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Zn(II), Pb(II) and Cd(II) levels in livers and muscles of wild duck (*Anas platyrhynchos*) hunting in El Melah Lagoon (NE Tunisia)

Cherif Ensibi[1,*], Francisco Soler Rodríguez[2], Prado Míguez Santiyán[2], Mohamed Nejib Daly Yahya[1], Marcos Pérez-López[2], David Hernández Moreno[2]

[1]Hydrology and Plonctology research group. Faculty of Sciences of Bizerte, Zarzouna, 7021, Tunisia.
[2]Toxicology Unit, Faculty of Veterinary Medicine, Avda de la Universidad s/n. 10071 Caceres, Spain.

** Corresponding author: Cherif.Ensibi@fsb.rnu.tn*

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Abstract: We investigated the levels of Zn(II), Pb(II] and Cd(II] in livers and muscles of the wild duck (*Anas platyrhynchos)* hunting in El Melah lagoon in northeastern Tunisia from December 2010. Analysis of variance shows that no significant differences in metals levels were found between samples, but it should be pointed out that the number of samples was small. HSD Tukey test show that the amount of Cd(II], Zn(II] and Pb(II] in the levers was higher than in the muscles. The results obtained suggest the importance of wild ducks as bioindicators of heavy metal pollution.

Keywords: Heavy metal, breast muscles, livers, Anas platyrhynchos, Eastern Tunisia.

I. Introduction

Pollutants such as Pb(II] and Cd(II] can have adverse effects on various physiological systems at environmentally relevant concentrations [1]. Previous studies have shown that heavy metals can also have a negative influence on the reproduction and general health of some birds [2, 3].

Because industrial and municipal waste streams, mining runoff, and other anthropogenic sources of metal contamination often end up in wetlands, waterfowl may be exposed to environmental pollutants. Exposure of waterfowl to heavy metals may occur through ingestion of Pb(II] shot gun pellets [4], contaminated sediments [5] or by food chain transfer [6]. As a result, waterfowl may accumulate metals in their tissues and could be used as bio-indicators of contamination [7].

One from the most visible and biodiverse group of animals living on water areas are birds. Mallard (*Anas platyrhynchos*) is the commonest duck in the world [8]. This species shows a great ecological plasticity and is observed in majority types of wetlands including urban habitats. Mallard is proposed as an effective biomonitor of metal pollution, because samples are easy to collect, the species is spread all over the world, the literature needed for comparison is available from different parts of the world [9]. Our study was undertaken in order to study concentrations of Zn(II), Pb(II), and Cd(II) metallic ions in muscle and liver of Mallards from El Melah lagoon (NE Tunisia).

I. 1. Study area

El Melah lagoon is a small coastal confined area (200 ha) located near Slimene (NE Tunis) and connected to the Mediterranean Sea through an artificial outlet. Diverse anthropogenic activities are present in El Melah Lagoon. The treatment station of Slimene drains directly to the western, confined area of the permanent lagoon waters. This station, constructed in 1992, has a capacity of 2500 m^3, insufficient for a mean daily volume (3000 m^3) of urban sewage actually produced by this town and the surrounding areas [10].

The industrial wastes of Grombalia, located in southeast Slimene, are also partially discharged to the catchment area of Oued El Bey, and can be transported to the lagoon by rain during the rainy season or through subterranean flows. Other industrial residues, derived from a parapharmaceutical plant, are also delivered to the northeastern sector of the lagoon. In addition, the southern border of the lagoon receives the liquid/solid residues of a broad area occupied by farming activities [10].

Other strong environmental impacts are produced by the solid residual deposits of Slimene, located in the southeastern corner of the lagoon. These residues can be washed in by the periodic rains and some dangerous metals could be transported to the aquifers and finally to the lagoon. In addition, remains of bricks, glazed tiles, concrete, cement or scrap-iron are dumped and stockpiled along the road that joins Slimene to the beach. Similar residues are also observed in the inner areas of the littoral dune strand, near the tourist resort of Solimar. Finally, some old, abandoned saltworks are found in the central lagoon [10].

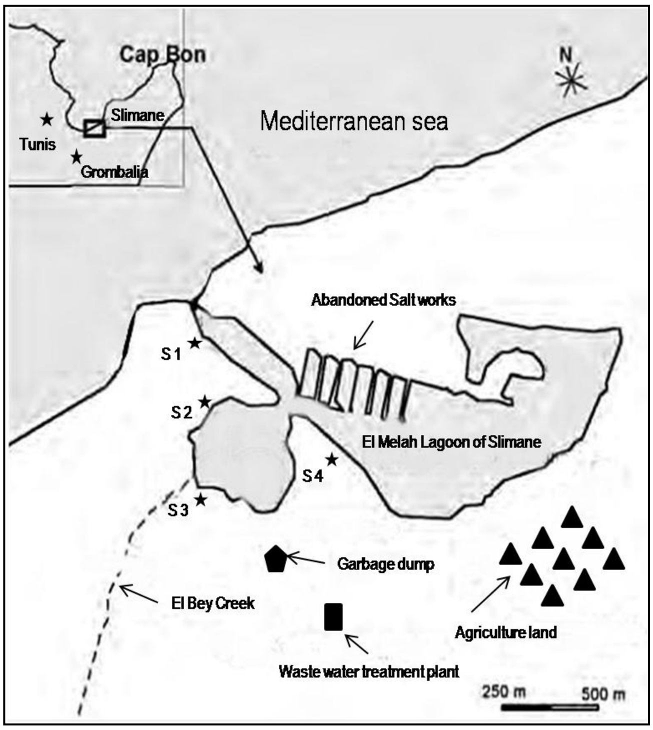

Figure 1: *Map of the El Melah Lagoon showing the four sampling sites from where mallards were hunted (S1, S2, S3, S4) and the pollution places around the lagoon.*

II. Experimental Section

Samples (about 50-70 g) of liver and muscles were taken from 14 mallards, collected in the hunting area at the El Melah lagoon (NE Tunis). All birds were shot using Pb(II] shot during the hunting season in 2009 and individually put in labeled plastic bags.

Liver and muscles samples were taken within 2 hours after death, carefully dissected to avoid external contamination, weighed, stored in chemically clean plastic bags, and kept at -20 ˚C until they were analyzed.

After defrosting, one aliquot (approximately 500mg of fresh hepatic tissue and muscle) was dried at 105 ˚C for 24 h and weighed. A volume of 2ml of an acid mixture (perchloric, nitric and sulphuric acids, 8:8:1, trace analysis quality, Scharlau) was added to the sample for mineralization. This process was realized in digestion tubes previously washed in a 10% solution of nitric acid, using an automatic digester, programmed to rise from room temperature to 370 ˚C in 5.5 h, according to the general method proposed by Garcia-Fernandez [11]. Digested samples were subsequently added with 200 ml of HCl Suprapurs (Merck), and diluted in deionized water to a final volume of 20 ml.

Concentrations of different inorganic elements (Zn, Pb and Cd) were determined by means of inductively coupled plasma-mass spectrometry (ICP-MS). Each analysis was carried out in duplicate. Final concentrations in samples were expressed referring to dry weight. All samples were run in batches that included blank and initial calibration standards.

A statistical package (SPSS for Windows, V. 13.0) was used to analyze the results. Comparisons among the different tissue for each heavy metal were realized using the non-parametric Kruskal Wallis's test. In order to determine which tissus were significantly different from each other, a post hoc comparison with the Dunn's test was carried out.

III. Results and Discussion

Cd(II] concentrations varied according to tissues (Figure 2). Liver was the main internal organ for Cd(II] accumulation in mallard. The high Cd(II] accumulation in Liver demonstrates the role of this organ in the detoxification process and storage of nonessential elements. In contrast, muscle represented minor sites of Cd accumulation, as previously described [12, 1]. Cd(II] is considered one of the most toxic metals. Cd(II] in the environment has mainly an anthropogenic origin. More than a thousand tonnes of Cd(II] have been emitted to the atmosphere in Europe, mining and smelting being the major sources [11]. In different habitats, birds accumulate varying amounts of Cd(II]. The metal concentrates mainly in kidneys, and also in the liver. This pattern was also confirmed by the Mallards from El Melah lagoon considered in this study. Chronic exposure to Cd(II] is known to increase birds susceptibility to disease or other kinds of stress and to reduce reproductive success [13].

Like Cd(II], Pb(II] is an element that plays no role in metabolic processes of animal organisms. It is an extremely toxic element with a wide range of harmful effects. Exposure to Pb(II] may cause kidney and nervous system problems. It can also inhibit heme synthesis. The mean of Pb(II] concentration determined for the mallards was 0.608 mg/kg w.w. in the liver and 0.467 mg/kg w.w. in muscles (Figure 3). Similar results were obtained by Kalisinska et *al*. [14], who investigated contents of iron, Zn(II], copper, manganese, nickel, Pb(II] and Cd(II] in selected tissues and organs of young and adult Mallards from two regions of northwestern Poland, and by Mãcinic et *al*. [15], who studied Mallard (*Anas platyrhynchos*) from a hunting ground in Romania.

It should be stressed that Pb is a serious neurotoxin; a small amount penetrating the brain may alter the bird's behaviour up to the extent of endangering the survival and precluding reproductive success. Higher Pb(II] contents result in visibly disturbed functioning of the central and peripheral nervous systems [16] Developing nervous system has long been recognized as a primary target site for Pb-induced toxicity [17, 18]. For those reasons, ecotoxicological research should pay more attention to the avian brain. In many cases the Mallard, have been used to assess wetland Pb(II] pollution [14, 19, 20]

Zn(II] belongs to the group of trace elements but in higher concentrations can be harmful to organisms [9, 21] Also, its deficiency causes loss of appetite, loss of body weight, impairment of epidermal products, impairment of reproductive functions and changes in bones, especially in birds due to osteogenesis [21]. The concentration of Zn(II] (figure 4) determined in the liver of the mallards was within the range of 93.8 mg/kg w.w to 302.7 mg/kg w.w. (mean 239 mg/kg w.w.). The Zn content in duck muscles ranged from 41.5 to 51.8 mg/kg w.w. Zn concentrations in the liver of the birds appear to have toxic levels. The highest concentrations of this metal occurred in liver what was observed also by Kalisinska et*.* *al*. [14] and Binkowski et *al*. [9]. Concentrations of Zn(II] in bird body may be influenced by the food quality and molting season.

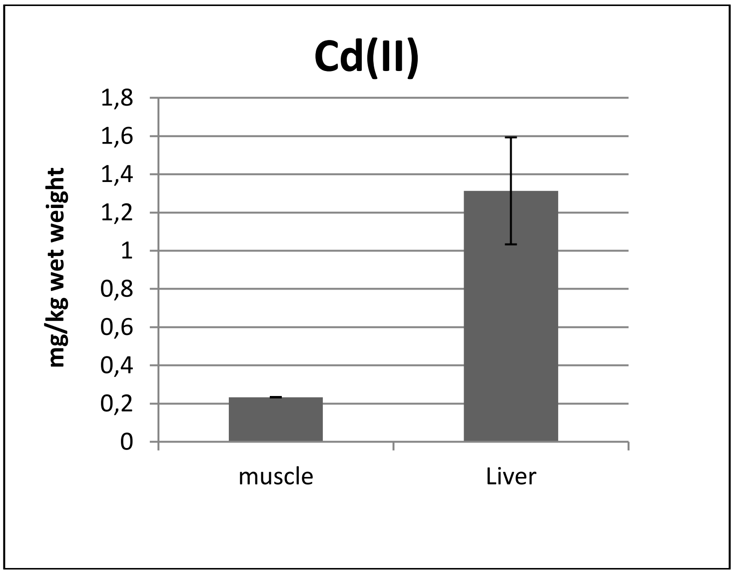

Figure 1: *Cd(II] concentrations (mg/kg w.w.) in Livers and muscles of Mallard (n=14)*

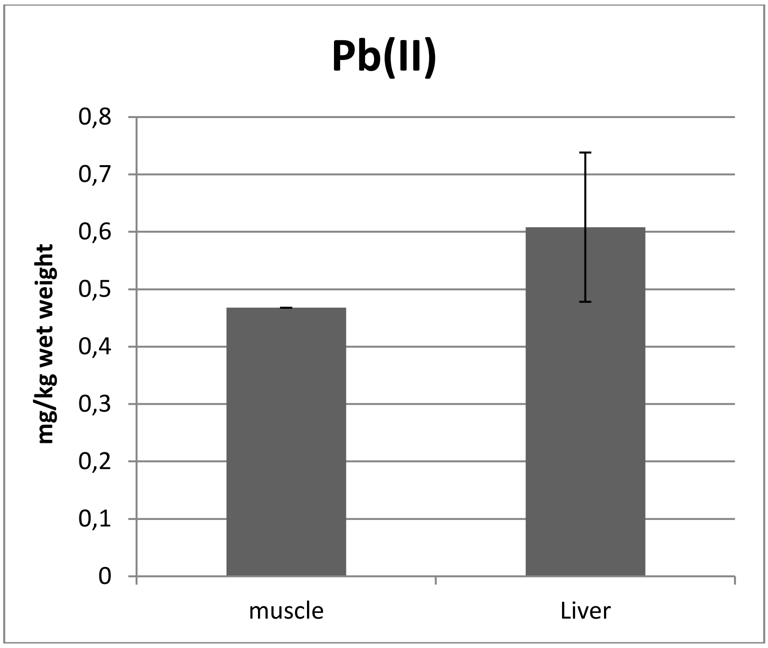

Figure 2: *Pb(II] concentrations (mg/kg w.w.) in Livers and muscles of Mallard (n=14)*

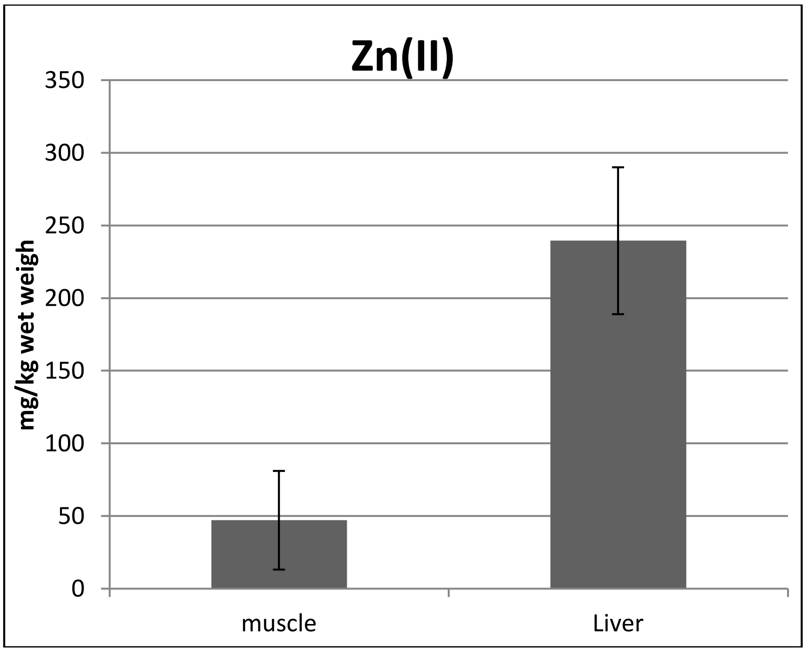

Figure 3: *Zn(II] concentrations (mg/kg w.w.) in Livers and muscles of Mallard (n=14)*

IV. Conclusion

The El Melah Lagoon's water has been observed to deteriorate in quality very rapidly because of the anthropogenic activities. Industrial, municipal and agricultural wastes are additionally discharged to El Melah Lagoon. These may be the possible causes of the high metal amounts observed in mallard's tissues. Levels of heavy metal varied depending on different tissues. The results of this study indicated that accumulation of heavy metals was higher in liver than muscles.

We can conclude there should be effort to protect El Melah Lagoon from pollution to reduce environmental risks and this study may provide valuable data for future research. The main topics that may be needed to be investigated are control of all discharges, regular observation of pollutants, evaluation of effect of pollutants on Lagoon's ecosystem over the long term, coordinating the pollution source and preventing inflow of pollutants to the Lagoon.

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