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Heavy metals in selected tissues and histopathological changes in liver and kidney of common moorhen (*Gallinula chloropus*) from Anzali Wetland, the south Caspian Sea, Iran

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ABSTRACT

The present study aimed to measure the concentrations of Sn, Pb, Zn, Hg, Cu, Ni and Cd in the muscle and liver of 40 Common Moorhens (*Gallinula chloropus*) hunted from four stations in Anzali Wetland (Pirbazar, Ghalam-Koudeh, Selkeh and Abkenar). The histopathologic alteration index (HAI) of liver and kidney was also assessed in these birds. The highest concentrations of selected metals were measured in the liver of birds collected from Ghalam-Koudeh (Pb: 4.59 ± 0.21, Sn: 6.663 ± 0.282, Zn: 29.867 ± 2.011, Cu: 24.07 ± 1.84, Hg: 7.5 ± 0.257, Ni: 6.85 ± 0.52, Cd: 1.879 ± 0.4 mg kg^{-1} dw). The lowest concentrations of metals were measured in the muscle of birds caught from Abkenar (Pb: 0.799 ± 0.207, Sn: 1.873 ± 0.066, Zn: 18.533 ± 1.582, Hg: 0.86 ± 0.08, Ni: 0.53 ± 0.117, Cu: 6.63 ± 1.114, Cd: 0.08 ± 0.002 mg kg^{-1} dw). Also the highest and lowest concentrations of metals were recorded in sediment of Ghalam-Koudeh and Abkenar stations, respectively. These stations were located next to multi-industry Anzali Port. However, the concentration of Sn and Zn in sediment and tissues of Common Moorhens collected from different stations was lower than the permissible limit suggested by WHO and Canadian Council of Ministers of the Environment (CCME). But, Pb, Hg and Ni concentration in sediment and birds caught from all stations was higher than the permissible limit defined by WHO and CCME. Cu and Cd concentration in tissue samples and sediment of Ghalam-Koudeh and Pirbazar was also higher than the permissible limit defined by WHO and CCME. Hemorrhage, melanomacrophage aggregations, sinusoidal congestion and hepatocyte vacuolation were the most pathological changes found in the liver. Reduction of the Bowman space, melanomacrophage aggregations and hemorrhage also were observed in the kidney. The HAI means of *G. chloropus* collected from Ghalam-Koudeh and Pirbazar were significantly higher than other sites. Based on the HAI values and metal bioaccumulation in the tissues of *G. chloropus*, Ghalam-Koudeh and Pirbazar could be considered as having the worst environmental quality.

1. Introduction

Wetlands are low-lying areas where water is at or just below the surface. These ecosystems are unique environments with many advantages. Many species of plants and animals use wetlands for feeding and breeding. The wetlands soak up all the water and then stop flooding in the areas surrounding the wetland. They also protect river banks and coastlines from being washed away.

Anzali Wetland is a large complex of fresh water lagoons with extensive reed-beds and seasonally flooded meadows that has been recorded on the Ramsar Convention (The Ramsar Convention is the only global environmental treaty that deals with a particular ecosystem. The treaty was adopted in the Iranian city of Ramsar in 1971 and the Convention's member countries cover all geographic regions of the planet). This wetland is internationally well-known as a good place for passing through the larval stages and spawning many kinds of aquatics. It is also known as a suitable place for migratory or terrestrial birds to pass cold winter (Vesali Naseh et al., 2012). In the last decades, Anzali Wetland has received a great deal of attention due to large amounts of various types of contaminants such as heavy metals and oil compounds loaded into it from surrounding cities and industries (Vesali Naseh et al., 2012). Only a few investigations have been conducted on the evaluation of pollution, especially in animals that live in this wetland. These studies confirmed that effluent discharge from surrounding aquaculture and agriculture farms, cities, oil jetty and shipbuilding industry is the main source of water contamination of Anzali Wetland (Hoseinizadeh et al., 2011; Vesali Naseh et al., 2012).

Heavy metal pollution in Anzali Wetland has become a great concern. Heavy metals bioaccumulate in the organisms that live in

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the wetlands over time; therefore, these animals are at risk of both lethal and sublethal effects of these elements (Aazami et al., 2010). Then it is necessary to understand the fate and effects of such chemicals to evaluate the health of ecosystem. In general, metallic tin is not toxic, not even in great quantities. The toxic effect of simple tin compounds and salts is low. Tin might be a necessary element in very, very, small quantities in rats. The toxic effect of tin compounds is based on the interference with the iron and copper metabolism. For example, it affects heme and cytochrome P450, and decreases their effectiveness (Blunden and Wallace, 2003). Lead has no biological role. It affects the gut, central nervous system and causes anemia (Park et al., 2008). Mercury is a heavy metal with no biological role that is widespread in the biosphere and in food chains. Mercury poisoning may lead to peripheral neuropathy, skin discoloration (pink cheeks, fingertips and toes), swelling, desquamation (shedding or peeling of skin), tachycardia (persistently faster-than-normal heart beat), increased salivation, and hypertension (high blood pressure) (Horowitz et al., 2002). Zinc is a trace element that is essential for human health, but large concentrations of zinc can cause eminent health problems, such as stomach cramps, skin irritations, vomiting, nausea and anemia. Very high levels of zinc can damage the pancreas and disturb the protein metabolism and cause arteriosclerosis (Bothwell et al., 2003). Copper is an essential trace element that is vital to the health of all living things (humans, plants, animals, and microorganisms). In humans, copper is essential to the proper functioning of organs and metabolic processes. However, like all essential elements, excess copper intake causes stomach upset, nausea, and diarrhea and can lead to tissue injury and disease. At high concentrations copper is known to produce oxidative damage to biological systems, including peroxidation of lipids or other macromolecules (Bremner, 1998). Cadmium is an extremely toxic metal commonly found in industrial workplaces. Due to its low permissible exposure limit, overexposures may occur even in situations where trace quantities of cadmium are found. Acute exposure to cadmium fumes may cause flu-like symptoms including chills, fever. More severe exposures can cause tracheo-bronchitis, pneumonitis, and pulmonary edema (Taylor, 1997). The adverse health effects of nickel depend on the route of exposure (inhalation, oral, or dermal) and can be classified according to systemic, immunologic, neurologic, reproductive, developmental, or carcinogenic effects. Nickel is the most observed cause of immediate and delayed hypersensitivity noticed in occupationally exposed as well in the general population. The metal is not only an allergen but also a potential immunomodulatory and immunotoxic agent (Das and Buchner, 2007).

Water birds, as important part of aquatic ecosystems, have been used as a suitable bioindicator to monitor the marine pollution (Braune et al., 1999), because these animals appear to be more sensitive to contaminants than other vertebrates (Furness, 1993). Various species of water birds live in or migrate to Anzali Wetland, many of which are very popular food consumed in the north of Iran. Common Moorhen (*Gallinula chloropus* Linnaeus, 1758) is a palearctic migratory bird and abundant species in the Anzali Wetland in winter (Aazami et al., 2010). It is a commercial species which serves as a favorite food. Common Moorhens are particularly reliable indicators because they are geographically widely distributed, highly available and of high nutritional value. These birds may be affected by both local conditions near their breeding colonies and/or by factors far from these locations (Mallory et al., 2010). The present study aimed to assess the accumulation of several heavy metals (Sn, Pb, Zn, Hg, Cu, Ni and Cd) in edible (muscle) and inedible (liver) tissues of Common Moorhens (*G. chloropus*) collected from different stations in Anzali Wetland and the histopathological changes of kidney and liver of these birds to assess the health of Anzali Wetland ecosystem.

2. Materials and methods

2.1. Chemicals

All chemicals including HNO_3, $HClO_4$, hematoxylin and eosin with a purity of greater than 97% were bought from Merck Company, Darmstadt, Germany.

2.2. Study area

Anzali Wetland, located in the southern coast of the Caspian Sea in Iran, is divided into more polluted area (eastern) and less polluted (western) part. The eastern part of Anzali wetland is located close to Anzali Port with industrialized characteristics (lat. 37°25′ N, lon. 49°28′ E). This port is an entrance of main rivers such as Zarjoub and Pirbazar rivers, which are famous due to their polluted bodies. Pirbazar station located at the end of Pirbazar River. The station of Ghalam-Koudeh also is the closest area to the Anzali Port with the same characteristics (lat. 37°48′ N, lon. 49°32′ E). Selkeh and Abkenar stations (located in the western part of the wetland) are surrounded by farms and aquaculture pools. Both eastern and western parts of the wetland surrounded by villages, small towns and large cities (Fig. 1, Table 1).

2.3. Sample collection

Adult males (n=26) and female (n=14) common moorhens (*Gallinula chloropus*) randomly were collected from four stations through late April 2011. The birds were hunted using cast net and were euthanized with benzocaine (0.1 g.L^{-1}). The birds were then dissected and the male and female birds were distinguished according to their genital system (Karimi et al., 2006). Permission from the collection of the birds was given by the Environmental Protection Organization of Iran.

The samples were taken from the liver and muscle tissues of the birds and were stored at –20°C for further analysis of heavy metals. Also the samples of liver and kidney were taken from all birds and were fixed in 10% formaldehyde for 48 h for histopathological study.

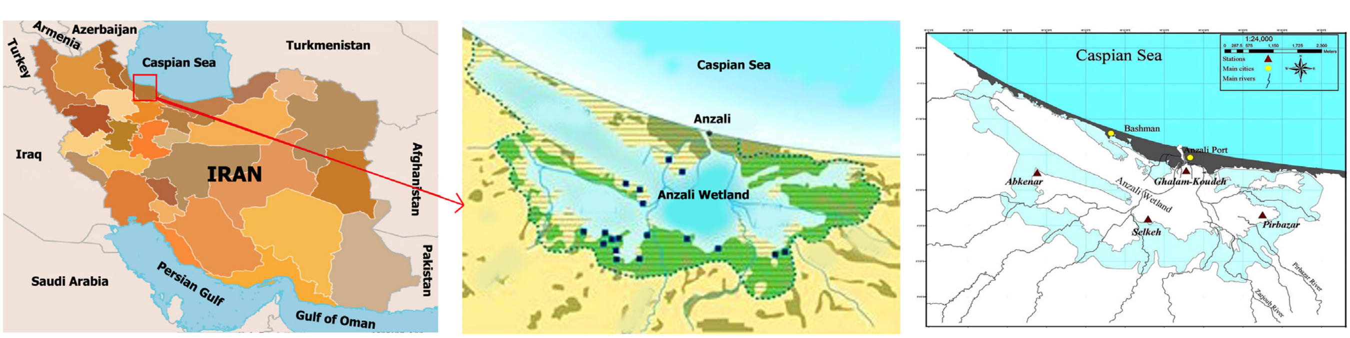

Fig. 1. Position of sampling stations in International Anzali Wetland.