Bull. Environ. Contam. Toxicol. (1997) 59:500-506

Bulletin of Environmental Contamination and Toxicology

Influence of Copper, Zinc, and Iron on Cadmium Accumulation in the Talitrid Amphipod, *Platorchestia platensis*

S. Casini,[1] M. H. Depledge[2]

[1]Department of Environmental Biology, University of Siena, Via Delle Cerchia 3, 53100 Siena, Italy
[2]Plymouth Environmental Research Centre, and Department of Biological Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, United Kingdom

Received: 5 September 1996/Accepted: 10 May 1997

Conventional ecotoxicological test procedures are designed to evaluate and rank the relative toxicities of a diverse range of chemicals singly, under controlled laboratory conditions. *In situ,* however, animals and plants are almost invariably exposed to complex chemical mixtures (Anderson *et al.* 1994). The extent to which components of such mixtures interact synergistically, antagonistically or additively with regard to uptake and toxicity has seldom been determined. This raises the question of how well routine laboratory-based tests are able to predict the extent of chemical impacts in natural ecosystems. Ecotoxicological assessment procedures are currently unable to address this problem since the infinite variety of mixture compositions and concentrations that might arise would demand an unreasonable allocation of resources. Therefore, safety factors are employed to guard against unexpected interactions. Although this precaution is necessary at present, there is a growing literature documenting interactive effects (or the lack of them) among various pollutants which may, in the future, allow key chemicals, that are likely to interact with strong synergisms or antagonisms, to be identified. In particular, certain trace metals have been shown to influence one another's uptake and toxicity. For example, the amelioration of mercury toxicity by selenium has been well documented (Koeman *et al.* 1973). Other metals which influence one another's uptake and/or toxicity include combinations of mercury, lead and zinc (Gray 1974) iron and mercury (Fowler *et al.* 1975) cadmium and selenium (Bjerregaard. 1988) and copper and manganese (Sunda & Huntsman 1983). Synergisms have also been documented between trace metals and natural environmental stress factors (Gray 1974).

Despite these and other documented cases of interactions (see Depledge *et al.* 1994) the implications of interactive effects have seldom been considered in the context of environmental biomonitoring (see for example, Bayne 1989). If mixtures of metals do influence one another's uptake, then biomonitor organisms might provide an inaccurate indication of the actual environmental concentrations of specific metals.

The purpose of the present study was to determine whether simultaneous exposures to two or more essential trace metals influences the uptake of non-essential cadmium via the seawater route in the putative biomonitor amphipod species, *Platorchestia platensis.*

Correspondence to: S. Casini

MATERIALS AND METHODS

Talitrid amphipods *(Platorchestia platensis)* were collected from Bøgebjerg Strand (North-East Fyn, Denmark) and transferred to the laboratory in polythene bags together with accompanying strandline material from the original site. Three days later, 120 individuals were chosen at random and rinsed in double-distilled water. They were then placed in glass aquaria (5 animals per aquarium) containing 0.5 l of continuously aerated seawater. Water temperature was maintained at 14.5 °C and salinity was held constant at 20‰. The trace metals cadmium, copper, iron and zinc were then added to the aquaria following the regime shown in Table 1. Nominal (analysis of test water were not performed) metal concentrations of 100 µg l^{-1} were obtained by the addition of the appropriate chloride salt of each metal.
Treatment groups comprised three replicate aquaria containing a total of 15 animals. Seawater was changed every three days, at which times new metal was added.

After 10 days of exposure to various metal combinations, test animals were rinsed in double-distilled water and frozen individually in polythene bags. They were then freeze-dried and digested in 65% nitric acid (analytical grade, Merck, FRG). The residue was dissolved in 2 ml of 0.2M HNO_3. Each sample was subsequently analyzed in duplicate for total cadmium by atomic absorption spectrophotometry using a Perkin-Elmer 2380 spectrophotometer. Air-acetylene flame and deuterium background correction were used (Bjerregaard 1988). The detection limits were 0.001 µg/ml. The accuracy of the results was verified by analysing an NBS oyster standard with a certified cadmium concentration of 3.5 ± 0.4 µg Cd g^{-1} dry wt that was found to contain 3.1 and 3.2 µg Cd g^{-1} dry wt (duplicate determinations). The precision of analysis was assessed by determination of 10 replicates of the same sample and was found to be below 10%. Added cadmium on the biological material used in the analysis (homogenate of amphipods) shows 98% of recovery.

Table 1. Trace metals' exposure regime. Amphipods were exposed to various combinations of cadmium, iron, copper and zinc at nominal concentrations of 100 µg /l for 10 days. The exposures were performed in triplicate.

Aquaria	Cd (100µg /l)	Fe (100µg/l)	Cu (100µg/l)	Zn (100µg/l)
1 - 3	+	-	-	-
4 - 6	+	+	-	-
7 - 9	+	-	+	-
10-12	+	+	+	-
13-15	+	-	-	+
16-18	+	+	-	+
19-21	+	-	+	+
22-24	+	+	+	+

(+) Presence of metal in the tank water; (-) Absence of metal in the tank water

Log transformation and analysis of variance of cadmium concentration data were performed using STATISTICA software (Statsoft Inc.).

RESULTS AND DISCUSSION

Mean cadmium concentrations for individuals from each aquarium were first determined. They ranged from ca. 3 to 23 ug Cd g dry weight^{-1}. These values were then log-transformed. This removed effects due to small differences in conditions among the replicate aquaria. Analysis of variance of the data (3 values per exposure regime) (Table 2) indicated that simultaneous exposure to copper and cadmium was associated significantly with decreased cadmium uptake compared to that seen in amphipods exposed to cadmium alone ($p < 0.001$) (median values Cd alone: 6.11 ug Cd g^{-1} dw and for Cd & Cu: 5.37 ug Cd g^{-1} dw; Fig.1).

In contrast, simultaneous exposure to Fe and Cd resulted in enhanced Cd uptake (8.97 µ Cd g^{-1} d.w.; Fig. 1) compared to that seen following exposure to cadmium alone ($p = 0.012$). No statistically significant differences in Cd uptake were detected when amphipods were exposed simultaneously to combinations of Cu & Zn, Fe & Zn and Cu & Fe & Zn.

Data presented above indicate that interactions occurred between copper and cadmium, and iron and cadmium that were reflected in altered cadmium uptake. It was also noted that interactions occurred between copper and iron. The influence of the presence or absence of iron and copper on cadmium uptake is shown in Figure 2A. Simultaneous exposure to Cd, Fe and Cu was associated with a marked decrease in Cd uptake ($p= 0.012$). Figures 2B and 2C indicate that the presence of Fe & Zn had little or no influence on cadmium uptake in the amphipods.

Table 2. Amphipods exposed to various combinations of cadmium, iron, copper and zinc at nominal concentrations of 100 µg/l for 10 days;analysis of variance of cadmium uptake log-transformed data.

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F RATIO	P
Cu	1.500	1	1.500	18.000	0.001
Fe	0.667	1	0.667	8.000	0.012
Zn	0.167	1	0.167	2.000	0.176
Cu-Fe	0.667	1	0.667	8.000	0.012
Cu-Zn	0.167	1	0.167	2.000	0.176
Fe-Zn	0.000	1	0.000	0.000	1.000
Cu-Fe-Zn	0.000	1	0.000	0.000	1.000
ERROR	1.333	16	0.083		

In recent years, the talitrid amphipod *Platorchestia platensis* has been proposed for use in biomonitoring studies of metal contamination (Weeks & Rainbow, 1991). This species accumulates trace metals from seawater in proportion to the ambient metal contamination, when exposed to metals singly (Weeks *et al.* 1992). However, the data presented here indicate that simultaneous exposure to copper or iron can significantly alter the uptake of cadmium in this species. This raises important questions about the value of the amphipod as a biomonitor, and indeed other organisms used in biomonitoring studies which are carried out to assess the extent of environmental contamination.

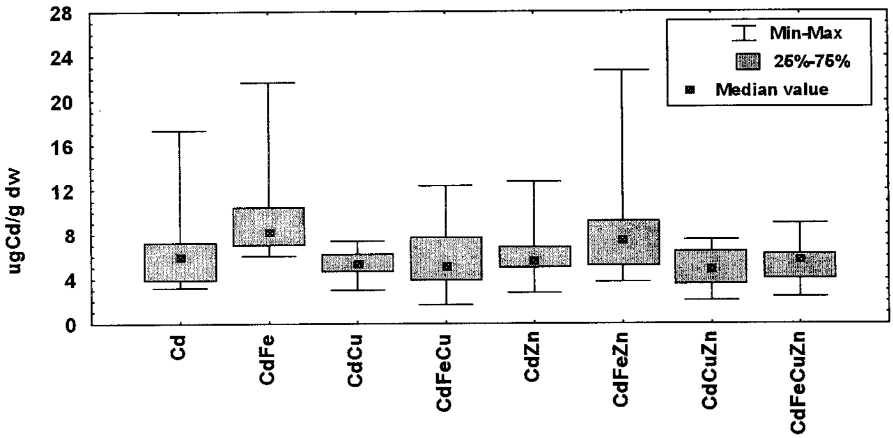

Figure 1. Median values, minimum and maximum values, and 25% - 75% cadmium concentrations in the amphipods following 10 days exposure to the 8 different experimental regimes, (Cd; Cd + Fe; Cd + Cu; Cd + Fe + Cu; Cd + Zn; Cd + Fe + Zn; Cd + Cu + Zn; Cd + Fe + Cu + Zn). The exposure concentration for each metal was nominally 100 µg/l.

Information regarding the mechanisms by which one metal influences the uptake of another is still sparse. Apparently, important factors are changes in metal speciation which influence bioavailability, and competition among bioavailable chemical species at sites of metal entry (Bjerregaard and Depledge 1994). Some metals appear to be taken up into organisms from solution only as free ions, while others are transported across biological membranes as inorganic complexes (see Depledge *et al.* 1994 and references therein). In experiments in which the free species of copper and cadmium were either carefully controlled by organic chelators or accurately measured using ion selective electrodes, the toxicity (and thus probably bioavailability) was correlated with the concentration of free metal ions rather than total dissolved metal concentration (Zamuda & Sunda 1982).

It has long been known that bulk metals, such as calcium, influence trace metal uptake concentrations in tissues. For example, Bjerregaard & Depledge (1994) demonstrated that for the gastropod *Littorina littorea,* the bivalve *Mytilus edulis* and the shore crab, *Carcinus maenas,* calcium ion concentration exerts differing degrees of influence on cadmium uptake and thereby partially or wholly counteracts changes in cadmium bioavailability as salinity changes. As chloride complexation of cadmium ions in seawater falls with reductions in salinity, one might expect cadmium uptake to increase. However, if the calcium concentration in seawater is increased as salinity is reduced, cadmium uptake also falls. This is thought to result from a competitive interaction of cadmium and calcium ions for active uptake *via* calcium pumps (Bjerregaard and Depledge 1994). It is not known to what extent trace metals such as copper or iron might influence cadmium uptake by similar mechanisms.

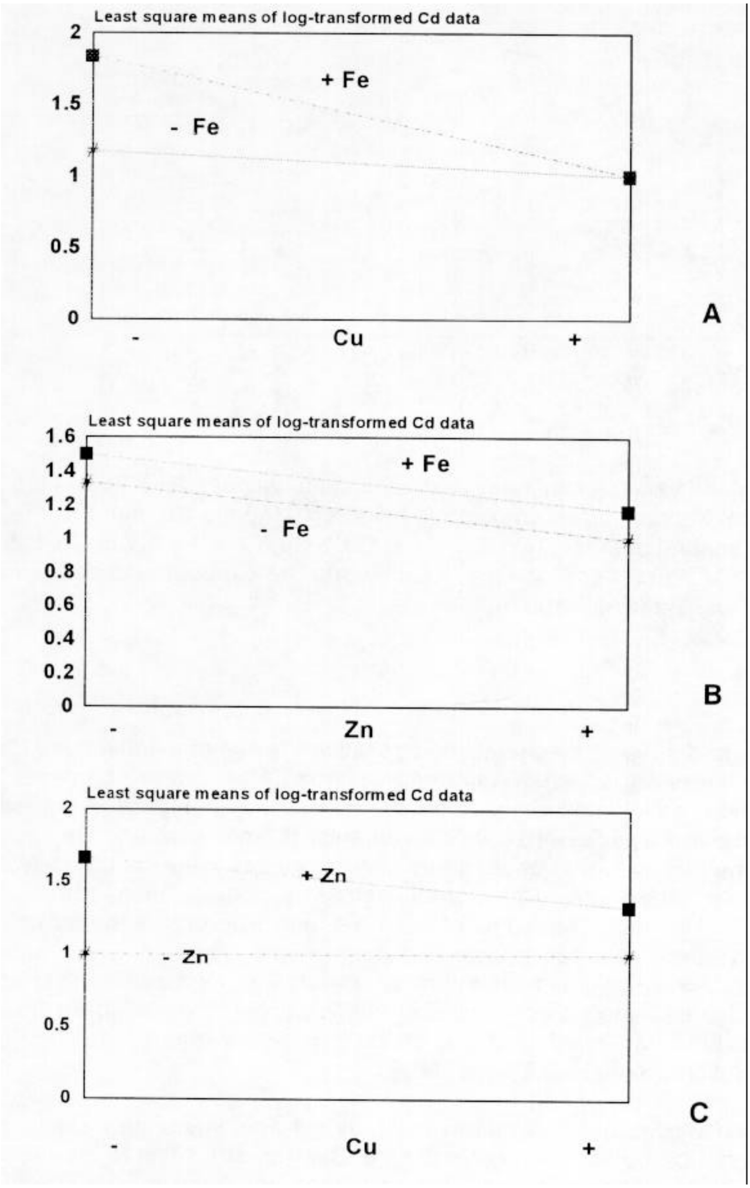

Figure 2. (**A**) Cu-Fe interaction in Cd uptake ($p<0.012$) and lack of interaction between Fe-Zn (**B**) and Zn-Cu (**C**); (-) absence of metal, (+) presence of metal.

As was mentioned earlier, mercury/selenium interactions are quite well documented. In many marine vertebrates, mercury and selenium concentrations are positively correlated in specific tissues (Koeman *et al.* 1973). Low concentrations of selenium have also been found to reduce mercury uptake in *Mytilus edulis* (Davies & Russell 1988).

Iron-mercury interactions have been described in the bivalve *Mercenaria mercenaria.* Mercury concentrations in the range 0.1 to 1.0 mg 1^{-1} resulted in reduced iron concentrations in the mantel fringe tissues of the clams (Fowler *et al.* 1975). Also, low concentrations of selenium reduce mercury uptake in *Mytilus edulis* (Davies & Russell 1988).

Copper-manganese interactions may have special significance with regard to phytoplankton growth. Thus, with rising manganese ion availability, the growth rate of *Thalassiosira pseudonana* increases. However, simultaneous addition of copper resulted in competitive inhibition of manganese uptake and consequently, slower growth rates (Sunda & Huntsman, 1983). Copper/manganese interactions have also been observed in lobsters, *Nephrops norvegicus* exposed to hypoxis *in situ* (Baden *et al* 1992).

Another way in which one metal may influence another's tissue concentration is *via* exchange. Engel & Brouwer (1989) proposed that during the molt cycle of the crab *Calinectes sapidus,* copper availability increased at ecdysis (perhaps resulting from haemocyanin catabolism and copper release) binding to metallothionein in the midgut gland displacing zinc as it does so. Consequently, as copper concentration in the midgut gland increases zinc concentration falls.

One final consideration when studying interactions among trace metals is that observed correlations between the concentrations of two metals in an organism may not be causative. Correlations may merely reflect a common dependency of the two metals on a third factor, such as the weight or age of the organism or the water content of a particular tissue (Depledge 1990). Statistical techniques are available for dealing with this problem.

In summary, the present study demonstrated that when amphipods are exposed to mixtures of trace metals, interactions may occur which influence metal uptake, and presumably toxicity. Further studies are required to elucidate the mechanisms involved and to quantify the extent of interactions among metals and with other pollutants. In the meantime, the results of biomonitoring studies should be treated with caution if complex mixtures or metals, and indeed other contaminants, are suspected at sampling sites. These considerations are especially relevant to the interpretation of data from major studies such as the Global Mussel Watch programme (Bayne 1989). Trace metal concentrations measured in mussels collected from coastal areas around the world may well have been influenced by metal-metal interactions, and, indeed, metal-xenobiotic interactions. This matter warrants further investigation.

REFERENCES

Anderson S, Sadinski W, Shugart L, Brussard P, Depledge M, Ford T, Hose J, Stegeman J, Suk W, Wirgin I, Wogan G (1994) Genetic and molecular toxicology: A research framework. Environ Hlth Perspec 102:3 - 8

Bayne BL (1989) Measuring the biological effects of pollution: the mussel watch approach. Wat Sci Tech 21:1089-1100

Baden SP, Depledge MH, Hagerman L (1992) Glycogen depletion and altered copper and manganese handling in *Nephrops norvegicus* following starvation and exposure to hypoxia. Mar Ecol Prog Ser 103:65-72

Bjerregaard P (1988) Effect of selenium on cadmium uptake in selected benthic invertebrates. Mar Ecol Prog Ser 48:17-28

Bjerregaard P, Depledge MH (1994) Cadmium accumulation in *Littorina littorea, Mytilus edulis* and *Carcinus maenas:* the influence of salinity and calcium ion concentrations. Mar Biol 119:385-395

Davies IM, Russel R (1988) The influence of dissolved selenium compounds on the accumulation of inorganic and methylated mercury compounds from solution by the mussel *Mytilus edulis* and the plaice *Pleuronectes platessa.* Sci Tot Environ 68: 197-205

Depledge MH (1990) Interactions between heavy metals and physiological processes in estuarine invertebrates. In:Chambers PL & Chambers CM (eds) Estuarine Ecotoxicology, Japaga, Wicklow, p 89

Depledge MH, Bjerregaard P, Weeks JM (1994) Heavy metals. In: Calow P (ed) Handbook of Ecotoxicology vol. 2, Blackwell Publ., Cambridge, p 79

Engel DW & Brouwer M (1989). Metallothionein and metallothionein-like proteins: Physiological importance. In: Advances in Comparative and Environmental Physiology, Springer-Verlag, Berlin, p 53

Fowler BA, Wolfe DA, Hettler WF (1975) Mercury and iron uptake by cytochromes in mantle epithelial cells of quahog clams *(Mercenaria mercenaria)* exposed to mercury. J Fish Res Bd Can 32:1767-1775

Gray JS (1974) Synergistic effects of three heavy metals on growth rates of a marine ciliate protozoan. In: Vernberg FJ, Vernberg WB (eds) Pollution and Physiology of Marine Organisms, Academic Press, London, p 465

Koeman JH, Peeters WHM, Koudstaal-Hol CHM, Tjioe PS, de Goeij JJM (1973) Mercury-selenium correlations in marine mammals. Nature 245:385-386

Sunda WG, Huntsman SA (1983) Effect of competitive interactions between manganese and copper on cellular manganese and growth in estaurine and oceanic species of the diatom *Thalassiosira.* Limnol Oceanogr 28:924-934

Weeks JM. Rainbow PS (1991) The uptake and accumulation of zinc and copper from solution by two species of talitrid amphipods (Crustacea). J Mar Biol Assoc U.K. 71:811-826

Weeks JM, Rainbow PS, Moore PG (1992) The loss, uptake and tissue distribution of copper and zinc during the moult cycle in an ecological series of talitrid amphipods (Crustacea: Amphipoda). Hydrobiologia 245: 15-25

Zamuda CD, Sunda WG (1982) Bioavailability of dissolved copper to the American oyster *Crassostrea virginica.* I. Importance of chemical speciation. Mar Biol 66:77-82.