

## Influence of Sediment on Heavy Metal Uptake by the Polychaete *Arenicola marina*

Levent BAT\*

Culterty Field Station, Department of Zoology, University of Aberdeen, Newburgh, Ellon, Aberdeenshire, AB41 0AA, United Kingdom.

Received: 03.04.1997

**Abstract:** In this study, the suitability of the polychaete *Arenicola marina* as a test organism using clean intertidal sediment contaminated with copper, zinc and cadmium was evaluated. Metal levels in casts were significantly lower than in unworked sediment indicating that the bioturbatory activity of lugworms might be significant in the distribution of the pollutants. This study showed that sediments contaminated with specific pollutants affected the uptake of these pollutants by *Arenicola* in a predictable and dose-dependant way and gave further justification for the use of this species in sediment toxicity tests.

**Key Words:** *Arenicola marina*, cast, lugworm, heavy metal, sediment.

### Poliket *Arenicola marina*'nın Ağır Metal Alımında Sedimanın Etkisi

**Özet:** Mevcut çalışmada poliket *Arenicola marina* bakır, çinko ve kadmiyumla kirlenilen temiz sedimanla yapılan deneylerde test organizması olarak kullanılmıştır. Cast adı verilen atıklardaki metal düzeyleri, organizma tarafından kullanılmamış sedimandaki metal düzeylerinden önemli ölçüde daha az bulunmuştur. Bu da halkalı deniz kurtlarının biyolojik aktivitelerinin kirleticilerin dağılımında önemli olabileceğini göstermektedir. Mevcut çalışma özel kirleticilerle kirlenmiş sedimanın, bu kirleticilerin *Arenicola* tarafından alınımını doza bağlı olarak etkilediğini göstermiş ve bu türün sediman zehirlilik deneylerinde kullanımlarını desteklemiştir.

**Anahtar Sözcükler:** *Arenicola marina*, atık, halkalı deniz kurdu, ağır metal, sediman.

### Introduction

Sediment toxicity tests are commonly used to determine the potential impact of sediment-associated contaminants (1). Although various guidelines and standards for bioassays concerning choice of species, time of exposure, and experimental design have been established (2), there has been little international standardisation of the protocols. The Environmental Protection Agency and the US Army Corps of Engineers (3) have proposed a list of polychaete genera including *Arenicola* sp. which they consider to be sensitive infaunal species and therefore suitable in bioassays. Reviews (e.g.4–6) on the use of polychaetes as test organisms for toxicity studies suggest that they are very useful organisms for monitoring the marine and estuarine environment. Because of their habitat in sediment, polychaetes are exposed to high concentrations of contaminants both in their food and in the ambient water. They are also important members of the benthic marine

community (7) and play an important role in the oxidation and recycling of sediment organic matter (8–11). The polychaete, *Arenicola marina* has been found to be sensitive and potentially useful for sediment monitoring (12–14), but the detailed ecotoxicology of this species is not well documented. *Arenicola* is also recognised as a significant bioturbator of marine sediments. The lugworm, *Arenicola marina*, is a benthic infaunal polychaete widely distributed in the shallow marine and estuarine benthic habitats of Europe, Norway, Spitzbergen, North Siberia, Iceland, Greenland, and along the northern coast of America from the Bay of Fundy to Long Island (15). Along the Mediterranean coast its distribution is limited to the Trieste area (16). It is a major prey for shorebirds (17) and fish (6) and is used as a bait for flatfish, cod, haddock and whiting. *Arenicola marina* lives in an L-shaped tube in the sediment (18) is 5mm wide and 10–20 cm long (19), feeding on detritus, bacteria, diatoms, flagellates, ciliates, planktonic

\* Present address: Ondokuz Mayıs University, Sinop Fisheries Faculty, 57000 Sinop, Turkey.

organisms and larger organisms (e.g. *Nereis*) (19). Sediment particles smaller than 300 to 400  $\mu\text{m}$  are preferred for ingestion (20). Sediment is ingested at the lower end of the column (about 10 to 15 cm) (10), the surface sediment of the funnel will reach the lugworm in only a few hours because of the small diameter of the column (19). Water, mainly for ventilation, is pulled down into the burrow through the tail shaft (21), the rate of oxygen diffusion is therefore greatly increased (9). Not surprisingly organic content and grain size of the sediment affect the abundance and distribution of the lugworm (22). Faecal pellets and surface porosity are high and easily suspended and compaction and cohesion are low (9). Moreover bioturbation both serves passively to transport microorganisms adhering to sedimentary particles and increases sediment water content (9), the metals in sediment can therefore be suspended or cycled.

Here, the potential of *Arenicola marina* as a bioassay organism is evaluated by exposing it to clean sediment contaminated with copper, zinc and cadmium.

## Materials and Methods

### Sample collection and experimental protocol

All *Arenicola* used in these experiments were collected from intertidal flats of the Ythan Estuary, Aberdeen (Scotland), by digging. The lugworms were maintained in a sediment-free darkened tank with running seawater for acclimatisation for a period of 24 h. Clean sediment (uncontaminated) was collected from the same area as the lugworms and washed through a 500  $\mu\text{m}$  mesh into a tank to remove any associated macrofauna and then washed again at least 6 times with seawater before use in subsequent experiments. This procedure ensured a standard particle size (<500  $\mu\text{m}$ ) for all experiments. These sediments were then treated by shaking solutions of copper (prepared from  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), or zinc (prepared from  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ), or cadmium (prepared from  $\text{CdCl}_2$ ) at the following concentrations: 0 (control), 10, 20, 50 and 90  $\mu\text{g g}^{-1}$  for copper, 0 (control), 30, 60, 90 and 120  $\mu\text{g g}^{-1}$  for zinc, 0 (control), 10, 35, 65 and 80  $\mu\text{g g}^{-1}$  for cadmium. The mixing time was limited to a few hours (3–4h) and temperatures were kept at about 4°C to minimise any alterations which occur in the sediment's physicochemical and microbiological characteristics with changes in temperature that might alter the bioavailability and toxicity of the contaminants (23). Sediments used as controls were treated as described above with uncontaminated seawater. Fifteen plastic tanks (32 cm x 21 cm wide x 20 cm deep) were used for each metal. They were first washed with

detergent, soaked in 15% HCl for 24 h, and rinsed with double-distilled water.

### Bioassay procedure

Test and control sediments were added to the bioassay tanks to a depth of 10 cm. The seawater used for the experiment was pumped from the estuary (32‰,  $9 \pm 1^\circ\text{C}$ ) through a biological filter and into a tank until 4 cm above the sediment. The sediment surface was then smoothed. After 24 h the overlaying water was removed to eliminate any copper, zinc or cadmium which may have leached from the sediment, and was replaced with clean seawater. The experimental set-up was maintained under constant aeration for a further 24 hours before any *Arenicola* were added. The seawater was aerated vigorously in order to maintain the oxygen levels above 60% in all containers, but without disturbing the sediment surface, providing acceptable conditions for sediment toxicity tests (2–3). Five adults *Arenicola* were randomly selected from the stock tanks and washed with uncontaminated seawater to remove any sediment adhering to the skin, blotted dry on paper towels and weighed and then placed into each concentration of metal (3 replicates). The average weight of the *Arenicola* was  $4.12 \pm 0.05$  g (wet wt). All *Arenicola* used in the experiments were adults. Damaged lugworms were discarded. The worms were not given any food during the course of the experiment. The test solutions were not changed. *Arenicola* can survive in this manner for six weeks or more if there is no contaminant in the sediment or the water (personal observations).

Each tank was checked daily and any dead worms were recorded but not replaced. Everyday casts and a sample of unworked sediment were removed from each tank. These samples were also analysed for metal content and the average measured concentrations were used in the subsequent data analysis.

After 4 days the contents of each tank were sieved and the number of surviving lugworms was recorded. These individuals were then transferred to aerated clean seawater without sediment, where they were held for 24 h to avoid any sediment in the gut. Their tissue was then analysed for the total copper, zinc and cadmium content. The concentrations of copper, zinc and cadmium in the whole tissues, in the casts and in the sediments were expressed as  $\mu\text{g}$  of copper, zinc and cadmium per g dry weight. Sediment samples were also analysed for total organic carbon.

Samples for total sediment organic carbon analysis were dried at 60°C in an oven for 48 h. A five gram sample was then treated with hydrochloric acid vapour

overnight in a desiccating jar to convert any calcium carbonate to chlorides. Weighed, dried samples were then placed in a muffle furnace at 600°C for four hours and the loss on ignition taken as the organic carbon content of the sediment (24).

During the course of the *Arenicola* bioassay, redox potential (Eh) was measured using a Russel platinum electrode (25–26) which was pushed stepwise down into an undisturbed sediment core to 4 cm depth to provide the most reasonable estimate of sediment conditions (27). The reading was allowed to equilibrate for 2 min (25) and corrected for hydrogen reference by adding +198 mv to the meter (Radiometer) reading (27).

All seawater (32‰) used in the experiments was pumped from the Ythan estuary into a large reservoir tank through a biological filter to avoid large amounts of suspended material and organisms larger than 10 µm. The water in the tank was continually aerated in order to maintain the dissolved oxygen levels above 60% of the air saturation value (2–3). 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.

The average total organic content of the sediment at the start of the experiment was 1.61% (SD 0.31). The mean temperature over the experimental period in all bioassays was 9°C±1, dissolved oxygen was 91%±4, salinity was 32‰±1 and pH was 7.28±0.16. The redox potential (Eh) of the sediments ranged from +264mV to +324mV, indicating oxygenated sediment in all tanks.

### Analysis for heavy metals

#### *Arenicola* tissues

After each experiment, the surviving individuals were placed for 24 hours in constantly aerated clean seawater at 9±1°C, then were rinsed in double-distilled water. Animal samples were dried to constant weight at 70°C. Drying of the animal samples was completed in 2 days. The samples were then transferred to Pyrex tubes for acid digestion. Twenty ml of concentrated nitric acid was added to the tubes for each dry weight of *Arenicola* and heated at 80°C for 3 to 4 days until a clear colourless liquid was formed. After digestion, the samples were diluted with distilled water, filtered through Whatman filter paper and made up to 50 ml for analysis on an Atomic Absorption Spectrophotometer (AAS).

#### Sediment samples, including *Arenicola* casts

Sediment samples from each tank were dried overnight at 105°C and sieved through a 500 µm mesh because *Arenicola* prefers particle sizes generally <500

µm (20). Thus this fraction of the sediment was analysed.

Twenty 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 concentrated 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. *Arenicola* cast samples were analysed in exactly the same way.

Flame AAS was chosen as the technique to determine the copper, zinc and cadmium content of tissues, sediment and casts. AAS is an analytical method based on the absorption of characteristic radiation by free atoms of the element when it is heated. The theory and application of AAS have been described in detail by Cresser (28). The sample solution is introduced as an aerosol into the flame, where the analyte ions are converted into free atoms. These atoms absorb radiation and this is related to the concentration of the metal in the sample solution. The most widely used flame is the air/acetylene (air/C<sub>2</sub>H<sub>2</sub>) flame, which is transparent over a wide spectral range and displays low emission, making it ideal for the determination of many elements including copper, zinc and cadmium (28).

The AAS used was a Varian SpectrAA10. The instrument was calibrated prior to use with standards of known concentration prepared on the day of analysis from stock solutions. The instrument was calibrated every hour. The operating conditions used for the analysis of copper, zinc and cadmium, are listed in Table 1.

Table 1. Operating conditions for Flame AAS.

Element	Copper	Zinc	Cadmium
Wavelength (mm)	324.8	213.9	228.8
Lamp current (mA)	8	8	6
Slit width (nm)	0.5	1	0.5
Flame	Air/C <sub>2</sub> H <sub>2</sub>	Air/C <sub>2</sub> H <sub>2</sub>	Air/C <sub>2</sub> H <sub>2</sub>
Background correction	on	on	on
Limit of detection (µg g <sup>-1</sup> )	0.03	0.025	0.02

All glassware was first washed with detergent (decon<sup>®</sup> 75) and rinsed with tapwater. They were then treated with 10% v/v HNO<sub>3</sub> (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 (supplied by BDH Chemicals Ltd.).

**Data analysis**

At the end of the experimental period between-treatment comparisons were made using one-way ANOVA and any significant differences further evaluated using the Tukey multiple-comparison test (29).

**Results**

None of the control lugworms died, demonstrating that the holding facilities, seawater, control sediment and handling techniques were acceptable for conducting such tests, as required in the standard EPA/COE protocol (3) where mean survival should be  $\geq 90\%$ . Lugworm mortality increased with increasing copper, zinc and cadmium sediment concentrations, this becoming more significant at higher concentrations ( $P < 0.05$ ). Dead lugworms were found on the sediment surface and were usually discoloured.

Figure 1 shows the average concentration of copper, zinc and cadmium in the tissues of *Arenicola* and in sediment after 4 days of exposure. Only live lugworms were analysed for metal content. No lugworms survived after being exposed to concentrations of  $20 \mu\text{g g}^{-1}$  Cu,  $60 \mu\text{g g}^{-1}$  Zn and  $35 \mu\text{g g}^{-1}$  Cd in sediment. Therefore these values were not shown in Figure 1. As expected, tissue metal concentrations increased with increasing copper, zinc and cadmium sediment concentrations. There were significant differences in concentrations of all metals in casts and unworked sediment (Tables 2–4), concentrations in casts always being lower than in unworked sediment. These differences decreased with decreasing metal concentrations indicating that ingestion of sediment by lugworms reduces sediment metal concentrations.

Test concentration ( $\mu\text{g g}^{-1}$ )	$\mu\text{gCu g}^{-1}$ in sediment (dry wt)	$\mu\text{gCu g}^{-1}$ in cast (dry wt)	Difference	% Change
Control	2.5 (1.9–4.5)	1.35 (<0.03–2.3)	1.15*	46
10	11 (6–14)	5.5 (3–7)	5.5**	50
20	21 (17–27)	10 (7–11)	11**	52
50	52 (46–60)	24 (21–33)	28**	54
90	87 (75–104)	(--)	--	--

(--): No cast was observed.

Table 2. Concentrations of copper in sediment and casts during the course of the experiment. All replicates are combined and averaged. Numbers in parentheses represent range of 3 replicate tanks during 4 days (n=12). (\* $P < 0.05$ , \*\* $P < 0.001$ ).

Test concentration ( $\mu\text{g g}^{-1}$ )	$\mu\text{gZn g}^{-1}$ in sediment (dry wt)	$\mu\text{gZn g}^{-1}$ in cast (dry wt)	Difference	% Change
Control	10 (6–13)	9 (5–11)	1*	10
30	31 (26–32)	18 (15–23)	13**	42
60	60 (55–67)	27 (21–32)	33**	55
90	85 (79–91)	36 (31–45)	49**	58
120	121 (99–130)	(--)	--	--

(--): No cast was observed.

Table 3. Concentrations of zinc in sediment and casts during the course of the experiment. All replicates are combined and averaged. Numbers in parentheses represent range of 3 replicate tanks during 4 days (n=12). (\* $P < 0.05$ , \*\* $P < 0.001$ ).

Test concentration ( $\mu\text{g g}^{-1}$ )	$\mu\text{gCd g}^{-1}$ in sediment (dry wt)	$\mu\text{gCd g}^{-1}$ in cast (dry wt)	Difference	% Change
Control	<0.02	<0.02	--	--
10	12 (7–15)	7 (5–10)	5*	42
35	32 (22–38)	18 (15–24)	14**	44
65	64 (58–71)	35 (31–39)	30**	45
80	76 (71–80)	(--)	--	--

(--): No cast was observed.

Table 4. Concentrations of cadmium in sediment and casts during the course of the experiment. All replicates are combined and averaged. Numbers in parentheses represent range of 3 replicate tanks during 4 days (n=12). (\* $P < 0.05$ , \*\* $P < 0.001$ ).

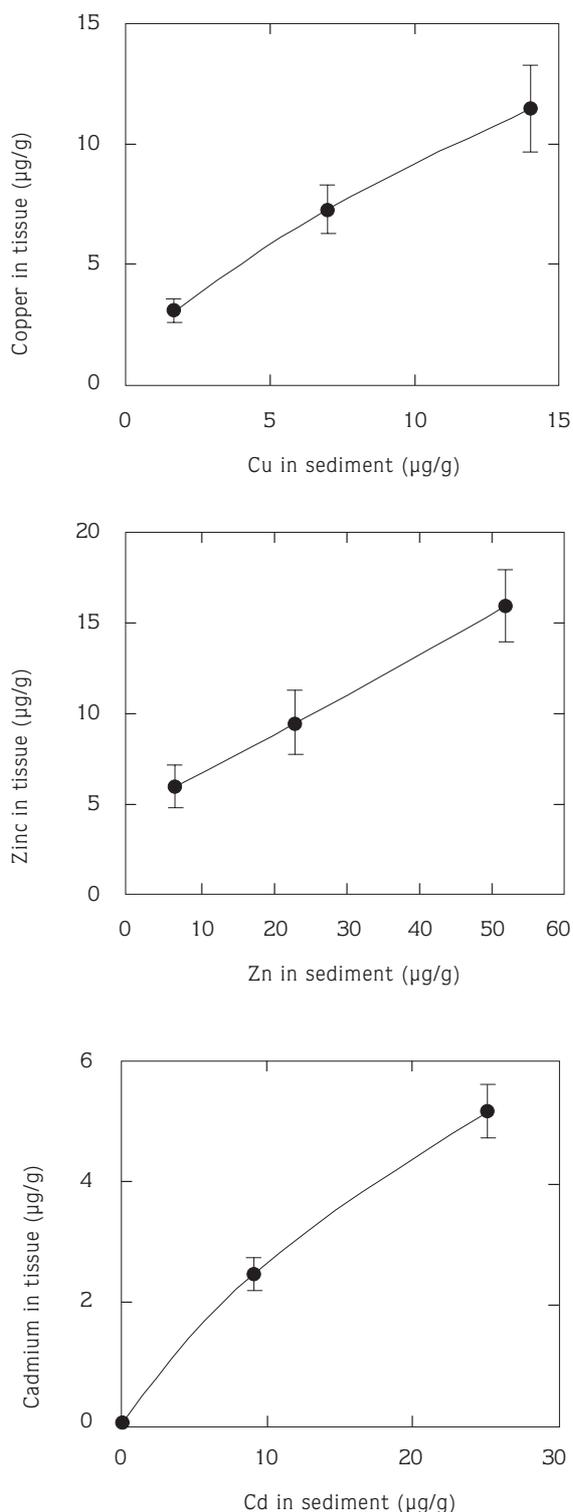


Figure 1. Mean concentrations of copper, zinc and cadmium in *Arenicola* at the end of 96-hour static sediment bioassay. Each value is based on 3 replicate tanks of 5 lugworms. Error bars=SE. Only live lugworms were analysed.

## Discussion

The macrobenthos like *Arenicola* may concentrate metals by absorption from solution through the outer body surface or through the gut from ingested food or sediment or water, and they eliminate contaminants via the gut in faeces, or via urine and excretion across the body surface and gills (30–31). In the present study, the concentration of copper, zinc and cadmium in *Arenicola* tissues increased with increasing metal sediment concentrations and cadmium was present in the lowest concentrations measured in the tissue. These observations are similar to those of Packer *et al.* (32) who studied cadmium, copper, lead, zinc and manganese in *Arenicola marina* and sediments around the coast of Wales. They pointed out that tissue concentrations were directly related to body weight for lead, zinc and manganese but not in case of cadmium and copper. They also found that there was a significant positive correlation between tissue concentrations and those in the sediment for cadmium and zinc but this was not so for copper, lead and manganese. In a similar study of several benthic species including *Arenicola marina*, Loring and Prosi (33) have shown that *Arenicola* accumulated less cadmium compared with other species including *Nereis diversicolor*, *Littorina littorea*, and *Mytilus edulis*, and they have concluded that *Arenicola* is more protected from the surrounding sediment than the other species. Similarly, americium and plutonium uptake by *Arenicola marina* from highly contaminated sediments was significantly lower than for other species including *Corophium volutator* and *Scrobicularia plana* (34). These authors have pointed out that the transfer of radionuclides from sediment to the organism could take place by two different processes: (a) direct uptake by desorption of the elements retained in the ingested sediment when passing through the digestive tract and (b) indirect transfer via interstitial water. Particulate metals from suspended particulate matter can also be a source and pathway of the contamination (33). Moreover, many biological, chemical and environmental factors may act in concert to regulate the relative importance of these pathways. However, burrow-dwelling organisms such as *Arenicola* maintain respiratory connections with overlying water (35–36) and may feed on suspended matter or bacteria (19), which potentially decreases their exposure to sediment-associated contaminants. It is also known that *Arenicola* can consume at the surface layer of the sediment as it collapses towards the mouth (19, 37) and metals are usually in an oxidised and less toxic state within the top few mm of sediment if the overlying water contains dissolved oxygen (38). In the present

study, the mean dissolved oxygen in all the tanks was higher than 83% and this may also affect the bioavailability of metals to *Arenicola*. Moreover, *Arenicola* may accumulate the metal which may be released into the overlaying water. Nevertheless it was not clear which uptake pathways prevailed in this study.

In this study metal concentrations in the ambient sediment were higher than those in casts, and other researchers have found similar results. For instance, Loring and Prosi (33) have found that the concentrations of cadmium, lead and zinc were lower in *Arenicola marina* casts and have suggested that this effectively diluted the metal concentrations in the top layer of the sediments. Similarly Prouse and Gordon (39) and Gordon *et al.* (40) have shown that oil concentrations in casts are lower than those in sediment, and have suggested four processes that might be responsible: (a) sediment sorting during feeding, (b) dissolution, (c) assimilation and (d) biodegradation.

#### Particle size sorting

Hylleberg (37) and Cadée (20) have noted that *Arenicola* selects sediment for ingestion, preferring particle sizes generally less than 500 $\mu$ m. It is also known that small particles of sediment (generally less than 63 $\mu$ m) are the most important sources of available metals (41–42). Sediments used in the present experiment had a particle size less than <500 $\mu$ m and the distribution of the particles (and therefore metals) is likely to be heterogeneous. If *Arenicola* ingests particles larger than >63 $\mu$ m or avoids contaminated sediments, this might account for the lower metal concentrations observed in the casts.

#### Dissolution

The sediment working activity of *Arenicola* (filling of headshaft, ingestion, digestion and defecation) takes place in the presence of water (18). Moreover, *Arenicola* strongly bioturbates its habitat (43) and overlaying water is pumped into the burrows at about 430 ml h<sup>-1</sup> (maximum) for a worm of 4 g (36) for respiratory purposes (44). Renfro (45) has found that artificial agitation of the sediment increased the loss of <sup>65</sup>Zn by factor of 3 compared with undisturbed sediment and burrowing, and irrigation by *Nereis diversicolor* was thought to contribute significantly to the diffusion loss of <sup>65</sup>Zn. Therefore loss by dissolution from the cast is possible.

#### Assimilation

Metals ingested in sediment are absorbed by the worms and in the present study, the concentrations of all

three metals in *Arenicola* increased with increasing sediment metal concentrations. This in itself would decrease the levels of metal in the faeces. Bryan and Hummerstone (46) have measured the concentrations of copper, zinc, lead, manganese and iron in *Nereis diversicolor* and in sediments from seven English estuaries influenced to varying degrees by a copper–tin mineral zone. They have reported that this species appeared to regulate its zinc concentrations, but its copper concentrations increased with increasing copper levels in sediment. Bryan (47) has found that the concentrations of copper, lead and cadmium in the same species (*Nereis diversicolor*) correlated strongly with levels in sediments from several English estuaries. From these studies it can be concluded that benthic organisms are able to remove metals from the sediments. However, when compared with abiotic processes, the accumulation of contaminants would seem to be a minor transport process (31). For example Renfro (45) has reported that *Nereis diversicolor* at a density of 50 worms per m<sup>2</sup> would accumulate only 0.08% of the <sup>65</sup>Zn of the upper 2 cm of the sediment layer, whereas 3% of the <sup>65</sup>Zn would be lost from the sediment by resuspension.

#### Biodegradation

Biodegradation of organically complexed metals by microorganisms could also affect metal loss or removal from sediments (48). Hylleberg (37) has reported that high concentrations of microorganisms in the sediment at the bottom of the *Arenicola* headshaft and their growth are stimulated by nutrients present in the sediments and by oxygen present in the irrigation water. Biodegradation may also be substantial in the casts after deposition (40). In a closely related species, *Abarenicola pacifa*, a gardening strategy is adapted whereby the undigested sediment which is passed out as faeces acts as a substrate for microorganisms which increase the nitrogen content of the sediment by their activity (37), thereby changing sediment characteristics and microbial activity (48). Similarly, Prouse and Gordon (39) and Gordon *et al.* (40) have shown that stimulation of microbial degradation by *Arenicola* might contribute to the loss of oil from contaminated sediments.

In contrast to the present study, heavy metals may also be concentrated in casts or faeces. Brown (49) has measured the concentrations of cadmium, copper, nickel and zinc in the faeces of intertidal benthic invertebrates, including *Arenicola marina* and in sediment from an estuary in South Wales. This author has reported that the average values for these metals in *Arenicola* casts were slightly higher than those in the sediment. However,

Brown's data are equivocal, because only a limited number of casts (two) were analysed compared with twenty–six sediment samples and the results for the latter were very variable. Brown (49) has also reported that all four metal concentrations in casts and sediments were inversely related to particle size and positively correlated with organic carbon content. This is quite normal, in particles of that size because of their relatively large surface area which can adsorb more heavy metals (41–42, 50) and more organic matter (19) per gram of sediment than large particles which are not utilised by *Arenicola* (18). *Arenicola* faeces can therefore contain more organic matter and microorganisms than the sediment (22, 37, 51) when part of the organic matter is indigestible. Brown's data (49) showed that the highest concentrations of these four metals were found in sediment, not in *Arenicola* casts, and the highest concentrations of the metals in Brown's study were found in sediment particles >250µm compared with smaller particles. This researcher has also reported that animals used in her study were collected from an area polluted by industrial sewage and affected by river discharges so that it is possible that the origin of the metals observed in the casts might have originated in the overlying water and not in ingested sediment.

Nevertheless, other studies have shown that metals may be concentrated in the faeces through the food. Boothe and Knauer (52) fed the decapod crab *Pugettia producta* on the kelp *Macrocystis pyrifera*, and followed the concentrations of nine metals in kelp tissue and crab faeces. Seven of the heavy metals (arsenic, cobalt, copper, iron, manganese, lead and zinc) were concentrated in the faeces above the levels in food, whilst the reverse was true for chromium and cadmium. They have also reported that the metal levels found in faecal samples were more variable, suggesting that this could be the result of sex and size differences among the animals or of variable contamination of faecal samples by small pieces of unconsumed algae. Their studies clearly show the importance of faecal material in the distribution and transfer of metals. Similarly, Nott and Nicolaidou (53) have shown that the faecal material of marine snails might play a significant role in the cycling of metals in tissues along food chains. These snails ingest organic matter preferentially from the sediment and incidentally take up any associated metals. In *Cerithium vulgatum* and *Monodonta mutabilis*, intracellular phosphate granules bound metals in the digestive gland, and the metals were then excreted via the gut in faecal pellets. The stability of these pellets also varied between species: magnesium

phosphate granules from *Monodonta mutabilis* dissolved, but calcium/metal phosphate granules from *Cerithium vulgatum* remained after 4 days of incubation in the same sediments (53).

All these studies suggest that the relative concentrations of metals in sediment and in casts or faeces depend not only on the organism and the metal but also on the sediment type and the food. According to Nott and Nicolaidou (53) there are two strategies for the excretion route into faecal pellets: either the metals are not assimilated when the organic material is digested and they pass through the gut, or, when they are assimilated, they are rendered insoluble within gut epithelial cells in granules and residual lysosomes and returned to the lumen of the gut during cell breakdown at the end of a digestive cycle. In the present study, the metal concentrations in casts were low compared with the original contaminated sediments. If the metals are not assimilated when *Arenicola* feeds on the sediment and the metals pass through the gut, this may be explained by *Arenicola* selecting particle sizes which are less contaminated or the metals in the cast are diluted by bioturbation, as discussed above. If the metals are assimilated, they may be rendered insoluble or accumulated in a specific organ or tissue. It is possible that *Arenicola* adopts both these strategies. For example, Amiard–Triquet (54) has shown that after 3 weeks of exposure to cesium, *Arenicola marina* eliminated 50% of the cesium after 3 days of decontamination (depuration) and most of the residue remained in the skin and the muscles. However, before depuration the cesium was mainly in the digestive tube. Cobalt was eliminated more slowly: even after 77 days not all the cobalt had been eliminated, and before and after depuration most cobalt remained in the digestive tube and blood (54).

Several bioassay methods have been developed since the EPA/COE testing protocol was devised, involving a great variety of test species. The polychaete *Arenicola marina* is now beginning to be used routinely as a standard bioassay organism for assessing the toxicity of marine sediments for regulatory purposes, but little is known about the effects of specific contaminants on the bioassay response. The present study has confirmed the potential of this species for sediment toxicity bioassays, with *Arenicola* meeting most of the criteria required for suitable estuarine sediment toxicity test organisms. They have a wide geographic distribution, ecological importance, high sensitivity to sediment contamination, broad salinity tolerance, ease of handling and maintenance in the laboratory, high survival under control

conditions, year-round availability from the field and a well-established, easily set up protocol.

In conclusion there are clear advantages of this bioassay as a means of assessing sediment toxicity, and it is hoped that *Arenicola* will continue to be employed routinely in monitoring programmes in coastal waters.

## References

1. Long, E.R. and Chapman, P.M., A sediment quality triad: measures of sediment contamination, toxicity and infaunal community composition in Puget Sound. *Mar. Pollut. Bull.*, 16, (10): 405–415, 1985.
2. ASTM, Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. ASTM E 1367–90. American Society for Testing and Materials, Philadelphia, PA., p. 1–24, 1990.
3. United States Environmental Protection Agency and Department of the Army (US Army Corps of Engineers). Evaluation of dredgen material proposed for ocean disposal. Testing manual. EPA–503/8–91/001, Washington DC, 1991.
4. Reish, D.J., Use of Polychaetous Annelids as test organism for marine bioassay experiments. *Aquatic Invertebrate Bioassays*. ASTM STP 715. A.L. Buikema, Jr., and John Cairns, Jr. (Eds.) American Society for Testing and Materials, p. 140–154, 1980.
5. Schulz-Baldes, M., Tiere als monitororganismen für schwermetalle im meer ein überlick. *Decheniana–Beihefte*, 26, 43–54, 1982.
6. Pocklington, P. and Wells, P.G., Polychaetes key taxa for marine environmental quality monitoring: review. *Mar. Pollut. Bull.*, 24 (12): 593–598 1992.
7. Fauchald, K. and Jumars, P.A., The diet of worms: A study of polychaete feeding guilds. *Oceanogr. Mar. Biol. Ann. Rev.*, 17: 193–284, 1979.
8. Rhoads, D.C. and Young, D.K., The influence of deposit-feeding organisms on sediment stability and community trophic structure. *J. Mar. Res.*, 28: 150–178, 1970.
9. Rhoads, D.C., Organism-sediment relations on the muddy sea floor. *Oceanogr. Mar. Biol. Ann. Rev.*, 12: 263–300, 1974.
10. Boon, J.J. and Haverkamp, J., Pyrolysis mass spectrometry of a benthic marine ecosystem—the influence of *Arenicola marina* on the organic matter cycle. *Neth. J. Sea Res.*, 13 (3/4): 457–478, 1979.
11. Paul, V., Snelgrove, R. and Butman, C.A., Animal-sediment relationships revisited: cause versus effect. *Oceanogr. Mar. Biol. Ann. Rev.*, 32: 111–177, 1994.
12. Matthiessen, P. and Thain, J., A method for studying the impact of polluted marine sediments on intertidal colonising organisms: test with diesel-based drilling mud and tributyltin antifouling paint. *Hydrobiologia*, 188/189: 477–485, 1989.
13. Hill, I.R., Matthiessen, P. and Heimbach, F., Guidance document on sediment toxicity tests and bioassays for freshwater and marine environments, 105 pp. Society of Environmental Toxicology and Chemistry, SETAC–Europe, 1993.
14. Thain, J., Matthiessen, P., Bifield, S. and McMinn, W., Assessing sediment quality by bioassay in UK coastal water and estuaries. *Proceedings of the Scientific Symposium on the North Sea Quality Status Report*, 10 p., 1994.
15. Ashworth, J.H., *Arenicola* (The lug-worm). In: W.A. Herdman (Ed.) *L.M.B.C. Memoires on Typical British Marine Plants & Animals XI*. 126 p. Williams & Norgatf, London, 1904.
16. Ashworth, J.H., *Catalogue of the Chaetopoda in the British Museum (Natural History). A Polychaete: Part I. Arenicolalidae*. Longmans, Green & Co., London, 1912.
17. Evans, P.R., Herdson, D.M., Knights, P.J. and Pienkowski, M.W., Short-term effects of reclamation of part of seal sand, Teesmouth, on wintering waders and shelduck. *Oecologia*, 41: 183–206, 1979.
18. Wells, G.P., The mode of life of *Arenicola marina* L.J. *Mar. Biol. Ass. UK*, 26 (2): 170–207, 1945.
19. Rijken, M., Food and food uptake in *Arenicola marina*. *Neth. J. Sea Res.*, 13 (3/4): 406–421, 1979.
20. Cadée, G.C., Sediment reworking by *Arenicola marina* on tidal flats in the Dutch Wadden Sea. *Neth. J. Sea Res.*, 10, (4): 440–460, 1976.
21. Baumfalk, Y.A., On the pumping activity of *Arenicola marina*. *Neth. J. Sea Res.*, 13 (3/4): 422–427, 1979.
22. Longbottom, M.R., The distribution of *Arenicola marina* (L.) with particular reference to the effects of particle size and organic matter of the sediments. *J. Exp. Mar. Biol. Ecol.*, 5: 138–157, 1970.

## Acknowledgements

The author wishes to thank Dr. David Raffaelli (University of Aberdeen) for his advice and constructive criticism during the preparation of the earlier drafts and to SNH for permission to work on the Ythan estuary. This study was carried out under the sponsorship of Ondokuz Mayıs University of Turkey.

23. ASTM, Standard guide for collecting, storage, characterization and manipulation of sediments for toxicological testing. ASTM E 1391–90. American Society for Testing and Materials, Philadelphia, PA, pp. 1105–1119, 1991.
24. Buchanan, J.B., Sediment analysis. In: *Methods for the Study of Marine Benthos*, (N.A. Holme and A.D. McIntyre, eds.), p.41–65. Blackwell Sci. Publ., 1984.
25. Jorgensen, B.B., Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. *Mar. Biol.*, 41: 7–17, 1977.
26. Revsbech, N.P., Sorensen, J., Blackburn, T.H. and Lomholt, J.P., Distribution of oxygen in marine sediments measured with microelectrodes. *Limnol. Oceanogr.*, 25 (3): 403–411, 1980.
27. Pearson, T.H. and Stanley, S.O., Comparative measurement of the redox potential of marine sediments as a rapid means of assessing the effect of organic pollution. *Mar. Biol.*, 53: 371–379, 1979.
28. Cresser, M.S., Flame spectrometry in environmental chemical analysis: A practical guide. N. Barnett (series ed.), RSC Analytical Spectroscopy, Monographs. Turpin Distribution Services Ltd., 1994.
29. Zar, J.H., Biostatistical analysis. Second edition. Prentice Hall, Int., New Jersey, 718 p., 1984.
30. Bryan, G.W., The effects of heavy metals (other than mercury) on marine and estuarine organisms. *Proc. Roy. Soc. Lond. B*, 177: 389–410, 1971.
31. Swartz, R.C. and Lee, H., Biological processes affecting the distribution of pollutants in marine sediments. Part I. accumulation, trophic transfer, biodegradation and migration. In: *Contaminants and sediments volume 2, analysis, chemistry, biology*, R.A. Baker (Ed.). Ann Arbor Science Publ., Inc., p. 533–553, 1980.
32. Packer, D.M., Ireland, M.P. and Wootton, R.J., Cadmium, copper, lead, zinc and manganese in the polychaete *Arenicola marina* from sediments around the coast of Wales. *Environ. Pollut.*, 22(A): 309–321, 1980.
33. Loring, D.H. and Prosi, F., Cadmium and lead cycling between water, sediment, and biota in an artificially contaminated mud flat on Borkum (F.R.G.). In: *Estuarine and coastal pollution: detection, research and control*, D.S. Moulder and P. Williamson (Eds.). *Wat Sci. Tech.* Vol. 18. Plymouth, pp. 131–139, 1986.
34. Miramand, P., Germain, P. and Camus, H., Uptake of americium and plutonium from contaminated sediments by the three benthic species: *Arenicola marina*, *Corophium volutator* and *Scrobicularia plana*. *Mar. Ecol. Prog. Ser.*, 7: 59–65, 1982.
35. Foster-Smith, R.L., An analysis of water flow in tube-living animals. *J. Exp. Mar. Biol. Ecol.*, 34: 73–95, 1978.
36. Baumfalk, Y.A., Heterogeneous grain size distribution in tidal flat sediment caused by bioturbation activity of *Arenicola marina* (Polychaete). *Neth. J. Sea Res.*, 13 (3/4): 428–440, 1979.
37. Hylleberg, J., Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vgabunda* and a concept of gardening in lugworms. *Ophelia*, 14: 113–137, 1975.
38. Lenihan, H.S., Kiest, K.A., Conlan, K.E., Slattery, P.N., Konar, B.H. and Oliver, J.S., Patterns of survival and behavior in Antarctic benthic invertebrates exposed to contaminated sediments: field and laboratory bioassay experiments. *J. Exp. Mar. Biol. Ecol.*, 192: 233–255, 1995.
39. Prouse, N.J. and Gordon, Jr., D.C., Interaction between the deposit feeding polychaete *Arenicola marina* and oiled sediment. In: *Source, Effects & Sinks of Hydrocarbons in the Aquatic Environment*, The American Institute of Biological Sciences. p. 407–422, Proc. Symposium American University, Washington, 1976.
40. Gordon, D.C. Jr., Dale, J. and Keizer, P.D., Importance of sediment working by the deposit-feeding polychaete *Arenicola marina* on the weathering rate of sediment-bound oil. *J. Fish. Res. Bd. Can.*, 35: 591–603, 1978.
41. 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. Pollut.*, 76: 86–131, 1992.
42. Langston, W.J. and Spence, S.K., Metal analysis. In: *Handbook of Ecotoxicology*. Volume 2 Peter Calow (Ed.) Ch. 4, p. 45–78. Oxford Blackwell Sci. Publ., London, 1994.
43. Reise, K., Experimental removal of lugworms from marine sand affects small zoobenthos. *Mar. Biol.*, 74: 327–332, 1983.
44. Reise, K. and Ax, P., A meiofaunal "thiobios" limited to the anaerobic sulfide system of marine sand does not exist. *Mar. Biol.*, 54: 225–237, 1979.
45. Renfro, W.C., Transfer of <sup>65</sup>Zn from sediments by marine polychaete worms. *Mar. Biol.*, 21: 305–316, 1973.
46. Bryan, G.W. and Hummerstone, L.G., Adaptation of the Polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of heavy metals. I. General observation and adaptation to copper. *J. Mar. Biol. Ass. UK*, 51: 845–863, 1971.
47. Bryan, G.W., Adaptation of an estuarine polychaete to sediments containing high concentrations of heavy metals. In: *Pollution and physiology of marine organisms*, F.J. Vernberg and W.B. Vernberg (Eds.). Academic Press London, p. 123–135, 1974.
48. Lee, H., II and Swartz, R.C., Biological processes affecting the distribution of pollutants in marine sediments. Part II. biodeposition and bioturbation. In: *Contaminants and sediments volume 2, analysis, chemistry, biology*, R.A. Baker (Ed.) Ann Arbor Science Publ., Inc., p. 555–606, 1980.

49. Brown, S.L., Faeces of intertidal benthic invertebrates: influence of particle selection in feeding on trace element concentration. *Mar. Ecol. Prog. Ser.*, 28: 219–231, 1986.
50. Phillips, D.J.H. and Rainbow, P.S., *Biomonitoring of trace aquatic contaminants*. Environmental Management Series, Chapman & Hall, London, 1994.
51. Jacobsen, V.H., The feeding of the lugworm, *Arenicola marina* (L.). Quantitative studies. *Ophelia*, 4: 91–109, 1967.
52. Boothe, P.N. and Knauer, G.A., The possible importance of faecal material in the biological amplification of trace and heavy metals. *Limnol. Oceanogr.*, 17 (2): 270–274, 1972.
53. Nott, J.A. and Nicolaidou, A., Kinetics of metals in molluscan faecal pellets and mineralized granules, incubated in marine sediments. *J. Exp. Mar. Biol. Ecol.*, 197: 203–218, 1996.
54. Amiard-Triquet, C., Etude de la décontamination d'*Arenicola marina* (annélide, polychète) après contamination expérimentale par le caesium 137 ou le cobalt 60. *Mar. Biol.*, 26: 161–165, 1974.