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Trace Element Contamination in Tissues of Four Bird Species from the Rift Valley Region, Ethiopia

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Abstract Concentrations of ten trace elements (Hg, As, Cd, Pb, Co, Cr, Cu, Ni, Se and Zn) were determined in different tissues (liver, kidney, muscle, heart and brain) of African sacred ibis (*Threskiornis aethiopicus*), Hamerkop (*Scopus umbretta*), marabou stork (*Leptoptilos crumeniferus*) and great white pelican (*Pelecanus onocrotalus*) inhabiting the Ethiopian Rift Valley region. There were differences in trace element patterns among the bird species. Significantly ($p<0.05$) higher concentrations of Cd (5.53 µg/g dw ± 2.94) in kidney and Hg (0.75 µg/g ww ± 0.30) in liver were observed in the great white pelican compared to the other species, and liver concentrations of these two elements showed positive correlations with trophic level. Concentrations of toxic elements (As, Cd, Pb and Hg) in liver were below their respective toxicological thresholds, indicating that the data may provide baseline information for future studies.

Keywords Bird · Tissue · Trace element · Ecological risk assessment · Ethiopian rift valley

Trace elements are highly persistent, have bioaccumulation and/or biomagnification potential along the food web, and depending upon their concentrations may be toxic to humans and wildlife. Owing to their wide distribution, feeding at different trophic levels and sensitivity to environmental changes, birds have been recognized as sentinel species for heavy metal contamination (Furness 1993; Zhang and Ma 2011). In particular, top-level piscivorous birds are liable to consume prey containing high level of pollutants, and can accumulate higher levels of contaminants than birds that are lower on the food chain (Burger and Gochfeld 2002). Chronic metal exposures can result in detrimental effects on growth, development, reproduction, behavior and physiological mechanisms (Scheuhammer 1987; Snoeijs et al. 2004). For example, lead (Pb) impairs the growth and survival of nestlings and causes haemolytic anaemia in wild Pb-poisoned birds (Mateo et al. 2007; Scheuhammer 1987). Mercury (Hg) correlates with decreased reproductive success (Varian-Ramos et al. 2014), and at high doses essential elements such as zinc (Zn) and selenium (Se) may have toxic effects on kidneys and impair reproduction, respectively (Carpenter et al. 2004; Heinz 1996). In general, effects of exposure to trace elements have been associated with declines in bird populations [http://www.birdlife.org]. However, despite the presence of the great biodiversity and numbers of birds, the African environment has received little attention from researchers in reference to environmental contamination up to the present day. As a consequence, there is a paucity of information about the contamination status and ecological impacts of pollutants like trace elements in birds inhabiting Africa. Thus, monitoring levels of

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environment pollutants in avian species may be of crucial importance in preventing potential risks to living beings.

The Ethiopian Rift Valley comprises seven principal lakes in a closed water basin. It is a highly productive agricultural region, and a major tourism attraction area for bird watching in the country. The region provides ideal habitat for a variety of avian species and wildlife. It serves as a breeding, and wintering ground, and as a migration stopover area for several resident and migratory bird species. Lake Ziway is one of the best sites in Ethiopia to see a diversity of bird species such as marabou stork, African fish eagle (*Haliaeetus vocifer*), white-breasted cormorant (*Phalacrocorax lucidus*), African sacred ibis and great white pelican. The wetland supports over 20,000 water birds (Bird life international 2013). However, in recent years it has been noted that the lake faces several anthropogenic threats from industrial and domestic wastewater, solid waste, and agricultural runoff. These pressures may adversely affect the lake ecosystem, potentially reducing populations of various fish, invertebrate and bird species. Nevertheless, no research has been performed on the potential ecological effects of trace elements in different bird species inhabiting the Ethiopian Rift Valley region. Thus, it is expected that the data generated here will serve as reference values and baseline data for future studies.

Therefore, this work was intended to (1) assess the bioaccumulation levels of ten elements (Hg, As, Cd, Pb, Co, Cr, Cu, Ni, Se, and Zn) in liver, kidney, muscle, heart and brain of four bird species, and (2) investigate potential ecological risks in birds to delineate the bird species at risk. The information will henceforth be highly useful for conservation research on avian species.

Materials and Methods

Detailed information about the studied bird species is described elsewhere (Yohannes et al. 2014). With the help of the local people, a total of 23 birds comprising four species; African sacred ibis (N = 7), hamerkop (N = 5), marabou stork (N = 6), and great white pelican (N = 5) were captured alive using nets in May 2012 at the shore of Lake Ziway (7°59′19″N; 38°50′30″E, surface area: 400 km^2, elevation: 1638 m). These bird species are widely distributed in the African ecosystems, and can be considered as potential bio-monitoring species for environmental contamination. Each bird was euthanized (using ether after capture) and necropsied. Samples of liver, kidney, muscle, heart and brain were placed in polyethylene bags and stored at −20°C. The frozen samples were then transported to Japan for trace element and stable isotope analyses. All analyses were carried out in the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan. Permission was granted from the Ethiopian Wildlife Conservation Authority (EWCA) (Permission No. DA/31/284/012) for capturing and sacrificing the birds.

Levels of nine elements (As, Cd, Pb, Co, Cr, Cu, Ni, Se, and Zn) were analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS; 7700 series, Agilent technologies, Tokyo, JP) in liver, kidney, muscle, heart and brain tissues. Briefly, approximately 1.0 g of individual samples were dried at 40°C, and digested using 70% (v/v) HNO_3 (5 mL) and 30% (v/v) H_2O_2 (1 mL) in a microwave system (Speed Wave MWS-2, Berghof, DE). After cooling, each mixture was transferred into a numbered plastic tube and topped to 10 mL with Milli Q-water. Analytical blanks were run in the same way as the samples. Total mercury (Hg) was determined directly without any pre-treatment using a fully automated thermal vaporization mercury analyzer (MA-3000, Nippon Instrument Corp., Osaka, JP).

The certified reference material, DOLT-4 (dogfish liver, National Research Council of Canada, Ottawa, CA) was used for method validation and quality control/quality assurance. Replicate analysis of DOLT-4 showed good recoveries ranging from 90% to 110%. The measured dry weight (dw) values were converted to wet weight (ww) for the threshold levels comparison based on their respective average water content, 68% ± 1.4% for liver and 74% ± 1.1% for kidney samples.

Stable nitrogen isotope analysis ($\delta^{15}N$) was analyzed using a Fisons NA1500 elemental analyzer (Fisons Instruments SpA, Strada Rivoltana, IT) coupled to a Finnigan MAT 252 mass spectrometer (Finnigan MAT GmbH, Bremen, DE) using dried and ground muscle subsamples. The procedure for the assessment of isotopic ratio was described in our previous study (Yohannes et al. 2014).

Statistical analyses were performed using JMP 9 (SAS Institute, Cary, NC, USA), and the level of significance was set at $p < 0.05$. Concentrations of trace elements in each tissue were used for analysis and presented as mean ± standard deviation. Data were log transformed to obtain normal distributions that satisfied the homogeneity of variance. Statistical differences in trace element concentrations in each tissue among the bird species were evaluated by one-way analysis of variance (ANOVA) followed by the Tukey HSD test. Linear regression analysis was employed to analyze relations between log-transformed liver concentrations of trace elements and $\delta^{15}N$.

Results and Discussion

Limited information is currently available for trace element concentrations in tissues of birds from Africa. This is the first study reporting on the levels and toxicity assessment of trace elements in birds from Ethiopia. Elemental concentrations in each of the five analyzed tissues of the four bird species are presented in Table 1. Of the ten elements tested, Zn followed by Cu were found at higher concentrations than the other trace elements in all bird tissues. Although concentrations were low, trace element concentrations showed significant differences ($p<0.05$) among the bird species in at least two tissues, indicating differences in tissue-specific accumulation of these elements. Hg and Pb in all tissues, Co in liver, heart and brain, Cr in heart, Cu in muscle and heart, Ni in liver and heart, Se in brain, and Zn in kidney and muscle showed significant differences ($p<0.05$) among the studied bird species. This might be caused by variations in diet, body condition, metabolic capacity, and detoxification ability among the bird species.

Essential elements such as Co, Cu, Ni, Se and Zn are of particular importance in cell metabolism because hundreds of known enzymes require these metals for their catalytic activities (Goyer 1997). Concentrations of Zn, Se, and Cu were higher than the non-essential elements (Hg, As, Pb, and Cd). Among the essential elements, concentrations of Ni and Co were much lower than the others. Higher concentrations of Hg, Pb, Cu, and Zn were found in liver; As and Cd in the kidney; Cr in the heart and Ni in the muscle. Element concentrations were generally higher in liver and kidney than in other tissues, which might be associated with normal homeostatic mechanisms, and which typically are found bound to metallothioneins for storage (Lucia et al. 2012).

Kidney, followed by liver, was the main organ for Cd accumulation in all bird species. The high Cd accumulation in these two internal organs demonstrates the role of these organs in the detoxification process and storage of nonessential elements (Lucia et al. 2012). Concentrations of Cd in kidney tissue of the great white pelican were high compared with other tissues, and significantly (F-ratio = 8.34, $p=0.001$) higher than the other studied bird species. The highest level of Cd (5.53 μg/g dw ± 2.94) was observed in the great white pelican, an aquatic bird species, followed by marabou stork (1.57 μg/g dw ± 1.07) (Table 1). Similar patterns of Cd accumulation in kidneys of aquatic birds were reported elsewhere (Kojadinovic et al. 2007; Lucia et al. 2010; Nam et al. 2005).

Mercury was most highly accumulated in the liver in all four species, followed by the kidney. The great white pelican exhibited the highest level of Hg (0.75 μg/g ww ± 0.30) compared to the other bird species (F-ratio = 8.63, $p<0.001$). This is in accordance with the findings of other authors that Hg predominantly accumulates in liver (Nam et al. 2005; Skoric et al. 2012). Birds demethylate organic Hg in tissues such as the liver and kidney, and store a large portion of their Hg burdens in inorganic form (Kim et al. 1996). The accumulation of this toxic element in the aquatic white pelican species might be related to diet and trophic levels. The pelican is a piscivorous bird which feeds primarily on fish and eats their prey whole, and fish are known to be a source of Hg contamination for aquatic birds.

Pb accumulated differently in the tissues and showed significant difference in kidney (F-ratio = 7.32, $p=0.001$), muscle (F-ratio = 4.05, $p=0.02$) and heart (F-ratio = 3.16, $p=0.04$) among the bird species (Table 1). In the present study, mean Pb levels in the liver ranged from 0.01 to 0.09 μg/g dw, and the marabou stork had the highest mean hepatic level, followed by the African sacred ibis. Ecological and feeding habitats of these bird species might be a plausible explanation for elevated Pb levels. They feed on a wide variety of food and their eating habits may have led them into urban areas to access garbage and waste from abattoirs and food waste from humans. With regard to As, there were no significant differences ($p>0.05$) among the studied bird species in all tissues. The highest level of As was observed in kidney. Nevertheless, all the bird species exhibited low As levels (<0.1 μg/g dw) (Table 1).

In this study, significantly high ($p<0.05$) concentrations of Cr ranging from 6.6 μg/g dw to 130.3 μg/g dw were observed in great while pelican heart samples (F-ratio = 4.78, $p=0.01$). Even though the source of elevated Cr in pelican heart samples could not be identified, further study is needed to speciate Cr and address concerns regarding this element; Cr(VI) is known to be highly toxic, while Cr(III) is an essential trace element (Levina et al. 2003). Nonetheless, Cr levels in liver and kidney of the studied bird species do not reach the level of adverse effects for internal tissues (Eisler 1986). Selenium presented the highest levels in kidney (ranged from 10.7 to 12.8 μg/g dw), followed by liver tissue (ranged from 6.28 to 7.03 μg/g dw) (Table 1). These levels of Se in liver and kidney observed in the present study were less than the accepted threshold levels for adverse biological effects in birds (Heinz 1996). Thus, this level of Se is probably beneficial, considering its importance in detoxification processes of other toxic elements (Ikemoto et al. 2004).

Exposure to trace elements is a hypothesis proposed to explain the decline in birds. At high concentrations, Cd can cause kidney damage, suppression of egg production, and testicular damage (Furness 1996; Lucia et al. 2009). Mercury disturbs the nervous system and may have a negative impact on growth, development, and reproduction (Scheuhammer 1987; Scheuhammer et al. 2007; Varian-Ramos et al. 2014). Lead can affect the brain and nervous system, and cause adverse effects on reproduction, such as decreased plasma calcium and egg production; and also cause behavioral impairments (Burger and Gochfeld 2000; Clark and Scheuhammer 2003). Arsenic, in its inorganic forms, may act as an endocrine disruptor, bring about the death of an individual, produce sublethal

Table 1 Trace element concentrations (mean ± SD, μg/g dry weight) in different tissues of four bird species from Ethiopia

Tissue	Species	Hg[§]	As	Cd	Pb	Co	Cr	Cu	Ni	Se	Zn
Liver	African sacred ibis	0.21 ± 0.10[b]	0.07 ± 0.02	0.11 ± 0.17[b]	0.05 ± 0.04	0.12 ± 0.04[a]	0.03 ± 0.02	32.4 ± 23.8	0.02 ± 0.004[ab]	6.71 ± 0.63	118 ± 43
	Hamerkop	0.26 ± 0.04[b]	0.03 ± 0.009	0.14 ± 0.09[b]	0.01 ± 0.003	0.06 ± 0.01[b]	0.02 ± 0.003	11.4 ± 3.82	0.01 ± 0.003[b]	7.03 ± 0.80	98 ± 39
	Marabou stork	0.44 ± 0.23[ab]	0.04 ± 0.01	0.19 ± 0.04[b]	0.09 ± 0.08	0.11 ± 0.03[ab]	0.07 ± 0.06	46.6 ± 47.7	0.05 ± 0.04[a]	6.28 ± 0.88	153 ± 84
	Great white pelican	0.75 ± 0.30[a]	0.05 ± 0.01	1.04 ± 0.08[a]	0.01 ± 0.005	0.09 ± 0.02[ab]	0.02 ± 0.004	31.2 ± 14.2	0.02 ± 0.007[ab]	6.44 ± 1.61	144 ± 33
Kidney	African sacred ibis	0.10 ± 0.08[b]	0.09 ± 0.03	0.99 ± 1.55[b]	0.05 ± 0.01[ab]	1.59 ± 1.89	0.04 ± 0.01	13.3 ± 1.28	0.07 ± 0.007	10.7 ± 2.79	94 ± 8[b]
	Hamerkop	0.19 ± 0.07[b]	0.07 ± 0.02	0.98 ± 0.78[b]	0.03 ± 0.01[b]	0.39 ± 0.15	0.04 ± 0.02	13.1 ± 1.90	0.06 ± 0.02	12.8 ± 1.46	84 ± 4[b]
	Marabou stork	0.28 ± 0.14[ab]	0.06 ± 0.02	1.57 ± 1.07[b]	0.07 ± 0.03[a]	0.39 ± 0.11	0.22 ± 0.21	16.9 ± 3.31	0.07 ± 0.04	11.4 ± 1.14	113 ± 13[a]
	Great white pelican	0.47 ± 0.22[a]	0.08 ± 0.02	5.53 ± 2.94[a]	0.03 ± 0.01[b]	0.64 ± 0.42	0.13 ± 0.14	13.8 ± 2.43	0.10 ± 0.04	11.9 ± 1.96	97 ± 15[ab]
Muscle	African sacred ibis	0.06 ± 0.04[b]	0.03 ± 0.02	0.01 ± 0.02[b]	0.02 ± 0.01[a]	0.06 ± 0.04	0.54 ± 1.22	6.63 ± 2.99[b]	0.23 ± 0.52	2.52 ± 0.46	45 ± 12[ab]
	Hamerkop	0.12 ± 0.01[b]	0.01 ± 0.005	0.005 ± 0.003[b]	0.01 ± 0.002[ab]	0.03 ± 0.006	0.14 ± 0.09	10.1 ± 1.53[b]	0.03 ± 0.01	2.70 ± 0.39	42 ± 9[b]
	Marabou stork	0.11 ± 0.05[b]	0.02 ± 0.006	0.005 ± 0.003[b]	0.01 ± 0.01[ab]	0.03 ± 0.01	0.10 ± 0.03	10.3 ± 3.24[b]	0.08 ± 0.05	2.45 ± 0.25	44 ± 11[b]
	Great white pelican	0.41 ± 0.12[a]	0.03 ± 0.02	0.04 ± 0.01[a]	0.01 ± 0.002[b]	0.03 ± 0.006	0.16 ± 0.08	19.9 ± 2.81[a]	0.10 ± 0.05	2.77 ± 0.11	63 ± 10[a]
Heart	African sacred ibis	0.07 ± 0.05[b]	0.05 ± 0.03	0.003 ± 0.003[b]	0.01 ± 0.008[ab]	0.16 ± 0.03[ab]	0.04 ± 0.01[b]	21.2 ± 2.37[a]	0.02 ± 0.01[b]	4.13 ± 1.04	116 ± 15
	Hamerkop	0.10 ± 0.02[b]	0.02 ± 0.006	0.003 ± 0.002[b]	0.01 ± 0.003[b]	0.09 ± 0.01[b]	0.08 ± 0.04[b]	20.7 ± 2.07[ab]	0.02 ± 0.01[b]	4.22 ± 0.44	99 ± 9
	Marabou stork	0.09 ± 0.04[b]	0.01 ± 0.004	0.003 ± 0.002[b]	0.01 ± 0.01[ab]	0.21 ± 0.07[a]	0.86 ± 0.72[b]	17.7 ± 1.24[ab]	0.02 ± 0.01[b]	3.67 ± 0.26	133 ± 12
	Great white pelican	0.27 ± 0.06[a]	0.06 ± 0.03	0.01 ± 0.008[a]	0.02 ± 0.007[a]	0.13 ± 0.06[ab]	50.2 ± 54.9[a]	16.0 ± 5.19[b]	0.36 ± 0.33[a]	3.49 ± 0.53	102 ± 39
Brain	African sacred ibis	0.02 ± 0.01[b]	0.03 ± 0.02	0.005 ± 0.007[b]	0.03 ± 0.01	0.06 ± 0.01[a]	0.05 ± 0.01	12.2 ± 1.70	0.02 ± 0.01	2.90 ± 0.23[b]	61 ± 13
	Hamerkop	0.04 ± 0.01[b]	0.01 ± 0.005	0.001 ± 0.001[b]	0.01 ± 0.002	0.05 ± 0.02[ab]	0.35 ± 0.65	9.65 ± 0.79	0.02 ± 0.01	3.35 ± 0.31[a]	53 ± 4
	Marabou stork	0.04 ± 0.01[b]	0.01 ± 0.002	0.001 ± 0.001[b]	0.03 ± 0.04	0.05 ± 0.007[ab]	0.10 ± 0.07	14.0 ± 2.70	0.02 ± 0.005	3.01 ± 0.08[ab]	62 ± 5
	Great white pelican	0.12 ± 0.02[a]	0.01 ± 0.006	0.01 ± 0.001[a]	0.01 ± 0.005	0.03 ± 0.008[b]	0.09 ± 0.02	14.4 ± 11.8	0.01 ± 0.004	2.63 ± 0.20[b]	47 ± 12
Toxicity thresholds*	Background levels			<5.0[d]	<2.0[e]						
	Reproductive impairment									>3.0[g]	
	Sublethal effects	>4.0[f]	>5.0[h]	>40.0[d]	>6.0[e]		>4.0[i]			>10.0[g]	
Instrumental detection limit[c]		0.002 ng/g	0.002 μg/L	0.001 μg/L	0.001 μg/L	0.001 μg/L	0.003 μg/L	0.07 μg/L	0.01 μg/L	0.02 μg/L	0.02 μg/L

In each tissue, means not sharing the same letters (a, b) among the bird species are significantly different (ANOVA and Tukey HSD test; $p < 0.05$)

*Toxicity thresholds—based on the clinical signs implicated in waterfowl, corresponding to liver concentrations (μg/g ww)

[§]Concentration expressed as μg/g wet weight

[c]Instrumental detection limit—calculated as three times the standard deviation of ten blank sample measurements divided by the slope of the calibration curve

[d]Furness (1996)

[e]Pain et al. (1995)

[f]Eisler (1987)

[g]Heinz (1996)

[h]Eisler (1994)

[i]Eisler (1986)

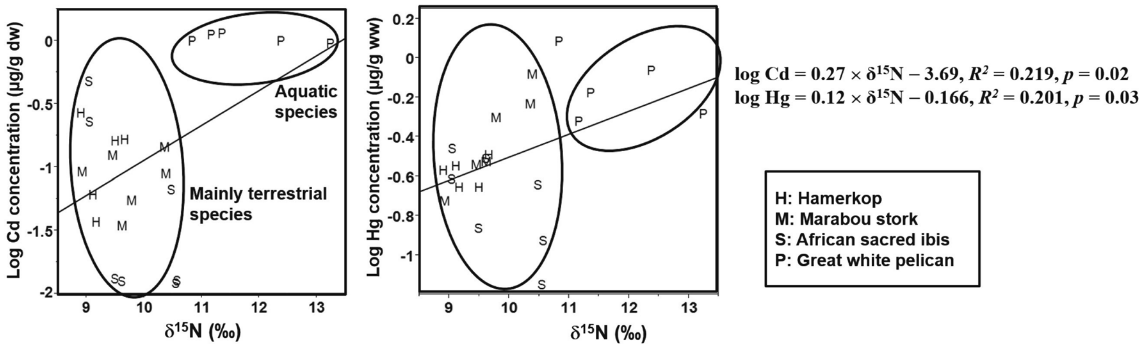

Fig. 1 Relationships between stable nitrogen signatures and log-transformed liver concentrations of Cd and Hg in four bird species from Ethiopia

effects (such as decreased body weight and feed intake), or disrupt reproduction (Eisler 1994; Kunito et al. 2008).

In the present study, the hepatic element concentrations were below the threshold values reported for waterfowl (Table 1). Concentrations of Cd ranged from 0.005 to 5.53 μg/g dw, and mean hepatic Hg concentrations ranged from 0.21 to 0.75 μg/g (ww) (~0.65 to 2.34 μg/g dw), revealing low exposure of these elements. Moreover, in this study the hepatic–Cd/renal–Cd concentration ratio was <1 (ranged from 0.03 to 0.44), indicating a low level of Cd exposure (Scheuhammer 1987). Meanwhile, hepatic Pb levels ranged from 0.01 to 0.09 μg/g dw and levels of As <0.1 μg/g dw were lower than their respective threshold limits.

At high exposure, Se and Hg can be individually toxic. However, because of the high binding affinity between Hg and Se, direct Hg sequestration by Se has often been assumed to be the mechanism for the protective effect of Se against Hg toxicity (Ralston et al. 2007). The existence of Se:Hg at 1:1 molar ratio suggests that detoxification might occur in the liver by forming insoluble mercury selenide (Ikemoto et al. 2004). In this study, molar ratios were always above one. Consequently, birds are protected against Hg toxicity, but the excess hepatic Se concentrations support a possible toxicity of this element for the studied bird species. Levels of Se in liver >3 μg/g ww are proposed to cause reproductive impairment (Heinz 1996).

Stable nitrogen isotope analysis can be used to establish trophic relationships and trophic transfer of environmental pollutants in both freshwater and marine ecosystems (Cabana and Rasmussen 1994). Thus, relationships between the $\delta^{15}N$ and the log-transformed concentrations of trace elements were examined to investigate the trophic level-dependent accumulation of trace elements (Fig. 1). The stable nitrogen isotope ($\delta^{15}N$) signatures ranged from 8.90‰ to 13.3‰, with the great white pelican showing a significantly higher $\delta^{15}N$ value (11.8‰ ± 1.0‰) compared to other bird species (F-ratio = 13.8, $p < 0.001$). There were significant correlations of $\delta^{15}N$ with Cd and Hg but not with other elements (data not shown). Concentrations of Cd and Hg increased with increasing $\delta^{15}N$ (Fig. 1). This relationship suggests that food intake may play an important part in Cd and Hg trophic transfer for the analyzed species, and the bioaccumulation potential of these two trace elements in the aquatic environment could be apparent, and related to trophic level.

In conclusion, our results give a first insight into the contamination of wild birds inhabiting the Ethiopian Rift Valley region. Meta-analyses using data from this study suggested that metal concentrations of the studied bird species were relatively low and below the toxic levels. Thus the data may serve as baseline data to facilitate management guidelines and conservation measures with the goal to ensure a healthy environment for all species in the Rift Valley region. However, even low concentrations of toxicants may harm the organisms by interacting and/or synergizing with other compounds. Thus, given the bioaccumulation potential of trace elements, and the importance of the wetland habitat for this region for breeding, wintering and migration stopovers of birds, future studies seem necessary for continued ecological risk assessment so that the region continues its role in global bird conservation.

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