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

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ABSTRACT

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Hepatic and renal concentrations of the elements arsenic, cadmium, cobalt, copper, lead, manganese, mercury, molybdenum, selenium and zinc were studied in samples collected from hippopotami from the Kafue River in the Kafue National Park and the Luangwa River in the Southern Luangwa National Park in Zambia. There were no significant differences between trace element concentrations in the tissues of the hippopotami taken in the Kafue River and the Luangwa River. The concentrations of copper and other essential elements were similar to those reported in normal domestic and wild ruminants. Judging by the results obtained in this study, pollution from the mining activity around the Kafue River drainage area in the Copperbelt region has not led to any accumulation of elements in tissues of the hippopotami in the Kafue National Park. The trace element concentrations observed may serve as reference for similar future studies on hippopotami.

Keywords: Hippopotami, Kafue River, Luangwa River, pollution, trace elements, Zambia

INTRODUCTION

Mining is the most important industry of Zambia. In 1997 three per cent of the world's annual production of copper (Cu) and twenty per cent of the annual production of cobalt (Co) were mined in Zambia, and most of this was smelted within the country (Stockwell, Hillier, Mills & White 2001). The mines and smelters in Zambia are concentrated within the drainage area of the Kafue River (Fig. 1) in the Copperbelt in the North Western Province (Mwase 1994). Downstream of the mines, the Kafue River meanders through relatively flat lands where large areas are flooded for several months during the rainy season. Metal pollutants from the mining industry upstream may be transported with the river to the ecologically important and protected regions further south, including Zambia's largest national park, the Kafue National Park.

A few studies on trace element pollution in the water and sediments of the Kafue River have been carried out (Sørensen & Ibrekk 1992; Petterson & Ingri 1993; Mwase 1994). The studies show that the concentration of elements mined in the area is enhanced in water and sediment collected in the Copperbelt, and that the concentration decreases downstream. In the Copperbelt area, the copper and cobalt concentrations in water, sediments and fish are extremely high, and sediments from the area

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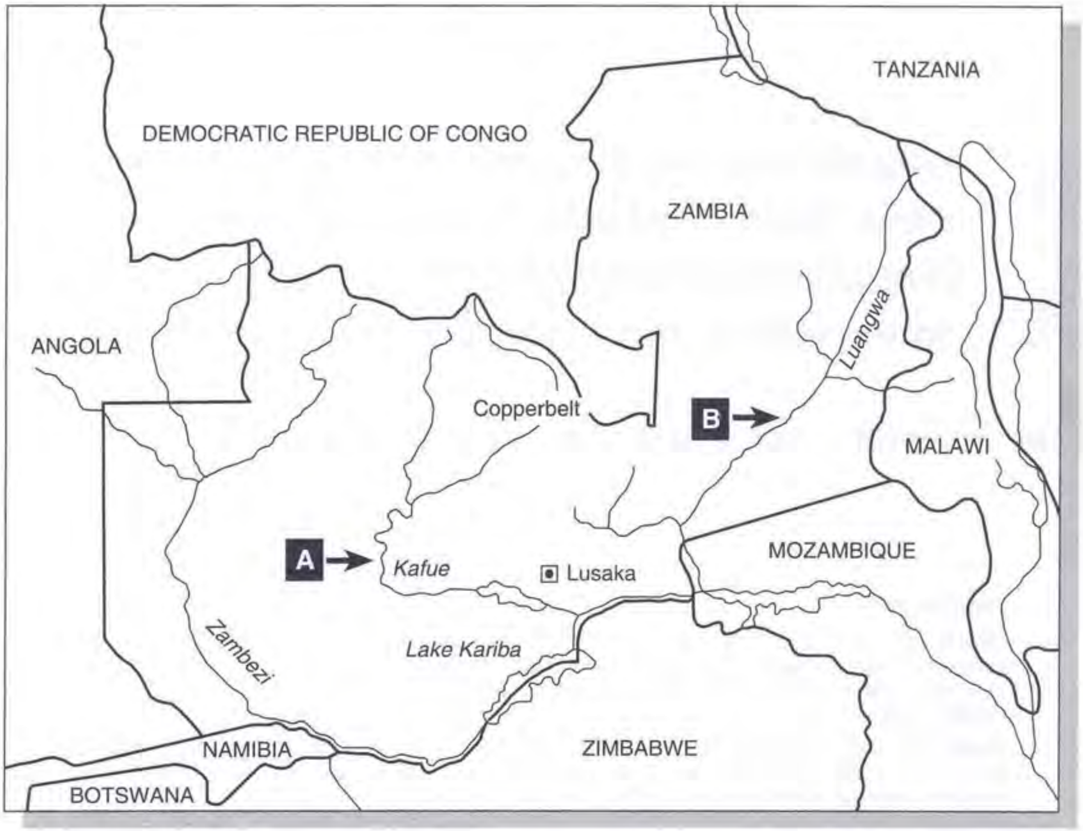

FIG. 1 Map of Zambia showing the two sites where the samples of tissues from the hippopotami were collected: Kafue River in Kafue National Park (A) and Luangwa River at Mfuwe in Southern Luangwa National Park (B)

are very toxic to the early life stages of fish (Mwase, Victor & Norrgren 1998).

There have, however, been no studies on the concentrations of trace elements in large animals living in and adjacent to the Kafue River. The role of this river as the most important water source for the wildlife in the Kafue National Park strongly justifies a study of his nature. The feeding habits of hippopotami make them a good indicator species for trace element contamination of the vegetation in the seasonal flooded areas along the river. With this as background, we studied hepatic and renal concentrations of copper and nine other essential and non-essential trace elements in samples of hippopotamus tissues. The animals were captured in two rivers of Zambia: In the Kafue River within the Kafue National Park (marked with an A in Fig. 1), and in the presumably uncontaminated Luangwa River at Mfuwe in the Southern Luangwa National Park (B in Fig. 1).

Most studies of trace elements in wild hoofed mammals have been done in cervids in Europe and North America. The only such study in hippopotami that we are aware of was restricted to lead levels in the blood of this species of the Kruger National Park (Dauth, Dreyer, Raubenheimer, Lemmer & De Vos 1988). An explanation for limited data on hippopotami may be that they are protected animals. Access to necropsy samples is therefore limited. However, in the Kafue and Luangwa National Parks they are numerous and culling is necessary to regulate the population. Through cooperation with the Parks' authorities we were able to collect a unique set of tissue samples.

The study had a dual purpose: To evaluate the possible impact of trace element contamination on hippopotami in the Kafue River, and to determine normal levels of essential and other trace elements in hippopotami living in Zambia. The elements analysed were those known to be important pollutants in the Copperbelt area (Petterson & Ingri 1993) and other trace elements of ecotoxicological or physiological importance in wild ungulates (Mertz 1986/1987; Frøslie, Sivertsen & Lochmiller 2001).

MATERIALS AND METHODS

Collection of samples

Tissue samples from hippopotami

Tissue samples were collected from 24 hippopotami (14 males and ten females) in the Luangwa River at Mfuwe in the Southern Luangwa National Park and six hippopotami (all females) in the Kafue River at Chunga in the Kafue National Park in Zambia between September and November 1998.

The animals were shot from the riverbanks while they were submerged in water with only the small upper part of the head exposed. The hunter was unable to determine the size or the gender of the animals beforehand. After being killed the animals sank, rising 3–8 h later as a result of the accumulation of gas in the gastrointestinal tract. They were then towed to the bank by local fishermen in canoes. The animals were exposed, the abdominal organs inspected, and samples of liver and kidney were taken. The mandibles were collected, identified with a metal tag and sent to the Wildlife Department Field Station for age determination. Age group of each hippopotamus was determined and converted to chronological age by the method of Laws (1968). The age of the hippopotami from the Luangwa National Park ranged from 7–43 years (median 25.5 years), and of those from the Kafue National Park from 17–35 years (median 27 years). The liver and kidney samples were collected using clean steel equipment, placed in clean polypropylene boxes and stored below 0 °C. During transportation to Norway, the samples were kept frozen on frozen carbon dioxide.

Samples of grass and river water

One sample of river water was taken from each river, contemporaneous with the hunting of the hippopotami. The water samples were taken by immersing a plastic container 30 cm below the surface. The collected water samples were filtered *in situ* through a Millipore XX 110500 / 0.45 mm filter directly into acid-washed 500 mℓ polyethylene bottles. To each sample 0.5 mℓ high purity concentrated nitric acid was added, and the samples were stored cold until analysis.

One sample of the common perennial grass *Cynodon dactylon* was likewise collected from the bank of each river, close to the sites of hunting of the hippopotami. The samples were collected in clean plastic bags. At the laboratory they were dried at 85 °C and weighed. The sample from Kafue weighed 0.3 kg DW and the sample from Luangwa 0.7 kg DW. Thereafter each sample was ground and homogenized before analysis.

Analytical methods

The concentrations of arsenic (As), cadmium (Cd), Co, Cu, lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), selenium (Se) and zinc (Zn) in livers, kidneys and grass were measured using atomic absorption spectrometry (AAS) after oxidative digestion with nitric acid or a mixed solution with nitric and perchloric acids. The water samples were analysed directly. Analyses for Cd, Co, Mn, Mo and Pb were performed with a Varian SpectrAA-300 with GTA-96 Graphite Tube Atomizer. The samples were digested with Romil Super Purity concentrated nitric acid in a Tecator Digestive System heating unit. Samples for the other determinations were digested in a mixture of nitric and perchloric acids. Arsenic, Hg and Se were determined with a Varian VGA-76 vapour generation accessory (Norheim & Haugen 1986), while those of Cu and Zn were determined by flame atomic absorption with Varian SpectrAA-600. Results were calculated and reported on a wet weight basis for the tissue samples, and on a dry matter basis for the two grass samples. Detection limits in the analyses of tissue and grass samples were 0.002 µg/g for As, 0.005 µg/g for Cd and Hg, 0.01 µg/g for Co, Pb and Se, 0.05 µg/g for Mn and Mo, and 0.5 µg/g for Cu and Zn. A quality control system including regular analyses of certified reference material (NBS bovine liver 1577 b, NRC-CNRC Dogfish DOLT-2, bovine liver BCR 185 and pig kidney BCR 186) was adopted. Statistical calculations were done with the statistical computer programme JMP (SAS). Comparisons between two different groups were performed by Wilcoxon rank sum test. Evaluations of possible variation in hepatic and renal element concentrations with age and of possible correlation between hepatic concentrations of different elements were conducted using the Pearson and Spearman correlation analyses. With one exception, only correlations showing statistical significance ($P < 0.05$) by both Pearson and Spearman analyses are reported.

RESULTS

Hippopotamus samples

The median values and the ranges for the trace elements analysed in the two animal groups are shown in Table 1. The overall picture is one of

TABLE 1 Medians and ranges of the hepatic and renal concentrations of arsenic, cadmium, cobalt, copper, mercury, manganese, molybdenum, lead, selenium and zinc in hippopotami from Kafue and Luangwa Rivers

Element	Liver		Kidney	
	Kafue ($n = 6$)	Luangwa ($n = 24$)	Kafue ($n = 6$)	Luangwa ($n = 24$)
As	0.016 (0.008–0.025)	0.008 (0.004–0.025)[1]	0.012 (0.010–0.020)	0.010 (0.006–0.029)[2]
Cd	0.03 (0.02–0.05)	0.04 (0.02–0.08)	0.44 (0.25–0.68)	0.58 (0.17–1.0)
Co	0.13 (0.11–0.22)	0.15 (0.07–0.37)	0.07 (0.05–0.08)	0.07 (0.03–0.15)
Cu	30 (0.7–110)	55 (2.2–89)	3.1 (2.3–3.3)	2.5 (1.9–4.0)
Hg	0.005 (0.005–0.01)	0.005 (< 0.005–0.01)[3]	0.02 (0.005–0.02)	0.02 (0.005–0.04)[4]
Mn	1.2 (1.0–1.5)	1.4 (0.94–2.9)	0.87 (0.74–1.1)	0.93 (0.25–2.1)
Mo	0.68 (0.41–2.4)	0.52 (0.32–1.2)	0.15 (0.12–0.20)	0.11 (0.03–0.19)
Pb	0.08 (0.05–0.12)	0.05 (0.02–0.12)	0.04 (0.03–0.08)	0.02 (0.01–0.16)
Se	0.38 (0.30–0.40)	0.54 (0.25–0.99)[2]	1.0 (0.68–1.5)	1.2 (0.64–1.6)[5]
Zn	29 (8.8–54)	26 (20–61)	20 (19–27)	18 (12–66)

[1] $n = 21$
[2] $n = 20$
[3] $n = 23$
[4] $n = 17$
[5] $n = 19$
All values in µg/g tissue (ww)

expected levels of essential elements and low concentrations of non-essential heavy metals and metalloids. Statistically significant differences between the samples from Kafue and Luangwa were recorded in liver for As ($P < 0.02$) and Pb ($P < 0.05$). In the kidneys, significant differences were found for Cu ($P < 0.05$), Mo ($P < 0.005$) and Pb ($P < 0.05$).

Significant gender differences were not observed for any of the parameters studied. A weak, but significant negative correlation of hepatic Cu concentration with age was found in the combined sample from both Luangwa and Kafue [$r = 0.43$ (Pearson)/ 0.44 (Spearman)]. According to the Spearman analysis, there was also a weak, significant positive correlation of renal Cd concentration with age ($r = 0.38$), but this correlation was not confirmed by Pearson analysis ($r = 0.05$). No other correlations between tissue element concentrations and the age of the animals were observed. A rather strong positive correlation was found between the hepatic concentrations of Cu and Se ($r = 0.58$ / 0.67) in the combined sample from both locations. This correlation was even stronger when analysed in the sample from Luangwa alone ($r = 0.72$ / 0.73). In the Luangwa sample weaker but still significant correlations (positive or negative) were also found between hepatic concentrations of As and Hg ($r = 0.41$ / 0.45), As and Se ($r = -0.46$ / -0.48), Cd and Cu ($r = -0.47$ / -0.52), Cd and Mo ($r = 0.47$ / 0.47)

TABLE 2 Concentrations of arsenic, cadmium, cobalt, copper, mercury, manganese, molybdenum, lead, selenium and zinc in water samples from Kafue and Luangwa Rivers, and in samples of *Cynodon dactylon* grass growing along the rivers

Element	Water µg/ℓ		Grass µg/g DM	
	Kafue	Luangwa	Kafue	Luangwa
As	0.5	< 0.1	12.6	0.19
Cd	< 0.04	0.27	0.04	0.03
Co	< 0.3	< 0.3	0.47	1.50
Cu	6.0	4.3	27	10
Hg	< 0.2	< 0.2	0.05	0.09
Mn	3.2	11	52	61
Mo	< 2	< 2	0.64	0.95
Pb	< 0.5	0.6	0.72	1.28
Se	< 0.5	< 0.5	0.04	0.09
Zn	< 0.01	< 0.01	39	74

and Hg and Se ($r = -0.49$ / -0.57). Of these, only the negative correlations between As and Se ($r = -0.48$ / -0.50) and between Hg and Se ($r = -0.38$ / -0.51) were significant with both statistical methods also in the combined sample. In the combined sample there was also a weak negative correlation between hepatic As and Cu concentrations ($r = -0.43$ / -0.42).

Table 2 shows the metal concentrations found in our spot samples of river water and grass.

DISCUSSION

The main finding in the present study is that the concentration of the trace elements studied in the liver and kidney of hippopotami did not differ substantially between the animals sampled in the Kafue River and those in the Luangwa River. The concentrations of the essential elements were similar to those reported in normal wild and domestic ruminants, while the concentrations of non-essential heavy metals were low.

Statistically significant differences in concentrations between Kafue and Luangwa samples were found for the elements As and Pb in the liver, and Cu, Mo and Pb in the kidneys. In all these cases the median concentrations were highest in the Kafue. However, in absolute values the differences were small (Table 1). With a possible exception for the As concentrations, the results do in our opinion not indicate any real difference in tissue trace elements levels between hippopotami from the two areas. This lack of distinct differences between the two areas is interesting, considering the substantial pollution in the upper part of the Kafue River system, especially with copper and cobalt (Petterson & Ingri 1993). By contrast, the areas along the Luangwa river are quite unexploited. Apart from the lodges and camps for tourists, few activities take place in and around this river that might contaminate it.

The lack of difference in tissue copper and cobalt concentrations between hippopotami from the two rivers may have several reasons. First, the effect of the upstream pollution level may be so much reduced at this site of the river that it does not substantially influence the element levels in the feed of the hippopotami. Though the pollution of copper and cobalt in the Kafue is very high in the Copperbelt region it decreases downstream. In tilapia fish (*Tilapia rendalli*) caught in the Itezhi-tezhi Dam adjoining the Kafue National Park Cu concentrations in liver and gills were several times higher than in those caught further downstream, but much lower than in a surviving fish species (*Alestes lateralis*) caught in the Copperbelt part of the Kafue River (Mwase 1994). Soil sediments collected at the Itezhi-tezhi Dam did show some residual toxicity to juvenile tilapia, but the toxic effect was limited compared to the effects of sediments from the Copperbelt area (Mwase *et al.* 1998).

Owing to a lack of time and resources we did not undertake an elaborate sampling of river water in the two rivers over time or of a range of relevant plants in the grazing area of the hippopotami. We had to restrict our sampling of the local environment to one spot sample of water and one sample of a representative grass species from each site. In the grass sample from Kafue we found a Cu concentration that was three times higher than in the sample from Luangwa and slightly above reported normal variation in feed crops for domestic animals (Davis & Mertz 1987). The Co concentration, on the other hand, was highest in the grass sample from Luangwa. The Cu and Co concentrations in the water samples from the two rivers were almost equal. The information value of these spot samples must not be overestimated, but in our opinion the results are reasonably in line with the previously published results for sediments and fish (Mwase 1994).

Secondly, the season and grazing habits of the hippopotami may have diminished the impact of the metal contamination in the river on the tissue concentrations in the hippopotami. In the rainy season and the first months thereafter the grass is found nearby, and the hippopotami will graze in areas that have been flooded by the river. However, in the dry season they have to seek further and can move up to 10 km from the river.

The concentrations of the other elements in the grass and water samples were within expected normal variation (Mertz 1986/1987; Andersen, Bratli, Fjeld, Faafeng, Grande, Hem, Holtan Korgh, Lund, Rosland, Rosseland & Aanes 1997), with a few exceptions. The As levels found in the water and grass samples from Kafue were much higher than in the samples from Luangwa, and considerably higher than expected. Arsenic concentrations in grass and other forage crops for domestic animals rarely exceed 1 µg/g DM, and a water As level of 0.5 µg/mℓ is usually found only in As-rich areas (Anke 1986). However, As enrichment has not been reported as a part of the pollution problem in the Copperbelt area in Zambia. River sediments from the Copperbelt area were low in As (Petterson & Ingri 1993). The Cd concentration found in the water sample from Luangwa River was high enough to classify the water as "severely polluted" by Cd according to standards in Scandinavia (Andersen *et al.* 1997). However, this finding was not reflected in the Cd concentration in the grass sample from Luangwa or in the tissue samples from the hippopotami. We therefore consider this value a stray result.

The median hepatic Cu concentrations in hippopotami sampled at Kafue and Luangwa are in agreement with those normally reported in wild and domestic ruminants (Davis & Mertz 1987; Frøslie, Holt, Høie & Haugen 1987). In general, healthy ruminants have average hepatic Cu concentrations that are about an order of magnitude higher than those found in other mammalian species (Davis & Mertz 1987). The hippopotamus is not a ruminant, but it is an artiodactyl herbivore closely related to ruminants. The wide range of hepatic Cu concentrations recorded in these hippopotami is also similar to findings in ruminants. In sheep it is common to find a surprisingly wide range of hepatic Cu values, also within one geographical area (Frøslie 1980), and even within the same flock. In Norwegian moose (*Alces alces*) Frøslie *et al.* (1987) found hepatic Cu concentrations varying between 3.5 and 640 µg/g. The hepatic and renal Co levels found in our study are also consistent with those recorded in healthy domestic and wild ruminants (Smith 1987; Sivertsen, Daae, Godal & Sand 1995).

The hepatic and renal As concentrations were similar to those in red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*) (Holm 1984) and in reindeer (*Rangifer tarandus*) from uncontaminated areas (Sivertsen *et al.* 1995). However, the As level in the hippopotamus livers in Kafue was significantly higher than in Luangwa ($P < 0.02$). Seen in relation to the high As concentrations found in the grass and water spot samples from Kafue, this may indicate a somewhat higher As level in the environment in the Kafue National Park than in the Southern Luangwa National Park.

Cd concentrations were generally low. Data on hepatic Cd concentrations in animals have been summarised by the Subcommittee on Mineral Toxicity of the US National Academy of Science (Kostial 1986). Hepatic and renal Cd levels below 1 µg/g are considered as background levels. As the Cd exposure levels in the environment increase, the Cd concentrations in the liver and especially in the kidneys of wild herbivores may increase considerably (Frøslie, Haugen, Holt & Norheim 1986). Renal Cd concentrations of up to 61 µg/g have been recorded in wild boar (Kleiminger 1983), and up to 34 µg/g in reindeer (Frøslie *et al.* 1986). Our results therefore indicate no significant Cd pollution in the Kafue and Luangwa national parks, despite the high Cd level found in our spot sample of river water from Luangwa.

The hepatic and renal concentrations of Hg similarly indicate a very low level of pollution in the feed of the hippopotami. Similar concentrations are found in moose and other herb-eating cervides from uncontaminated areas (Lusky, Bohm, Stoyke, Hecht, Luthardt & Lippert 1992; Sivertsen *et al.* 1995). In contaminated areas, hepatic Hg concentrations of up to 4.9 µg/g and renal concentrations up to 50 µg/g have been reported from roe deer and even higher values in samples from wild boar (Kleiminger 1983).

The hepatic and renal concentrations of Pb recorded in the hippopotamus samples were also low, and similar to levels found in herb-eating wild cervids in uncontaminated areas (Hecht 1986; Frøslie, Norheim, Rambæk & Steinnes 1984). The Hg and Pb tissue concentrations in the hippopotami are thus comparable to concentrations in other ungulates foraging on grass, herbs and foliage. The lichen-eating reindeer and caribou have higher tissue concentrations of these elements, even in seemingly uncontaminated areas (Elkin & Bethke 1995; Sivertsen *et al.* 1995). Lead contamination in hippopotami has also been studied by Dauth *et al.*

1988. They investigated blood concentrations in hippopotami from the Kruger National Park in South Africa, and found no indications of Pb contamination in that area.

The hepatic and renal Mn concentrations were slightly lower than typical concentrations in healthy domestic ruminants (Hurley & Keen 1987; Blood & Radostits 1989). The hepatic and renal concentrations of Mo were in the lower normal range, compared to domestic ruminants (Mills & Davis 1987). The hepatic and renal concentrations of Se were also well within the normal range for domestic ruminants (Levander 1986; Blood & Radostits 1989). However, the variation in Se concentrations among these hippopotami was less than previously reported for some of the wild cervids (Frøslie *et al.* 1987). Hepatic and renal Zn concentrations observed also fell within the normal range in domestic and wild ungulates (Hambidge, Casey & Krebs 1986; Frøslie *et al.* 1987).

Originally we intended to include investigation of nickel (Ni) and chromium (Cr) concentrations in the hippopotamus tissues. However, due to logistical reasons we had to use steel knives in the collection of the samples. In analysing the samples for Ni and Cr we found strange variations that most probably resulted from Ni and Cr contamination from the steel equipment. To avoid this kind of contamination special measures must be taken in the collection and preparation of tissue samples (Godal, Langseth, Sivertsen & Lund 1995). We therefore consider our Ni and Cr results unreliable and have not reported them. However, the results did not indicate any significant Ni or Cr contamination of the environment of the hippopotami.

The reduced number of samples analysed for some of the elements (Table 1) is also due to logistical restraints in the fieldwork. A few of the samples obtained were too small to conduct all the planned analyses with reliable quality. In these cases priority was given to the elements considered most important for the study.

The weak negative correlation observed between hepatic Cu concentration and age is difficult to interpret, and may be fortuitous. On the other hand, the correlation between renal Cd concentration and age obtained with the non-parametric Spearman analysis was expected. In most mammalian species, there is a strong effect of age especially in renal Cd concentrations (Kostial 1986; Frøslie *et al.* 1986). In the present study this correlation was weak, and not observed with the parametric Pearson analysis. This may be a result of the low overall level of Cd in these hippopotami, indicating low concentrations in their environment. At these low concentrations, individual and sample variations may tend to overshadow the age effect on renal Cd accumulation.

The strongest and most consistent inter-element correlation obtained was a positive correlation between hepatic Cu and Se concentrations. A similar correlation has previously been observed in Norwegian reindeer, in two independent studies (Frøslie *et al.* 1987; Sivertsen *et al.* 1995). The weak, but consistent negative correlations between As and Se and between Hg and Se were surprising. At higher contamination levels strong positive correlations between Hg and Se are regularly found, both in humans and in wild marine mammals (Clarkson 1987). The other inter-element correlations obtained were weaker and less consistent, and may be incidental.

In summary, the analyses of the tissue samples from the hippopotami have provided interesting information. From an ecotoxicological point of view, our results indicate that the trace element pollution of the Kafue River originating from the industrial activity in the Copperbelt region does not affect the hippopotami in the Kafue National Park. From the point of view of general ecological science, this study contains the first information we are aware of on hepatic and renal levels of a range of essential and other trace elements in free-living hippopotami. The results obtained may serve as reference values for further scientific work in this field.

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