitation still can be traced through fish by their liver DNA damage (Stüber and Zahn, 1985).

Yet there are many pollutants, such as polycyclic aromatic hydrocarbons, (PAH) which, when an animal first encounters them, are taken up in high concentrations without causing any toxic signs. However, in the course of hours or days they provoke the synthesis of a large spectrum of proteins, mostly enzymes. Among these are the Mixed Function Oxygenases (MFO). These convert the practically insoluble PAH by oxidation into more water-soluble compounds that can be excreted. Among the PAH however are some, which, upon oxidation, become very dangerous compounds that are able to couple to high molecular cell components, including DNA. This modified DNA gives rise to many mutants; among them are mutations to cancer. Therefore the dangerous forms of the PAH and many other substances with similar properties, are called "ultimate carcinogens".

So besides the direct carcinogens, there are compounds, procarcinogens, such as some PAH, that by the aid of MFO are enzymatically transformed into ultimate carcinogens. This capability of enzymatic change is different in different tissues, often highest in liver and different in different animals. Remarkably, it is inducible, i.e. it is practically absent in those animals that have never met pollution and it is high in animals living in polluted conditions. We have shown that the state of induction in river fish (the MFO activity) is a function of the pollutional state of the river (Kezic et al ., 1983). Using Blennius pavo, a fish that throughout its life stays in a small area, we could show the change of MFO activity brought about by an oil spill in the Northern Adriatic (Kurelec et al., 1977). So one can say that there is a second method for the detection of pollutional risk by determination of MFO induction.

A third approach is to measure the mutation-inducing capability of pollutants, e.g. by the Salmonella typhimurium or Ames test (Ames et al., 1973).

One of the advantages seems to us that when used properly these tests allow work under actual conditions of pollution. Their sensitivity can be brought to levels below the one permitted.

When DNA damage is calculated it comes as a surprise that the numbers of strand breaks are so high, and that even controls show damage. One has to realize that these methods open new aspects.

Thus we have to accept from medical epidemiology (Doll and Peto, 1981) that in humans, where 20 - 25% of all deaths are by tumors, 35% are caused through carcinogenicity of our diet (solanin and chaconin of potatoes; caffein, chlorogenic acid, atractylosides from coffee; latex with terpenoids from salad; quercetine, safrol,

phorbol esters, quinones from plants in teas; protein and amino acid pyrolytic products, endoperoxides, epoxides from lipids, genotoxins from browning reactions from bread crust, meat etc. (Ames, 1983). 30% are caused by smoking. Compared to this, environmental poisons contribute relatively little. In the human diet (where avoidance reactions are possible) the "natural" genotoxins contribute far more than thousand times as much as the artificial poisons as DDT, dioxin etc. which we find in our food.

In animals in their natural habitat the situation is somewhat similar. Since, however, in most cases avoidance is not possible, animals are more affected by the environmental quality. Yet still a substantial contribution is by allelochemicals (poisons from the food etc.); (Brattsten et al., 1977; Brattsten, 1979; Ames, 1983), leading to a non-zero control level of DNA damage. Alteration tests and MFO determinations should therefore best be conducted as differential tests, where an ideal ambient is compared to the ambient under assay. The ideal ambient should be standardized. Under such conditions meaningful results can be achieved and comparisons among different research groups are possible.

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