Bioaccumulation of Heavy Metals (Zn, Pb, Cu and Cd) by *Tympanotonus fuscatus* var. *radula* (L.) exposed to Sublethal Concentrations in Laboratory Bioassays

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Abstract

The exposure of the edible periwinkle, *Tympanotonus fuscatus* var. *radula* to sublethal concentrations (1/100th and 1/10th of 96 h LC_{50} values of zinc, lead, copper and cadmium compounds, respectively) of heavy metals resulted in the bioaccumulation of the test metals to varying degrees that was dependent on the type of metals and concentration of the metal compound in the test media. Post treatment analysis of the body tissues of the animals revealed that the exposed animals accumulated higher concentrations of Zn and Pb ions that were about 2-6 times higher than the levels accumulated in control animals. With regards to Cu ions, it was observed that the concentration of Cu ions accumulated by the exposed animals fluctuated significantly over the 30-days exposure period while there was little differences between the concentrations of Cd ions accumulated by animals exposed to the treated media and control. Comparisons between the concentration of heavy metals in whole body tissues of *T. fuscatus* and the sediment of the media showed that the concentration of the metals accumulated in tissues of *T. fuscatus* were about 2–729 times higher than that in the sediment. The significance of these results and the need to include bioaccumulators of heavy metals such as *T. fuscatus* in monitoring programmes aimed at establishing the environmental levels of such pollutants in aquatic ecosystems were discussed.

Introduction

Industrial wastes or effluents are a complex admixture of several classes of pollutants such as synthetic chemicals of various types, hydrocarbons and heavy metals (Van-Den-Heever & Frey, 1994). Some of these pollutants, particularly the heavy metals, are non-degradable hence they persist in the recipient environment for a long period of time. Although these metals may occur at levels below their toxic thresholds in natural bodies of water (Oyewo, 1998), the low concentrations may still pose risk of damage *via* uptake and subsequent bioaccumulation by organisms which cannot effectively metabolize and excrete the absorbed metals. Due to the high risk of biological damage, e.g. Minamata and Itai-Itai diseases posed by heavy metals from industrial and domestic sources over long period in aquatic and terrestrial ecosystems, a lot of research on metal pollution has taken place in the industrialized countries of Europe, America and Asia (Panigrahi & Misra, 1980; Khangarot *et al.*, 1982; Bryan & Langston, 1992).

In Nigeria, however, most studies on heavy metal pollution have concentrated on levels of occurrence and distribution of these pollutants in sediment and water column of aquatic resources (Fodeke, 1979; Bhalerao & Adeeko, 1981; Akinola *et al.*,

1981; Okoye, 1989; Ogunsua *et al.*, 1991) without relating the observed level of occurrence to biological action, such as acute toxicity and sublethal chronic action including bioaccumulation.

Many research efforts in other parts of the world have shown increases in concentrations of metals and other pollutants in whole body of organisms inhabiting the waterbodies following observed increases in ambient metal concentrations. For example, Kiffney & Clement (1993) reported increases in metal concentration of Cd, Cu and Zn by about two to three fold over a period of 1year in *Periphyton, Baetis, Arctopysche* and *Rhyacophila* species in a heavy metal contaminated station on the Arkansas river. Furthermore, the authors reported that the metal concentrations in the animals remained high at the end of the 1-year monitoring period, even though the ambient concentrations of heavy metals had decreased. Nehring (1976) reported that the aquatic insects *Ephemeralla grandis* (mayfly) and *Pteronarcys california* (stonefly) accumulated heavy metals to levels over a 100-fold higher than the concentrations of the same metals in the surrounding waters. The results showed a strong and positive correlation ($r = 0.97$) between amounts of metals in the aquatic insects and the surrounding water medium, indicating that most of the accumulated metals were from the water medium.

The significance of bioaccumulation studies lies in the potential disruption of ecological balance which had been attained over the years and the public health risk which may occur when organisms including man, which occupy higher levels in the food chain, feed on highly contaminated prey and become exposed to the toxic effects of the metals. Real life tragic experiences such as the Minamata and Itai-Itai diseases (Varma *et al.*, 1976) have shown to the whole world the devastating effects that the accumulation of heavy metals in animal/plant tissues which serve as food sources could have on higher predators, particularly man. Furthermore, the observation of very high concentrations of heavy metals in animal/plant tissues inhabiting water bodies with low metal concentration in their sediment and water column have necessitated the inclusion of such bioaccumulators in monitoring programmes aimed at establishing the environmental levels of such pollutants in aquatic ecosystems (Bryan & Langston, 1992). Therefore, studies on bioaccumulation of heavy metals, particularly in organisms that serve as food for man, have been a focus of many research efforts all over the world (Kiffney & Clement, 1993; Van-Den-Heever & Frey 1994; Oyewo, 1998).

In view of the above-mentioned, the objective of this paper is to determine the importance of *Tympanotonus fuscatus* as a bioaccumulator of heavy metals that can, therefore, be included in biomonitoring programmes of heavy metals in aquatic ecosystems.

Materials and methods

Test animals

Tympanotonus fuscatus var. *radula* L. (Periwinkle) (Mollusca; Gastropoda, Mesogastropoda, Potamididae) of similar sizes (shell length of 28-32 mm) were collected by hand–picking into a bucket (12.6 *l*) from the lagoon edge at low tide. The periwinkles were always collected from the same site, in order to reduce variability in biotype. The animals were transported to

the laboratory and kept in holding glass tanks (30 cm × 30 cm × 30 cm) which contained aerated lagoon water (6 *l*) at measured salinity that was variable (ranging from 2 psu to 21 psu) depending on time of year. Mud was collected from the same site and placed in the holding tank as substrate.

Laboratory animal cultures, acclimatization and selection of test animals for bioassays

Tympanotonus fuscatus var. *radula* collected as described above were left in holding tanks with a thin layer of sediment serving as substrate and food source for 5–6 days to allow them acclimatize to laboratory and experimental conditions (R.H. 70 ± 2%; temperature 26 ± 2 °C; salinity 16 psu) before using them in bioassays. The test animals were acclimatized to higher or lower salinities by diluting or concentrating.the lagoon water by 2.5 psu once every 24 h using dechlorinated tap water or sea water of known salinity strength so that after the required number of changes, the desired pre-determined test salinity (16 psu) for all bioassays was achieved. This approach of gradual change in salinity was adopted to allow the periwinkles time to gradually adapt to the new salinity and so prevent a sudden osmotic change, which could result in stress that may distort responses to the test toxicants. *T. fuscatus* var. *radula* of similar sizes depending on shell length (32-38 mm) were selected for all experiments.

Test chemicals

The heavy metals investigated in this work were obtained as metallic salts of Fisons laboratory reagents, analar grades of the following types:

(a) Copper as $CuS0_4.5H_20$
(b) Zinc as $ZnC0_3.3H_20$
(c) Lead as $Pb(N0_3)_2$
(d) Cadmium as $3CdS0_4.8H_20$

The choice of heavy metals for this study was based on the available, common and abundant metals from the results of a chemical survey of effluents of industrial establishments in Lagos carried out by Oyewo (1998) and a survey of the Lagos lagoon water and sediment for heavy metals (Otitoloju, 2001).

General bioassay technique
Salinity of test media

All bioassays were carried out at salinity of 16 psu. To achieve this, sea water was diluted with dechlorinated tap water to a salinity of 16 psu and served as test media and also as dilution water for mixing toxicants. The animals were acclimatized to 16 psu. This salinity condition was used in all bioassays in order to standardize and simulate a typical brackish water medium, since changing salinity have been observed to affect toxicity (Tokolo, 1988).

Preparation of substrate

In an attempt to simulate the natural environment of benthic test animals, sediment from the site of animal collection was used as substrates. The sediment also served as food for the test animals during the bioassays.

Preparation of test media including application of toxicant

A pre-determined amount of each heavy metal compound was weighed (using an oertling 30TD top loading balance) out and to this was added a given volume of diluent to obtain a stock solution of known strength.

The resultant stock solution was serially diluted to obtain solutions of required concentrations.

Bioaccumulation studies

Bioaccumulation of heavy metals (Zn, Pb, Cu and Cd) by T. fuscatus *exposed to sublethal concentration of single metal compounds*

In this series of experiments, *T. fuscatus* was exposed to only sublethal concentrations of selected metal salts (fractions of 96 h LC_{50}) values derived from experiments on acute toxicity of heavy metals against benthic animals (Otitoloju, 2001). A total of 120 test animals were exposed per sublethal concentration or control in five replicates (24 periwinkles per replicate). Each bioassay container (plastics) held a thin layer of standardized sediment substrate and test media at salinity of 16 psu. In this series of bioassays that went on for 30 days in order to investigate the rate of bioaccumulation, the semi-static bioassay procedure was always adopted to avoid drastic changes in concentration of test media via evaporation and excessive reduction in dissolved oxygen level. In this semi-static procedure, each test media was changed into a fresh solution once every 4 days, at exactly the same concentration of heavy metal salt or untreated control as the case may be. The same exposed test animals were transferred into the freshly prepared test media over a 30-days period of experimentation.

At pre-determined time intervals (day 0, 4, 10, 20 and 30), four live *T. fuscatus* per replicate, making 20 per treatment, including control, were randomly selected, cleaned thoroughly with distilled water and placed in labelled polyethylene bags in which they were kept frozen awaiting digestion of the extracted whole animal tissues and analysis for test metals by atomic absorption spectrophotometer (AAS). Sediment samples in the test media were also collected at the pre-determined period, digested and analysed for metal content by AAS. Sublethal concentrations under which bioaccumulation of test metals by *T. fuscatus* was investigated were as follows:

a. Zn ($ZnC0_3$. $3H_20$) was tested at:
   8.310 mg/l ($1/10^{th}$ of the 96 h LC_{50})
   0.8317 mg/l ($1/100^{th}$ of the 96 h LC_{50})
   and untreated control.

b. Pb ($Pb(N0_3)_2$) was tested at:
   37.077 mg/l ($1/10^{th}$ of the 96 h LC_{50})
   3.7077 mg/l ($1/100^{th}$ of the 96 h LC_{50})
   and untreated control.

c. Cu ($CuS0_4.5H_20$) was tested at:
   3.925 mg/l ($1/10^{th}$ of the 96 h LC_{50})
   0.3925 mg/l ($1/100^{th}$ of the 96 h LC_{50})
   and untreated control.

d. Cd ($3CdS0_4.8H_20$) was tested at:
   2.825 mg/l ($1/10^{th}$ of the 96 h LC_{50})
   0.2825 mg/l ($1/100^{th}$ of the 96 h LC_{50})
   and untreated control.

Analysis of samples (sediment and animal) for heavy metal content by atomic absorption spectrophotometry

Sample preparation

Sediment samples. These samples were subjected to low temperature (50 ± 2 °C) oven drying for 72-120 h. After drying, visible remains of organisms and debris were removed. The dried samples were then tritured using a mortar and pestle and sieved using a 200 µm sieve to normalize for particle size. From the dried sieved sediment sample, 5 g was placed in plastic beaker

along with 25 ml of 0.5 *M* HCl. This mixture was stirred for $2^1/_2$ h with a magnetic stirrer instead of a mechanical shaker in a slight modification of the method of Whitehead *et al.* (1984). This cold extraction method is sufficient to release the loosely-held fraction of heavy metals into the solution. Metals in this loosely-held fraction are known to be most readily bioavailable (Ageiman & Chau, 1976; Bryan & Langston, 1992).

Animal samples. Whole animal samples (deshelled) of *T. fuscatus* were properly cleaned (by copiously rinsing all exposed and partially enclosed parts) with distilled water to remove debris, plankton and other external adherents before they were homogenized. A portion (10 g wet weight basis) from the homogenate of each animal species was digested using a freshly prepared mixture 1:1 of hydrogen peroxide and perchloric acid (FAO/SIDA, 1986).

AAS determination of heavy metals in samples (sediment and animals)

All digestates and extracts obtained were filtered through Whatman No. 1 filter paper and made up to the mark in appropriate volumetric flasks (25 cc for animal samples, 50 cc for water samples and 100 cc for sediment samples). The heavy metal content of each sample was then determined by comparing their absorbances with those of standard AAS solutions using an alpha–4 cathodeon atomic absorption spectrophotometer.

Data analysis

Regression analysis (using Microsoft Excel 2000) was carried out to determine correlation coefficient (r^2) between concentrations of test metals accumulated in the test animals *T. fuscatus* or sediment with period of exposure. Bioaccumulation factor (BAF) was also estimated as the ratio of the concentration of the metal in animal tissue after 30 days of exposure to the concentration of metals in the sediment.

$$\text{Bioaccumulation factor (BAF) \{steady state -30 days\}} = \frac{\text{Concentration in animal tissue}}{\text{Concentration in sediment}}$$

Results

Bioaccumulation of Zn ions by T. fuscatus

At the highest sublethal concentration (4.325 mg/*l*, 1/10th of 96 h LC_{50}) tested, the amount of Zn accumulated in the test animals increased steadily with time of exposure to a maximum level of 32.28 µg/g which was found to be four times higher than the amount of 7.29 µg/g accumulated by animals in the untreated control (Table 1). The result also shows that at the lower sublethal concentration (0.4325 µg) tested, the amount of Zn accumulated in the test animals increased with time to a maximum of 15.36 µg/g which was found to be 2.0 times higher than the amount of 7.29 µg/g accumulated by the control. Additionally, slight fluctuations and a tendency towards leveling off in levels of Zn accumulated in test animals exposed to sublethal concentrations 1/100th of 96 h LC_{50}) of Zn were observed during the 30-days period of observation (Fig. 1).

Furthermore, at the end of the 30-days exposure period, there was an overall gain of 11.07 µg/g and 27.99 µg/g of Zn over the respective initial amount of the metal in the animal tissues exposed to the sublethal concentration of 0.4235 mg/l (1/100th of 96 h LC_{50}) and 4.235 mg/l (1/10th of 96 h LC_{50}), respectively (Table 1). There were

TABLE 1

Accumulation of heavy metals [Zn, Pb, Cu or Cd] by T. fuscatus *exposed to sublethal concentrations of each metal over a 30-day period under laboratory conditions*

Treatments [mg/l]	*Mean concentrations of heavy metals in whole animal tissue [μg g⁻¹ dry weight basis]*					*Overall net gain **
	0 days	*4 days*	*10 days*	*20 days*	*30 days*	
Zinc						
Untreated control	4.29	7.37	8.09	7.82	7.29	
+0.4325 [1/100th LC_{50}]	4.29	8.42	7.44	8.93	15.36	
Net gain**	-	4.13	-0.98	1.49	6.43	11.07
Untreated control	4.29	7.37	8.09	7.82	7.29	
++4.325 [1/10th LC_{50}]	4.29	7.84	12.27	26.48	32.28	
Net gain**	-	3.55	4.43	14.21	5.80	27.99
Lead						
Untreated control	29.90	29.19	49.84	58.64	60.55	
+3.9635 [1/100th LC_{50}]	29.90	31.54	39.54	160.75	173.95	
Net gain**	-	1.64	8.00	121.21	13.20	144.05
Untreated control	29.90	29.19	49.84	58.64	60.55	
++39.635 [1/10th LC_{50}]	29.90	80.79	130.55	203.43	368.93	
Net gain**	-	50.89	49.76	72.88	160.50	339.03
Copper						
Untreated control	11.04	14.63	17.62	10.69	6.55	
+0.1021 [1/100th LC_{50}]	11.04	12.96	13.05	7.65	11.48	
Net gain**	-	1.92	0.09	-5.4	3.83	0.44
Untreated control	11.04	14.63	17.62	10.69	6.55	
++1.021 [1/10th LC_{50}]	11.04	21.69	18.89	12.39	18.27	
Net gain**	-	10.65	-2.8	-6.5	5.88	7.23
Cadmium						
Untreated control	0.02	0.02	0.03	0.06	0.08	
+0.0904 [1/100th LC_{50}]	0.02	0.03	0.11	0.32	0.58	
Net gain**	-	0.01	0.08	0.21	0.26	0.56
Untreated control	0.02	0.02	0.03	0.06	0.08	
++0.904 [1/10th LC_{50}]	0.02	0.26	0.91	1.22	1.26	
Net gain**		0.24	0.65	0.31	0.04	1.24

*Overall net gain = Concentration in animal after 30 days – Concentration in animal at zero day

**Net gain = difference in concentration between the immediate preceding days of harvesting, e.g. 4 – 0 day, 10 – 4 day, etc.

+ 1/100th 96-h LC_{50} values of the metal ions in the test compounds

++1/10th 96-h LC_{50} values of the metal ions in the test compounds

significant and positive correlations (r^2 = 0.818 and 0.9789; n=3) between the amount of Zn accumulated by *T. fuscatus* with time of exposure for the 1/100th and 1/10th 96 h LC_{50} test media, respectively (Fig.1). Similarly, there was a significant and positive correlation (r^2 = 0.9529; n=3) between the concentration of Zn accumulated by the test animals and the concentration detected in sediment from the various test media (control, 1/100th and 1/10th 96 h LC_{50}) at the steady state (day 30).

On the basis of the computed bioaccumulation factor (BAF), with reference to sediment concentration, the levels of Zn accumulated in the test animals exposed to sublethal concentrations (1/100th and 1/10th of 96 h LC_{50} values) were

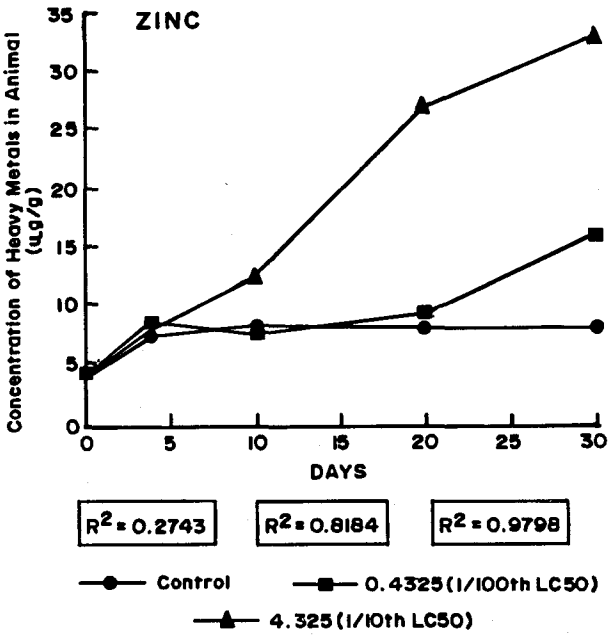

Fig. 1. Profile of accumulation of zinc ions by *T. fuscat*exposed to varying sublethal concentrations of the metals over a 30-days period in laboratory bioassays.

found to be lower than the concentration detected in the sediment substrate (BAF = 0.879 and 0.213 for 1/100th and 1/10th of 96 h LC_{50} test media, respectively) (Table 2). However, in the control medium, the amount of Zn ions accumulated by the test animals was found to be about 729 times higher than the level detected in the sediment substrate.

Biaccumulation of Pb ions by T. fuscatus

Post treatment analysis of whole body tissue of *T. fuscatus* showed that the animals exposed to sublethal concentrations (3.964 mg/l and 39.635 mg/l) of the lead compound accumulated measurable quantities of the metal that were approxi-mately 3-6 times above the levels accumulated by the animals in untreated control media (Table 1, Fig. 2). Furthermore, there was an overall gain of the 144.05 µg/g and 339.03 µg/g of Pb over the respective initial amount of the metal in the animal tissues exposed to the sublethal concentrations of 3.964 mg/l ($^{1}/_{100}^{th}$ of 96 h LC_{50}) and 39.635 mg/l ($^{1}/_{10}^{th}$ of 96 h LC_{50}) of Pb compound, respectively (Table 1).

For all the sublethal concentrations tested, the amount of Pb accumulated in the animal tissues increased steadily with the time of exposure (Table 1 and Fig. 2) and the concentration of Pb in the test media. For example, *T. fuscatus* exposed for 30 days to sublethal concen-tration of 39.635 mg/l (1/10th of 96 h LC_{50}), 3.9635 (1/100th of 96 h LC_{50}) and control media accumulated an amount of 368.93 µg/g, 173.95 µg/g and 60.55 µg/g of Pb in their body tissues, respectively, at the end of the 30-days observation period (Table 1).

There were significant and positive correlations (r^2 = 8.8859 and 0.9715; n=3) between the amount of Pb accumulated by *T. fuscatus* with time of exposure for 1/100th of 96 h LC_{50} and 1/10th of 96 h LC_{50} test media, respectively (Fig. 2). Similarly, there was a significant and positive correlation (r^2 = 0.9085; n=3) between the concentration of Pb accumulated by the test animals and the concentration detected in sediment from the various test media (control, 1/100th and 1/10th 96 h LC_{50}) at the steady state (day 30).

On the basis of the computed bioaccumulation factor (BAF), with reference to the sediment concentration, the amount of Pb accumulated by the animals exposed to sublethal concentrations (1/100th of 96 h LC_{50} and 1/10th of 96 h LC_{50}) and control media were found to be 16

TABLE 2

Bioaccumulation factors of heavy metals [Zn, Pb, Cu and Cd] by* T. fuscatus *exposed to sublethal concentrations of the metals over a 30-day period under laboratory conditions*

Treatments metals	*Mean concentration of metals in sediment** (μgg^{-1})*	*Mean concentration of metals in animal tissues after 30 days (μgg^{-1})*	*BAF**
Control			
Zn	0.01	7.29	729
Pb	0.623	60.55	97.19
Cu	0.014	6.55	467.86
Cd	0.001	0.08	80
1/100th of 96-h LC_{50} mg/l			
Zn {0.832}	17.13	15.36	0.897
Pb {6.098}	10.72	173.95	16.227
Cu {0.393}	1.69	11.48	6.793
Cd {0.283}	0.033	0.58	17.576
1/10th of 96-h LC_{50}mg/l			
Zn {8.32}	151.665	32.28	0.213
Pb {60.98}	140.43	368.93	2.627
Cu {3.93}	15.32	18.27	1.193
Cd {2.83}	0.07	1.26	18

*BAF = Bioaccumulation factor {steady state-30 days} = $\frac{\text{Concentration in animal tissue}}{\text{Concentration in sediment}}$

** Values for three replicates of sediment samples and 10 animal tissues samples

times, three times and 97 times higher than the level detected in the sediment substrate, respectively (Table 2).

Bioaccumulation of Cu by T. fuscatus

Post treatment analysis of wholebody tissues of *T. fuscatus* showed that the animals exposed to sublethal concentrations (0.1021 mg/l and 1.021 mg/l) of the test Cu compound accumulated measurable quantities of the metal ions that were approximately two to three times higher than the level accumulated by animals in the untreated control media (Table 1, Fig. 3).

Furthermore, at end of the 30-days exposure period, there was an overall gain of 0.44 µg/g and 7.23 µg/g of Cu ions over the respective initial amount of Cu ions in the wholebody tissues of animals exposed to sublethal concentrations of 0.1021 mg/l (1/100th of 96 h LC_{50}) and 1.021mg/l (1/10th of 96 h LC_{50}), respectively (Table 1). For all the sublethal concentrations tested, the amount of Cu accumulated by the exposed animals fluctuated significantly over the 30-days exposure period (Table 1, Fig. 3). For instance, animals exposed to the highest sublethal concentration (1/10th of 96 h L C_{50}) of the metal lost an amount of 2.8 mg/g and 6.5 mg/g of Cu gained initially after the 10th and 20th days of exposure.

There were no significant positive correlations (r^2 = 0.1351 and 0.0069; n=3) between the amount of Cu accumulated by *T. fuscatus* with time of exposure for 1/100th and 1/10th of 96 h LC_{50} test media, respectively (Fig. 3). However, there was a significant and positive correlation (r^2 = 0.8936; n=3) between the concentration of

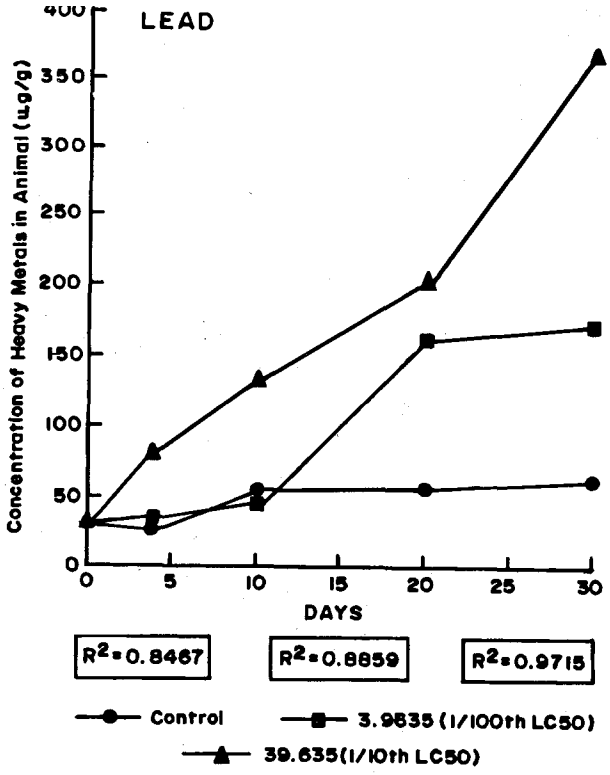

Fig. 2. Profile of accumulation of lead ions by *T. fuscatus* exposed to varying sublethal concentrations of the metals over a 30-days period in laboratory bioassays.

Cu accumulated by the test animals and the concentration detected in sediment from the various test media (control, 1/100th and 1/10th 96 h LC_{50}) at the steady state (day 30).

On the basis of the computed bioaccumulation factor (BAF), with reference to sediment concen-tration, the amount of Cu accumulated by the test animal exposed to sublethal concentrations, 1/100th and 1/10th of 96 h LC_{50} and untreated control media were found to be about 7 times, 1.2 times and 468 times higher than the levels detected in the sediment of the corresponding test media, respectively (Table 2).

Bioaccumulation of cadmium ions by T. fuscatus

Post-treatment analysis of wholebody tissue of *T. fuscastus* showed that the concentration of Cd accumulated in tissues of animals exposed to sublethal concentrations of 0.0904 mg/l and 0.904 mg/l were 0.58 µg/g and 1.2 µg/g, respectively, compared to the amount of 0.08 µg/g accumulated in the body tissues of animals in the control medium (Table 1, Fig. 4). Furthermore, at the end of the 30-days exposure period, there was an overall gain of 0.56 µg/g and 1.24 µg/g over the respective initial amount of Cd ions in the animals exposed to the sublethal concentration of 0.0904 mg/l (1/100th of 96 h LC_{50}) and 0.904 mg/l (1/10th of 96 h LC_{50}), respectively (Table 1).

Although the magnitude of Cd accumulated by the test animals was not substantial, there were significant and positive correlations between the amount of Cd accumulated by *T. fuscatus* with time of exposure (r^2 = 0.966 and 0.9712; n=3) for 1/100th and 1/10th of 96 h LC_{50} test media, respectively (Fig. 4). Similarly, there was a significant and positive correlation (r^2 = 0.9979; n=3) between the concentration of Cd accumulated by the test animals and the concentration detected in sediment from the various test media (control, 1/100th and 1/10th 96 h LC_{50}) at the steady state (day 30).

On the basis of the computed bioaccumulation factor (BAF), with reference to sediment concentration, the amount of Cd ions accumulated by the test

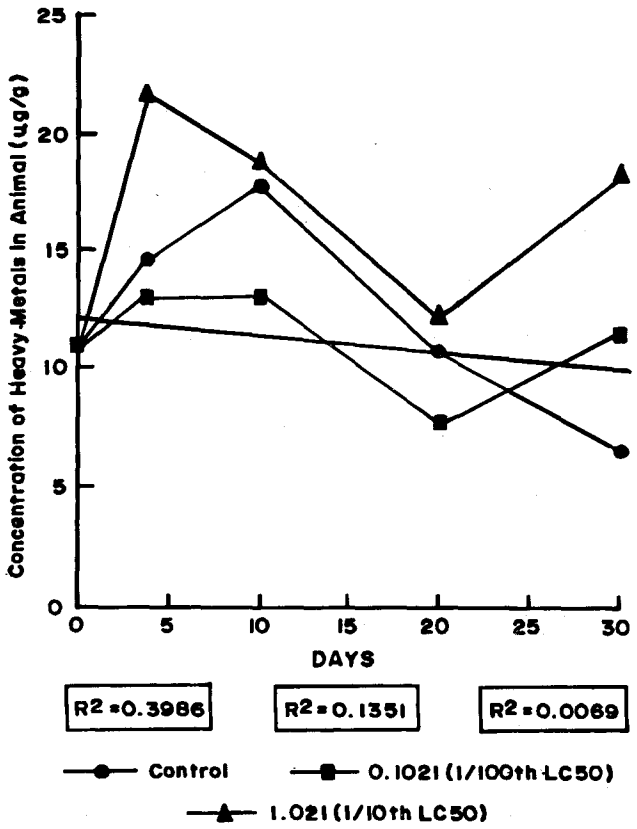

Fig. 3. Profile of accumulation of copper ions by *T. fuscatus* exposed to varying sublethal concentrations of the metals over a 30-days period in laboratory bioassays.

animals exposed to sublethal concentrations, 1/100th and 1/10th of 96 h LC_{50} and untreated media were found to be about 17 times, 18 times and 80 times higher than the levels detected in the sediment, respectively (Table 2).

Discussion

The exposure of *T. fuscatus* to sublethal concentrations of heavy metals (Zn, Pb, Cu or Cd) resulted in a varying degree of accumulation of the metals by the exposed organism. The level of heavy metals accumulation in the animals was found to depend largely on the type of heavy metals and its concentration in the test media. For example, the concentration of Zn and Pb ions accumulated by *T. fuscatus* was found to increase steadily with period of exposure and concentration of the metal compound in the test medium. Chukwu (1991) and Oyewo (1998) have also demonstrated that concentrations of these metals in exposed animals such as *Palaemonestes africanus*, *Tympanotonus fuscatus*, *Tilapia guineensis* and *Clibanarius africanus* increased with time of exposure and test medium concentration.

Furthermore, exposure of *T. fuscatus* to the sublethal concentrations (0.1021 mg/l and 0.021 mg/l (1/10th and 1/100th of 96 h LC_{50}) of Cu revealed that the amount of Cu accumulated in the tissues of these animals fluctuated significantly over the experimental period; also indicating the capability of the test animal to regulate its tissue concentration of copper. This observed ability of *T. fuscatus* to regulate its body concentration of Cu is rather expected because copper is a component of haemocyanin, which is a respiratory pigment in the body fluids of *T. fuscatus*. Bryan & Gibbs (1987) have also reported the ability of polychaete worms, *Melinna palmata* (which naturally has a high concentration of Cu in its tissues) and *Tharyx marioni* to regulate their tissue concentration of Cu. The significance of this observation and those of other researchers is that in biomonitoring programmes, the concentration of Cu in

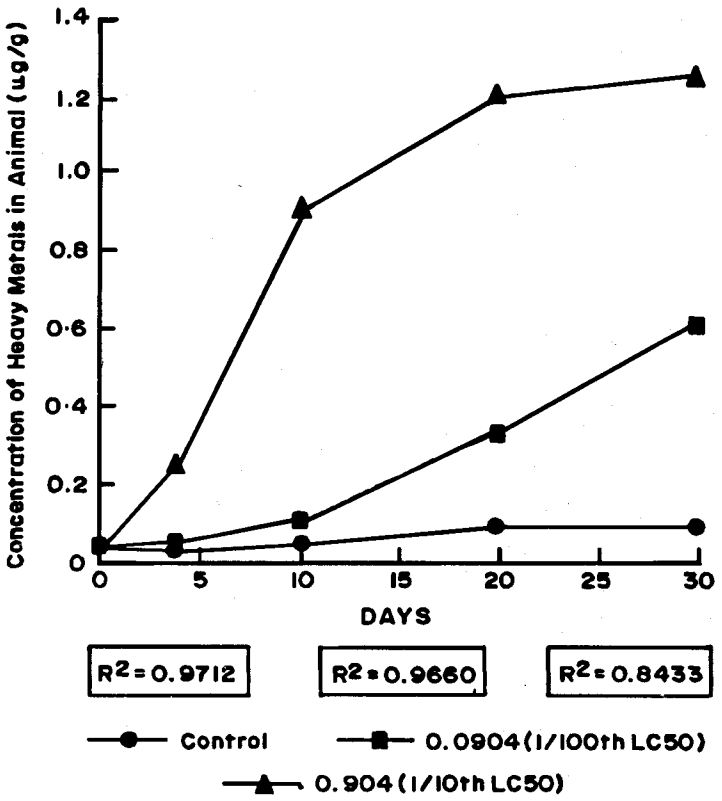

Fig. 4. Profile of accumulation of cadmium ions by *T. fuscatus* exposed to varying sublethal concentrations of the metals over a 30-days period in laboratory bioassays.

tissues of aquatic animals such as *T. fuscatus*, *M. palmate* and *T. marioni* may not serve as a good estimate of environmental levels of the metal (Cu) in the sediment and water column.

With regards to cadmium accumulation, the exposure of the test animal to sublethal concentrations of cad-mium was found to result in slight changes in the body tissue concen-tration of cadmium in the exposed animals over the 30-days experimental period. Similar observation of low level of cadmium accumulation in the body tissues of *T. fuscatus* and *T. guineensis* has been reported by Oyewo (1998). Therefore, the mechanism(s) by which *T. fuscatus* and *T. guineensis* excrete cadmium rapidly from their bodies or ensure that the levels in their body tissues do not build up relative to the ambient concentrations would merit future investigations.

Comparisons between the concentration of heavy metals in the body tissue of the *T. fuscatus* and the sediment of the test media showed that there were positive correlations between the concentration of the test heavy metals (Zn, Pb, Cu and Cd) accumulated by *T. fuscatus* and the concentration of the metals in the sediment in that, as the concentration in the sediment increased, the concentration accumulated by the test animals also increased. This observation confirms the importance of metal concentration in the sediment habitat on the amount of metals bioaccumulated by benthic animals, particularly the deposit feeders such as *T. fuscatus* which normally ingest the sediment particles in order to obtain its food materials. It also justifies the importance attached to benthic animals (bottom dwelling animals) as an important and useful group of organisms for assessing heavy metal toxicity and bioaccumulation in this study. Furthermore, in some instances, the concentration of the metals in the body

tissue of *T. fuscatus* was found to be about two to 729 times higher than the concentrations detected in the sediment.

The observation of the ability of the edible periwinkle, *T. fuscatus* to accumulate heavy metals to levels that are several folds higher than the amount in its surrounding environment demonstrates how biological systems can render unsafe an otherwise seemingly low and apparently safe prevalent level of persistent pollutants in ecosystems. Moreso, when most monitoring programmes of pollutants usually concentrate on measuring the pollutants levels in the sediment and water column only (Schumacer *et al.*, 1992 and Sastry & Shukla, 1993). Furthermore, it should also necessitate the inclusion of such bioaccumulators like *T. fuscatus* in monitoring programmes aimed at establishing the environmental levels of such pollutants in aquatic ecosystems.

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