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Estimation of Hepatic Levels of Heavy Metals and Metalloids in Aquatic Birds from a Wetland Irrigated with Residual Water in the City of Durango, Mexico

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Abstract: Concentrations of zinc, lead, cadmium and arsenic were determined in hepatic tissue of three groups of aquatic birds (ducks, geese and shorebirds) from a wetland irrigated with water from the wastewater treatment plant of the city of Durango, Mexico. Researchers collected liver portions from 50 birds obtained by hunters during the 2010-2011 hunting season. Metals and metalloid concentrations were determined by atomic absorption spectrophotometry. The four chemicals were detected in 100% of the samples. The results of a Kruskal-Wallis test were not significant ($p>0.05$) among mean concentrations of Zn, Cd and As for the three groups of birds but the average concentration of Pb in geese was higher ($p<0.05$) than in the other two bird groups. An individual of the species shoveler (*Anas clypeata*) has a maximum Cd concentration of 31.98 ppm (dry weight) and the highest value of Zn was 256 ppm (dw) that belonged to an individual of snow goose (*Chen caerulescens*). Values of metals and metalloids obtained in this study do not represent a commitment on health of birds.

Key words: Metal, metalloid, aquatic birds, biomonitoring, Durango, Mexico

INTRODUCTION

Wetlands are among the most productive ecosystems of the world in terms of the biological diversity they support, wetlands play an important role as filters of nutrients and contaminants, besides having the ability to function as buffer zones against flooding and groundwater recharge.

Biological monitoring or biomonitoring is used to detect and evaluate the changes caused by human activity on the biota. These biomonitoring techniques have gained significant importance since they are used to determine the health status and toxicological conditions of water bodies and wetlands.

Wastewater Treatment Plants (WWTPs) are capable of removing a large proportion of different pollutants through various treatment processes but not all compounds are eliminated completely with removal efficiencies varying according to the wastewater treatment processes employed at individual facilities, resulting in potential discharge to receiving waters (Ramirez *et al*., 2009).

In Mexico as in other countries of the world, water from WWTPs is used to irrigate parks and gardens, agricultural areas and remaining is discharged into wetlands close to urban areas. This has attracted the attention of various researchers who have conducted studies to assess the health of wildlife living in these ecosystems. These studies have focused on detecting the presence in animal organisms of pollutants such mercury, cadmium, copper, zinc, lead, aluminum, nickel, arsenic, selenium (Lucia *et al*., 2010; Ohlendorf *et al*., 1986), Pharmaceuticals and Personal Care Products (PPCPs) (Ramirez *et al*., 2009).

Taking the aforementioned as reference, the objective of this study was to evaluate the presence of metals and metalloids in aquatic birds of a wetland irrigated with water from the Wastewater Treatment Plant (WWTP) in the city of Durango, Mexico.

MATERIALS AND METHODS

Study site and sampling: This study was conducted in the Management Unit for Wildlife Conservation area (UMA) Los Alamos in the municipality of Durango, (24° 05'382"N and 104°30'043"W). The area is flooded by residual waste water after being treated at the WWTP facility in the city of Durango. The area represents a lake-type wetland, not tidal. The dominant vegetation includes plants of the genera *Acacia* and *Prosopis* and herbaceous layers of grasses and halophytes of the genus *Sporobolus*.

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The UMA occupies an area of 900 ha and is licensed for hunting of migratory aquatic birds. Upon solicitation and cooperation by hunters, portions of liver were collected from 50 birds during the 2010-2011 hunting season (October-March) and were labeled according to procedures of the field manual of wildlife diseases (Friend and Franson, 1999). Sampling of birds depended on the birds collected by hunters (approved permit SEMARNAT document SGPA/DGVS/03850/10, dated May 11, 2010) which consisted of: ducks (subfamily Anatinae), geese (subfamily Anserinae) and shorebirds. Age and sex of the birds were not determined. The collection of hepatic tissue was taken from dead and injured birds. Injured birds were sacrificed by cervical dislocation. The samples were stored in 100 mL sterile glass containers and they were placed immediately on ice until taken to the Veterinary Diagnostics Laboratory where they were stored at -20°C.

Metal determination: The liver tissue samples were prepared for analysis as follows: approximately 9 g were dried at 60°C during 7 days, then charred in a muffle furnace for 1 h at 600°C the dry residues were then subjected to a nitric acid digestion and hydrochloric acid over a hot plate. After digestion, concentrations of metals and metalloids were determined using an atomic absorption spectrophotometer (Varian) of the laboratory of the Regional Livestock Union of the State of Durango.

Statistical analysis: Statistical analysis of data was performed using the JMP 9.0.0 program obtaining the main descriptive statistics for each group of birds. Due to the lack of normality of the data, nonparametric (Kruskall-Wallis) tests were performed to compare the average values of the concentrations of metals and metalloids.

RESULTS AND DISCUSSION

Total of 50 livers of birds were collected which were organized into three groups according to their trophic affinity (Table 1).

The Shapiro-Wilk test was performed to evaluate the normality of the data, from the above it was observed that the values of Pb and As are normally distributed while the opposite occurred with Zn and Cd. These results helped to choose nonparametric test Kruskall-Wallis to compare the values of the concentration of each element, thus obtaining the values shown in Table 2.

Generally the highest concentrations of Zn were observed in the group of ducks while the lowest finding was in Cd in groups of ducks and geese. The four elements studied were present in all samples collected.

Table 1: Bird group's composition

Groups	Scientific name	n
Ducks	*Anas clypeata*	15
	Anas crecca	2
	Anas discors	8
	Anas platyrhynchos	5
	Anas strepera	3
Geese	*Chen caerulescens*	8
	Chen ross	4
Shorebirds	*Hymantopus mexicanus*	1
	Limodromus scolopaecus	3
	Recurvirostra Americana	1

Table 2: Metal and metalloids concentration on liver of different bird species. Values are ppm, dry weight

Metal and metalloids concentration	Ducks	Geese	Shorebirds
Zn			
Mean	93.96^{A}	110.92^{A}	77.00^{A}
SE	5.82	16.34	9.43
Minimum	44.84	46.63	44.83
Maximum	181.56	256.92	94.26
Pb			
Mean	2.74^{A}	2.90^{B}	1.91^{AC}
SE	0.15	0.29	0.44
Minimum	1.51	1.36	0.86
Maximum	4.71	4.11	3.46
Cd			
Mean	2.66^{A}	1.71^{A}	1.95^{A}
SE	0.92	0.17	0.22
Minimum	0.45	0.45	1.15
Maximum	31.90	2.35	2.35
As			
Mean	4.76^{A}	3.80^{A}	3.55^{A}
SE	0.36	0.57	0.96
Minimum	0.46	0.59	0.84
Maximum	9.51	7.24	6.38
n	33.00	12.00	5.00

Values with different capital letter superscripts are significantly different within the row (Kruskall-Wallis test ($p<0.05$))

Zinc: Zinc is part of >100 metalloenzymes is essential in the synthesis of DNA in processes involved in immunity, wound healing (Sullivan *et al*., 1980; Tate *et al*., 1999). Zinc is present in the earth's crust at an average concentration of 70 mg kg^{-1} which is not normally found in free form but forming complexes with sulfides, carbonates and oxides. The main contamination of Zn in the environment comes from industrial waste derived from their use.

As a result of physiological importance, Zn is normally observed at concentrations higher than the other metals and metalloids. In the study there were no significant differences (Kruskal-Wallis test $p>0.05$) in concentrations of metals and metalloids in livers of the different bird groups, mean values in liver are lower than those reported by De Mendoza *et al*. (2006) who reported 45 ppm Zn fresh weight in Mallards (*Anas platyrhynchos*) and 54 ppm (ww) reported by Gassaway and Buss (1972) also these researchers suggest that in aquatic birds, hepatic levels of Zn can reach 200 ppm dw, without being considered pathological. However in the present study the highest value of Zn was 256 ppm (dw) that belonged to an individual of snow goose. This value seems to indicate some exposure to this metal. But similar results were found by Beyer *et al.* (2004) showed that waterfowl were the birds with significantly increased Zn concentrations in livers (440 ppm dw, N = 17, SD = 710). According to their research, tissue concentrations of Zn are imperfect indicators of exposure in birds because birds regulate Zn effectively with a wide range of exposure. In experimental studies, the proportional increase in Zn concentrations in kidneys and pancreases has been shown to be greater that in livers of dosed birds.

According to different studies on Zn poisoning in mallards, liver reported amounts ranging from 770-1100 ppm dw (National Wildlife Health Center Necropsy Report, Case 17088, ACC: 001, 002, 003). In some instances where Zn poisoning is diagnosed, the main findings at necropsy of mallards had mild to severe degenerative abnormalities of the exocrine pancreas with Zn concentrations in livers and pancreases (Sileo *et al.*, 2003). Gassaway and Buss (1972) suggest that decreased motor function in bird legs is a clinical sign of Zn poisoning.

Lead: The lead contamination of wetlands does come from several sources as mining, milling and smelting (Beyer *et al.*, 2004). Also consequential levels of contamination could be the result of the ingestion of Pb-shot from ammunition used in hunting areas (Lucia *et al.*, 2010; Rattner *et al.*, 1989; Zwank *et al.*, 1985).

There is evidence that all metallic Pb of the sinkers for fishing and hunting shot rounds are transformed in particulate and molecular portions of Pb that are dispersed in the environment. When Pb in form of shot rounds or sinkers is exposed to air or water, produces Pb oxide, Pb carbonates and other compounds (Hoffman *et al.*, 2003). There are previous research like Beyer *et al.* (2004) who conducted a study to determine if waterfowl habitat has been contaminated by lead, based on tissue concentrations, found that several birds should be considered Pb poisoned. Also Pain (1996) developed criteria for evaluating Pb concentrations in tissues of individual waterfowl and found that certain functions may be impaired and that external signs of poisoning may occur when hepatic Pb is between 6 and 15 mg kg^{-1} (ww) (approximately 20 and 50 ppm dw, respectively).

Lead was detected in 100% of the samples collected. The results of a Kruskal-Wallis test were significant ($p<0.05$) and the mean ranks of values found in the hepatic tissue of birds was higher in the group of geese while the concentration of this metal in ducks and shorebirds was similar ($p>0.05$). Studies reported by Pain *et al.* (1995) consider that the levels of Pb in livers of birds with a concentration <2 ppm in dry weight do not represent toxicological risk however, it is considered an evidence of moderate exposure. Irwin and Karstad (1972) in a study to evaluate the toxicity of lead in mallard ducks found that in moderate exposure scenarios where the hepatic levels reached 3.6 ppm (SD = 0.59) wet weight, there was no decrease in food intake or loss of body weight. However, there is some degree of absorption of lead in blood causing the fluorescence of erythrocytes as consequence of disorder in the synthesis of the heme factor, due to the increased release of erythrocytic protoporphyrin which is a result of the inhibitory effect of lead on the combination of iron with protoporphyrin in the final stage of the heme factor.

Cadmium: The Cd is a natural constituent in some rocks, found in the form of sulphide, carbonate and oxide of Cd. They are practically insoluble in water but in nature can be transformed to more soluble compounds such as nitrates and halides. According to a report from the World Health Organization (WHO, 1992), the washing of the rocks enriches with significant amounts of Cd the aquatic environments, estimating the annual influx of 15,000 tons. Water pollution by cadmium is caused by copper, lead and zinc mining and is also found in industrial sludges and phosphate fertilizers.

The Cd was present in 100% of the samples collected, the maximum concentration of this metal was 31.98 ppm (dry weight) and was found in a duck (*Anas clypeata*).

There were no significant differences in Cd concentrations among species (Kruskal-Wallis test $p>0.05$). When the Cd is in the blood it is evidence of recent exposure (Fernande *et al.*, 1996) in the case of birds, chronic exposures are manifested by accumulation in tissues of kidney and liver. The hepatic tissue can accumulate up to half of all the existing Cd in the organism (De Mendoza *et al.*, 2006; King and Cromartie, 1986). Cd suppresses egg production in mallards (Hoffman *et al.*, 2003) also can cause a wide spectrum of toxic effects in mammals and birds. When its concentrations in kidney and liver tissue exceeded 20 ppm (fresh weight) histo-pathological damages can be observed (King and Cromartie, 1986). Previous research suggests that some birds such as gulls (*Larus* sp.) may have high concentrations of Cd when they feed on insects and worms from sites where the land has been contaminated by this element (Burger, 1996).

Arsenic: This metalloid and its compounds are used in the manufacture of different products which include pesticides, preservatives for wood, desiccants and herbicides. It is also released during processes related to the extraction of gold and lead. As is toxic and carcinogenic (Newman, 2010). In its inorganic form As is highly toxic and has been found in some seabirds where its effect is related to reproductive problems and endocrine dysfunction resulting in the death of individuals (Lucia *et al*., 2010; Eisler, 1994; Kunito *et al*., 2008). The presence of As is generally low (3 μ g g^{-1} dry weight) in most living organisms (Braune and Noble, 2009). The studies done by Martin and Nickerson (1973) constitute a benchmark for the contents of As in birds that as a whole had concentrations of As <0.04 ppm (wet weight). Results obtained in this study present average concentration similar for the three groups of birds (Kruskal-Wallis test $p>0.05$) although, individuals in the group of ducks were observed with concentrations up to 9.51 ppm dry weight. These values have some similarity with those obtained in raptors (1.06-5.8 ppm dw) by De Mendoza *et al*. (2006) who also notes that the As is an element that tends to bio-accumulate and apparently is not biomagnified. Values of As obtained in this study do not represent a commitment on health of birds in accordance with Eisler (1994) as it has been found that their bodies can tolerate sub-lethal exposure to this metalloid.

CONCLUSION

Results from this study provide a preliminary assessment of the presence of heavy metals and metalloids in different species of aquatic birds in the city of Durango, Mexico. This information is important since, there are not many wildlife contaminant studies in Mexico. Some individuals of the species shoveler (*Anas clypeata*) and snow goose (*Chen caerulescens*) appeared to be slightly contaminated mainly by Cd and Zn, respectively. However, liver concentrations of metals and metalloids obtained in this study do not represent a commitment on health of birds. Further studies should increase the sample size to assess metal and metalloids levels and age or sex-related differences in concentrations.

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