

**Studies on the uptake of copper, zinc and cadmium by
the amphipod *Corophium volutator* (Pallas) in the
laboratory**

**Laboratuvar Koşullarında Amfipod *Corophium volutator*
(Pallas)'un bakır, çinko ve kadmiyumu alımı üzerine
çalışmalar**

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Abstract: In this study, accumulation of copper, zinc and cadmium at varying concentrations from sea water and sediment under the laboratory conditions was assessed by bioaccumulation tests, using the marine crustacean *Corophium volutator* (Pallas). Concentrations of these metals in the whole tissues of *C. volutator* were determined at intervals of 3, 6, 24, 48, 72 and 96 h. There is positive correlation between metal levels in *C. volutator* and metal levels in sea water and sediment. The results also show that the accumulation of copper, zinc and cadmium in *C. volutator* from the sea water is higher than those from sediment.

Keywords: *Corophium volutator*, heavy metal, sediment, bioaccumulation

Introduction

Amphipods are an important and an abundant component of the soft bottom marine and estuarine benthic community. *Corophium volutator* in particular offers many advantages for toxicological research. It is widely distributed in coastal waters (Meadows and Reid,1966 ; Muus,1967) and can reach extremely high densities of up to 60.000 m⁻² (Gorman and Raffaelli,1993). *C. volutator* is also a principle prey of many estuarine fish (Jaquet and Raffaelli,1989), shorebirds (Raffaelli and Milne,1987) and some larger invertebrates (Hall and Raffaelli,1991) and its susceptibility to pollutants has implications for the entire estuary food web. Finally, *C. volutator* can survive for 3 months or longer under

normal laboratory conditions, has a quick response time to pollutants (Erdem and Meadows, 1980) and is easily collected all the year-round from intertidal flats. Amphipods like *C. volutator* have been recommended for testing the toxicity of marine sediments by the United States Environmental Protection Agency (1991) and Swartz *et al.* (1985), but there have been no studies to date where the responses outlined in these protocols have been examined in relation to specific and separate contaminants. Following the standard EPA protocol, present study was designed to determine contaminant uptake by *C. volutator* exposed to three metals.

Materials and Methods

1. Sample collection and experimental protocol

C. volutator were collected from the mudflats of the Ythan estuary, Aberdeenshire, Scotland by sieving sediment through a 500 µm mesh. Large pieces of debris and any other macrofauna were discarded. The sea water used for the bioassay experiments was pumped from the estuary through a biological filter and into a sediment-free tank and continually aerated (30‰ salinity, 11±1°C). The amphipods were stored here for acclimatisation for a period of at least 7 days. All *C. volutator* used in the experiments were adults (4-7mm) with equal numbers of males and females.

Bioassay methodology was based on that outlined by the American Society for Testing and Materials (1990) and the US Environmental Protection Agency and the US Army Corps of Engineers (EPA/COE) (1991) as developed by Swartz *et al.* (1985). Sediments were collected from an area of the estuary known to be clean and to support a healthy population of *C. volutator* (Raffaelli, pers. comm.). The sediment was washed with clean sea water through a 500 µm mesh into a tank to remove any *C. volutator* and associated macrofauna, and then washed again with clean sea water through a 300 µm mesh to ensure a standardised sediment particle size for all experiments.

2. Bioassay procedure

2.1. Experiment 1: Accumulation from sea water

A stock solution of 1000 ppm of each metal was prepared by dissolving copper ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), or zinc ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) or cadmium (CdCl_2) in distilled water. Test solutions were prepared by diluting the stock solution with sea water. *C. volutator* were exposed to three concentrations (0.1, 1.0 and 10.0 ppm) of copper, zinc and cadmium in sea water, as well as controls (uncontaminated sea water), in set-ups with clean sediment. Each set-up consisted of four replicate containers (90 mm in diameter 80 mm deep) of each of three concentrations and three controls. 175 ml of clean sediment was added to these containers to create a 2 cm deep layer. All containers were aerated in order to maintain the dissolved oxygen levels above 60% of the air saturation value (ASTM, 1990 ; US EPA/COE Manual, 1991). All containers were covered by black material to exclude direct light except from directly above, and 20 *C. volutator* placed in each. No food was

supplied during the course of the experiment nor were the test solutions changed. Each container was examined daily and any dead organisms was removed.

2.2. Experiment 2: Accumulation from sediment

Clean or uncontaminated sediment as described above is treated by shaking with solutions of copper ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), or zinc ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), or cadmium (CdCl_2) at the following concentrations: 10, 30 and 50 $\mu\text{g g}^{-1}$ for copper and zinc, 5, 10 and 30 $\mu\text{g g}^{-1}$ for cadmium. Clean sediment used as control sediment is resuspended four times in clean sea water. All experimental containers (9 cm in diameter, 8 cm deep) used were covered by black material to exclude light except from directly above. The test and control sediment were transferred to the containers to a depth of 2 cm. The surface of the sediment was smoothed, and uncontaminated (clean) sea water carefully added to about 5 mm from the top of the container. Aeration, at a rate of approximately two or three bubbles per second, was supplied by a Pasteur pipette without disturbing the sediment surface. The experimental set-up was maintained under constant aeration for 48 hours before any *C. volutator* were added.

Twenty *C. volutator* were added to each container with a wide-mouthed pipette. After 1 hour any *C. volutator* that were dead or showed abnormal behaviour were removed and replaced. The numbers of individuals that were dead noted at 3, 6, 24, 48, 72 and 96 h and dead amphipods removed but not replaced.

In both experiments, at 3, 6, 24, 48, 72 and 96 h, living *C. volutator* were transferred to clean sea water without sediment to evacuate guts for 48h and were deep frozen until analysis for the metals could be carried out. To determine how much copper, zinc and cadmium had accumulated in *C. volutator* tissues over the course of the experiment, the animals were dissolved in 70 % HNO_3 and diluted with distilled water. Then this solution analysed for metals by atomic absorption spectrophotometry (see detail below). The concentrations of the metals in the amphipod tissues were expressed as μg of copper or zinc or cadmium per g of dry weight. Samples of each test solution and the sediments were also analysed for copper, zinc and cadmium at the beginning and at the end of the experiment and the average of the concentrations of the metals was used in subsequent data analysis.

Temperature, dissolved oxygen, salinity and pH were measured in all experiments and the design of the experiments ensured that all replicates and treatments were exposed to the same factors.

Sodium citrate was added to complex copper and zinc solutions for the prevent precipitation of copper and zinc in sea water. Second control was used that contained sodium citrate at the highest concentrations used in these solutions (Reish *et al.*, 1974; Reish and Carr, 1978). There was no effect on the sodium citrate on *C. volutator* survival. Another method involves slight acidifications of solutions, but the pH never dropped below 7 (Ahsanullah, 1976; Ahsanullah *et*

al., 1981). There was no differences between these methods with respect to *C. volutator* survival. In the present study both methods were used as appropriate.

2.3. Analysis for heavy metals

2.3.1. *C. volutator* tissues

After each experiment, surviving individuals were placed for 48 hours in constantly aerated clean sea water at $11\pm 1^\circ\text{C}$, and then rinsed in double-distilled water. The samples were dried to constant weight at 70°C , weighed and dissolved in concentrated nitric acid at 80°C . Caparis and Rainbow (1994) noted that approximately 0.2 ml of concentrated nitric acid was enough to completely digest individual *C. volutator* with dry weights up to 4.7 mg. After digestion, the samples were diluted with distilled water and filtered through Whatman filter paper for analysis on a Varian SpectraAA10 Atomic Absorption Spectrophotometer (AAS).

2.3.2. Sediment samples

Sediment samples from each jar were dried overnight at 105°C and were sieved through a $63\ \mu\text{m}$ mesh to select for particles smaller than this. This fraction was analysed because these particles are the most important sources of available metals contained in sediments (Bryan and Langston, 1992; Langston and Spence, 1994). Moreover *C. volutator* can ingest only particles between 4 and $63\ \mu\text{m}$ diameter (Fenchel *et al.*, 1975).

20 ml of concentrated nitric acid was added to 1 g of each of the dried sieved sediments and allowed to stand overnight. Digestion mixtures were heated on a hot plate set at 80°C for 3-4 days. After digestion the beakers were removed from the hot plate and allowed to cool. The residue was dissolved in conc. nitric acid (1 ml per 1 g of dry sediment or cast), diluted with double-distilled water and made up to 10 ml for analysis.

2.3.3. Water samples

Water samples for metal analysis were taken from the centre of each container and acidified with 0.1 % nitric acid. The samples were then filtered through a $0.45\ \mu\text{m}$ filter and analysed by AAS.

2.4. Preparation of standard solutions

All glassware was first washed with detergent (decon[®] 75) and rinsed with tapwater. They were then treated with 10% v/v HNO_3 (analytical reagent grade) for 24 hours, and then rinsed at least three times with double distilled water before use. All reagents were of analytical reagent grade (AristaR or AnalaR, BDH).

The accuracy of a determination by AAS is only as good as that of the set of calibration standards used. Some standard solutions can be prepared directly by weighing out an appropriate compound of the element. But in most cases it is preferable to determine their exact concentrations (Marr, personal

communication). The initial approximate 1000 ppm solutions were prepared using the following procedures, as used routinely in the Chemistry Department, University of Aberdeen (Marr, 1992 and 1993).

2.4.1. Copper: 1000 µg/ml: 0.10 g of AnalaR copper foil was trimmed and accurately weighed. It was dissolved in 1 ml of HNO₃-H₂O (1:1) and then diluted to 100 ml with 1% v/v HNO₃.

2.4.2. Zinc: 1000 µg/ml: 0.50 g of granulated zinc metal was accurately weighed and dissolved in 25 ml of HNO₃-H₂O (1:1). This solution was boiled to expel any dissolved oxides of nitrogen, before being made up to 500 ml with distilled water to give a stock 1000 ppm zinc solution.

EDTA : 0.01 M: 3.7224 g of EDTA disodium salt was dissolved and made up to 1 l with distilled water.

Standardisation: Four ml of 10% (w/v) hexamine solution and 2 drops of xylenol orange indicator were added to 10 ml of 1000 µg/ml zinc solution. This was titrated with 0.01M EDTA.

2.4.3. Cadmium: 1000 µg/ml: 0.2032 g of cadmium chloride (CdCl₂.2½H₂O) was dissolved in distilled water and made up to 100 ml.

Standardisation: 35 ml of 0.01M EDTA, 5 ml of NH₃:NH₄Cl buffer and 2 drops of Eriochrome Black T indicator were added to 10 ml of cadmium solution. This was back-titrated with 0.01M zinc solution.

The concentrations of these stock solutions were determined by titration. Working standards were prepared with the same acid matrix as acid-digested samples to be analysed as well as a blank. If the concentrations of the metals in the test material exceeded the highest standard, the test material was diluted with the appropriate matrix.

2.5. Data analysis

The data were analysed statistically for differences in mean metal concentrations using analysis of variance (ANOVA) for differences amongst all means. If differences were found a Tukey test was used to determine differences between means (Zar, 1984).

3. Results

Temperature, oxygen, salinity and pH for each of the replicates used in the bioassay were monitored daily to ensure these were similar in all experiments. Samples of sediments were analyzed for total organic carbon (Buchanan, 1984). The mean temperature for the 96h experimental period in all bioassay was 10°C ± 1, dissolved oxygen was 92% ± 5, salinity was 30‰ ± 1 and pH was 8.03 ± 0.25. The total organic content of the sediment was 2.3% ± 0.42.

Table 1 and 2 show the toxic effect of copper, zinc and cadmium concentrations in sea water on the amphipod *C. volutator*. Survival decreased with increasing

copper, zinc and cadmium concentrations both in sea water and in sediment. At the three concentrations used in the first experiment, 0.1, 1.0 and 10.0 ppm, only 10.0 ppm was found to be toxic in the experimental period for the metals. At 10.0 ppm copper, zinc and cadmium in sea water, 10%, 30% and 45% of amphipods had died after 96 hours exposure, respectively (Table 1). In the second experiment at 50 ppm copper and zinc and 30 ppm cadmium in sediment 45%, 60% and 75% of amphipods had died after 96 hours exposure, respectively (Table 2).

The amphipod *C. volutator* accumulated copper, zinc and cadmium from sea water and sediment (Figures 1-6). The concentration in the tissues was consistently less in the sediment treatment than those in the sea water treatment ($P < 0.05$).

Table 1. Effect of increasing copper, zinc and cadmium concentration in sea water on mortality of *Corophium volutator*. Each value included 3 replicates.

Number of amphipods alive												
Time (hours)	copper (ppm)				zinc (ppm)				cadmium (ppm)			
	Control	0.1	1.0	10.0	Control	0.1	1.0	10.0	Control	0.1	1.0	10.0
0	20	20	20	20	20	20	20	20	20	20	20	20
3	20	20	20	20	20	20	20	20	20	20	20	20
6	20	20	20	20	20	20	20	20	20	20	20	20
24	20	20	20	19	20	20	20	19	20	20	20	17
48	20	20	20	19	20	20	18	18	20	20	20	13
72	20	20	19	18	20	20	18	16	20	20	18	12
96	20	20	19	18	20	20	17	14	20	20	17	11

Table 2. Effect of increasing copper, zinc and cadmium concentration in sediment on mortality of *Corophium volutator*. Each value included 3 replicates.

Number of amphipods alive												
Time (hours)	copper (ppm)				zinc (ppm)				cadmium (ppm)			
	Control	10	30	50	Control	10	30	50	Control	5	10	30
0	20	20	20	20	20	20	20	20	20	20	20	20
3	20	20	20	20	20	20	20	20	20	20	20	20
6	20	20	20	20	20	20	20	20	20	20	20	18
24	20	20	20	17	20	20	18	15	20	20	20	14
48	20	20	19	16	20	20	17	11	20	19	16	8
72	20	20	18	12	20	20	17	10	20	18	16	6
96	20	20	18	11	20	19	16	8	20	18	15	5

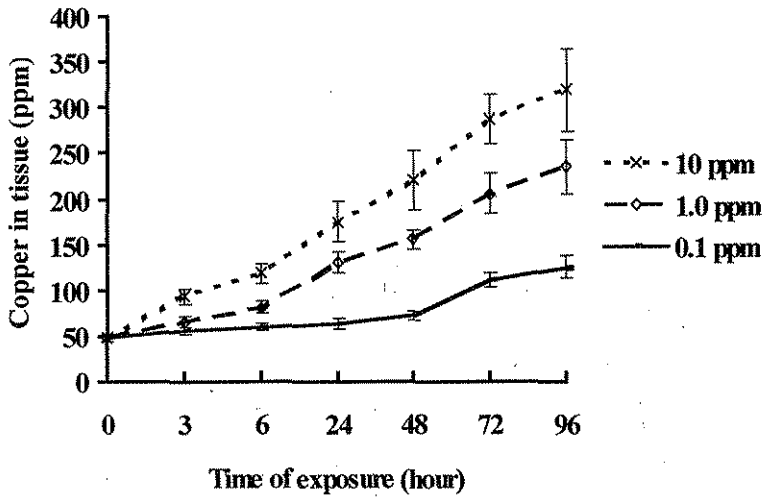


Figure 1. Mean concentrations of copper in *Corophium* at 0, 3, 6, 24, 48, 72 and 96h static bioassay of sea water with uncontaminated sediment. Each value shows the mean and standard error of three replicates.

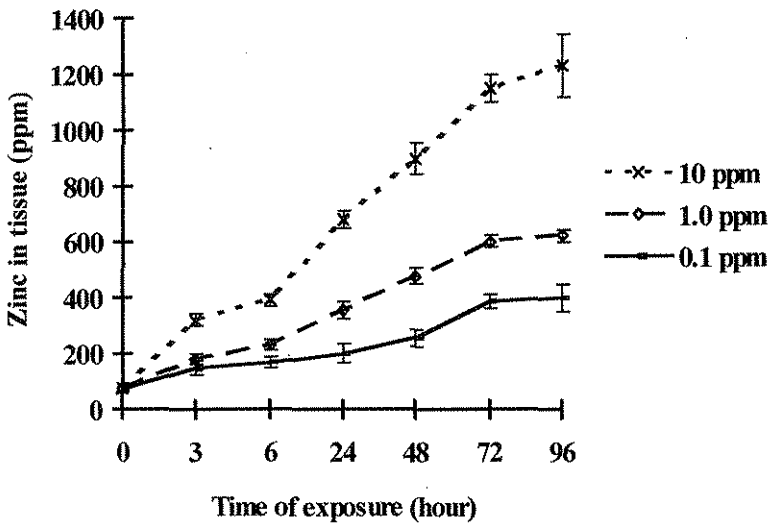


Figure 2. Mean concentrations of zinc in *Corophium* at 0, 3, 6, 24, 48, 72 and 96h static bioassay of sea water with uncontaminated sediment. Each value shows the mean and standard error of three replicates.

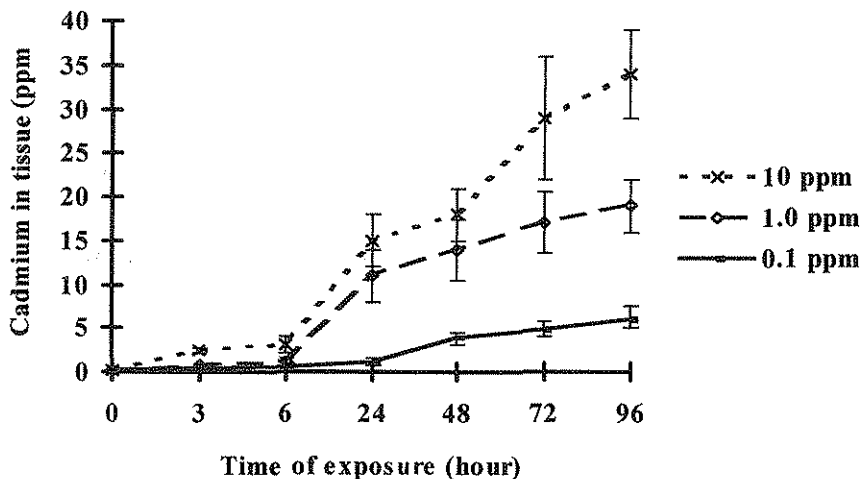


Figure 3. Mean concentrations of cadmium in *Corophium* at 0, 3, 6, 24, 48, 72 and 96h static bioassay of sea water with uncontaminated sediment. Each value shows the mean and standard error of three replicates.

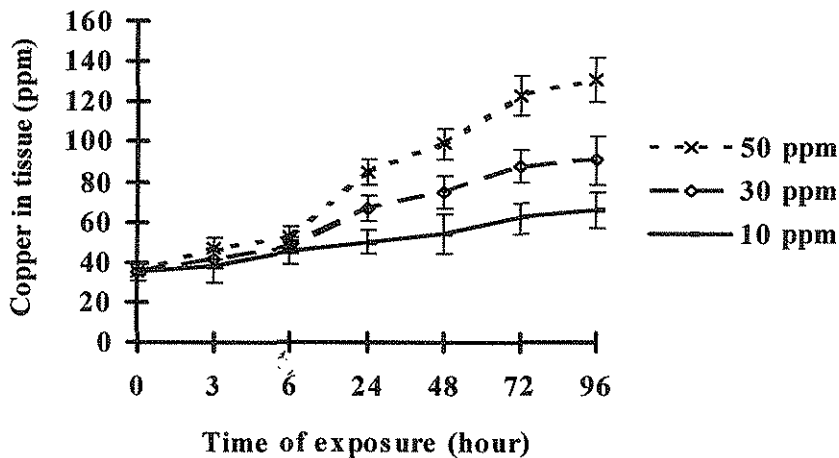


Figure 4. Mean concentrations of copper in *Corophium* at 0, 3, 6, 24, 48, 72 and 96h static bioassay of sediment with uncontaminated sea water. Each value shows the mean and standard error of three replicates.

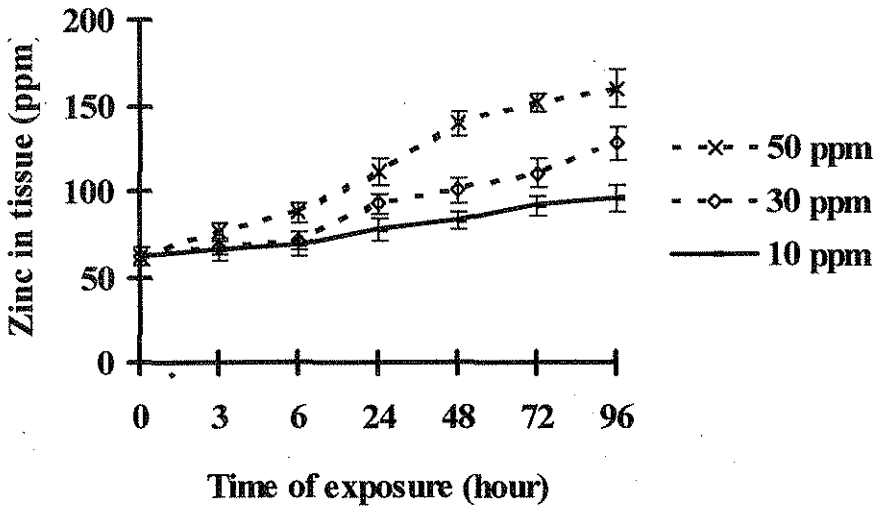


Figure 5. Mean concentrations of zinc in *Corophium* at 0, 3, 6, 24, 48, 72 and 96h static bioassay of sediment with uncontaminated sea water. Each value shows the mean and standard error of three replicates.

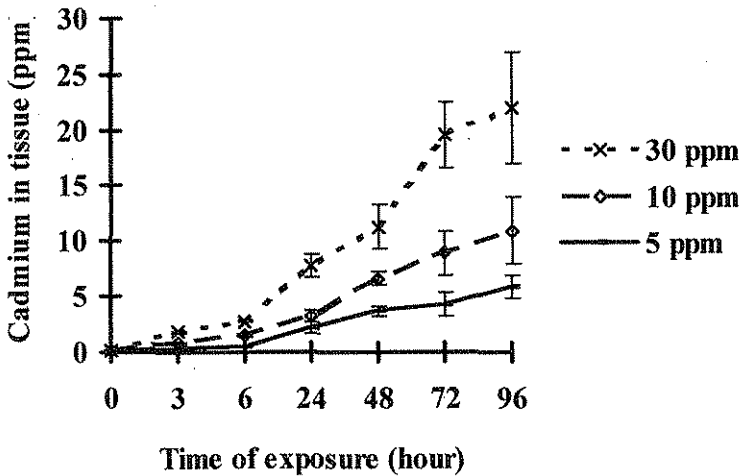


Figure 6. Mean concentrations of cadmium in *Corophium* at 0, 3, 6, 24, 48, 72 and 96h static bioassay of sediment with uncontaminated sea water. Each value shows the mean and standard error of three replicates.

4. Discussion

The present study showed that copper was less toxic to *C. volutator* than either zinc or cadmium. Bryan (1976a and 1976b) reported one of the most Cu resistant organisms is the estuarine amphipod *C. volutator*. Bryan and Hummerstone (1971) pointed out that animals including the amphipod *C. volutator* from sediments containing high concentrations of copper had developed a resistance to its toxic effects. Icely and Nott (1980) suggested that the tolerance of *C. volutator* to high concentrations of copper in the environment might be partially attributable to the formation of intracellular granules within the cells of the alimentary canal.

The present study showed that the amphipod *C. volutator* would accumulate copper, zinc and cadmium from both water and sediment. The uptakes of copper, zinc and cadmium from sediments and solutions by *C. volutator* have taken place either by direct uptake of these metals through the body cuticle or by ingestion of sediment during feeding. Marine invertebrates can take up passively heavy metals even when external total metal concentrations are low (Rainbow, 1990). Pesch and Morgan (1978) suggested that the animals in the sediment might regulate the Cu better, perhaps by controlling the flow of water circulated through the burrows, thus, being exposed to less Cu, or by being able to excrete Cu from the body more effectively. McLusky and Phillips (1975) showed that it was the rate of copper uptake and not the actual amount of copper which determined acute effects on the polychaete *Phyllodoce maculata*. Pesch and Morgan (1978) found similar results for copper in the polychaete *Neanthes arenaceodentata*, but they analysed only live worms unlike McLusky and Phillips (1975) and this difference in protocol makes comparisons between the two studies difficult. Milanovich *et al.* (1976) also obtained similar results with the polychaete *Ciriformia spirabrancha* and their conclusion was identical to McLusky and Phillips' (1975) hypothesis; that is, mortality was related not to the total amount of the metal accumulated but rather to the rate of uptake. These findings are consistent with the present study.

Direct uptake rate from sea water occurs both by adsorption of the elements across the surface, such as gut wall. The uptake rate generally decreases until a steady is reached between the element in the water and the organism's tissue. It is not known whether this type of behaviour is due to the regulation of metal against changes in the environment or whether it is because the organism is basically very impermeable to metal and the initial uptake is mainly due to saturation of the body surface with the metal (Bryan, 1976a). Accumulation from water tends to be more important in smaller organisms than larger forms principally because of the greater relative surface to volume (weight) ratio for adsorption in the former, and also higher metabolic rates of these organisms. Absorption and tissue distribution of ingested metals depend of the bioavailability of the element. Biologically essential elements such as Cu and Zn are rapidly absorbed across the gut and assimilated into tissues of organisms. Heavy metals in marine invertebrates are eliminated in either soluble or particulate forms (Rainbow,

1990). Marine invertebrates can remove metals from their bodies variety of routes (Bryan, 1968 and 1971 ; Bryan and Hummerstone, 1971 and 1973 ; Rainbow, 1990). For example copper in *C. volutator* might be released into the lumen of the alimentary tract when the epithelial cells complete their cell cycle (Icely and Nott, 1980). Despite the multitude of parameters affecting the rate of elimination from aquatic biota, most studies indicate that pollutants are lost more slowly than they are accumulated.

Comparisons with controls indicated that amphipods accumulated more metals from the water than those from the sediment, and the concentrations of the all metals in animals continued to increase as the concentrations of the metals in the water and in the sediment and the duration of the experiment increased. However, it is difficult to determine experimentally the relative importance of uptake from solution, food or organic particles (Bryan,1976a). It may be suggested that copper, zinc and cadmium from sediment was less available to the amphipod *C. volutator* than from sea water. There are at least two possible reasons for this. The dilution of toxic sediment with clean or uncontaminated sea water may reduce the amount of toxicant available to *C. volutator*, thus reducing the dose of contaminants that amphipods may obtain directly from the sediments.

Alternatively due to their requirement for a suitable sediment type in which to burrow, exposure to contaminated sea water through the burrows may have been stressful in the first experiment. *C. volutator* is frequently exposed to overlying water as water is pumped through the tube. The limited animal-sediment contact thus reduces the changes of direct exposure of this animal to the particle-bound contaminant in the sediment. Animal-sediment relations must be considered in evaluating the relative sensitivity if different species to sediment contaminants. Pesch and Morgan (1978) showed that the present of uncontaminated sediment affected copper concentrations in the polychaete *Neanthes arenaceodentata*. It was suggested that the animals in the sediment might regulate the copper better or were able to excrete copper from their body more effectively (Pesch and Morgan, 1978), but in some related polychaetes such as *Nereis diversicolor* and *Nephtys hombergi* copper appeared not to be regulated (Bryan and Hummerstone, 1971; Bryan, 1976a). Similarly, Jackim *et al.* (1977) found that the bivalve *Mya arenaria* took up the greatest amount of cadmium when kept without a substrate and progressively less when kept in sediment. However in case of the bivalve *Mulina lateralis*, the difference was not statistically significant, indicating that different environmental habits and feeding types within a taxonomic group appear play a significant role (Jackim *et al.*, 1977). Pesch (1979) found that when the polychaete *Neanthes arenaceodentata* was exposed to copper-spiked sand, silt and a mixture of the two, survival was a function of sea water copper levels which were dependent on sediment grain size. These findings emphasise the importance of the organic content of sediment in reducing metal toxicity to infaunal organisms. Fowler (1982) and Jackim *et al.* (1977) have pointed out that metal uptake processes vary with environmental conditions such as temperature, salinity and sediment type and it would be useful to repeat these experiments on *C. volutator* over a range of these variables.

Amphipods, crustaceans, particularly those of the genus *Corophium* have been used as test animals in aquatic and sediment toxicity (Chapman, 1992, Chapman *et al.*, 1992; Hill *et al.*, 1993; Tay *et al.*, 1992; Bat, 1995a,b). Amphipods including *C. volutator* are important components of marine food web and toxicity tests with these animals can therefore be seen to have considerable environmental relevance. On account of their size, amphipods are easier to handle than microcrustacea, whilst still requiring considerably less laboratory space and aquarium volume than fish.

In conclusion, the amphipod *C. volutator* was a suitable test species to assess toxicity tests. The advantages of the bioassay included its rapid toxicity assessment and screening method, short duration, inexpensive method and precision for statistical analysis.

Özet:

Bu çalışmada, laboratuvar koşullarında hem deniz suyundan hemde sedimentten farklı konsantrasyonlardaki bakır, çinko ve kadmiyumun birikimleri deniz kabuklularından *Corophium volutator*'u (Pallas) kullanarak biyolojik birikim testleriyle tayin edilmiştir. *C. volutator*'lardaki bu metallerin konsantrasyonları 3, 6, 24, 48, 72 ve 96 saat aralıklarla ölçülmüştür. *C. volutator*'lardaki metal düzeyleri ile deniz suyu ve sedimentteki metal düzeyleri arasında pozitif ilişki mevcuttur. Ayrıca sonuçlar *C. volutator*'un bakır, çinko ve kadmiyumun sudan alınımının sedimentten alınımına göre daha yüksek olduğunu göstermiştir.

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