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LEVENT BAT

DAVE RAFFAELLİ

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Effects of Gut Sediment Contents on Heavy Metal Levels in the Amphipod *Corophium volutator* (Pallas)

Levent BAT*, Dave RAFFAELLI
Culterty Field Station, Department of Zoology, University of Aberdeen ,Newburgh, Ellon, Aberdeenshire, AB41 OAA, UK

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Abstract: Concentrations of copper, zinc and cadmium were determined in *Corophium volutator* (with and without gut contents) exposed for 4 and 10 days to contaminated sediment. Significant differences were observed in metal concentrations among individuals, where gut contents were removed and those there they were left intact. On the other hand, the concentrations of these metals in *Corophium* were always high when no time was given for amphipods to depurate their guts. Therefore, it is recommended that either depuration in seawater or removal of the gut should be carried out before analysing amphipods for metal levels.

Key Words: *Corophium volutator*, copper, zinc, cadmium, sediment, gut.

Barsakdaki Sediman İçeriklerinin Amfipod *Corophium volutator* (Pallas)'lardaki Ağır Metal Düzeylerine Etkisi

Özet: Kirletilmiş sedimanlara 4 ve 10 gün süreyle maruz bırakılan *Corophium volutator*'ların barsak içeriği alınmış ve alınmamış bireylerinde bakır, çinko ve kadmiyum konsantrasyonları ölçülmüştür. Barsak içeriği olan ve olmayan bu örneklerdeki metal konsantrasyonları önemli derecede farklı bulunmuştur. Diğer bir ifadeyle barsak içeriği alınmamış örneklerde bu metallerin konsantrasyonları daima yüksek olmuştur. Sonuç olarak amfipodların metal düzeylerini ölçmeden önce deniz suyuna konarak barsaklarını temizlemelerinin sağlanması veya barsaklarının alınması önerilmiştir.

Anahtar Sözcükler: *Corophium volutator*, bakır, çinko, kadmiyum, sediman, barsak.

Introduction

Gut contents can significantly influence the researcher's impressions of contaminant levels in marine invertebrates whenever whole organism assays are conducted (1-3). The levels of metals in the material within the gut must be accounted for if accurate estimates of tissue concentrations are to be made (4) and a range of protocols has been developed. For example, Miramand et al. (5) found that 16 hours in running seawater was sufficient to eliminate the gut contents of the amphipod *Corophium volutator,* the bivalve *Scrobicularia plana* and the polychaete *Arenicola marina* after 14 days exposure to contaminated sediment. In contrast, Icely and Nott (6) estimated that only 60% of *Corophium* depurate their guts in filtered seawater after 48 h, which is three times as long as the period recommended by Miramand et al. (5). Moreover, Icely and Nott (7) calculated that the average time for coarse material to pass through the gut was 9 min (range 4 to 24 min) so that contaminated fine material in the gut, which is usually retained for longer, could be eliminated by allowing the animals to replace the finer gut contents with clean coarse sediment. A third approach is to physically remove the gut of the test organism, although this is often difficult with small species. Here, we compare the effect of each of these different protocols on metal concentrations recorded for *Corophium volutator.*

Materials and Methods

Sample collection and experimental protocol

Corophium were collected from the Ythan Estuary, Aberdeenshire, Scotland, by sieving mud through a 0.5 mm mesh sieve. The seawater used for the experiments was pumped from the estuary (32 ppt, 11±1°C) through a biological filter and into a tank with continual aeration. The amphipods were placed in sediment-free tanks for an acclimatisation period of at least one week. Sediments used in the experiments were taken from an area known to support a healthy population of *Corophium.*

* Present address: Ondokuz Mayıs University, Sinop Fisheries Faculty, 5700, Sinop-TURKEY.

The clean sediments were washed through a 500 µm mesh sieve into a tank to remove any associated macrofauna, including *Corophium,* and then washed again through a 300 µm mesh sieve to ensure a standard particle size for the sediment in all experiments. These sediments were then treated by shaking with solutions of copper (added as $CuSO_4.5H_2O$), or zinc (added as $ZnSO_4.7H_2O$), or cadmium (added as $CdCl_2$) at the following concentrations: 10, 30 and 50 µg g^{-1} for both copper and zinc, 1.5, 10 and 30 µg g^{-1} for cadmium. All experimental containers (9 cm in diameter, 8 cm deep) used were covered by black material. The sediments were then added to the containers to a depth of 2 cm. Clean seawater was added to the containers to 5 mm from the top and the sediment surface smoothed. The experimental setup was maintained under constant aeration for 48 hours before any *Corophium* were added. Aeration, at a rate of approximately two or three bubbles per second, was provided with a Pasteur pipette without disturbing the sediment surface. This maintained dissolved oxygen levels above 60% of the air saturation value, acceptable conditions for such tests (8). Three replicate containers were set up for each concentration of each metal. Twenty adult *Corophium* were randomly selected from the tanks and placed in each jar using a wide-mouthed pipette. After 1 hour any *Corophium* that were dead or showed abnormal behaviour were removed and replaced. The amphipods were not given any food during the course of the experiments and the test solutions were not changed. The photoperiod was 16:8 h light:dark. All *Corophium* used in the experiments were adults (4-7 mm) with the sexes in equal proportion. *Corophium* were exposed to different concentrations of copper, zinc and cadmium in sediment for 4 and 10 days.

The sediments were analysed for copper, zinc and cadmium at the beginning and at the end of the experiment. Sediment samples from each container were dried overnight at 105˚C and sieved through a 63 µm mesh to select for particles smaller than 63 µm, which *Corophium* can ingest (9). Analysis of metal concentrations in the <63 µm sediment fraction is also recommended because these particles are the most important sources of bioavailable metals in sediments (10, 11). Twenty ml of concentrated nitric acid was added to 1 g of each of the dried fine sediments and allowed to stand overnight. Digestion mixtures were heated on a hot plate at 80˚C. After digestion the mixtures were allowed to cool and the residue was diluted with double-distilled water to 10 ml for analysis on a Varian Spectra AAIO Atomic Absorption Spectrophotometer (AAS).

After 4 and 10 days, living amphipods were removed from the three replicate containers and subjected to one of the following treatments:

a) Animals were rinsed with distilled water to remove any adhering sediment, blotted, and frozen (-20˚C) immediately for metal analysis. No time was given for these animals to depurate their guts.

b) Animals were rinsed with clean seawater and then placed in constantly aerated clean seawater at 11˚C for 48h to allow depuration, were rinsed with distilled water, blotted and then frozen (-20˚C) for metal analysis.

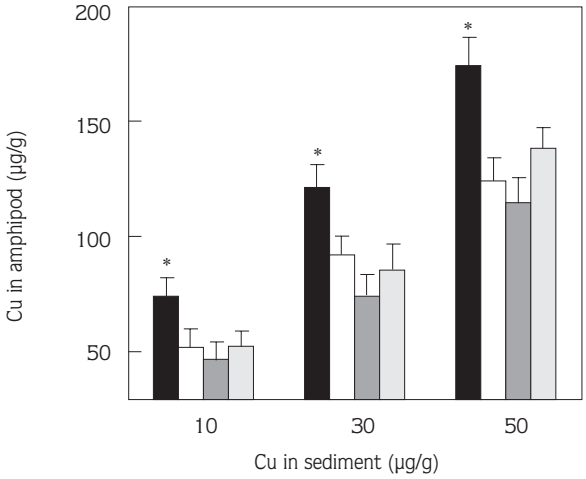
c) Animals were rinsed with clean seawater then placed in constantly aerated clean seawater with clean sediment at 11˚C. They were allowed to ingest the clean sediment for 48h in order to displace any treated sediment in the gut. The amphipods were then sieved from the sediment, rinsed in distilled water, blotted and then stored at -20˚C for metal analysis.

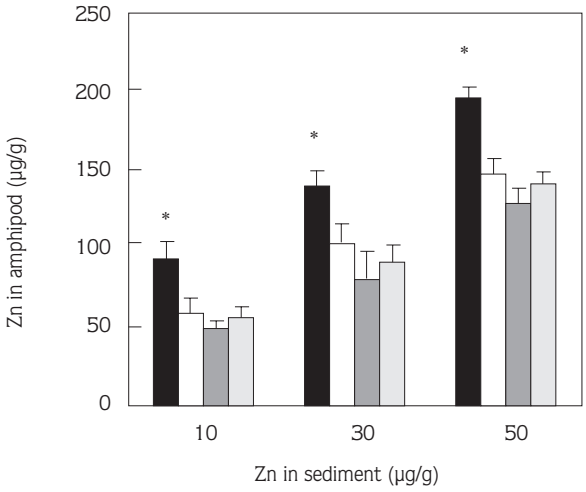
d) Animals were rinsed with distilled water then immediately frozen. After thawing, the amphipods were dissected under a microscope to remove the gut (and its contents) in 2 ml of distilled water. The gut content of each individual was discarded and the amphipods, without their guts, were rinsed in distilled water, blotted, and prepared for metal analysis.

For metal analysis the animals were oven-dried to contants weight at 70˚C, weighed and dissolved in concentrated nitric acid (0.2 ml / 5 mg dry wt) at 80˚C. After digestion, the samples were diluted with 10 ml of distilled water for analysis as described above for sediments.

Results

As might be expected, tissue concentrations of all metals increased with increasing metal concentrations in sediment after 4 and 10 day experiments (Figs. 1 and 2), the concentrations being higher in the 10 day experiment. There were no statistically significant differences between procedures b, c or d for each metal concentration (Figs. 1 and 2). However, animals analysed with contaminated sediment in their guts (procedure a) had significantly higher concentrations, except for cadmium at low concentrations (Figs. 1 and 2). This was true for both 4 and 10 day exposures. Whilst there were no statistical differences between the three procedures b, c and d, it is interesting that method c consistently yielded the lowest concentration (Figs. 1 and 2). The possible significance of this is discussed below.





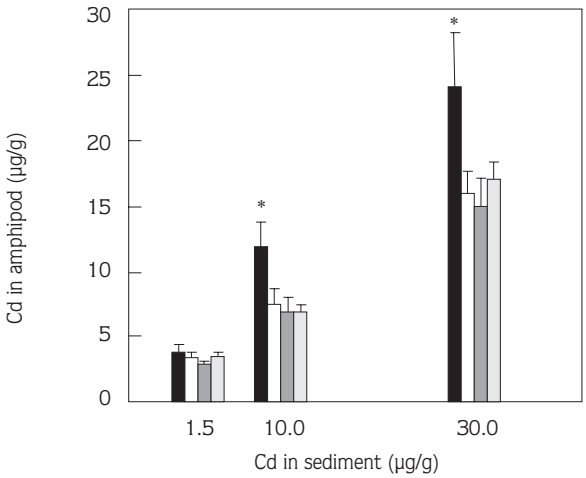
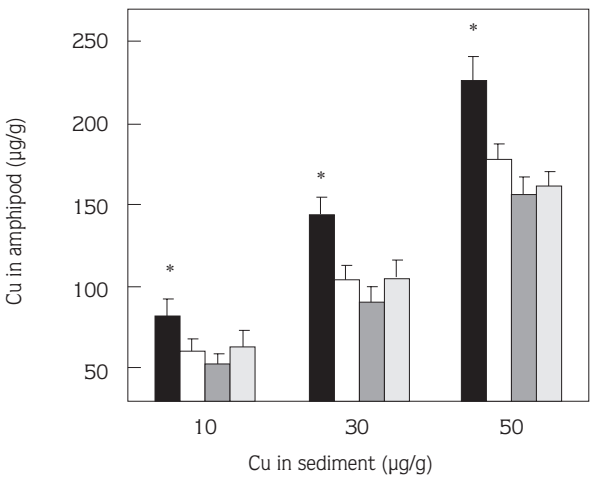
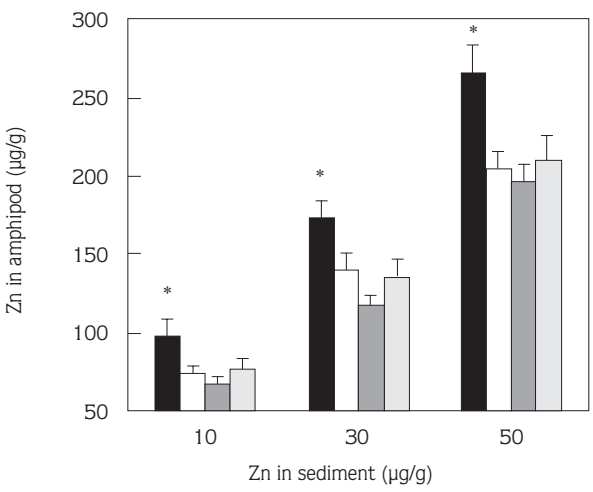


Figure 1. Mean copper, zinc and cadmium levels in *C. volutator* exposed for 4 days to various concentrations of these metals and treated by procedures a= ■ , b= □ , c= ▒ , d= ▒ , For treatments see Materials and Methods. (error bars=SE). (*P<0.05).





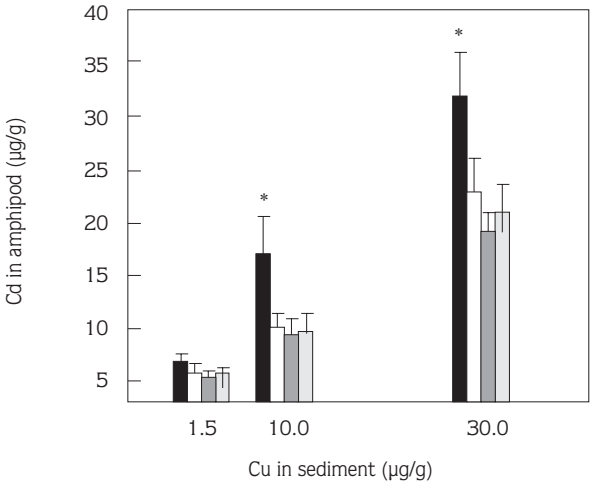


Figure 2. Mean copper, zinc and cadmium levels in *C. volutator* exposed for 10 days to various concentrations of these metals and treated by procedures a= ■ , b= □ , c= ▒ , d= ▒ , For treatments see Materials and Methods. (error bars=SE). (*P<0.05).

Discussion

Flegal and Martin (2) found that the amount of inorganic matter present in benthic invertebrates (when expressed as a percentage of the sample weight) and presumed to be ingested sediment, was often significantly correlated with their apparent elemental concentrations measured; the more material within the gut, the higher the concentration. Depuration, where by the organism is allowed to purify its gut in clean seawater for a period up to 48 h, has therefore been recommended by several authors (3, 6). Chapman (12) questioned whether this procedure was effective in the case of the marine clam *Yoldia* sp. ind it is also ineffective in the case of tubificid oligochaetes (*Limnodrilus hoffmeisteri* and *Tubifex tubifex*) which, along with many other benthic organisms (family Chironomidae and Lamprey larvae), engage in coprohagy. Phillips and Rainbow (13) noted that depuration was not recommended for samples destined for hydrocarbon or organochlorine analysis because of the short biological half-life of these pollutants, and their high elimination rates from tissues. However, Latouche and Mix (14) found that after depuration, manganese levels decreased significantly in both gonadal and somatic tissues of the bivalve *Mytilus edulis* while, copper and nickel concentrations increased in somatic tissues. They suggested that these unexpected findings may indicate that copper and nickel were taken up from seawater in response to stress. These authors also showed that zinc and cadmium levels declined slightly in both somatic and gonadial tissues of mussels but this was not statistically significant. Rainbow and Moore (15) found that defecation had no effect on copper concentrations (whole animals) in seven species of amphipods but the presence of material in the gut artificially lowered the zinc concentrations of individuals and altered the relationship between zinc concentration and animal dry weight. In the case of lead, the presence of gut material resulted in a significant increase in total concentrations in *Gammarus pulex* (15). It was concluded that the ingested material has a lower zinc concentration and a higher lead concentration than the amphipods themselves. However, Rainbow (16) noted that the gut contents of deposit-feeding crustaceans often present a significant component of the total body load because they ingest potentially metal-rich sediments. Of course, in the present study, the metal-rich sediments to which the animals had been exposed were found in the guts and it is not surprising that the metal content was high in *Corophium* with sediment in the gut.

It is clear that if contaminated sediment in the gut is included in whole-body estimates of metal burdens, then the apparent concentrations will be elevated. A period of 48 h in constantly aerated clean seawater with or without clean sediment, or dissection and removal of the gut is sufficient to remove this effect, and the concentrations of metals in individuals 'cleaned' by placing them in clean sediment are consistently lower, albeit to a small degree (10-20 $\mu g\ g^{-1}$ in the case of Cu and Zn, and 1-3 $\mu g\ g^{-1}$ in the case of Cd). This is probably due to the additional weight of the clean sediment in the gut the inclusion of which in the sample weight effectively lowers the overall concentration in the animal. It is recommended therefore that either depuration in seawater or removal of the gut should be carried out before analysing invertebrates for their true metal burdens.

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References

1. Elwood, J.W., Hildebrand, S.G. and Beauchamp, JÖ.J., "Contribution of gut contents to the concentration and body burden of elements in Tipula spp. from a spring-fed stream", J. Fish. Res. Bd. Canada. Vol. 33, pp. 1930-1938, 1976.
2. Flegal, A.R. and Martin, J.H., "Contamination of biological samples by ingested sediment", Mar. Pollut. Bull., Vol. 8, pp. 90-92, 1977.
3. Hare, L., Campbell, P.G.C., Tessier, A. and Belzile, N., "Gut sediments in a burrowing mayfly (Ephemeroptera, Hexagenia limbata): their contribution to animal trace element burdens, their removal and the efficacy of a correction for their presence", Can. J. Fish. Aquat. Sci., Vol.46, pp. 451-456, 1989.
4. Chapman, P.M., Churchland, L.M., Thomson, P.A. and Michnowsky, E., "Heavy metal studies with oligochaetes", In: R.O. Brinkhurst and D.G. Cook (Eds.), Aquatic Oligochaete Biology. Plenum Press New York and London. pp. 477-502, 1980.
5. Miramand, P., Germain, P. and Camus, H., "Uptake of americium and polutonium from contaminated sediments by the three benthic species: Arenicola marina, Corophium volutator and Scrobicularia plana", Mar. Ecol. Prof. Ser., Vol.7, pp. 59-65, 1982.
6. Icely, J.D. and Nott, J.A., "Accumulation of copper within the "Hepatopancreatic" caeca of Corophium volutator (Crustacea: Amphipoda)", Mar. Biol., Vol. 57, pp. 193-199, 1980.

7. Icely, J.D. and Nott, J.A., "Feeding and digestion in Corophium volutator (Crustacea: Amphipoda)", Mar. Biol., Vol. 89, pp. 183-195, 1985.

8. American Society for Testing and Materials, "Standard guide for collecting, storage, characterization and manipulation of sediments for toxicological testing", American Society for Testing and Materials, Philadelphia, PA, pp. 1105-1119, 1991.

9. Fenchel, T., Kofoed, L.H. and Lappalainen, A., "Particle size-selection of two deposit feeders: the Amphipod Corophium volutator and the Prosobranch Hydrobia ulvae", Mar. Biol., Vol. 30, pp. 119-128, 1975.

10. Bryan, G.W. and Langston, W.J., "Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review", Environ. Pllut., Vol. 76, pp. 89-131, 1992.

11. Langston, W.J. and Spence, S.K., "Metal analysis", In: P. Calow (Ed.), Handbook of Ecotoxicology. Oxford Blackwell Sci. Publ., London. Vol. 2, pp. 45-78, 1994.

12. Chapman, P.M., "Effects of gut sediment contents on measurements of metal levels in benthic invertebrates - a cautionary note", Bull. Environ. Contam. Toxicol., Vol. 35, pp. 345-347, 1985.

13. Phillips, D.J.H. and Rainbow, P.S., "Biomonitoring of trace aquatic contaminants", Environmental Management Series, Chapman & Hall, London, 1994.

14. Latouche, Y.D. and Mix, M.C., "The effects of depuration size and sex on trace metal levels in bay mussels", Mar. Pollut. Bull., Vol. 13, pp. 27-29, 1982.

15. Rainbow, P.S. and Moore, P.G., "Comparative metal analyses in amphipod crustaceans", Hyrobiologia, Vol. 141, pp. 273-289, 1986.

16. Rainbow, P.S., "The significance of trace metal concentrations in decapods", Symp. Zool. Soc. Lond., Vol. 59, pp. 291-313, 1988.