



Title	Metal and metalloid levels and bio-accumulation characteristics in soil, sediment, land plants and hippopotami ( <i>Hippopotamus amphibius</i> L) from the South Luangwa National Park, Zambia
Author(s)	Nakayama, Shouta M. M.; Ikenaka, Yoshinori; Muzandu, Kaampwe; Choongo, Kennedy; M'kandawire, Ethel; Yasuda, Jun; Ishizuka, Mayumi
Citation	Ecotoxicology and Environmental Safety, 80, 333-338 <a href="https://doi.org/10.1016/j.ecoenv.2012.04.003">https://doi.org/10.1016/j.ecoenv.2012.04.003</a>
Issue Date	2012-06-01
Doc URL	<a href="http://hdl.handle.net/2115/49400">http://hdl.handle.net/2115/49400</a>
Type	article (author version)
File Information	EES80_333-338.pdf



[Instructions for use](#)

Metal and metalloid levels and bio-accumulation characteristics in soil, sediment, land plants and hippopotami (*Hippopotamus amphibius* L) from the South Luangwa National Park, Zambia

Shouta M.M. Nakayama<sup>a\*</sup>, Yoshinori Ikenaka<sup>a\*</sup>, Kaampwe Muzandu<sup>b</sup>, Kennedy Choongo<sup>b</sup>, Ethel M'kandawire<sup>c</sup>, Jun Yasuda<sup>d</sup>, and Mayumi Ishizuka<sup>a</sup>

<sup>a</sup>Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan

<sup>b</sup>Department of Biomedical Sciences, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia

<sup>c</sup>Department of Disease Control, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia

<sup>d</sup>Department of Veterinary Clinical Medicine, Faculty of Agriculture, Iwate University, Morioka 020-8550, Japan

Corresponding author

Mayumi Ishizuka

Email address: [ishizum@vetmed.hokudai.ac.jp](mailto:ishizum@vetmed.hokudai.ac.jp)

Tel: +81-11-706-6949 / Fax: +81-11-706-5105

Note: \*The first and second authors equally participated in this work

## **Abstract**

Hippopotami (*Hippopotamus amphibius* L) are large semi-aquatic mammals that can be exposed to metals and metalloid from both terrestrial and aquatic environments. Therefore, knowledge of metal and metalloid accumulation characteristics in hippopotami living in the national park is important from ecotoxicological point of view. Levels of toxic metals (Cd, Pb and Hg) and metalloid (As) in hippopotami liver from the South Luangwa National Park in Zambia were far lower compared to the established values of toxic levels in cattle. No temporal variations of metal levels in hippopotami were observed, probably because of good management condition and the lack of anthropogenic activities around the national park. However, hippopotami liver accumulated significantly higher concentrations of Hg compared to soil, sediment and their food (plants), most likely due to a process of biomagnifications throughout a trophic chain. Moreover, hippopotami liver and land plants showed significantly higher Cd levels than those of soil. These results strongly suggest that hippopotami liver accumulate higher levels of these metals if surrounding environment is contaminated. Levels of Cr and Ni in hippopotami liver were higher compared to other toxic metals. Since this is the first report to show the Cr and Ni levels and bio-accumulation characteristics of Hg and Cd in hippopotami, we concluded that continuous monitoring and evaluation of toxic effects of these metals on hippopotami should be conducted.

**Keywords:** Bio-accumulation, Hippopotamus, Metal, Plant, Sediment, Soil

## 1. Introduction

Africa is known for its rich diversity of wildlife including birds, amphibians, reptiles and large mammals. In recent years, there have been concerns about significant environmental pollution caused by anthropogenic activities like mining and metallurgical activities in most African countries (Yabe et al., 2010; Oelofse 2008). In particular, African aquatic environments are experiencing increases in metal pollution as a result of expansions in agricultural and industrial activities (Yabe et al., 2010; Lwanga et al., 2003; Berg et al., 1995), as well as the sustained use or spillage of leaded gasoline from watercraft (Muohi et al., 2003). For ecosystem conservation, there is a need to clarify metal bio-accumulation characteristics throughout a trophic chain in wildlife prior to potential further contamination.

Zambia is well known as a mining country (Stockwell et al., 2001) and metal pollution has been reported in aquatic environments around and downstream of mining areas (Nakayama et al., 2011, 2010; Ikenaka et al., 2010; Choongo et al., 2005; Norrgren et al., 2000). However, the South Luangwa National Park (including the Luangwa River) is considered to be a relatively unexploited and uncontaminated area because of limited traffic within the national park and distance from industrial, agricultural and mining areas (Almli et al., 2005; Mwase et al., 2002). Therefore, investigation of metal levels in this area could provide information on the reference metal levels in wildlife, which could be compared with metal levels in animals from polluted environments.

Among the many wildlife species in the South Luangwa National Park, hippopotami (*Hippopotamus amphibius* L) have a unique lifestyle. They are large, semi-aquatic animals that spend most of their time in river water but regularly emerge to

graze land plants along riverbanks (Mwase et al., 2002). Consequently, they are exposed to environmental pollutants in both terrestrial and aquatic environments. At present, there is little information available about the levels of essential and non-essential elements in wild hippopotami (Mwase et al., 2002) and there have been no studies relating metal levels in hippopotami to levels in environmental samples such as soil, river sediment and plants. Therefore, the present study is the first report to evaluate the bio-accumulation of metals and metalloid in hippopotami.

The aims of the current study were to: (1) compare metal levels among hippopotami, soil, river sediment and land plants in order to assess the bio-accumulation characteristics of metals and metalloid in hippopotami; and (2) evaluate the health risks by accumulation of metals and metalloid in hippopotami living in the protected national park. The current study also discussed the temporal variation of metal levels in hippopotami in order to evaluate the management condition and occurrence of anthropogenic disturbance in the national park.

## 2. Materials and Methods

### 2.1. Study area and sampling

The study was conducted in the South Luangwa National Park of Zambia, including the Luangwa River, in Mfuwe in 2007 (Fig. 1). We collected samples of soil (n = 22), sediment from the Luangwa River (n = 4), land plants (n = 11), and hippopotami liver (n = 16, 8 male and 8 female). We collected several plant species that hippopotami graze (*Sacciolepis africana*, *Panicum repens*, *Echinochloa colonum*, *Burnatia enneandra*, *Trichoneura grandiglumis*). River sediment samples were collected using an Ekman grab sampler. Each soil and sediment sample was air-dried in the laboratory at room temperature and passed through a 2 mm sieve prior to metal extraction. The water content of each sample was measured after 12 h of drying in an oven at 105°C.

Liver samples of hippopotami were collected after a culling program that was conducted by the Zambia Wildlife Authority (ZAWA) between 2007 and 2008 for population control. None of our research team members participated in the culling program. Both adult (n = 14) and young (n = 2) samples were obtained from ZAWA in October 2007 at South Luangwa National Park. Therefore, the exact age, body length and weight of the hippopotami could not be determined in this study. Samples were transported to the laboratory and kept frozen at the School of Veterinary Medicine, University of Zambia. Then, samples were imported in to Japan after seeking authorization from both the Zambian and Japanese governments. Metal analysis was done at the Graduate School of Veterinary Medicine, Hokkaido University, Japan. The liver samples were stored at -20°C for heavy metal analysis and water content

measurement, which was determined after drying in an oven at 105°C for 24 h.

## *2.2. Metal extraction and analysis*

Metals in the soil and sediment samples were extracted using a method by Nakayama et al. (2011 and 2010). Briefly, 1 g of each soil sample was placed in a 200 mL flask. Nitric acid (15 mL, atomic absorption spectrometry grade, 60 %, Kanto Chemical Corp., Tokyo, Japan) was added. The mixture was then heated at 180 °C for 5 h on a hotplate. After cooling, 1 g of ammonium chloride (Wako Pure Chemical Industries Ltd., Osaka, Japan) was added. The samples were reheated at 180 °C for 1 h and evaporated to approximately 5 mL. After the samples cooled, they were filtered into plastic bottles using ash-less filter paper 5B (Advantec, Tokyo, Japan). Lanthanum chloride (1 mL, atomic absorption spectrometry grade, 100 g La/L solution, Wako) was added. The sample volume was standardized to 100 mL using 2 % HNO<sub>3</sub>. A reagent blank was prepared using the same procedures.

Metals were extracted from the plant samples using nitric acid digestion with a slight modification from the method by Hseu (2004). Briefly, plant samples including both parts of stock and leaf were washed in distilled water in order to remove soil contamination and were air-dried at room temperature. Then 1 g of each plant sample was placed in a 200 mL flask and 20 mL of nitric acid was added. The samples were gradually heated up to 225 °C on a hotplate, and left for 12 h to evaporate to approximately 5 mL. Then, 0.2 mL of lanthanum chloride (100 g La/L solution) was added. The volume was then made up to 20 mL with 2 % HNO<sub>3</sub>. A reagent blank was prepared using the same procedures.

Metals in the hippopotamus liver samples were extracted by digestion using a method by Nakayama et al. (2011). Briefly, 1 g of fresh tissue was placed in a 200 mL flask and 20 mL of nitric acid was added. The samples were gradually heated up to 225°C on a hotplate, and left for 12 h to evaporate to approximately 5 mL. After the liquid mixture became clear, 0.2 mL of lanthanum chloride (100 g La/L solution) was added. The volume was then made up to 20 mL with 2 % HNO<sub>3</sub>. A reagent blank was prepared using the same procedures.

We measured the concentrations of 8 metals (Cr, Co, Cu, Zn, Cd, Pb and Ni) and metalloid (As) in soil, river sediment, plant and hippopotamus liver using an atomic absorption spectrophotometer (AAS) (Z-2010, Hitachi High-Technologies Corporation, Tokyo, Japan) with either an acetylene flame or argon non-flame method, after preparation of the calibration standard. Analytical quality control was performed using four certified reference materials as follows; BCR-320R (channel sediment, Community Bureau of Reference of European Commission, Brussels, Belgium), SRM 1944 (New York/New Jersey Waterway Sediment, National Institute of Standards and Technology, New York, USA), DOLT-4 (Dogfish liver, National Research Council of Canada, Ottawa, Canada) and DORM-3 (Fish protein, National Research Council of Canada, Ottawa, Canada). Recovery rates (%) of soil and sediment using certified reference materials (BCR-320R and SRM 1944) were acceptable; Cr (80-115), Co (96-111), Cu (88-95), Zn (90-95), Cd (113-120), Pb (91-114), Ni (85-87) and As (113-121). For liver tissue, recovery rates (%) of all elements were acceptable; Cr (91-108), Co (96-111), Cu (88-90), Zn (78-83), Cd (91-108), Pb (89-98) and Ni (98-111) except for As (50-67%). The detection limits (µg/kg) of Cr, Co, Cu, Zn, Cd, Pb, Ni and As were 0.5, 0.5, 1.0, 0.1, 0.2, 1.0, 0.5 and 2.0, respectively. Each metal concentration was converted from mg/kg

wet-weight (wt) to mg/kg dry-wt using the water content.

### *2.3. Analysis of mercury (Hg)*

The concentration of Hg in each sample was measured by thermal decomposition, gold amalgamation, and atomic absorption spectrophotometry (Mercury Analyzer, MA-3000, Nippon Instruments Corporation, Tokyo, Japan) after preparation of the calibration standard. Recovery rates of Hg for the four certified reference materials (BCR-320R, SRM 1944, DOLT-4 and DORM-3) ranged from 92 to 103%. The detection limit of Hg was 2.0 pg of total Hg. Concentration of Hg was converted from mg/kg wet-wt to mg/kg dry-wt using the water content.

### *2.4. Soil/sediment pH and organic matter measurement*

Each soil and sediment sample pH was measured with a soil (or sediment):water ratio of 1:2.5 (Ge et al., 2000). To determine the soil/sediment organic matter (SOM) content, the ignition loss of each sample was measured after 5 h in an oven at 600°C.

### *2.5. Bio-accumulation factor (BAF)*

The bio-accumulation factor (BAF) is the ratio between the metal concentration in the soil/sediment and that in the plant/liver (Abdallah and Abdallah, 2008). We separately calculated BAF values for soil to plant, plant to hippopotami liver as well as soil/sediment to hippopotami liver (Gnamus et al., 2000). The following formulas were

used:  $BAF_1 = C_p/C_s$ , where  $C_p$  = metal concentration in plant and  $C_s$  = metal concentration in soil;  $BAF_2 = C_h/C_s$ , where  $C_h$  = metal concentration in hippopotami and  $C_s$  = metal concentration in soil or sediment;  $BAF_3 = C_h/C_p$ , where  $C_h$  = metal concentration in hippopotami and  $C_p$  = metal concentration in plant. Since we collected terrestrial plants, we did not calculate BAF values between plant and sediment.

## *2.6. Statistical analysis*

Statistical analyses were performed using JMP 7.0.1 (SAS Institute, Cary, NC, USA). Data were normalized by base 10 logarithm transformations. We analyzed for differences in metal accumulations among the groups using a Student's t-test (between sex or between adult and young hippopotami) or Tukey test (among soil, sediment, plant and hippopotami) ( $p < 0.05$ ). Since there were no differences by sex or age in metal levels in hippopotami, we treated these samples as the same group. Correlation coefficients among soil/sediment pH, organic matter and metal concentrations were analyzed. We also analyzed correlation coefficients among metal concentrations in hippopotami liver. Principal component analysis (PCA) was performed on the basis of normalized values of each metal.

## **3. Results and Discussion**

### *3.1. Soil/sediment characteristics and relationships with metal concentrations*

Soil/sediment characteristics such as pH and organic matter (SOM) are shown in Table 1. There was no correlation between soil pH and metal levels (data not shown). As shown in Table 2, there were significant positive correlations between Cu-SOM, Zn-SOM and Hg-SOM in soil. Lin and Chen (1998) similarly reported that the adsorbabilities of metals (Cu, Zn, Pb and Cr) in sediments increased with increasing organic matter content.

### *3.2. Metal concentrations in soil and sediment samples*

Metal concentrations in soil and sediment are summarized in Table 1. Although several research papers have reported contamination of Cu, Co, Zn, Pb, Cd and As around mining areas in Zambia (Nakayama et al., 2011, 2010; Ikenaka et al., 2010; Choongo et al., 2005; Norrgren et al., 2000), concentrations of all metals and metalloids in soil and sediment in the current study were below the US EPA benchmark values (2004, 2003) and previously published results for global ranges in non-polluted soils (Kabata-Pendias and Pendias, 1992). Therefore, we believe that soil and sediment in this national park are not affected by pollution resulting from mining and other anthropogenic activities at present. However, continuous monitoring of metal levels (especially Cd and Hg) in soil and sediment should be conducted because we found bio-accumulation characteristics of these metals in hippopotami as mentioned below (section 3.4.).

### *3.3. Metal concentrations in hippopotami liver*

In the current study, the age, body length and weight of the hippopotami are not available. In an earlier study (Mwase et al., 2002), a negative correlation between liver Cu concentration and age in hippopotami [ $r = -0.43$  (Pearson)/  $-0.44$  (Spearman)] was

observed but this result was considered difficult to interpret. In the present study, there were no differences in the metal levels in liver between male and female hippopotami (Student's t-test, data not shown). Our result is in accordance with previous findings in hippopotami conducted by Mwase et al. (2002). Moreover, other studies indicate that male and female marine mammals generally show no differences in the accumulation of metals (Agusa et al., 2008; Seixas et al., 2008; O'Shea 1999).

Table 3 shows a comparison of the metal levels in the present study and those in hippopotami liver collected from the same area in 1998 (Mwase et al., 2002). The concentrations of metals were similar in both studies, indicating that metal levels in hippopotami liver have not changed in the past 10 years, most likely due to few anthropogenic activities and contamination sources around the national park. This lack of temporal variation suggested good management condition and absence of anthropogenic disturbances in this national park.

However, we found that Cr and Ni levels in hippopotami liver were higher compared to other toxic metals such as Cd, Pb and Hg. Due to scarcity of information on metal accumulation levels in hippopotami, we compared our results with previous studies on metal levels in wild deer, which are also grazing animals. Levels of Cr and Ni in hippopotami liver were approximately 7 times higher than the levels in wild reindeer exposed to contamination attributed to industrial activities in Norway (Cr: <0.01-0.09 mg/kg wet-weight and Ni: 0.01-0.18) (Sivertsen et al., 1995). Since the previous study did not measure Cr and Ni levels in hippopotami (Mwase et al., 2002), our findings are new and provide basic values for these metals in hippopotami. Further monitoring in this national park and risk assessment of toxic effects on hippopotami should be performed since excess Cr and Ni levels are known to be toxic in mammals.

In the present study, Zn and Cu concentrations in hippopotami liver were much higher compared with other metals (Table 1). Moreover, hippopotami liver contained higher levels of Zn and Cu compared to those of soil, sediment and plants (Tables 1 and 4). Similar to our results, high concentrations of Zn and Cu were observed in wild reindeer and cattle liver (Nriagu et al., 2009; Lopez-Alonso et al., 2002; Miranda et al., 2005; Sivertsen et al., 1995). Since Zn and Cu are essential elements in animals and play important roles in many physiological functions such as catalytic centers of liver Cu/Zn superoxide dismutase (Cu/Zn-SOD) (Zelco et al., 2002), we suggested that a wider range of Cu and Zn concentrations can be accumulated in hippopotami liver compared to non-essential elements. Regarding Cu deficiency, multiple clinical symptoms were reported with the liver Cu concentration below 25 mg/kg in moose (*Alces alces* L) and cattle (Frank 1998; Mattioli et al., 1996). In the current study, 75% of hippopotami liver showed lower Cu concentration than the established value in wild moose and cattle. Future studies should focus more on the Cu deficiency status in hippopotami since no information on mineral deficiency is available in this animal.

Levels of toxic metals such as Pb, Cd, As and Hg in hippopotami were low, probably due to lack of anthropogenic activities around the national park. Similar levels of Pb (0.10-0.93 mg/kg) and Cd (0.11-0.40 mg/kg) in liver of wild red deer (*Cervus elaphus*) from a non-contaminated area were reported in Spain (Santiago et al., 1998). Concerning the health risk posed by metals, it has been established that kidney Cd levels of 80 to 200 mg/kg can cause renal damage in cattle (Nordberg et al., 2007), whereas Pb levels exceeding 10 mg/kg in liver and 40 mg/kg in kidneys can cause damage to the nervous and the hemopoietic systems (Skerfving and Bergdahl, 2007; NAS, 1980). In addition, As levels of 3.5 to 60.4 mg/kg in the liver were reported to cause poisoning in

cattle (NAS, 1980). These established values are quite higher than the metal concentrations observed in hippopotami in the present study. Given these findings, it is clear that the concentrations of toxic metals in our study did not reach intoxication levels, although differences in metal tolerance between hippopotami and cattle should be considered.

#### *3.4. Metal interactions in hippopotami liver and relationships of metal levels among soil, sediment, plants and hippopotami liver*

Correlations among metal concentrations in hippopotami liver are presented in Table 2. We found significant positive correlations among essential metals including Cr-Zn, Cr-Ni and Ni-Co. Positive correlation between Se and Cu in hippopotami was previously reported by Mwase et al. (2002). Similarly, Lopez-Alonso et al. (2004) reported positive correlations among essential metals in cattle. Interestingly, Hg-Co (toxic-essential) and Hg-Pb (toxic-toxic) were negatively correlated in this study. As far as we are aware, there are no reports on negative relationships between these metals. Further investigations are required to explain the mechanisms of these interactions and implications in hippopotami.

Hippopotami liver accumulated significantly higher concentrations of Hg compared to levels in soil, sediment and plants (Tables 1 and 4, Fig. 2). The BAF<sub>1</sub> value of Hg (1.0) suggests a low transfer of Hg from soil to plants. This result is in accordance with a previous study that reported low Hg transfer, most likely as a result of the block system in root of plant (Gnamus et al., 2000). In an earlier study (Gnamus et al., 2000), the bio-accumulation profile of Hg from plant to terrestrial herbivorous roe deer (*Capreolus capreolus* L.) was reported. This report supports the current findings of a high

BAF<sub>3</sub> value of Hg (5.0), which implies bio-accumulation from plant to hippopotami. In addition to consumption of plants, uptake of Hg from river water is one of the exposure routes, although we did not measure Hg levels in the river water in the present study. In the case of Cd, hippopotami liver and plants accumulated significantly higher levels of Cd compared to those of soil (Tables 1 and 4, Fig. 2). BAF<sub>1</sub> and BAF<sub>2</sub> values of Cd were very high (Table 4). Cadmium is known to be mobile in soils and available to plants (Alloway 1990). In contrast to these values, the BAF<sub>3</sub> value of Cd in hippopotami liver was low (1.3). Similarly, in grazing animals, relatively low BAF values of Cd were observed between pasture grass/hay and cattle liver (0.1-3.3) and sheep liver (0.6-1.5) from eastern Kazakhstan (values were calculated from Farmer and Farmer (2000)).

Higher concentrations of Cr, Ni, Co, Pb and As were observed in soil and sediment compared to levels in plants and hippopotami liver (Tables 1 and 4). Our results suggest that these metals do not bio-accumulate in hippopotami. Generally, unlike Cd and Hg, Pb is not a bio-accumulative metal (Chen and Folt., 2000). However, Almli et al. (2005) did report a bio-magnification effect of Pb in crocodiles (*Crocodylus niloticus*) from the same river as the hippopotami in the current study. The authors noted that crocodiles are carnivorous and consume the whole body of fish, birds and mammals, including bones that are known to accumulate Pb (Almli et al., 2005). Although they inhabit the same area, diet differences would most likely cause bio-magnification of Pb in crocodiles but not in hippopotami that only graze land plants.

PCA was performed in order to characterize metal compositional patterns (Fig. 3). PCA clearly divided into three groups (1: soil/sediment, 2: plant, 3: hippopotami) indicating different metal accumulation patterns among these groups. As shown in Fig. 3, metal compositional patterns in hippopotami were mainly influenced by Cd, Hg and Zn,

while those in soil/sediment were influenced by Co, Cr, Ni, Pb and As. The plant group located between the hippopotami group and the soil/sediment group. These results were supported by the results of BAF values (Table 4).

#### **4. Conclusions**

Metal concentrations, especially toxic metals such as Cd, Pb and Hg in hippopotami liver from the South Luangwa National Park in Zambia were generally low compared to established toxic levels in cattle. Temporal variations of metal levels in hippopotami were not found. However, we observed that Cr and Ni concentrations in hippopotami liver were higher than those of other toxic metals such as Cd, Pb and Hg. Furthermore, hippopotami liver had higher levels of Cr and Ni compared to other grazing animals like wild deer from contaminated areas. Higher concentrations of Cd and Hg in hippopotami than in the surrounding environment suggests that these metals can bio-accumulate in hippopotami. Given these results, we concluded that continuous monitoring in hippopotami as well as surrounding environmental samples in this national park should be conducted.

## **Acknowledgments**

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan awarded to M. Ishizuka (No. 19671001) and Y. Ikenaka (No. 23710038) and foundations of JSPS AA Science Platform Program, Sumitomo, Heiwa Nakajima, and Mitsui & Co., LTD. One of the authors (S. Nakayama) is a Research Fellow of the Japan Society for the Promotion of Science (No. 2200517701). We would like to acknowledge the financial support by The Naito Foundation and Sompo Japan Environment Foundation.

## References

- Abdallah, M.A.M., Abdallah, A.M.A., 2008. Biomonitoring study of heavy metals in biota and sediments in the South Eastern coast of Mediterranean sea, Egypt. *Environ. Monit. Assess.* 146, 139-145.
- Agusa, T., Nomura, K., Kunito, T., Anan, Y., Iwata, H., Miyazaki, N., Tatsukawa, R., Tanabe, S., 2008. Interelement relationships and age-related variation of trace element concentrations in liver of striped dolphins (*Stenella coeruleoalba*) from Japanese coastal waters. *Mar. Pollut. Bull.* 57, 807-815.
- Alloway, B.J., 1990. Cadmium. In: Alloway BJ (ed) *Heavy Metals in Soils*, Blackie, Glasgow. p 100-124.
- Almli, B., Mwase, M., Sivertsen, T., Musonda, M.M., Flåøyen, A., 2005. Hepatic and renal concentrations of 10 trace elements in crocodiles (*Crocodylus niloticus*) in the Kafue and Luangwa rivers in Zambia. *Onderstepoort J. Vet. Res.* 337, 75-82.
- Berg, H., Kibus, M., Kautsky, N., 1995. Heavy metals in tropical lake Kariba, Zimbabwe. *Water. Air. Soil. Pollut.* 83, 237-252.
- Chen, C.Y., and Folt, C.L., 2000. Bioaccumulation and diminution of arsenic and lead in a freshwater food web. *Environ. Sci. Technol.* 34, 3878-3884.
- Choongo, C.K., Syakalima, S.M., Mwase, M., 2005. Coefficient of condition in relation to copper levels in muscle of serranochromis fish and sediment from the Kafue river, Zambia. *Bull. Environ. Contam. Toxicol.* 75, 645-651.
- Farmer, A.A., and Farmer, A.M., 2000. Concentrations of cadmium, lead and zinc in livestock feed and organs around a metal production centre in eastern Kazakhstan. *Sci. Total. Environ.* 257, 53-60.
- Frank, A., 1998. 'Mysterious' moose disease in Sweden. Similarities to copper deficiency and/or molybdenosis in cattle and sheep. Biochemical background of clinical signs and organ lesions. *Sci. Total. Environ.* 209, 17-26.
- Ge, Y., Murray, P., Hendershot, W.H., 2000. Trace metal speciation and bioavailability in urban soils. *Environ. Pollut.* 107, 137-144.
- Gnamus, A., Byrne, A.R., Horvat, M., 2000. Mercury in the Soil-Plant-Deer-Predator Food Chain of a Temperate Forest in Slovenia. *Environ. Sci. Technol.* 34, 3337-3345.
- Hseu, Z.Y., 2004. Evaluating heavy metal contents in nine composts using four digestion methods. *Bioresour. Technol.* 95, 53-59.
- Ikenaka, Y., Nakayama, S.M.M., Muzandu, K., Choongo, K., Teraoka, H., Mizuno, N., Ishizuka, M., 2010. Heavy metal contamination of soil and sediment in Zambia.

- African J Environ Sci Tech. 4(11), 29-739.
- Kabata-Pendias, A., and Pendias, H., 1992. Trace elements in soils and plants. 2nd ed. CRC Press., London, pp. 413.
- Lin, J.G., and Chen, S.Y., 1998. The relationship between adsorption of heavy metal and organic matter in river sediments. Environ. Int. 24, 345-352.
- Lopez-Alonso, M., Benedito, J.L., Miranda, M., Castillo, C., Hernandez, J., Shore, R.F., 2002. Cattle as biomonitors of soil arsenic, copper and zinc concentrations in Galicia (NW Spain). Arch. Environ. Contam. Toxicol. 43, 103–108.
- Lopez-Alonso, M., Montana, F.P., Miranda, M., Castillo, C., Hernandez, J., Benedito, J.L., 2004. Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. Biometals. 17, 389-397.
- Lwanga, M.S., Kansime, F., Denny, P., Scullion, J., 2003. Heavy metals in Lake George, Uganda, with relation to metal concentrations in tissues of common fish species. Hydrobiologia. 499, 83-93.
- Mattioli, G.A., Ramirez, C.E., Giuliadori, M.J., Tittarelli, C.M., Yano, H., Matsui, T., 1996. Characterization of cattle copper deficiency in the Magdalena district. Livest Product Sci. 47, 7-10.
- Miranda, M., Lopez-Alonso, M., Castillo, C., Hernandez, J., Benedito, J.L., 2005. Effects of moderate pollution on toxic and trace metal levels in calves from a polluted area of northern Spain. Environ. Pollut. 31, 543–548.
- Muohi, A.W., Onyari, J.M., Omondi, J.G., Mavuti, K.M., 2003. Heavy metals in sediments from Makupa and Port–Reitz Creek systems: Kenyan Coast. Environ. Int. 28, 619-647.
- Mwase, M., Almlı, B., Sivertsen, T., Musonda, M.M., Flåøyen, A., 2002. Hepatic and renal concentrations of copper and other trace elements in hippopotami (*Hippopotamus amphibius* L) living in and adjacent to the Kafue and Luangwa Rivers in Zambia. Onderstepoort J. Vet. Res. 69, 207-214.
- Nakayama, M.M.S., Ikenaka, Y., Muzandu, K., Choongo, K., Oroszlany, B., Teraoka, H., Mizuno, N., Ishizuka, M., 2010. Heavy Metal Accumulation in Lake Sediments, Fish (*Oreochromis niloticus* and *Serranochromis thumbergi*), and Crayfish (*Cherax quadricarinatus*) in Lake Itzhi-tezhi and Lake Kariba, Zambia. Arch. Environ. Contam. Toxicol. 59 (2), 291-300.
- Nakayama, S.M.M., Ikenaka, Y., Hamada, K., Muzandu, K., Choongo, K., Teraoka, H., Mizuno, N., Ishizuka, M., 2011. Metal and metalloid contamination in roadside soil and wild rats around a Pb-Zn mine in Kabwe, Zambia. Environ. Pollut. 159,

175-181.

- National Academy of Sciences (NAS)., 1980. Mineral Tolerance of Domestic Animals. National Academy Press. Washington, D.C, 256-276.
- Nordberg, G.F., Nogawa, K., Nordberg, M., Friberg., L.T., 2007. Cadmium. In: Gunnar F. Nordberg, Bruce A. Fowler, Monica Nordberg and Lars T. Friberg. (eds). Handbook on the toxicology of metals, (3rd edition), Vol. III, Elsevier, Amsterdam, 445-486.
- Norrgrén, L., Pettersson, U., Örn, S., Bergqvist, A.P., 2000. Environmental monitoring of the Kafue River, located in the Copperbelt, Zambia. Arch. Environ. Contam. Toxicol. 38, 334-341.
- Nriagu, J., Boughanen, M., Linder, A., Howe, A., Grant, C., Rattray, R., Vutchkov, M., Lalor, G., 2009. Levels of As, Cd, Pb, Cu, Se and Zn in bovine kidneys and livers in Jamaica. Ecotoxicol. Environ. Safety. 72, 564– 571.
- Oelofse, S., 2008. Mine water pollution—acid mine decant, effluent and treatment: a consideration of key emerging issues that may impact the state of the environment. Emerging Issues Paper: Mine Water Pollution 2008.
- O’Shea, T.J., 1999. Environmental contaminants and marine mammals. In: Reynolds III, J.E., Rommel, S.A. (Eds.), Biology of Marine Mammals. Smithsonian Institution Press, Washington, pp. 485-563.
- Santiago, D., Matas-Guzman, M., Reja, A., Maria-Mojica, B., Rodero, B., Garcia-Fernandez, A.J., 1998. Lead and Cadmium in Red Deer and Wild Boar from Sierra Morena Mountains (Andalusia, Spain). Bull. Environ. Contam. Toxicol. 61, 730-737.
- Seixas, T.G., Kehrig, H.A., Costa, M., Fillmann, G., Benedetto, A.P.M., Secchi, E.R., Souza, C.M.M., Malm, O., Moreira, I., 2008. Total mercury, organic mercury and selenium in liver and kidney of a South American coastal dolphin. Environ. Pollut. 154, 98-106.
- Sivertsen, T., Daae, H.L., Godal, A., Sand, G., 1995. Ruminant uptake of nickel and other elements from industrial air pollution in the Norwegian-Russian border area. Environ. Pollut. 90, 75-81.
- Skerfving, S., Bergdahl, I.A., 2007. Lead. In: Gunnar F. Nordberg, Bruce A. Fowler, Monica Nordberg and Lars T. Friberg. (eds). Handbook on the toxicology of metals, (3rd edition), Vol. III, Elsevier, Amsterdam, 599-643.
- Stockwell, L.E., Hillier, J.A., Mills, A.J., White, R., 2001. World mineral statistics 1995-99, Keyworth, Nottingham. British Geological Survey 2001.
- United States Environmental Protection Agency, 2003. Guidance for Developing

Ecological Soil Screening Levels. OSWER-Directive 9285.7-55. <http://www.epa.gov/ecotox/ecossl/>.

United States Environmental Protection Agency, 2004. Framework for Inorganic Metals Risk Assessment. Draft EPA/630/P-04/068B, 20460. Washington DC.

Yabe, J., Ishizuka, M., Umemura, T., 2010. Current levels of heavy metal pollution in Africa. *J. Vet. Med. Sci.* 72(10), 1257-1263.

Zelco, I.N., Mariani, T.J., Folz, R.J., 2002. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic. Biol. Med.* 33(3), 337-349.

## Figure captions

Fig. 1. Map of Zambia showing the South Luangwa National Park including the Luangwa River.

Fig. 2. Vertical and box plot of Cd and Hg concentrations (mg/kg dry-wt) in soil, sediment, plants and hippopotami liver. Different letters indicate significant differences (Tukey test,  $p < 0.05$ ).

Fig. 3. Principal Component Analysis (PCA) of heavy metal concentrations (mg/kg dry-wt) in hippopotami (asterisk), soil (square), sediment (cross) and plants (diamond) showing loading and score plot.

Table 1: Metal concentrations (median and range, mg/kg dry-wt), soil/sediment pH and organic matter (SOM, %).

Element, pH and SOM	Soil (n=22)	Sediment (n=4)	Plant (n=13)	Hippo (n=16)	Benchmark value of soil <sup>(c)</sup>	World range in non-polluted soils <sup>(d)</sup>
Cr	20 (4-49)	15 (14-39)	1 (0.5-3)	0.7 (0.1-3)	240	5-120
Co	9 (1.2-21)	1 (0.5-7)	2 (0.5-5)	0.6 (0.3-1)	-	0.1-20
Cu	13 (2-26)	7 (6-14)	14 (6-22)	10 (1-270)	70	6-60
Zn	37 (0.4-68)	9 (2-33)	52 (16-131)	164 (100-495)	50-120	17-125
Cd	0.007 (0.001-0.1)	0.04 (0.03-0.05)	0.1 (0.007-0.6)	0.1 (0.1-0.4)	1.6	0.07-1.1
Pb	21 (10-130)	2 (0.9-6)	0.4 (0.02-2)	0.2 (0.1-0.8)	120	10-70
Ni	14 (3-30)	12 (9-27)	3 (0.8-8)	0.5 (0.2-5)	30-200	1-200
As	0.6 (0.2-1)	1 (0.8-3)	0.02 (0.006-0.1)	0.03 (0.01-0.09)	18	1-15
Hg	0.004 (0.0001-0.01)	0.003 (0.0004-0.006)	0.004 (0.001-0.02) <sup>(b)</sup>	0.02 (0.008-0.05)	32	-
pH	7.25 (5.86-9.55)	7.32 (7.21-7.42)	-	-	-	-
SOM <sup>(e)</sup>	0.05 (0.23-33.3)	1.1 (0.1-3.8)	-	-	-	-

(a) ND: not detected, (b) n=9, (c) Soil cleanup criteria and ECO-Soil Screening Levels (US EPA, 2004, 2003),

(d) Kabata-Pendias and Pendias (1992), (e) soil/sediment organic matter content (%)

Table 2: Correlation coefficient (r) for each metal in hippopotami and between metal level and organic matter in soil (SOM).

	<b>Element or SOM</b>	<b>Correlation coefficient (r)</b>	<b>p value</b>
<b>hippo</b>	Cr - Zn	0.57	0.02
	Cr - Ni	0.66	0.006
	Ni - Co	0.59	0.016
	Hg - Co	-0.63	0.008
	Hg - Pb	-0.50	0.046
<b>soil</b>	Cu - SOM	0.47	0.026
	Zn - SOM	0.47	0.026
	Hg - SOM	0.53	0.011

Table 3: Comparison of metal concentrations (mg/kg wet-wt) in hippopotami liver between the present study and the reference study (Mwase et al., 2002).

<b>Element</b>	<b>Median (range, mg/kg wet-wt)</b>		<b>Ratio of median value (2007/1998)</b>
	<b>Values in 1998 (Mwase <i>et al.</i> 2002)</b>	<b>Values in 2007 (present study)</b>	
Cr	-	0.17 (0.05-0.82)	-
Co	0.15 (0.07-0.37)	0.13 (0.07-0.34)	0.87
Cu	55 (2.2-89)	2.4 (0.4-66)	0.04
Zn	26 (20-61)	40 (26-124)	1.5
Cd	0.04 (0.02-0.08)	0.03 (0.01-0.10)	0.75
Pb	0.05 (0.02-0.12)	0.06 (0.03-0.20)	1.2
Ni	-	0.13 (0.07-1.2)	-
As	0.008 (0.004-0.03)	0.007 (0.002-0.02)	0.88
Hg	0.005 (<0.005-0.01)	0.006 (0.002-0.01)	1.2

Table 4: Bio-accumulation factor (BAF) values for soil, sediment, plant and hippopotami.

Element	Plant/Soil (BAF <sub>1</sub> )	Hippo/Soil (BAF <sub>2</sub> )	Hippo/Sediment (BAF <sub>2</sub> )	Hippo/Plant (BAF <sub>3</sub> )
Cr	0.1	0.03	0.05	0.7
Co	0.2	0.1	0.5	0.3
Cu	<b>1.1</b>	0.8	<b>1.5</b>	0.7
Zn	<b>1.4</b>	<b>4.5</b>	<b>18.5</b>	<b>3.2</b>
Cd	<b>13.9</b>	<b>18.1</b>	<b>3.1</b>	<b>1.3</b>
Pb	0.02	0.01	0.09	0.6
Ni	0.2	0.04	0.04	0.2
As	0.03	0.05	0.02	1.3
Hg	<b>1.0</b>	<b>5.6</b>	<b>7.5</b>	<b>5.0</b>

Bold indicates that value is more than 1.

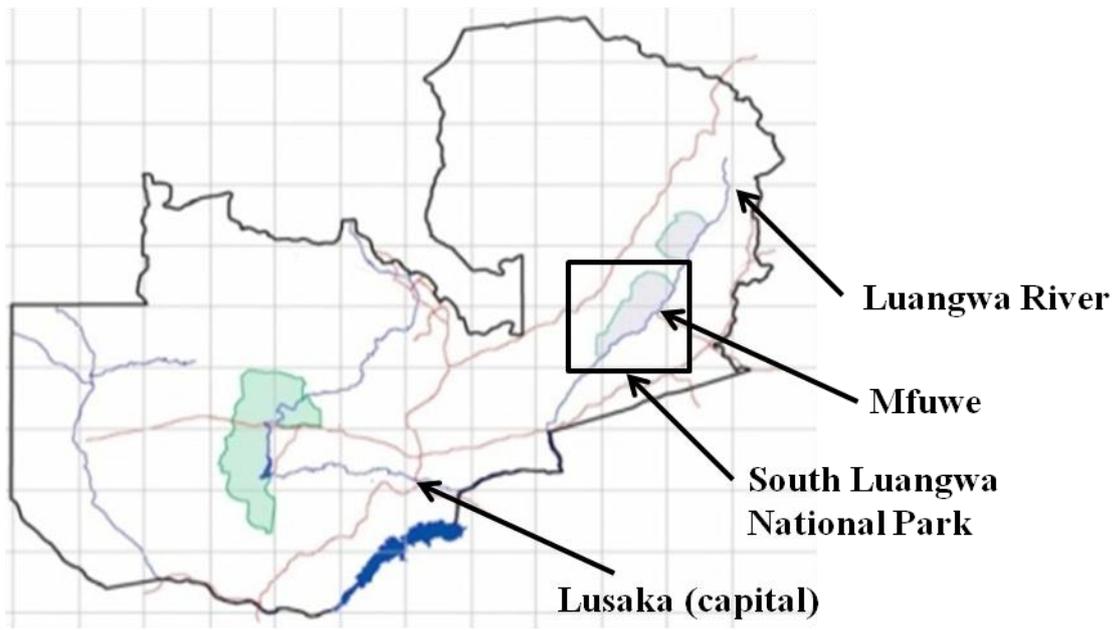


Fig. 1

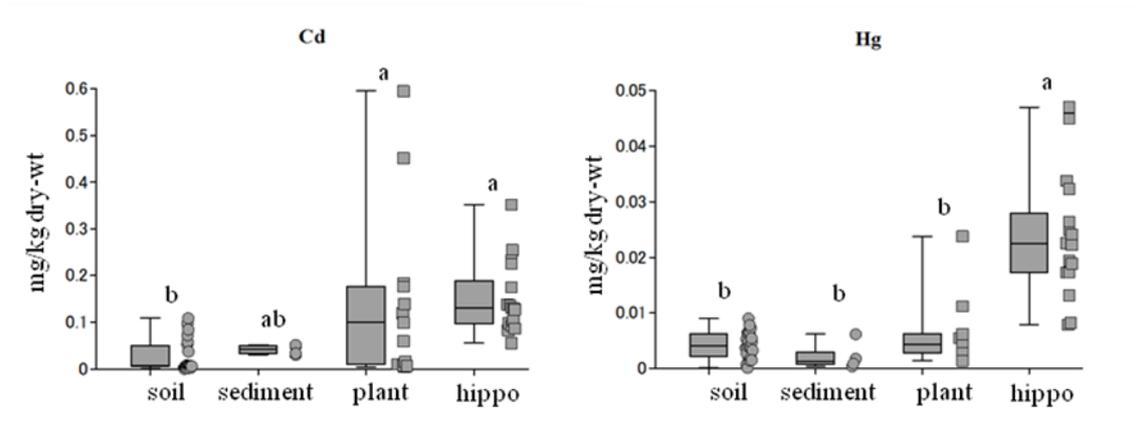


Fig. 2

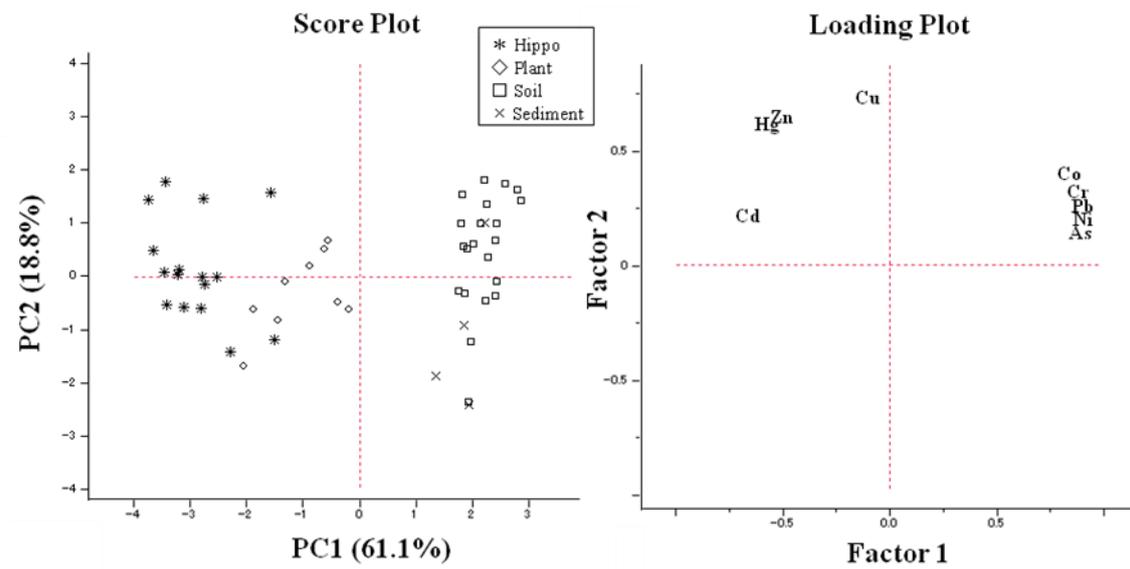


Fig. 3