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A comparison of metal levels and antioxidant enzymes in freshwater snails, *Lymnaea natalensis*, exposed to sediment and water collected from Wright Dam and Lower Mguza Dam, Bulawayo, Zimbabwe

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**A R T I C L E  I N F O**

Article history:
Received 2 June 2010
Received in revised form 26 July 2010
Accepted 2 August 2010
Available online 1 September 2010

**Keywords:**
Antioxidant enzymes
Heavy metals
*Lymnaea natalensis*

**A B S T R A C T**

We compared the bioaccumulation of lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), nickel (Ni) and iron (Fe) with antioxidant enzyme activity in tissues of the snails, *Lymnaea natalensis*, exposed to elements of two differently polluted dams. 45 snails were exposed to sediment and water collected from Wright Dam (reference) whilst another 45 snails were also exposed to sediment and water collected from Lower Mguza Dam (polluted dam). Except for Fe in sediment and Pb in water, metal concentrations were statistically higher in sediment and water collected from Lower Mguza Dam. Lead, Cd and Zn were two times higher in tissues of snails exposed to Lower Mguza Dam elements. On one hand, superoxide dismutase (SOD), diphosphotriphosphodiaphorase (DTD) and catalase (CAT) activities were significantly lower whilst malondialdehyde (MDA) levels were significantly higher in tissues of snails exposed to Lower Mguza Dam sediment and water. On the other hand, selenium-dependent glutathione peroxidase (Se-GPX) activity was significantly elevated in tissues of snails exposed to Lower Mguza Dam sediment and water. Snails exposed to Lower Mguza Dam elements seem to have responded to pollution by increasing CAT and Se-GPX specific activity in an effort to detoxify peroxides produced as a result of metal induced oxidative stress.

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1. Introduction

High levels of trace metals in freshwater may occur as a result of natural weathering of minerals in the sediments and bedrocks or as a result of anthropogenic activities such as mining, industrial, municipal and agricultural discharges (Olsvik et al., 2001). These high metal levels pose stress to aquatic organisms in particular and the ecosystem in general (Kotze et al., 1999). Monitoring of these metals in the environment has become imperative. As aquatic and terrestrial molluscs have been shown to have a high capacity for metal accumulation (Cossu et al., 2000; Pyatt et al., 2003), they have become important in biomonitoring of the metals (Gomot, 2000). Most of the trace metals are essential in small concentrations for normal metabolic processes in most flora and fauna. At abnormally high concentrations, metals such as copper can cause death in aquatic snails (Masola et al., 2003). Other metals such as cadmium (Cd), mercury (Hg), silver (Au), zinc (Zn) and chromium (Cr) have been shown to be toxic to *Lymnaea luteola* (Khangarot and Ray, 1988) and *Lymnaea stagnalis* (Gomot, 1998).

Sublethal and chronic concentrations of metals exert their toxicity by generating free radicals and some non-radicals that are reactive oxygen species (Halliwell and Gutteridge, 2001). Examples of free radicals are the hydroxyl-radical (OH), peroxyl radical (RO₂) and superoxide (O₂⁻) whilst hydrogen peroxide (H₂O₂) is a non-radical reactive oxygen species. These reactive oxygen species can trigger oxidative damage to proteins, nucleic acids and lipids. This usually occurs when the metal detoxification process involves redox cycling reactions (Livingstone, 2001). However, defensive antioxidant enzymes, which detoxify reactive oxygen species, are present in most organisms and their various tissues (Buhler and Williams, 1988). In marine and freshwater organisms, antioxidant enzymes have been used as biomarkers of pollution by metals and organic compounds that generate oxidative stress (Regoli et al., 1997; Dooytse et al., 1997). Malondialdehyde levels have also shown respond to oxidative stress (Zapata-Vivenes and Nusetti, 2007).

Since antioxidant enzyme activities and malondialdehyde levels can be used as biomarkers of pollution (Regoli et al., 1997; Ait Alla et al., 2006), this study was undertaken to determine bioaccumulation of metals, antioxidant enzyme activities and malondialdehyde levels in aquatic snails exposed to sediment and water collected from four randomly chosen sites of Wright Dam (35 km south-east of Bulawayo), a privately owned

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0147-6513/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2010.08.001
dam with no history of pollution and Lower Mguza Dam (which receives domestic and industrial effluent from Bulawayo City). This effluent would contain discharges from several industries including paint manufacture, printing, electroplating and battery manufacture. We chose Lymnaea natalensis, an indigenous species, as it is widely distributed in Zimbabwe where it acts as an agricultural pest.

2. Materials and methods

2.1. Snail exposure and tissue processing

Three replicates of 15 snails, Lymnaea natalensis, (10–15 cm in diameter), were exposed for 30 days to about 20 L water and 3–5 kg surface sediment collected from 4 randomly chosen sites of Lower Mguza and Wright Dams. The snails were fed on lettuce for 30 days after which the surviving snails were sacrificed and stored at either –20 °C for metal analysis or –70 °C for biochemical analysis. The snails had been bred and maintained outdoors in 100 L cement aquaria and fed on

2.2. Heavy metal analysis

2.2.1. Metal analysis in water

Water was filtered through Whatman no. 40 ashless filter paper. Distilled water was spiked with known concentration of the metals for recovery studies. A 100 ml aliquot was digested using HCl on a hot plate until about 10 ml was left. The digest was left on the hot plate to evaporate to about 1 ml and removed to cool after which it was quantitatively washed and filtered using an ashless Whatman no. 540 filter paper into a 25 ml volumetric flask. A 100 ml aliquot of water was spiked with known concentration of the metals for recovery studies. A 100 ml aliquot was digested using HCl on a hot plate until about 10 ml was left. The digest was left on the hot plate to evaporate to about 1 ml and removed to cool after which it was quantitatively washed and filtered using an ashless Whatman no. 540 filter paper into a 25 ml volumetric flask.

2.2.2. Metal analysis in snail tissue and snail shells

About 1 g whole snail soft fresh tissue and 0.5 g snail shells in a 50 ml conical flask (spiked and non spiked) were digested in 30 ml HCl+HNO3 (3+1) for 5 h on a hot plate until the vapor and acid fluid inside the flask (digest) turned clear (Smith et al., 1996). The digest was left on the hot plate to evaporate to about 1 ml and removed to cool after which it was quantitatively washed and filtered using an ashless Whatman no. 540 filter paper into a 25 ml volumetric flask.

2.2.3. Analytical quality control and determination of accuracy

All assays were performed in duplicate or triplicate. Reagent blanks were inserted after every 10 samples. In addition, a known concentration of the standard solution was spiked and inserted after every 10 samples to verify the analytical quality of the result since no standard reference material was available. The recovery rate ranged from 81% to 110% depending on the metal and sample.

2.3. Biochemical analysis

2.3.1. Catalase activity

Catalase activity was determined spectrophotometrically according to the method described by Clairborne (1989). The reaction mixture contained 50 mM potassium phosphate buffer, pH 7 and 19 mM H2O2 in the phosphate buffer. The mixture was incubated at 25 °C and the change in absorbance at 240 nm recorded for 60 s. Catalase activity was calculated using the extinction coefficient for H2O2 (ε=43.6 M−1 cm−1).

2.3.2. Diphosphophosphodiesterase activity

Diphosphophosphodiesterase activity was measured according to the method described by Lind et al. (1990), modified and adapted for a 96 well microplate reader in our laboratory. The reaction mixture was in a final volume of 200 μl and a decrease in absorbance at 600 nm over 3 min at 30 °C was recorded. DTD activity was calculated using the extinction coefficient for dichloropheno-lindophenol (ε=21 M−1 cm−1).

2.3.3. Se-Glutathione peroxidase activity

Hydrogen peroxide was used as a substrate to determine selenium-dependent glutathione peroxidase (Se-GPX) activity and consumption of NADPH monitored at 340 nm after 3 min at 30 °C as described by Scholz et al. (1981) after adapting the method for a 96 well microplate reader. The total reaction mixture volume was 160 μl. The activity of Se-GPX was calculated using the coefficient extinction for NADPH (ε=6.22 × 103 M−1 cm−1).

2.3.4. Superoxide dismutase activity

Superoxide dismutase activity was measured according to the method of Sun et al. (1988). Standard SOD solutions (0–200 ng/tube) in isotonic saline and the SOD assay reagent containing xanthine, EDTA, Na2CO3 and nitrobluetetrazolium were made. The reaction was started by adding xanthine oxidase and allowed for 30 min. The amount of the bovine liver SOD that inhibited NBT reduction by 50% was defined to be 1 unit enzyme activity.

2.3.5. Lipid peroxidation

Lipid peroxidation was measured by determining MDA concentration in snail tissue according to the method of Draper and Hadley (1990). Tissue was homogenized in aqueous TCA and methanolic BHT and heated in a capped tube in a boiling water bath for 30 min. The cooled samples were centrifuged to remove particulate matter and absorbance read at 535 nm. Concentration of MDA was calculated using the coefficient of extinction, ε=1.5 × 105 M−1 cm−1.

2.3.6. Protein measurements

Protein was measured according to Lowry et al. (1951), using bovine serum albumin as the standard. All assays were performed in either triplicate or quadruplicate using either a Perkin Elmer UV/VIS Spectrometer, Lambda 2 (Perkin Elmer Corporation, Uberlingen, FRG) or a Spectra Max 96 well plate reader (Spectra Max 340, Molecular Devices Corporation, California, USA).

2.3.7. Statistical analysis

Results were expressed as mean ± SD. Statistical analysis was performed using Graph Pad Instat Program, Graph Pad Prism, San Diego, USA. One way analysis of variance (ANOVA) was used to find any interaction amongst treatments and the Student’s t-test used to find differences between groups.

3. Results

Dry weight trace metal concentrations of sediment collected from Wright and Lower Mguza dams are shown in Table 1. Except for Fe concentration, there were significantly higher concentrations of Pb, Zn, Cu, Ni (p < 0.001) and Cd (p < 0.01) in the sediment collected from Lower Mguza Dam when compared with concentrations in sediment collected from Wright Dam. The concentration of Fe, Ni, Zn, Cu, Pb and Cd in the sediment from both dams are all shown in the ranking of Fe > Ni > Zn > Cu > Pb > Cd. Statistically higher levels of Cd, Ni, Fe (p < 0.001), Cu (p < 0.01) and Zn (p < 0.05) were found in water collected from Lower Mguza compared with water collected from Wright Dam whilst the concentration of Pb in water collected from Lower Mguza was not quite significantly higher (p=0.06) than that in water collected from Wright Dam (Table 1).

A comparison of mean values of metal concentrations in the tissue of L. natalensis exposed to Wright and Lower Mguza Dam sediment and water is shown in Table 2. Except for Fe and Cu significantly higher concentrations of Pb, Cd, Zn (p < 0.01) and Ni (p < 0.001) were found in tissues of snails exposed to Lower Mguza Dam sediment and water compared with those exposed to Wright Dam sediment and water. The order of metal concentration in tissues of snails exposed to Lower Mguza Dam elements was Fe > Zn > Cu > Pb > Ni = Cd whilst that of tissues of snails exposed to Wright Dam elements was Fe > Ni > Zn > Cu > Pb > Cd.

Selected heavy metal concentrations in shells of snails exposed to Wright Dam and Lower Mguza Dam elements are shown in Table 3. There were significantly higher concentrations of Zn, Cu (p < 0.001) and Cd (p < 0.01) in shells of snails exposed to Lower
Table 1
A comparison of heavy metal concentration in surface sediment (mg/kg dry weight) and water (mg/L) collected from Wright and Lower Mguza Dams.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Sediment</th>
<th></th>
<th></th>
<th>Water</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wright Dam</td>
<td>Lower Mguza Dam</td>
<td>Lower Mguza Dam</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.32 ± 0.03</td>
<td>5.17 ± 0.90***</td>
<td>0.10 ± 0.05</td>
<td>0.18 ± 0.05</td>
<td></td>
<td></td>
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<tr>
<td>Cd</td>
<td>0.10 ± 0.02</td>
<td>0.25 ± 0.05**</td>
<td>0.02 ± 0.01</td>
<td>0.09 ± 0.02***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.82 ± 0.11</td>
<td>5.92 ± 1.25***</td>
<td>0.03 ± 0.02</td>
<td>0.09 ± 0.04*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.56 ± 0.10</td>
<td>4.09 ± 0.78***</td>
<td>0.04 ± 0.01</td>
<td>0.07 ± 0.01**</td>
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</tr>
<tr>
<td>Ni</td>
<td>2.34 ± 0.42</td>
<td>5.36 ± 0.43***</td>
<td>0.12 ± 0.03</td>
<td>0.75 ± 0.03***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>3485 ± 609</td>
<td>3640 ± 744</td>
<td>2.92 ± 0.49</td>
<td>0.22 ± 0.05**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Asterisks indicate that results are significantly different from each other (Student’s t-test, *p < 0.05; **p < 0.01; ***p < 0.001).

Table 2
A comparison of heavy metal concentration in tissues (mg/kg wet weight) of snails exposed to differently polluted dams.

| Parameter measured | Wright Dam (reference) | Lower Mguza Dam (polluted) | | | | |
|--------------------|------------------------|----------------------------|---|---|---|
| Pb                 | 0.11 ± 0.03**          | 0.26 ± 0.05**              | | | |
| Cd                 | 0.06 ± 0.01**          | 0.12 ± 0.01**              | | | |
| Zn                 | 0.24 ± 0.03**          | 0.51 ± 0.05**              | | | |
| Cu                 | 0.23 ± 0.03            | 0.30 ± 0.05                | | | |
| Ni                 | 0.44 ± 0.04***         | 0.15 ± 0.16***             | | | |
| Fe                 | 8.19 ± 2.12            | 9.92 ± 3.54                | | | |

Asterisks indicate that results are significantly different from each other (Student’s t-test: *p < 0.05; **p < 0.01; ***p < 0.001).

Table 3
A comparison of heavy metal concentration in shells of snails exposed to Wright Dam and Lower Mguza Dam sediment and water.

| Metal | Wright Dam (reference) | Lower Mguza Dam (polluted) | | | | |
|-------|------------------------|----------------------------|---|---|---|
| Cd    | 0.05 ± 0.02**          | 0.12 ± 0.45**              | | | |
| Zn    | 0.18 ± 0.06***         | 0.47 ± 0.03***             | | | |
| Cu    | 0.13 ± 0.05***         | 0.45 ± 0.03***             | | | |

Asterisks indicate that results are significantly different from each other (Student’s t-test: *p < 0.01; ***p < 0.001).

Mguza water and sediments compared with those of snails exposed to Wright Dam.

Significantly higher Se-GPX and CAT activities (p < 0.001) were observed in snails exposed to Lower Mguza Dam water and sediment compared with the snails exposed to Wright Dam sediment and water (Fig. 1). Higher levels of MDA (p < 0.05) were also observed in tissues of snail exposed to the polluted dam elements (Fig. 2) whilst the activity of SOD (p < 0.01) and DTD (p < 0.001) was lower in snails exposed to elements collected from the polluted dam (Fig. 1).

4. Discussion

Higher levels of heavy metals were bioaccumulated in tissues and shells of snails exposed to water and sediment of the polluted Lower Mguza Dam when compared with the reference Wright Dam. This is consistent with laboratory studies that have reported accumulation of metals such as Cu in the tissues and shells of the snails, Helisoma duryi and L. natalensis, when compared with controls (Masola et al., 2003). It has been also reported that snails sequestrate and store heavy metals in their shells in contaminated environments (Everand and Denny, 1989; Pyatt et al., 2003). It is however interesting to note that no significant differences in the concentration of heavy metals in shells and tissues of snails were found in those snails exposed to either of the dams sediment and water suggesting equal bioconcentration of metals in the tissues and the shells.

The observed elevated levels in GPX activity and MDA concentration is in agreement with results obtained in our previous fish studies (Siwela et al., 2009) where increases in GPX activities and MDA levels and decreases in DTD in livers of fish exposed to the polluted Lower Mguza Dam were observed. Data presented in the present study is in contrast to reports that molluscs exposed to metals (Rodrigues-Ariza et al., 1992) and fish from areas polluted by metals and organic compounds.
(Rodrigues-Ariza et al., 1993) display high GPX activities in parallel with low MDA. In the present study, MDA coincides with the significantly higher metal (whole) body burden in snails exposed to Lower Mguza Dam sediment and water. This is consistent with studies which found significant elevated levels of thiobarbituric acid reactive substances in fish collected from a polluted stream when compared with those from an unpolluted site (Sanchez et al., 2007) and increased lipid peroxidation in the snail, *Theba pisana* (Radwan et al., 2010) and the green lipped mussel *Perna viridis* (Zapata-Vivenes and Nusetti, 2007) exposed to heavy metals

As Fe and Ni have similar chemistries and are metabolized similarly (Tallkvist and Tjalve, 1997), leading to reduced accumulation and toxicity in aquatic and terrestrial animals (Ptashynski et al., 2001). Zn could be considered as the most accumulated metal in aquatic snails in this study. In comparison, laboratory studies have shown land snails to be sensitive to Zn (Rodrigues-Ariza et al., 1993) display high GPX activities in parallel with low MDA. In the present study, MDA... 


**Fig. 2.** A comparison of malondialdehyde concentration *L. natalensis* exposed to Wright and Lower Mguza dam sediment and water. *p* < 0.05 and ||***||p < 0.001 significantly different from Wright Dam sediment and water exposure.

5. Conclusion

The results presented in this study not only indicate that that *L. natalensis* is a suitable indigenous bioaccumulation indicator species when assessing bioaccumulation risk in freshwater ecosystems or environments at sublethal metal concentrations but the increased activities of CAT and Se-GPX as well as the increased levels of MDA can be used as relevant biomarkers of freshwater pollution.

Acknowledgments

This work was supported by Grants from International Program for Chemical Sciences, Sweden and Research Board, National University of Science and Technology, Zimbabwe. The authors wish to thank the Bulawayo City Council and Mr Dave Wright for allowing them to collect samples from their dams.

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