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Concentrations of Heavy Metals in tissues of the Mallard *Anas platyrhynchos* in Kanibarazan, northwestern Iran

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Abstract

Concentrations of cadmium, lead, copper and zinc were measured in the liver, kidneys, pectoral muscle and feathers of the Mallard *Anas platyrhynchos* (*N* = 16) in Kanibarazan Wetland, northwestern Iran. The concentration of metals was estimated using Varian 220 graphite furnace atomic absorption spectrometer. The highest mean concentrations of Cd, Pb, Cu and Zn were observed in the liver at 1.05, 2.37, 10.69 and 59.63 µg g-1 dw, respectively. The lowest mean concentration of Cd, Pb and Zn were found in muscle tissue at 0.46, 0.91 and 20.6 µg g-1 dw, respectively, while the lowest mean concentration of Cu was measured in feathers at 6.44 µg g-1 dw. The concentration of metals follows the sequence: Zn>Cu>Pb>Cd. In this study, heavy metal concentrations in the studied tissues of the Mallard were lower than the background levels.

1. Introduction

Contamination by heavy metals has become one of the serious concerns in different countries. The discharge of heavy metals from natural processes (erosion, sedimentation and decomposition) and anthropogenic activities (industrial, agricultural and mining activities) cause the accumulation of metals into the biota (Ferreira 2010; Licata *et al*. 2010; Alipour *et al*. 2013). Heavy metals such as Cu and Zn are essential nutrients for the animal health, but they can be harmful, when their maximum level exceeds their physiological needs. Cd and Pb are non-essential that are potentially toxic, even at trace amounts (Ebrahimpour *et al.* 2010; Jiang *et al.* 2016). Metals after entering the water may precipitate or be absorbed on the surface of solids, remain soluble or suspended in it or may be taken up by fauna and flora (Palaniappan & Karthikeyan 2009).

Wetlands are among important ecosystems on the earth. Migratory birds use wetlands as a habitat both for food and shelter (Alipour *et al*. 2013). However, anthropogenic activities may cause wetland degradation. The adverse effects of anthropogenic pollutants may pose some risks to waterbird species (Liang *et al*. 2016).

Birds are useful as bioindicators of pollution because they are observable, sensitive to pollutions, and are in different trophic levels. Consequently, studies assessing bird population status and toxicological importance of metal exposures can be extrapolated to other wildlife and probably humans (Ferreira 2011). Toxic concentration of metals can cause teratogenic, mutagenic and carcinogenic effects on biological organisms including birds (Hashmi *et al*. 2013). Metal contamination can also influence habitat, food chains, community structure, morphological or neurological changes, molting, migration and alteration in biochemical processes of birds (Kim & Oh 2013). Metal concentrations can be assessed in

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birds by using the kidneys, liver, muscle tissues, feathers, bones, eggs and excrement (Markowski *et al*. 2013).

The Mallard is the most common and widely distributed dabbling duck, having a widespread global distribution throughout the northern hemisphere with its population estimated about 2.4 million birds in Asia and over 30 millions around the world (Wetlands International 2012). Most often, they prefer wetlands, where highly productive water produces large amounts of floating, emergent and submerged vegetation. Mallards are omnivore, and their diet consists not only of terrestrial and aquatic invertebrates (such as insects, molluscs, crustaceans and worms), but also of tadpoles, fish egg, small fish, or all sorts of plant materials (Baldassarre 2014). The objective of this study was to examine the concentration of Cd, Pb, Cu and Zn in the liver, kidneys, muscle tissue and feathers of the Mallard.

2. Materials and methods

2.1. Study area

Kanibarazan wetland is located in the north-west region of Iran and consists of a freshwater lake surrounded by diverse plant communities. This site is located to the south of Lake Urmia (927 ha; 36°59'N 45°46'E), and is surrounded by seasonal wetlands which become dry during summer and autumn seasons (Fig. 1). Kanibarazan wetland was designated as a Ramsar Site in February 2011. Kanibarazan wetland is one of the most important habitats for waterbirds in the region, supporting more than 20,000 birds with more than 144 bird species recorded at this site, including a number of important species such as the White-headed Duck *Oxyura leucocephala* and Mallard *Anas platyrhynchos* (Ramsar Site 2015).

Fig. 1. Map showing Kanibarazan wetland, Iran

2.2. Analytical method

During the period of November and December 2014, 16 Mallards were collected from Kanibarazan wetland. All the birds were shot, then weighed and stored in plastic bag, and kept at −20 °C until samples were prepared for analysis (AOAC 1990). The average weight (±SD) of the sampled birds was 1155.25± 165.43 g and the average body length was 56.75± 2.81 cm. Liver, kidneys, pectoral muscle and breast feathers were carefully washed with deionized water. Samples were dried in a porcelain crucible at 135 ºC for 2 hours to reach their dry weight. The tissue samples were accurately weighed (0.1 g) into separate 25 ml Erlenmeyer flasks. We added 10 ml of nitric acid 65% to each sample, then samples were left overnight to be digested slowly. Thereafter, 5 ml perchloric acid 72% was added to each sample. Digestion was performed in a sand bath on a hot plate at 150 ºC for 6 h or until solutions were clear and near to dryness (Ebrahimpour *et al*. 2011, Alipour *et al*. 2013). After cooling, mixtures were diluted to 25 ml in polyethylene bottles with deionized water. Then, the solutions were filtered using 0.45 µm nitrocellulose membrane filters. The concentration of metals was estimated using Varian 220 graphite furnace atomic absorption spectrometer. The limits of detection were as follows: Zn (0.75), Pb (0.2), Cd (0.01) and Cu (0.3) µg g^{-1}. Results for Zn, Pb, Cd and Cu gave a mean recovery of 106, 97.9, 99.7 and 99%, respectively. The concentration of metals in tissues is reported in µg g^{-1} dry weight.

Data analysis

Data analyses were performed using the statistical package SPSS (version 19). The Kolmogorov–Smirnov test was accomplished to analyze the normality of data distribution. The concentration of metals was compared between the liver, kidneys, muscle tissue and feathers using one-way ANOVA, followed by Tukey's test. The significance level was 0.05. The Pearson correlation coefficient was applied to assess relationships between metal concentrations with the body mass and total length of examined birds.

3. Results and Discussion

The highest concentrations of Cd, Pb, Cu and Zn were observed in the liver (Table 1). The lowest concentrations of Cd, Pb and Zn were found in muscle tissue while the lowest mean concentration of Cu was measured in feathers (Table 1). The metal concentrations were in descending order of Zn>Cu>Pb>Cd. The distribution patterns of Cd, Pb and Zn in tissues follow the sequence: liver> kidney> feather> muscle, while for Cu follows the sequence: liver> kidney> muscle> feather. Concentrations of Cd, Pb, Cu and Zn differed significantly among tissues (one-way ANOVA, $P<0.05$, Table 1). The Pearson correlation coefficient of the total length and body mass with metal concentrations in the Mallard are presented in Table 2. Significant positive correlations ($P<0.01$, $r=0.709$) were observed between the total length and body mass of the Mallard. Table 2 shows that there was a negative correlation between Cd with the total length ($P<0.01$, $r=-0.693$) and body mass ($P<0.05$, $r=-0.555$) and between Zn and Cu ($P<0.05$, $r=-0.500$) in muscle tissue. There was a negative correlation between Cu and Pb ($P<0.01$, $r=-0.709$) in the kidneys.

Table 1. Mean metal concentrations (μg g^{-1}dw) in tissues of the Mallard in Kanibarazan wetland (*N*=16).

Tissue		Cadmium	Lead	Copper	Zinc
Liver	Mean(SD)	1.05(0.49)[a]	2.37(1.84)[a]	10.69(3.58)[a]	59.63(13.2)[a]
	Min-Max	0.41-2.1	0.07-5.78	4.3-16.1	45.3-86.5
Kidneys	Mean(SD)	0.67(0.45)[ab]	1.93(1.42)[ab]	8.63(2.51)[ab]	30.28(8.42)[bd]
	Min-Max	00-1.73	0.01-4.48	3.88-13.37	15.21-41.7
Muscle tissue	Mean(SD)	0.46(0.33)[b]	0.91(0.56)[b]	8.27(5.96)[ab]	20.6(6.94)[c]
	Min-Max	0.02-0.89	00-1.9	2.67-22.8	8.87-31.21
Feathers	Mean(SD)	0.64(0.37)[bc]	1.62(0.74)[ab]	6.44(1.87)[b]	25.04(7.65)[bd]
	Min-Max	0.01-1.03	0.36-2.66	3.56-9.45	10.9-36.21

Notes: [a,b,c]Means with the same letters in each column for each metal are significantly different according to Tukey's test. Significant differences at $P<0.05$.

Table 2. Statistical significant relations (Pearson correlations) between metal concentrations, body mass and the total length in the tissues of the Mallard (*N*=16).

Tissue	Metal	Cd	Pb	Cu	Zn	Body mass	total length
Liver	Cd		0.105	0.182	-0.279	0.272	-0.026
	Pb			-0.275	0.287	0.306	0.464
	Cu				-0.099	0.094	-0.239
	Zn					-0.218	0.030
Kidneys	Cd		0.122	0.294	-.038	0.317	0.205
	Pb			-0.709**	0.035	0.059	0.391
	Cu				-0.224	-0.008	-0.192
	Zn					0.317	0.223
Muscle tissue	Cd		0.276	0.131	-0.162	-0.555*	-0.693**
	Pb			-0.110	-0.375	-0.281	-0.212
	Cu				-0.500**	-0.067	-0.151
	Zn					0.356	0.233
Feathers	Cd		-0.213	-0.456	0.033	0.199	-0.163
	Pb			-0.019	0.202	0.235	0.418
	Cu				-0.188	-0.202	-0.083
	Zn					0.342	0.198
	Body mass						0.709**

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

Cadmium is a strongly toxic and non-essential element. If it absorbed into the organism through the digestive and pulmonary systems, it can forms complexes with proteins, in which it is easily transported and stored, mainly in the liver and kidneys (Szymczyk & Zalewski 2003). The liver and kidneys are most frequently used for monitoring of Cd levels in birds. The liver was considered to be a better indicator of chronic Cd exposure than the kidneys (Beyer & Meador 2011). In our study, the mean concentrations of Cd in the liver, kidneys, muscle tissue and feathers were generally lower compared to data in the literature and there were significant differences for Cd between the liver and muscle tissue and feathers. For instance, the study conducted by Binkowski *et al.* (2013) (3.44 µg g^{-1}), Kalisińska *et al.* (2004) (1.41 µg g^{-1}) and Lucia *et al.* (2010) (14.79 µg g^{-1}) reported higher concentrations of Cd in the liver of the Mallard as compared to the present study. Cd concentration in the kidneys of the Mallard in this study was lower than in other areas (Zator, Binkowski *et al.* 2013 (8.42 µg g^{-1}), Szczecin, Kalisińska *et al.* 2004 (12.94 µg g^{-1}) from Poland and Southwest Atlantic from France, Lucia *et al.* 2010, 39.66 µg g^{-1}). Scheuhammer (1987) argued that the liver accumulates about half of the body burden of Cd −the Cd content of the liver is extremely stable, and the liver is generally resistant to the toxic effects of Cd unlike the kidneys, in which Cd concentrations decline after the production of renal tubular dysfunction. Mallards tolerate 200 ppm of Cd in diets for protracted periods, the kidneys cadmium exceeded 130 ppm fresh weight under this regime, a concentration considered life-threatening to some organisms. Sublethal effects of Cd in birds included growth retardation, anemia, and testicular damage (Eisler 2000). According to Scheuhammer (1987), a liver to the kidneys concentration ratio >1 indicates acute exposure to relatively high doses of Cd. In this study, this ratio in the Mallard was >1. Scheuhammer (1987) suggested that the background levels of Cd at <3 and <8 mg/kg dw in the liver and kidneys might indicate increased environmental exposure. In this study, the mean concentrations of Cd in the liver and kidneys were lower than the background levels. Lucia *et al.* (2010) found a mean Cd concentration of feathers and muscle tissue of the Mallard in Southwest Atlantic of 0.03 and 0.12 µg g^{-1}, respectively, that is lower than the mean concentration observed for the Mallard in the present study. Mansouri & Majnoni (2014) reported mean Cd concentrations of 0.71 and 0.52, in feathers and muscle tissue, respectively, in the Mallard from Zarivar wetland. These values were higher than those of observed in the present study. Feathers are often used as a non-injurious and non-invasive means of assessing relative cadmium exposure in birds. Feathers reflect the amount of metals present in the blood at the time of the feather growth, either from current dietary sources or from mobilisation of metals from internal organs. Concentrations in feathers have sometimes been correlated with those in the kidneys or liver (Beyer & Meador 2011; Costa *et al.* 2012) although such correlations were not found in these studies. According to Burger & Gochfeld (2000), Cd concentration of 2 µg g^{-1}dw in bird feathers might be associated with the adverse effects. Feathers and muscle tissue Cd contents in this study never exceeded 1 µg g^{-1}dw.

Pb is a non-essential, highly toxic heavy metal and acts as a non-specific poison affecting all the body systems and has no known biological requirement. Pb can cause toxic effects on a long series of organs and tissues (Beyer & Meador 2011). Waterfowls retain them during feeding as grit in the gizzard; the pellets are eroded and soluble Pb is absorbed from the digestive tract. Species such as the Mallard that feeds in shallow water through bottom mud and are more likely to encounter shot than other species that feed on submerged vegetation or on the surface (Eisler 2000). The Mallard's bills are between 3.5 and 4.5 cm long and can probe up to 5 cm into the sediment or soil. The Mallard is an omnivore and feeds mostly gastropods, invertebrates, crustaceans, worms, many varieties of seeds and plant matters, roots and tubers (Dawson *et al.* 2011). Alipour *et al.* (unpublished data) studied heavy metal accumulation in the sediments of Kanibarazan Wetland and reported the mean levels of Cd, Cu and Pb for sediment samples as 5.24, 27 and 8.5 µg g^{-1}, respectively. These values were generally higher than those observed in the present study. The mean concentration of Pb in the liver, kidneys, feathers and muscle tissue were 2.37±1.84, 1.93±1.42, 1.62±0.74 and 0.91±0.56 µg g^{-1} dw, respectively. There was significant differences for Pb between the liver and muscle tissue (P<0.01). The mean concentrations of Pb in the liver and kidneys of the Mallard in this study were higher than in the liver and kidneys of the Mallard in Izumi coast from Japan (0.72 and 0.72 µg g^{-1}, Nam *et al*. 2005) and Zarivar wetland (2.20 and 1.83 µg g^{-1}, Mansouri & Majnoni 2014), respectively. Lucia *et al* (2010) reported the mean liver and kidneys Pb concentrations of the Mallard in southwest Atlantic of 3.47 and 17.98 µg g^{-1}, respectively. These values were higher than those observed in the present study. Normal background levels of Pb in tissues of adult bird species living in relatively uncontaminated areas are 1−10 and 0.5−5.0 µg g^{-1} in the kidneys and liver, respectively. Species living in more contaminated environments can accumulate 50 times more than these values and demonstrate evidence of renal and hematological toxicity (Scheuhammer 1987). In the present study, Pb concentrations in the kidneys and liver of the Mallard did not exceed the background level, that could be indicative of a safe environmental exposure without toxicological risk and could be considered as the background level. Pb concentrations in feathers and muscle tissue of the Mallard in this study were higher than in feathers (0.26 µg g^{-1} dw) and muscle tissue (0.08 µg g^{-1} dw) of the Mallard in southern Poland (Zator fish ponds) (Binkowski & Sawicka-Kapusta 2015). Binkowski *et al* (2013) and Mansouri & Majnoni (2014) reported the mean Pb concentrations in muscle tissue of the Mallard at 0.95 and 1.08 µg g^{-1}, respectively. These values were generally higher than those observed in the present study. Muscle tissue Pb contents in this study never exceeded 1 µg g^{-1}dw. Burger & Gochfeld (2000) suggested that Pb concentration of 4 µg g^{-1} dw in bird feathers might be associated with the adverse effects, although seabirds can often tolerate higher levels. The level of Pb in feathers of the Mallard was well below this threshold level. Burger & Gochfeld (2000) noted that Pb levels of 4 µg g^{-1} in feathers are associated with delayed parental and sibling recognition, impaired thermoregulation, locomotion, depth perception, feeding behaviour, and lowered chick survival in birds.

Cu is a micronutrient and toxin. Its toxic effects on birds include reduced growth rates, lowered egg production and developmental abnormalities (EPA 2014). Cu is an essential element for the normal growth, metabolism for living cells and structure and the function of many proteins vital for cells (Malik & Zeb 2009). In our study, the average concentrations of Cu in the liver, kidneys, muscle tissue and feathers were 0.69±3.58, 8.63±2.51, 8.27±5.96 and 6.44±1.87 µg g-1 dw, respectively. There was significant differences for Cu between the liver and feathers (P<0.05). Several studies stated that mean Cu concentration in the liver is higher than the kidneys (Binkowski *et al*. 2013; Szymczyk & Zalewski 2003; Bojar & Bojar 2009; Kalisińska *et al*. 2004; Mansouri & Majnoni 2014; Nam *et al*. 2005) in accordance with our results. Eisler (2000) reported excessive dietary intakes of Cu by 20 to 50 folds over normal levels, however, causing serious adverse effects on birds and mammals. Mansouri & Majnoni (2014) measured concentrations of Cu in the liver (13.82 µg g^{-1}), kidneys (8.90 µg g^{-1}), muscle tissue (7.19 µg g^{-1}) and feathers (6.28 µg g^{-1}), respectively. A study on the Mallard in Poland (Kalisińska *et al.* 2004) showed that Cu in muscle tissue, kidneys and liver was 3 to 10 times, respectively, higher than those revealed in our study. Okati (2013) reported that the mean concentration of Cu in feathers of the Mallard (32.85 µg g^{-1}) from Shadegan wetland, southwest Iran was five times higher as compared to the present study.

The levels of essential metals such as Zn in tissues of birds are regulated metabolically. Zn plays an important role in many metabolic processes, especially in the activation of enzymes and the regulation of gene expression (Ferreira 2010). However, an excess of Zn leads to poisoning, described also in waterfowls, mainly from the examination of the liver or pancreas of affected birds (Kalisińska *et al*. 2007). The mean concentrations of Zn in the liver, kidneys, feathers and muscle tissue were 59.63±13.2, 30.28±8.42, 25.04±7.65 and 20.6±6.94 µg g^{-1} dw, respectively. There were significant differences for Zn between the liver, kidneys, muscle tissue and feathers (P<0.001) and kidneys with muscle tissue ($P<0.05$). Levengood *et al*. (1999) reported clinical signs of Zn poisoning in Mallards with the liver concentrations of 473–1990 µg g^{-1} dw. Also, Sileo *et al*. (2003) diagnosed Zn poisoning in wild waterfowls with the liver concentrations of 280–2900 µg g^{-1} dw. Some studies indicated that Zn levels in the kidneys of the Mallard are normally less than 200 µg g^{-1} dw (Binkowski *et al*. 2013; Szymczyk & Zalewski 2003; Bojar & Bojar 2009; Mansouri & Majnoni 2014; Kalisińska *et al*. 2004; Nam *et al*. 2005). Gasaway & Buss (1972) reported that the threshold level of Zn toxicosis in birds is 1200 µg g^{-1}. In the present study, the Zn concentrations in the Mallard were lower than the Zn toxicosis level. Because Zn play an important role in many metabolic processes, it is not surprising to find a high concentration of Zn in tissues of waterfowls (Simonetti *et al*. 2013). Zn levels in our samples were similar to those detected by Mansouri & Majnoni (2014) in muscle tissue of the Mallard. However, Zn concentrations in the liver and kidneys of the Mallard in the present study were lower than in other studies (Nam *et al*. 2005; Binkowski *et al*. 2013; Kalisińska *et al*. 2004; Mansouri & Majnoni 2014; Bojar & Bojar 2009; Szymczyk & Zalewski 2003). Study conducted by Okati (2013) (56.35 µg g^{-1}) reported higher concentrations of Zn in feathers of the Mallard as compared to the present study.

The difference in the accumulation levels in different organs/tissues of a bird can be attributed to the difference in the physiological role of each organ. In this study, the highest concentrations of Cd, Pb and Zn were observed in the liver of the Mallard, followed by the kidneys, feathers and muscle tissue while Cu concentrations was the highest in the liver, followed by the kidneys, muscle tissue and feathers. The concentration of heavy metals in the liver, kidneys and muscle tissue can be considered indicative of metals chronic exposure, according to the diet and pollution in a habitat, because the liver and kidneys are organ for detoxification in the body but muscle tissue for deposition and accumulation (Naccari *et al*. 2009). Target organs such as the liver and kidneys are metabolically active tissues and accumulate heavy metals in higher levels. Muscle tissue, in the present study, contained the lowest levels of heavy metals. These results agree with many authors who reported that muscle tissue is not an active tissue in accumulating heavy metals (Szymczyk & Zalewski, 2003; Kim & Oh 2013; Mansouri & Majnoni, 2014; Nam *et al*. 2005). Feathers are very interesting indicator of heavy metals in birds because feathers can store and eliminate metals, especially into the growing feathers during moulting process. It is also proportional to the body mass of animals that may increase with the age due to exogenous contamination of atmospheric depositions and feathers reflect the local contamination of a particular habitat (Naccari *et al*. 2009). Levels of heavy metals examined in the liver, kidneys and muscle tissue can be considered indicative of chronic exposure, whereas the presence of metals in feathers could be due to stored metals or deposition process of metals (Licata *et al*. 2010).

4. Conclusion

The findings of this study have shown that the Mallard have different capabilities to accumulate metals in its tissues. Generally, essential elements (Zn and Cu) occur in high levels compared to the non-essential elements (Cd and Pb) in the tissues of the Mallard. In this study, heavy metal concentrations in the tissues of the Mallard were lower than the background levels. More studies are needed for an accurate assessment of the hazards associated with the contaminant concentrations observed in tissues of the Mallard.

References

Alipour H., Pourkhabbaz A. & Hassanpour M. (2013). Assessing of heavy metal concentrations in the tissues of *Rutilus rutilus caspicus* and *Neogobius gorlap* from Miankaleh international wetland. *Bulletin of Environmental Contamination and Toxicology*, 91(5): 517–521.

AOAC. (1990). Official methods of analysis of the Association of Official Analytical Chemists. 15th edition. Washington, DC.

Baldassarre G. (2014). Ducks, Geese, and Swans of North America, JHU Press, 2-vol, revised, pp 1088.

Beyer W.N. & Meador J.P. (2011). Environmental contaminants in biota: interpreting tissue concentrations, 3rd edn. CRC Press, Taylor and Francis Group, pp 751.

Binkowski Ł.J., Stawarz R.M. & Zakrzewski M. (2013). Concentrations of cadmium, copper and zinc in tissues of mallard and coot from southern Poland. *Journal of Environmental Science and Health, Part B*, 48(5): 410–415.

Binkowski Ł.J., & Sawicka-Kapusta K. (2015). Lead poisoning and it's in vivo biomarkers in Mallard and Coot from two hunting activity areas in Poland. *Chemosphere*, 127: 101–108.

Bojar H, & Bojar I. (2009). Monitoring of contamination of the Lublin region wetlands using mallards (*Anas platyrhynchos*) as a vector of the contamination by various conditionally toxic elements. *Annals of Animal Science*, 9(2): 195–204.

Burger J. & Gochfeld M. (2000). Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. *Science of the Total Environment*, 257(1): 37–52.

Costa R.A., Eeva T., Eira C., Vaqueiro J. & Vingada J.V. (2012). Assessing heavy metal pollution using Great Tits (*Parus major*): feathers and excrements from nestlings and adults. *Environmental Monitoring and Assessment*, 185(6): 5339–5344.

Dawson M.M, Metzger K.A, Baier D.B. & Brainerd E.L. (2011). Kinematics of the quadrate bone during feeding in mallard ducks. *The Journal of Experimental Biology*, 214: 2036–2046.

Ebrahimpour M., Alipour H. & Rakhshah S. (2010). Influence of water hardness on acute toxicity of copper and zinc on fish. *Toxicology and Industrial Health*, 26(6): 361–365.

Ebrahimpour M., Pourkhabbaz A.R., Baramaki R., Babaei H. & Rezaei M. (2011). Bioaccumulation of heavy metals in freshwater fish species, Anzali, Iran. *Bulletin of Environmental Contamination and Toxicology*, 87(4): 386–392.

Eisler R. (2000). *Handbook of Chemical Risk Assessment: Health hazards to humans, plants, and animals.* Lewis Publishers, Boca Raton, FL, pp 1821.

EPA. (2014). *Ecological toxicity information*, http://www.epa.gov/region5/superfund/ecology/toxprofiles.htm.

Ferreira A.P. (2010). Estimation of heavy metals in little blue heron (*Egretta caerulea*) collected from sepetiba bay, rio de janeiro, brazil. *Brazilian Journal of Oceanography*, 58(4): 269–274.

Ferreira A.P. (2011). Assessment of heavy metals in *Egretta thula*: case study: Coroa Grande mangrove, Sepetiba Bay, Rio de Janeiro, Brazil. *Brazilian Journal of Biology*, 71(1): 77–82.

Gasaway W.C. & Buss I.O. (1972). Zinc toxicity in the mallard duck. *Journal of Wildlife Management*, 36:1107–1117.

Hashmi M.Z., Malik R.N. & Shahbaz M. (2013). Heavy metals in eggshells of cattle egret (*Bubulcus ibis*) and little egret (*Egretta garzetta*) from the Punjab province, Pakistan. *Ecotoxicology and Environmental Safety*, 89: 158–165.

Jager L.P., Rijnierse F.V.J., Esselink H. & Baars A.J. (1996). Biomonitoring with the Buzzard *Buteo buteo* in the Netherlands: heavy metals and sources of variation. *Journal of Ornithology*, 137: 295–318.

Jiang H., Qin D., Chen Z.H., Tang S.H., Bai S.H. & Mou Z.H. (2016). Heavy metal levels in fish from Heilongjiang River and potential health risk assessment. *Bulletin of Environmental Contamination and Toxicology*, 29: 97(4): 536–542.

Kalisińska E., Salicki W., Kavetska K.M. & Ligocki M. (2007). Trace metal concentrations are higher in cartilage than in bones of scaup and pochard wintering in Poland. *Science of the Total Environment*, 388(1-3): 90–103.

Kim J. & Oh J.M. (2013). Assessment of trace metals in four bird species from Korea. *Environmental Monitoring and Assessment*, 185(8): 6847–54.

Levengood J.M., Sanderson G.C., Anderson W.L., Foley G.L., Skowron L.M., Brown P.W. & Seets J.W. (1999). Acute toxicity of ingested zinc shot in game-farm mallards. *Illinois Natural History Survey Bulletin*, 36: 1–36.

Liang J., Liu J., Yuan X., Zeng G., Yuan Y., Wu H. & Li F. (2016). A method for heavy metal exposure risk assessment to migratory herbivorous birds and identification of priority pollutants/areas in wetlands. *Environmental Science and Pollution Research*, 23(12): 11806–13.

Licata P., Naccari F., Lo Turco V., Rando R., Di Bella G., & Dugo G. (2010). Levels of Cd (II), Mn (II), Pb (II), Cu (II), and Zn (II) in Common Buzzard (*Buteo buteo*) from Sicily (Italy) by Derivative Stripping Potentiometry. *International Journal of Ecology*, 2010: 1–7.

Lucia M., André J.M., Gontier K., Diot N., Veiga J. & Davail S. (2010). Trace element concentrations (mercury, cadmium, copper, zinc, lead, aluminum, nickel, arsenic, and selenium) in some aquatic birds of the southwest Atlantic coast of France. *Archives of Environmental Contamination and Toxicology*, 58(3): 844–853.

Malik R.N. & Zeb N. (2009). Assessment of environmental contamination using feathers of *Bubulcus ibis* L. as a biomonitor of heavy metal pollution, Pakistan. *Ecotoxicology*, 18(5): 522–36.

Mansouri B. & Majnoni F. (2014). Comparison of the metal concentrations in organs of two bird species from western of Iran. *Bulletin of*

Environmental Contamination and Toxicology, 92(4): 433–9.

Markowski M., Kaliński A., Skwarska J., Wawrzyniak J., Bańbura M., Markowski J., Zieliński P. & Bańbura J. (2013). Avian feathers as bioindicators of the exposure to heavy metal contamination of food. *Bulletin of Environmental Contamination and Toxicology*, 91(3): 302–305.

Naccari C., Cristani M., Cimino F., Arcoraci T. & Trombetta D. (2009). Common buzzards (*Buteo buteo*) bio-indicators of heavy metals pollution in Sicily (Italy). *Environment International*, 35(3): 594–598.

Nam DH, Anan Y, Ikemoto T, & Tanabe S (2005) Multielemental accumulation and its intracellular distribution in tissues of some aquatic birds. *Marine Pollution Bulletin*, 50(11): 1347–62.

Okati N. (2013). Biomonitoring of heavy metals in birds in Iran in relation to trophic levels. *International Research Journal of Applied and Basic Sciences*, 4(11): 3478–3485.

Palaniappan P.L.R.M. & Karthikeyan S. (2009). Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish *Cirrhinus mrigala* individually and in binary solutions with nickel. *Journal of Environmental Sciences*, 21(2): 229–236.

Ramsar Site. (2015). Ramsar Sites Information Service, Kanibarazan Wetland — URL https://rsis.ramsar.org/ris/1940?language=en.

Scheuhammer A.M. (1987). The chronic toxicity aluminium, cadmium, mercury and lead in birds: a review. *Environmental Pollution*, 46: 263–295.

Sileo L., Beyer W.N. & Mateo R. (2003). Pancreatitis in wild zinc-poisoned waterfowl. *Avian Pathology*, 32: 655–660.

Simonetti P., Bottéa S.E., Beltzerd A.H. & Marcovecchio J.E. (2013). Tissue distribution of Cd, Cu, Zn, Ni, Cr, Pb and Hg in striated heron, *Butorides striatus* (Aves: Ardeidae), in a fluvial ecosystem. *Chemistry and Ecology*, 30(3): 197–205.

Szymczyk K. & Zalewski K. (2003). Copper, zinc, lead and cadmium content in liver and muscles of mallards (*Anas Platyrhychnos*) and other hunting fowl species in Warmia and Mazury in 1999-2000. *Polish Journal of Environmental Studies*, 12(3): 381–386.

Wetlands International. (2012). *Waterbird Population Estimates.* — URL wpe.wetlands.org.

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