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

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19
20 **Abstract**

21 Concentrations of 10 trace elements (Hg, As, Cd, Pb, Co, Cr, Cu, Ni, Se and Zn) were determined
22 in different tissues (liver, kidney, muscle, heart and brain) of African sacred ibis (*Threskiornis*
23 *aethiopicus*), Hamerkop (*Scopus umbretta*), marabou stork (*Leptoptilos crumeniferus*) and great
24 white pelican (*Pelecanus onocrotalus*) inhabiting the Ethiopian Rift Valley region. There were
25 differences in trace element patterns among the bird species. Significantly ($p < 0.05$) higher
26 concentrations of Cd ($5.53 \mu\text{g/g dw} \pm 2.94$) in kidney and Hg ($0.75 \mu\text{g/g ww} \pm 0.30$) in liver were
27 observed in the great white pelican compared to the other species, and liver concentrations of these
28 two elements showed positive correlations with trophic level. Concentrations of toxic elements (As,
29 Cd, Pb and Hg) in liver were below their respective toxicological thresholds, indicating that the data
30 may provide baseline information for future studies.

31
32 Key words: Bird; Tissue; Trace element; Ecological risk assessment; Ethiopian Rift Valley

33
34 Trace elements are highly persistent, have bioaccumulation and/or biomagnification potential
35 along the food web, and depending upon their concentrations may be toxic to humans and wildlife.
36 Owing to their wide distribution, feeding at different trophic levels and sensitivity to environmental
37 changes, birds have been recognized as sentinel species for heavy metal contamination (Furness
38 1993; Zhang and Ma 2011). In particular, top-level piscivorous birds are liable to consume prey
39 containing high level of pollutants, and can accumulate higher levels of contaminants than birds
40 that are lower on the food chain (Burger and Gochfeld 2002). Chronic metal exposures can result in
41 detrimental effects on growth, development, reproduction, behavior and physiological mechanisms
42 (Scheuhammer 1987; Snoeijs et al. 2004). For example, lead (Pb) impairs the growth and survival
43 of nestlings and causes haemolytic anaemia in wild Pb-poisoned birds (Mateo et al. 2007;

1 Scheuhammer 1987). Mercury (Hg) correlates with decreased reproductive success (Varian-Ramos
2 et al. 2014), and at high doses essential elements such as zinc (Zn) and selenium (Se) may have
3 toxic effects on kidneys and impair reproduction, respectively (Carpenter et al. 2004; Heinz 1996).
4 In general, effects of exposure to trace elements have been associated with declines in bird
5 populations [<http://www.birdlife.org>]. However, despite the presence of the great biodiversity and
6 numbers of birds, the African environment has received little attention from researchers in reference
7 to environmental contamination up to the present day. As a consequence, there is a paucity of
8 information about the contamination status and ecological impacts of pollutants like trace elements
9 in birds inhabiting Africa. Thus, monitoring levels of environment pollutants in avian species may
10 be of crucial importance in preventing potential risks to living beings.

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

25 Therefore, this work was intended to (1) assess the bioaccumulation levels of 10 elements (Hg,
26 As, Cd, Pb, Co, Cr, Cu, Ni, Se, and Zn) in liver, kidney, muscle, heart and brain of four bird species,
27 and (2) investigate potential ecological risks in birds to delineate the bird species at risk. The
28 information will henceforth be highly useful for conservation research on avian species.

29

30 **Materials and Methods**

31 Detailed information about the studied bird species is described elsewhere (Yohannes et al.
32 2014). With the help of the local people, a total of 23 birds comprising four species; African sacred
33 ibis (N = 7), hamerkop (N = 5), marabou stork (N = 6), and great white pelican (N = 5) were
34 captured alive using nets in May 2012 at the shore of Lake Ziway (7°59'19"N; 38°50'30"E, Surface
35 area: 400 km², Elevation: 1638 m). These bird species are widely distributed in the African
36 ecosystems, and can be considered as potential bio-monitoring species for environmental
37 contamination. Each bird was euthanized (using ether after capture) and necropsied. Samples of
38 liver, kidney, muscle, heart and brain were placed in polyethylene bags and stored at -20 °C. The
39 frozen samples were then transported to Japan for trace element and stable isotope analyses. All
40 analyses were carried out in the Laboratory of Toxicology, Graduate School of Veterinary Medicine,
41 Hokkaido University, Japan. Permission was granted from the Ethiopian Wildlife Conservation
42 Authority (EWCA) (Permission No. DA/31/284/012) for capturing and sacrificing the birds.

1 Levels of 9 elements (As, Cd, Pb, Co, Cr, Cu, Ni, Se, and Zn) were analyzed using an
2 inductively coupled plasma-mass spectrometer (ICP-MS; 7700 series, Agilent technologies, Tokyo,
3 JP) in liver, kidney, muscle, heart and brain tissues. Briefly, approximately 1.0 g of individual
4 samples were dried at 40°C, and digested using 70% v/v HNO₃ (5 mL) and 30% v/v H₂O₂ (1 mL) in
5 a microwave system (Speed Wave MWS-2, Berghof, DE). After cooling, each mixture was
6 transferred into a numbered plastic tube and topped to 10 mL with Milli Q-water. Analytical blanks
7 were run in the same way as the samples. Total mercury (Hg) was determined directly without any
8 pre-treatment using a fully automated thermal vaporization mercury analyzer (MA-3000, Nippon
9 Instrument Corp., Osaka, JP).

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

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

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

26 27 **Results and Discussion**

28 Limited information is currently available for trace element concentrations in tissues of birds
29 from Africa. This is the first study reporting on the levels and toxicity assessment of trace elements
30 in birds from Ethiopia. Elemental concentrations in each of the five analyzed tissues of the 4 bird
31 species are presented in Table 1. Of the 10 elements tested, Zn followed by Cu were found at higher
32 concentrations than the other trace elements in all bird tissues. Although concentrations were low,
33 trace element concentrations showed significant differences ($p < 0.05$) among the bird species in at
34 least two tissues, indicating differences in tissue-specific accumulation of these elements. Hg and
35 Pb in all tissues, Co in liver, heart and brain, Cr in heart, Cu in muscle and heart, Ni in liver and
36 heart, Se in brain, and Zn in kidney and muscle showed significant differences ($p < 0.05$) among the
37 studied bird species. This might be caused by variations in diet, body condition, metabolic capacity,
38 and detoxification ability among the bird species.

39 Essential elements such as Co, Cu, Ni, Se and Zn are of particular importance in cell
40 metabolism because hundreds of known enzymes require these metals for their catalytic activities
41 (Goyer 1997). Concentrations of Zn, Se, and Cu were higher than the non-essential elements (Hg,
42 As, Pb, and Cd). Among the essential elements, concentrations of Ni and Co were much lower than
43 the others. Higher concentrations of Hg, Pb, Cu, and Zn were found in liver; As and Cd in the

1 kidney; Cr in the heart and Ni in the muscle. Element concentrations were generally higher in liver
2 and kidney than in other tissues, which might be associated with normal homeostatic mechanisms,
3 and which typically are found bound to metallothioneins for storage (Lucia et al. 2012).

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

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

22 Pb accumulated differently in the tissues and showed significant difference in kidney (F -ratio =
23 7.32, $p = 0.001$), muscle (F -ratio = 4.05, $p = 0.02$) and heart (F -ratio = 3.16, $p = 0.04$) among the
24 bird species (Table 1). In the present study, mean Pb levels in the liver ranged from 0.01 to 0.09
25 $\mu\text{g/g dw}$, and the marabou stork had the highest mean hepatic level, followed by the African sacred
26 ibis. Ecological and feeding habitats of these bird species might be a plausible explanation for
27 elevated Pb levels. They feed on a wide variety of food and their eating habits may have led them
28 into urban areas to access garbage and waste from abattoirs and food waste from humans. With
29 regard to As, there were no significant differences ($p > 0.05$) among the studied bird species in all
30 tissues. The highest level of As was observed in kidney. Nevertheless, all the bird species exhibited
31 low As levels ($<0.1 \mu\text{g/g dw}$) (Table 1).

32 In this study, significantly high ($p < 0.05$) concentrations of Cr ranging from $6.6 \mu\text{g/g dw}$ to
33 $130.3 \mu\text{g/g dw}$ were observed in great white pelican heart samples (F -ratio = 4.78, $p = 0.01$). Even
34 though the source of elevated Cr in pelican heart samples could not be identified, further study is
35 needed to speciate Cr and address concerns regarding this element; Cr(VI) is known to be highly
36 toxic, while Cr(III) is an essential trace element (Levina et al. 2003). Nonetheless, Cr levels in liver
37 and kidney of the studied bird species do not reach the level of adverse effects for internal tissues
38 (Eisler 1986). Selenium presented the highest levels in kidney (ranged from 10.7 to $12.8 \mu\text{g/g dw}$),
39 followed by liver tissue (ranged from 6.28 to $7.03 \mu\text{g/g dw}$) (Table 1). These levels of Se in liver
40 and kidney observed in the present study were less than the accepted threshold levels for adverse
41 biological effects in birds (Heinz 1996). Thus, this level of Se is probably beneficial, considering its
42 importance in detoxification processes of other toxic elements (Ikemoto et al. 2004).

1 Table 1 Trace element concentrations (mean \pm SD, $\mu\text{g/g}$ 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	^b 0.21 \pm 0.10	0.07 \pm 0.02	^b 0.11 \pm 0.17	0.05 \pm 0.04	^a 0.12 \pm 0.04	0.03 \pm 0.02	32.4 \pm 23.8	^{ab} 0.02 \pm 0.004	6.71 \pm 0.63	118 \pm 43
	Hamerkop	^b 0.26 \pm 0.04	0.03 \pm 0.009	^b 0.14 \pm 0.09	0.01 \pm 0.003	^b 0.06 \pm 0.01	0.02 \pm 0.003	11.4 \pm 3.82	^b 0.01 \pm 0.003	7.03 \pm 0.80	98 \pm 39
	Marabou stork	^{ab} 0.44 \pm 0.23	0.04 \pm 0.01	^b 0.19 \pm 0.04	0.09 \pm 0.08	^{ab} 0.11 \pm 0.03	0.07 \pm 0.06	46.6 \pm 47.7	^a 0.05 \pm 0.04	6.28 \pm 0.88	153 \pm 84
	Great white pelican	^a 0.75 \pm 0.30	0.05 \pm 0.01	^a 1.04 \pm 0.08	0.01 \pm 0.005	^{ab} 0.09 \pm 0.02	0.02 \pm 0.004	31.2 \pm 14.2	^{ab} 0.02 \pm 0.007	6.44 \pm 1.61	144 \pm 33
Kidney	African sacred ibis	^b 0.10 \pm 0.08	0.09 \pm 0.03	^b 0.99 \pm 1.55	^{ab} 0.05 \pm 0.01	1.59 \pm 1.89	0.04 \pm 0.01	13.3 \pm 1.28	0.07 \pm 0.007	10.7 \pm 2.79	^b 94 \pm 8
	Hamerkop	^b 0.19 \pm 0.07	0.07 \pm 0.02	^b 0.98 \pm 0.78	^b 0.03 \pm 0.01	0.39 \pm 0.15	0.04 \pm 0.02	13.1 \pm 1.90	0.06 \pm 0.02	12.8 \pm 1.46	^b 84 \pm 4
	Marabou stork	^{ab} 0.28 \pm 0.14	0.06 \pm 0.02	^b 1.57 \pm 1.07	^a 0.07 \pm 0.03	0.39 \pm 0.11	0.22 \pm 0.21	16.9 \pm 3.31	0.07 \pm 0.04	11.4 \pm 1.14	^a 113 \pm 13
	Great white pelican	^a 0.47 \pm 0.22	0.08 \pm 0.02	^a 5.53 \pm 2.94	^b 0.03 \pm 0.01	0.64 \pm 0.42	0.13 \pm 0.14	13.8 \pm 2.43	0.10 \pm 0.04	11.9 \pm 1.96	^{ab} 97 \pm 15
Muscle	African sacred ibis	^b 0.06 \pm 0.04	0.03 \pm 0.02	^b 0.01 \pm 0.02	^a 0.02 \pm 0.01	0.06 \pm 0.04	0.54 \pm 1.22	^b 6.63 \pm 2.99	0.23 \pm 0.52	2.52 \pm 0.46	^{ab} 45 \pm 12
	Hamerkop	^b 0.12 \pm 0.01	0.01 \pm 0.005	^b 0.005 \pm 0.003	^{ab} 0.01 \pm 0.002	0.03 \pm 0.006	0.14 \pm 0.09	^b 10.1 \pm 1.53	0.03 \pm 0.01	2.70 \pm 0.39	^b 42 \pm 9
	Marabou stork	^b 0.11 \pm 0.05	0.02 \pm 0.006	^b 0.005 \pm 0.003	^{ab} 0.01 \pm 0.01	0.03 \pm 0.01	0.10 \pm 0.03	^b 10.3 \pm 3.24	0.08 \pm 0.05	2.45 \pm 0.25	^b 44 \pm 11
	Great white pelican	^a 0.41 \pm 0.12	0.03 \pm 0.02	^a 0.04 \pm 0.01	^b 0.01 \pm 0.002	0.03 \pm 0.006	0.16 \pm 0.08	^a 19.9 \pm 2.81	0.10 \pm 0.05	2.77 \pm 0.11	^a 63 \pm 10
Heart	African sacred ibis	^b 0.07 \pm 0.05	0.05 \pm 0.03	^b 0.003 \pm 0.003	^{ab} 0.01 \pm 0.008	^{ab} 0.16 \pm 0.03	^b 0.04 \pm 0.01	^a 21.2 \pm 2.37	^b 0.02 \pm 0.01	4.13 \pm 1.04	116 \pm 15
	Hamerkop	^b 0.10 \pm 0.02	0.02 \pm 0.006	^b 0.003 \pm 0.002	^b 0.01 \pm 0.003	^b 0.09 \pm 0.01	^b 0.08 \pm 0.04	^{ab} 20.7 \pm 2.07	^b 0.02 \pm 0.01	4.22 \pm 0.44	99 \pm 9
	Marabou stork	^b 0.09 \pm 0.04	0.01 \pm 0.004	^b 0.003 \pm 0.002	^{ab} 0.01 \pm 0.01	^a 0.21 \pm 0.07	^b 0.86 \pm 0.72	^{ab} 17.7 \pm 1.24	^b 0.02 \pm 0.01	3.67 \pm 0.26	133 \pm 12
	Great white pelican	^a 0.27 \pm 0.06	0.06 \pm 0.03	^a 0.01 \pm 0.008	^a 0.02 \pm 0.007	^{ab} 0.13 \pm 0.06	^a 50.2 \pm 54.9	^b 16.0 \pm 5.19	^a 0.36 \pm 0.33	3.49 \pm 0.53	102 \pm 39
Brain	African sacred ibis	^b 0.02 \pm 0.01	0.03 \pm 0.02	^b 0.005 \pm 0.007	0.03 \pm 0.01	^a 0.06 \pm 0.01	0.05 \pm 0.01	12.2 \pm 1.70	0.02 \pm 0.01	^b 2.90 \pm 0.23	61 \pm 13
	Hamerkop	^b 0.04 \pm 0.01	0.01 \pm 0.005	^b 0.001 \pm 0.001	0.01 \pm 0.002	^{ab} 0.05 \pm 0.02	0.35 \pm 0.65	9.65 \pm 0.79	0.02 \pm 0.01	^a 3.35 \pm 0.31	53 \pm 4
	Marabou stork	^b 0.04 \pm 0.01	0.01 \pm 0.002	^b 0.001 \pm 0.001	0.03 \pm 0.04	^{ab} 0.05 \pm 0.007	0.10 \pm 0.07	14.0 \pm 2.70	0.02 \pm 0.005	^{ab} 3.01 \pm 0.08	62 \pm 5
	Great white pelican	^a 0.12 \pm 0.02	0.01 \pm 0.006	^a 0.01 \pm 0.001	0.01 \pm 0.005	^b 0.03 \pm 0.008	0.09 \pm 0.02	14.4 \pm 11.8	0.01 \pm 0.004	^b 2.63 \pm 0.20	47 \pm 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	
^c Instrumental detection limit	0.002 ng/g	0.002 $\mu\text{g/L}$	0.001 $\mu\text{g/L}$	0.001 $\mu\text{g/L}$	0.001 $\mu\text{g/L}$	0.001 $\mu\text{g/L}$	0.003 $\mu\text{g/L}$	0.07 $\mu\text{g/L}$	0.01 $\mu\text{g/L}$	0.02 $\mu\text{g/L}$	0.02 $\mu\text{g/L}$

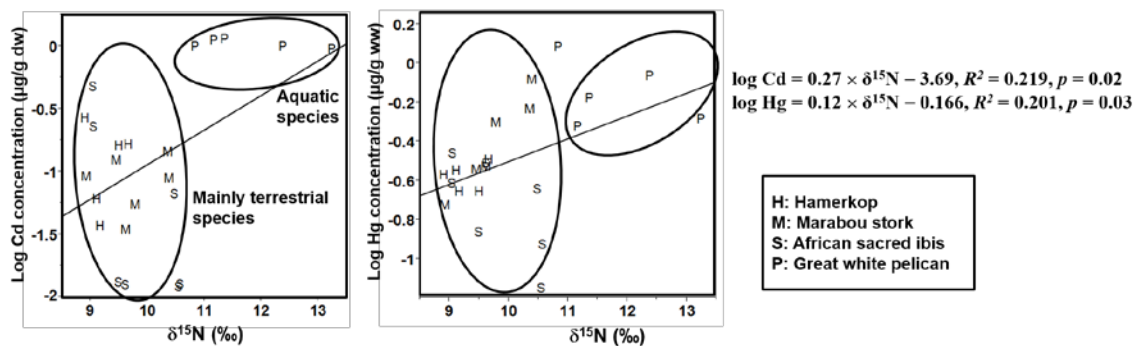
- 2
- 3 § Concentration expressed as $\mu\text{g/g}$ wet weight
- 4 In each tissue, means not sharing the same letters (a, b) among the bird species are significantly different (ANOVA and Tukey HSD test; $p < 0.05$)
- 5 ^c Instrumental detection limit – calculated as 3 times the standard deviation of 10 blank sample measurements divided by the slope of the calibration curve
- 6 *Toxicity thresholds – based on the clinical signs implicated in waterfowl, corresponding to liver concentrations ($\mu\text{g/g}$ ww)
- 7 ^dFurness (1996), ^ePain et al. (1995), ^fEisler (1987), ^gHeinz (1996), ^hEisler (1994), ⁱEisler (1986)

1 Exposure to trace elements is a hypothesis proposed to explain the decline in birds. At high
 2 concentrations, Cd can cause kidney damage, suppression of egg production, and testicular damage
 3 (Furness 1996; Lucia et al. 2009). Mercury disturbs the nervous system and may have a negative
 4 impact on growth, development, and reproduction (Scheuhammer 1987; Scheuhammer et al. 2007;
 5 Varian-Ramos et al. 2014). Lead can affect the brain and nervous system, and cause adverse
 6 effects on reproduction, such as decreased plasma calcium and egg production; and also cause
 7 behavioral impairments (Burger and Gochfeld 2000; Clark and Scheuhammer 2003). Arsenic, in
 8 its inorganic forms, may act as an endocrine disruptor, bring about the death of an individual,
 9 produce sublethal effects (such as decreased body weight and feed intake), or disrupt reproduction
 10 (Eisler 1994; Kunito et al. 2008).

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

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

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



1 Fig. 1 Relationships between stable nitrogen signatures and log-transformed liver concentrations of
2 Cd and Hg in 4 bird species from Ethiopia.

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

13
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