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THE BIOLOGICAL ASSESSMENT OF TOXIC METAL IMPACTS ON RECEIVING WATER QUALITY, UK

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ABSTRACT Mortality rates and metal uptake rates were determined for total and selected body areas of *Gammarus pulex* and *Asellus aquaticus* under both in- stream and laboratory test conditions. Metal equilibrium concentrations are achieved in the soft-tissue fraction within 5 to 6 weeks of field exposure with four to five fold increases over background control levels noted for caged organisms exposed to intermittent polluted urban discharges. The controlled toxicity tests show higher metal uptake rates in comparison to the field bioassays, with the latter indicating a possible discrimination between chronic and acute impacts. The importance of using sediment water quality criteria as distinct from ambient water phase quality is also stressed in determining chronic exposure rates for organisms in polluted urban receiving waters. The use of LC_{20} values for very short term exposures and storm event periods is proposed for acute criteria which possess adequate safety margins.

INTRODUCTION

Environmental legislation is primarily based on a toxicity analysis approach with ambient pollutant concentrations for specified exposure periods forming the basis for the development of water quality criteria. The methods used to identify such standards for environmental protection are essentially based on fixed dose-response rates determined from conventional bioassays. The importance of establishing links between (laboratory and field) toxicological and ecological data as a prerequisite for environmental management applications has been emphasized by many workers including Williams *et al.* (1984).

This paper provides an assessment of an in-stream testing approach employing benthic organisms for the development of toxic metal criteria and compares this field experience with conventional laboratory toxicity procedures. Martin & Coughtrey (1982) have outlined the criteria for selecting organisms as either indicators or monitoring agents of heavy metal pollution and suggest that the tissue concentrations of the latter group should be related to contamination levels. Given the importance placed on benthic detritivore species as base components of the food web in aquatic organisms (Mance, 1987), *Gammarus pulex* (L) and *Asellus aquaticus* (L) were selected as appropriate organisms for the monitoring of in-stream metal uptake. *Asellus* is widely distributed in urban waters, whereas *Gammarus* is rarely recorded in routine enumerations of sites subject to transient urban discharges, but has been consistently found on colonization samplers installed at such sites (Bascombe *et al.*, 1988). This suggests that its absence may be related to loss of habitat from channelization and either acute stresses imposed by extreme episodic events and/or to chronic exposures following storm disturbance of benthal sediments which have been contaminated by the intermittent polluted discharges. Both *Gammarus* and *Asellus* are detritivores but their contrasting levels of activity make them particularly suitable complementary biomonitors of metal uptake at the critical sediment-water interface and their respective responses are discussed in this paper.

MATERIALS AND METHODS

To investigate the site-specific behavior of macroinvertebrates exposed to ambient metal stress, *Gammarus pulex* and *Asellus aquaticus* were collected from an unpolluted upstream site (Site 1) on a tributary of the Salmon's Brook which is situated in northeast London. Fifty individuals of each species were transferred to separate cages fixed at the first site and four downstream locations. Site 2 is located on the Salmon's Brook on the outer fringes of the urban area and has few contributing point sources, whereas sites 3 and 4 are located downstream of surface water discharged from a busy highway and an industrial area, respectively. Site 5 is situated immediately below a major storm sewage overflow.

The cages consist of small plastic containers (1 l internal volume) with windows of nylon mesh (1 mm pore size) to allow throughflow of water. The cages were firmly secured by clips and plastic coated wire to a basal concrete slab which was anchored to the river bed by two stakes.

TABLE 1 Mean sediment and water concentrations for Cu, Pb, and Zn.

Location	Metal	Sediment ($\mu g\ g^{-1}$)	Water insoluble ($\mu g\ l^{-1}$)	Water soluble ($\mu g\ L^{-1}$)
Site 2	Pb	42	12	7
	Cu	30	11	15
	Zn	91	44	121
Site 3	Pb	112	12	7
	Cu	82	10	16
	Zn	182	47	157
Site 4	Pb	186	15	8
	Cu	99	11	18
	Zn	228	56	161
Site 5	Pb	232	23	9
	Cu	251	18	24
	Zn	495	118	318

At weekly intervals, four individuals of each species were recovered from the cages and the separate samples were analyzed for copper (Cu), lead (Pb), and zinc (Zn) to assess the dry-weight effects of exposure to ambient metals. The total tissue metal levels were determined and three target areas were differentiated; the weakly adsorbed surface fraction of available metal which was initially removed by a dilute acid wash; absorbed metal within the body tissues which was separated by dissection of invertebrates into soft tissue and hard exoskeleton fractions. After digestion in a 9:1 (v/v) nitric: perchloric acid mixture, samples were taken up in dilute nitric acid, filtered and analyzed for the above metals by anodic stripping voltammetry. Sediment particulate and dissolved Cu, Pb, and Zn concentrations were determined by atomic absorption spectrophotometry for samples collected at monthly intervals from sites 2-5.

In addition to the in-stream experiments, laboratory toxicity tests were carried out in which *Gammarus* and *Asellus* individuals from the background site (site 1) were exposed to fixed metal concentrations. A continuous-flow toxicity testing apparatus was designed for this purpose, based on that described by Green and Williams (1983). Invertebrates were simultaneously exposed in parallel tests to concentrations of 0, 50, 100 and 500 $\mu g\ l^{-1}$ of each of the metals as solutions of their nitrate salts in water collected from the background site. When mortality in the control reached 5% of the test organisms, the associated series of tests was terminated. At least one duplicate of each test run was carried out. After 0.5, 1, 2, 4, 8, 12, 24 hours, and subsequently at 24 hourly intervals, any dead individuals were counted and removed and tissue metal concentrations were determined in living individuals at 24 hour intervals. The uptake behavior of the metals concerned was assessed in relation to the mortality of both species.

RESULTS AND DISCUSSION

In-stream metal uptake

The changes in mean total tissue concentrations of Pb and Zn in caged *Gammarus* over 7 week exposure periods are shown in Fig. 1a. Pb concentrations (25-30 $\mu g\ g^{-1}$) remain low at the control site and at sites 2 and 3 in both species. At site 4, some bioaccumulation is apparent in *Gammarus* but a marked progressive elevation of tissue Pb concentration occurs in *Asellus*, reaching equilibrium levels in excess of 90$\mu g\ g^{-1}$ over five week exposure periods. At site 5 there is a rapid initial metal uptake rate to establish equilibrium concentrations of 130$\mu g\ g^{-1}$ and 110$\mu g\ g^{-1}$ in *Gammarus* and *Asellus* after four and five weeks respectively.

Tissue Zn concentrations in both species show a pronounced elevation in the first week of exposure at the two most polluted downstream sites followed by a slower linear uptake to equilibrium values of 100 and 190 $\mu g\ g^{-1}$ for site 4, and 200 and 240 $\mu g\ g^{-1}$ for site 5 in *Gammarus* and *Asellus*, respectively. Broadly similar trends to those observed for Zn were also obtained for Cu with *Gammarus* and *Asellus* attaining equilibrium concentrations of 100 $\mu g\ g^{-1}$ and 90 $\mu g\ g^{-1}$, respectively. At the downstream site there is an overall increase in maximum equilibrium tissue concentrations of between four and five times compared to the background levels for Pb, Cu and Zn.

The enhancement of ambient water and sediment metal levels at site 5 (Table 1) clearly produces biomagnifications in caged *Gammarus* as indicated by the total tissue metal levels (Fig. 1a and Table 2). A comparison between caged organisms transferred from the background control site (site 1) and from site 3 to site 5 below the storm sewer overflow indicates very similar total tissue metal equilibrium concentrations, irrespective of the original location of the organism (Table 2). As stated in the introduction, natural populations of *Gammarus* were absent from the polluted sites 4 and 5. Additionally there was no marked variation between the observed cumulative percentage mortalities of either of the transferred caged sets over the exposure period. The data would suggest that there is no significant acquired metal pollution tolerance shown by the organisms after they had been transferred downstream.

TABLE 2 Mean Total Tissue Concentrations for Pb, Cu and Zn in (a) Free living organisms and (b) caged organisms; NF is not found.

Organisms	Site	*Gammarus* (μg g^{-1}) Pb	Cu	Zn	*Asellus* (μg g^{-1}) Pb	Cu	Zn
(a)	2	42	44	94	65	75	126
(a)	3	61	59	106	67	77	130
(a)	4	NF	NF	NF	85	94	227
(a)	5	NF	NF	NF	212	220	525
(b)	1 to 5	130	100	200	110	90	240
(b)	3 to 5	130	102	215	-	-	-

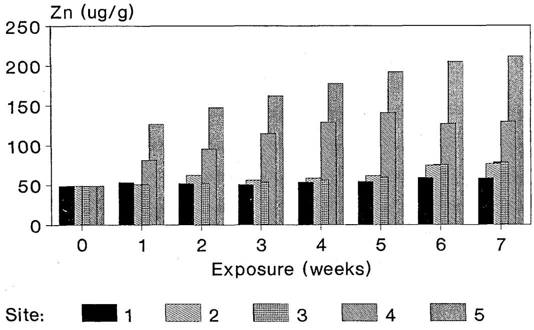


FIG. 1a Zn uptake by caged Gammarus pulex at increasingly urbanized sites.

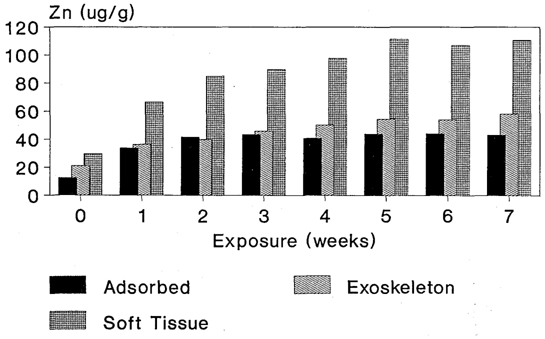


FIG. 1b Tissue distribution of Zn uptake in caged Gammarus pulex at site.

Gammarus species have been shown to filter some 0.16 l day^{-1} of water to satisfy their oxygen demands (Abel & Barlocher, 1988). This throughput is compatible with the observed rapid bioaccumulation of Zn at site 5, as concentrations of this metal are correspondingly higher than for Pb or Cu in the aqueous phase (Table 1). A *Gammarus* individual of 10 mg wet weight has been estimated to require about 0.15 mg day^{-1} of food (Abel & Barlocher, 1988), although there would be variation with food quality. Hence, the high Pb bioaccumulation rate can be explained by the observed elevation in metal concentrations of the particulate phase (Table 1).

All the metals, but particularly Zn, demonstrate a higher and equivalent tissue level in both caged and free-living *Asellus* at site 4 (Table 2) compared to site 3, despite only marginally elevated sediment, particulate and dissolved metal concentrations at the former site (Table 1). This may be explained by a change in substrate composition from gravel at site 3 to unconsolidated silts downstream which facilitate metal uptake through improved feeding rates. However, only a small elevation occurs in caged *Asellus* at site 5, whereas free-living organism tissue levels double in comparison to site 4. This discrepancy can be explained by the increased flow rates at site 5 and the consequent reduction of settled particles in the cages which are consumed by this substrate feeding detritivore. Furthermore, the nylon mesh of the cage windows limits access to particles with a diameter of < 1 mm, whereas natural populations have access to larger detrital particles. It is clearly desirable to compare tissue metal levels where possible in both captive and free-living populations to satisfactorily interpret metal uptake behavior. Both river channelization and intermittent hydraulic shocks are also comparable in importance to ambient pollution levels in determining the availability of habitats and the distribution of species in the natural environment. Such physical factors are likely to have a significant effect on spatial variation in metal uptake rates. These parameters have been effectively ignored in the experimental technique.

Separate tissue metal uptake

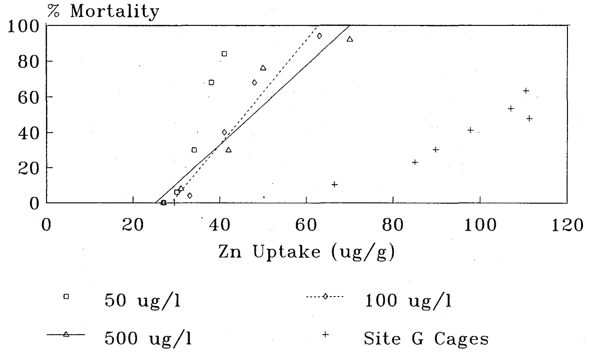
The differential uptakes of Pb and Zn by soft tissue, exoskeleton and surface adsorption at site 5 are shown for *Gammarus* in Fig. 1b. Equilibrium concentrations in caged *Gammarus* and *Asellus* for adsorbed Pb (25 and 25 µg g^{-1}), Zn (42 and 50 µg g^{-1}) and Cu (13 and 15 µg g^{-1}) are reached after 2-4 weeks, suggesting that the exoskeleton surface area is the limiting factor. The exoskeleton tissue metal concentrations, which normally exceed adsorbed concentrations, appear to reach equilibrium after five to six weeks attaining values of 32 and 25 µg g^{-1} for Pb, 54 and 62 µg g^{-1} for Zn, and 13 and 15 µg g^{-1} for Cu in *Gammarus* and *Asellus*, respectively. A progressive and rapid bioaccumulation of Pb and Zn is apparent in soft tissue for *Gammarus* (Fig. 1b) with equilibrium concentrations in of 90, 70 and 110 µg g^{-1} and in *Asellus* of 55, 50 and 130 µg g^{-1} for Pb, Cu and Zn respectively.

The equilibrium metal distributions in the different tissue components shown in Table 3 indicate the greater affinity of Pb and Cu for the adsorbed and exoskeleton fractions in *Asellus* compared to *Gammarus*. However, the high proportion and rapid rate of soft tissue metal bioaccumulation, particularly Pb and Cu in *Gammarus*, confirms the suitability of this target area for monitoring metal toxicity over relatively short exposure periods. It is possible that metal diffusion across the exoskeleton to the soft tissue may be complementing contaminated particle ingestion and uptake through the gills.

Comparison of mortality and metal uptake for the in-stream and laboratory experiments

The previous field observations regarding the soft tissue metal concentrations in *Gammarus* and *Asellus* provide an indication of the dose-response interaction between the organism and the ambient source metal levels. Therefore, in situations typical of urban river systems where intermittent inputs and changes in soluble metal levels can occur as a result of flow changes during storm conditions, the metal uptake values give an "averaged" indication of the impact of these short term variations. The relationships between metal uptake and mortality for *Gammarus* and *Asellus* at site 5 over seven week exposure periods are shown for Zn in Figs. 2a and b. After an initial tolerance which is consistent with their more

(a) Gammarus pulex



(b) Asellus aquaticus

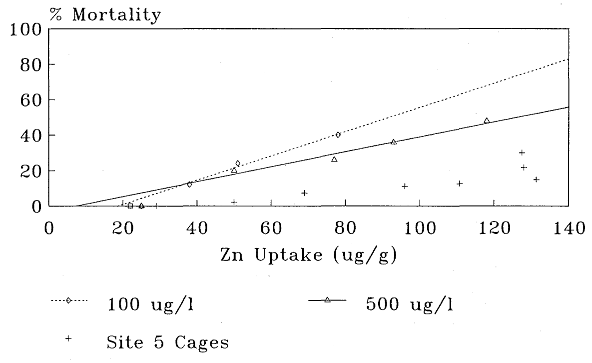


FIG. 2 Relationship between soft tissue Zn uptake and mortality under laboratory and field conditions.

efficient Zn compared to Pb uptake, both *Gammarus* and *Asellus* show higher mortality rates in response to small increases in the soft tissue concentration of this metal; this transition occurring at a higher tissue level in the case of *Asellus*. *Gammarus* shows a greater sensitivity to Cu uptake than either Zn or Pb and in-stream metal tissue results contrast with the toxicity order of Zn>Cu>Pb, which has been established for *Gammarus* dosing experiments (Bascombe *et al.*, 1990). *Asellus* also exhibits an enhanced sensitivity to Cu and Pb compared to Zn, which corresponds to laboratory toxicity test results for these metals.

The laboratory based metal uptake experiments show that the variation in mortality is virtually independent of the solution metal concentrations, with the exception of the most dilute Zn solutions (Figs. 2a and 2b). The lack of a marked discrimination between solutions of different metal concentrations is unexpected and suggests, in the case of the laboratory experiments, the existence of a threshold concentration above which a uniform - metal uptake relationship exists. The gradients of the plots for each of the metals are similar and considerably steeper than those for the in-stream experiments showing a greatly enhanced toxicity over the one week period of these tests. The slopes of the lines for *Asellus* are less steep than for *Gammarus*, indicating the greater tolerance of *Asellus* to elevated levels of these metals, in agreement with previous toxicity studies (Cowley, 1985; Martin & Holdich, 1986; Rehwoldt *et al.*, 1973). In this study the hardness remained at 230 mg l^{-1} $CaCO_3$ throughout the laboratory experiments and mean field levels were consistently of similar values, which would be expected to alleviate the toxic impact of the metals.

The increased toxicity demonstrated by the laboratory experiments may be partly explained by a greater metabolic activity resulting from the higher temperatures to which the test species were subjected. In addition, the organisms were being exposed almost entirely to free metal ions whereas only a small proportion of the soluble metal at site 5 would be present in this most bioavailable and toxic form. Previous studies of Cu, Pb and Zn speciation in urban river systems (Morrison *et al.*, 1984) have shown that only Zn has a tendency to exist in the free ionic or very weakly complexed forms with up to 50% of the soluble metal occurring as these species. The metal uptake and toxicity characteristics at site 5 are not, therefore, being controlled entirely by the bioavailable metal concentration but are being more generally influenced by the physico-biochemical interactions between the organism and its microhabitat at the sediment - water interface. The form of the metal uptake graphs at Site 5, as shown in Figs. 2a and 2b, can be interpreted in terms of an initial linear accumulation to critical uptake levels followed by a more rapid increase in mortality rates, as equilibrium levels are subsequently achieved. The tissue levels of Pb and Zn associated with an in-situ mortality rate of 20% are 60 and 80 $\mu g\ g^{-1}$ for *Gammarus* and 55 and 130 $\mu g\ g^{-1}$ for *Asellus* (Bascombe et al 1990). These trends may well be reflecting chronic exposure effects, whereas the laboratory toxicity tests indicate the influence of acute events.

TABLE 3 Percentage equilibrium metal tissue distribution in caged organisms at site 5 (After 5-6 weeks).

	Pb		Cu		Zn	
	G	A	G	A	G	A
Absorbed	15.9	22.9	13.4	17.1	20.3	21.0
Exoskeleton	21.4	29.4	15.4	26.4	27.4	26.0
Soft Tissue	62.7	47.6	71.1	56.5	52.2	53.0

G = *Gammarus pulex* A = *Asellus aquaticus*

CONCLUSIONS

The results described in this paper demonstrate the inherent problems associated with the prediction of toxicity or mortality rates from tissue pollutant uptake rates. Water quality standards have traditionally been derived by consideration of LC_{50} values for a chosen test species over a 96 h period but such laboratory experiments cannot accurately simulate all aspects of the natural sediment-water environment particularly for short duration periods of between 1 to 12 h. Relationships between metal tissue levels and mortality can provide a realistic indication of how the organism interacts with variable metal concentrations over varying time periods. Laboratory based experiments are also inadequate in reproducing characteristic metal concentrations for the critical water, suspended particulate and sediment phases. The in-stream metal uptake experiments therefore provide a much more realistic indication of metal-induced mortality. A knowledge of the soft tissue metal level which corresponds to the transition in mortality between the chronic and acute phases in an appropriate monitoring organism may therefore provide an advanced warning of a potentially severe toxic pollution problem in the receiving water system. The importance of sediment quality criteria as distinct from ambient water phase quality is also of significance in determining chronic exposure rates for benthic organisms in polluted urban receiving waters. Further work is needed to develop realistic acute criteria which possess adequate safety margins such as LC_{20} values for very short term exposures and storm event return periods.

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