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Original Article

Assessment of heavy metals on some tissues of Marbled Teal, *Marmaronetta Angustirostris* in Al-Dalmaj Marsh, Iraq

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Abstract: The study was conducted in the Al-Dalmaj marsh which is one of the most important ecosystems in Iraq and the Middle East and represents one of the most important environments for the reproduction of Marbled Teal. The study evaluated the accumulation of heavy elements of Nickel, Cadmium, and Lead in the liver and small intestine tissues of MT during the summer and winter. The results showed that the concentration of heavy metals in the MT organs is consequent as small intestine > Liver. The average concentration of Ni was the highest as 6.6973 ppm in the small intestine, and the highest percentage was recorded at 4.3272 ppm in the liver during the summer. The highest cumulative rate of the element during both seasons was 5.95±0.54 in the intestine, and 1.1681 ppm was the highest rate of Cd in the liver during summer, and 0.9308 ppm was the highest concentration in small intestine during winter. The highest cumulative rate of concentration of elements in the liver during both seasons was 0.982±0.27 ppm. The highest level of Pb was 8.0715 ppm in summer, and 7.566 ppm in the small intestine. The highest cumulative average of the element during both seasons in the small intestine was 6.93±0.55 ppm.

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Introduction

The Tigris and Euphrates River system provides biodiversity, economic productivity, human health, and cultural resources in Turkey, Iran, Syria, and Iraq. It is also known for its high productivity, providing biodiversity habitats for birds, fish, plankton, benthic, and others, and the most important natural source for fishing, bird and wildlife monitoring in Iraq. In addition, this river is being used as a warehouse to store water flowing from the Tigris and Euphrates rivers during the flood and contributes to reducing desert encroachment, dust storms, and tempering the climate in southern Iraq (Ahmed, 2012). The health and vitality of the Tigris and Euphrates River ecosystems are heavily affected by water pollution, unsustainable management of hydrological systems, and climate change (Bachmann et al., 2019).

The Al-Dalmaj Wetlands are important at the national, regional, and global levels (Evans, 1994; Scott, 1995). The Al-Dalmaj marsh was originally listed as an important bird area (IBA 032) (Evans, 1994). The marsh represents an important area for Iraqi biodiversity located within a semi-desert area including terrestrial and aquatic habitats. It supports a wide variety of species, including birds, and many threatened and endemic bird species have made these areas eligible as a Key Biodiversity Area (KBA) and an Important Bird Area (IBA).

These wetlands are breeding habitats for Marbled Teal, *Marmaronetta angustirostris*, Ferruginous Duck, *Aythya nyroca* (both are Near Threatened), Common Pochard, *Aythya ferina*, Basra Reed Warbler, *Acrocephalus griseldis* (both are Endangered) and other bird species. In addition, many waders and waterfowl have also been recorded at the site, in addition to passerines, where most of them breed (Nature Iraq, 2017). Bird hunting, especially duck species, is part of the means that the inhabitants of the marshes have practiced widely for a long time (Maxwell, 1957) as a key activity (Fawzi et al., 2016). Bird hunting in the marshes has always taken place, and the decrease of water in the marshes significantly

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during the seventies, contributed to the declining size of the wetlands and made many bird species, especially migratory birds more exposed to hunting (Garstecki et al., 2011).

Iraq's marsh's environment faces many challenges, including a lack of water supply, increased pollutants, and a lack of attention to the ecosystem, all of which lead to an increased concentration of various chemical elements including heavy metals (Usero et al., 2004). Heavy metal pollution has become a major environmental problem due to its toxicity. This has led to their accumulation in plants, microorganisms, and other aquatic organisms from which they are transported to humans through the food chain, causing multiple health problems (Varol and Sen, 2012). The increase of heavy metals in aquatic environments is a crucial problem because they are non-biodegradable, and most of them carry potential toxic risks to living organisms (Öztürk et al., 2009), and many environmental and seasonal conditions affect their bioaccumulation (Fallah et al., 2011). These metals have received great attention in different countries because they lead to accumulation especially in soft and hard tissues of animals such as bones and liver (Hassan et al., 2007). In addition, they are highly toxic and cumulatively susceptible because they are not biodegradable as not integrate into the natural cycle of the ecosystem, (Purves, 1985). Waterfowl are exposed to pollutants that are deposited and associated with sediment in wetlands, because they consume sediments when eating, as in species that feed on invertebrates or benthic plants (Taggart et al., 2009), and these metals are concentrated on plant surfaces and submerged parts, which are also diet components of some waterfowl (Taggart et al., 2005).

Marbled Teal (MT) is a congregator species with a sparse distribution in the West Arctic and North Africa (Algeria, Senegal, Tunisia, and Morocco), Eastern Mediterranean (Turkey, Syria, and Egypt), and Southwest Asia (Iraq, Iran, India, and China) and has a relatively wide global distribution (Green, 1993). This bird is either a resident or migratory bird in some countries in the Middle East (Porter and Aspinall, 2010), and in Iraq, this species is a local breeding resident in wetlands in Central Iraq, more widespread in the southern marshes, where wintering population is probably largest in the world (Salim et al., 2012). This species was recently assessed by the IUCN Red List of Threatened Species (2022) and listed as Near Threatened according to A2cd + 4cd + 3cd criteria, after being vulnerable and the rate of decline approaching the threshold of threat due to a number of factors driving the decrease, namely the destruction and habitats loss, degradation and disturbance of wetlands, in addition to climate change, the direct killing contributes to the decrease in its numbers in geographical distribution areas (IUCN Red List, 2023). Brochet et al. (2019) estimated that 5,000-15,000 birds are killed or taken illegally annually in the Arabian Peninsula; Iran and Iraq, this is approximately 17% of the total number in the world. Marbled Teal in the region and Iraq in particular exposed to several threats such as illegal hunting, habitat pollution, habitat loss, environmental destruction, and drought, in addition to the impact of climate change. Our study aimed to evaluate some heavy metals and their accumulation in some tissues of MT in the Al-Dalmaj marshes.

Materials and Methods

Study area: The Al-Dalmaj Marsh or Hor Al-Dalmaj is a large isolated wetland located in the heart of the alluvial plain of Mesopotamia, 65 km northeast of Diwaniyah city and 35 km southeast of Kut city. The length of the Al-Dalmaj marsh is about 50 km and it has about 10 km width (Carp, 1980), located between the latitudes of 32°05′ to 32°23′ and longitudes of 45°10′ to 45°38′ (Faris Beg et al., 2021). Its area is estimated at 100,000 hectares, and 20 m above sea level (Mohammad, 2014). The marsh is fed mainly by water through the Main Outfall Drain Canal (MOD) (Mousa, 2013) and runoff water during rainy seasons from the surrounding valley basins in the northern and northwestern marshes (Faris Beg et al., 2021), and its total storage capacity is about 429 million m^3 (Russian, 1984). Al-Dalmaj area contains both terrestrial habitats represented by arid areas to desert with sandy dunes, and a large part of the wetlands divided into an open water lake that exceeds 2 meters in depth, which is a suitable habitat for many birds and fish and marsh with dense reeds and shallow and salt water (less than 1 m). The southern part of the marsh includes mainly clay surfaces, including Reed Phragmites and Typha plants and submerged plants with large dry land with scattered shrubs (Nature Iraq, 2017; BirdLife, 2023).

Sample collection: The samples were collected seasonally during summer and winter representing months from August 2022 until February 2023. Eight samples of the MT were collected (males and females) from the Al-Dalmaj marsh in each season, obtained from hunters. Bird samples were identified based on Allouse (1960) and Porter and Aspinall (2010). The samples were placed in containers filled with crushed ice until they reached the laboratory. Then, they were dissected by making a longitudinal incision in the body to remove the organs i.e. liver and small intestines, and then the concentration of heavy metals was measured.

Extraction of heavy metals: One-gram dry weight was taken from the liver and small intestines, and after being dried using a convection oven at 80℃ for 24 hours, they were grounded and sieved by a sieve of mesh size of 0.5 mm. Then, they were placed in a 250 ml beaker and 6 ml of concentrated HCL (4.5 ml) and HNO_3 acid (1.5 ml) were added to the mixture and heated on a thermal plate with 80℃, and afterward, 4 ml of a mixture of concentrated perchloric acid and hydrofluoric acid was added in a ratio of 1:1 and evaporated to near dryness (Ropme, 1983). The concentration of heavy metals in tissues was calculated using the formula of Econ = (A x B x df) / D, where Eco = Element concentration in sample (µg/g dry weight), A = concentration of the element extracted from the titration curve (mg/L), B = final sample volume (ml), D = Dry weight of sample (g) and df = dilution factor that calculated using the formula of df = volume of dilution sample solution in ml / volume of a liquid taken for dilution in ml.

Measurement of heavy metals in tissues: Heavy element ions in tissues were measured using the Flame Atomic Absorption Spectrophotometer, AA-7000 Shimadzu model after the standard solution of the examined elements was prepared according to APHA (2003).

Statistical analysis: The results were analyzed using the Statistical Analysis System - SAS (2018) to study the effect of different factors on the studied traits according to the design of complete random sectors (RCBD), and significant differences between the averages were compared with the test of the least significant difference (LSD), and the Pearson correlation coefficient was extracted between the different variables. Significant differences were determined at a probability level of $P<0.05$.

Results and Discussion

Water pollution with heavy metals has recently become one of the most important environmental problems in developing countries. This pollution has increased significantly due to natural and human sources (Goher et al., 2014; Vardanyan and Trchounian, 2015). Heavy metals are hazardous materials because of their interactions with environmental materials and their ability to accumulate in the tissues of living organisms (Tete et al., 2014). They accumulate in living organisms when ingested and stored faster than they are broken down (metabolized) or excreted. These metals enter the water supply from industrial and other waste or even from acid rain after soil fragmentation and release into streams, lakes, rivers, and groundwater (Roberts, 1999), Heavy metal pollution of the aquatic environment may be a result of agricultural residues, human activities, mining, and other activities (Lau et al., 1995). The danger of these elements is due to the inability of bacteria to destroy them (Al-Saadi, 2002). Surface water quality is of great importance, especially in arid and semi-arid regions (Jazaa, 2009). Heavy metal pollution in the environment has attracted great attention due to their toxicity, multiple sources, and non-biodegradable properties, and animals can precipitate these metals from the wetland environment across the food supply chain (Salamat et al., 2014; Hong et al., 2020).

Ni concentration in liver and intestine tissues: The

Table 1. Ni concentrations in the liver and small intestine tissues in both seasons.

	Constriction (ppm)	
Season	Liver	Small intestines
Summer	4.3272	6.6973
Winter	1.9939	5.2007
Rates	3.16 ±0.77	5.95±0.54
LSD	1.784 *	1.209 *
LSD value of Interaction	1.792 *	
*$P \leq 0.05$.		

Table 2. Cd concentrations of liver and small intestine tissues in both seasons.

	Constriction (ppm)	
Season	Liver	Small intestines
Summer	1.1681	0.7134
Winter	0.7961	0.9308
Rates	0.982 ±0.27	0.822 ±0.23
LSD	0.2447 *	0.239 NS
LSD value of Interaction	0.251 *	
*$P \leq 0.05$		

results showed significant differences in Ni concentration between the small intestines and liver during the summer and winter. A high percentage was found in the intestine in summer ($P \leq 0.05$), while its concentration in the intestine during winter was the second highest value. Ni concentration (4.3272 ppm) in the liver comes third during summer. While the lowest concentration in the liver was recorded during the winter (Table 1; Fig. 1). This finding is similar to the results of Jaleel (2020) in Common moorhen, *Gallinula chloropus* in southern Iraq, that Ni had accumulated in high concentration in the liver. Green (1998) also expressed significant differences in the concentration of heavy metals in the liver and bones of Marbled Teal and White-headed duck, *Oxyura leucocephala* in Spain. These differences can be due to long-term exposure or the degree of accumulation of these metals by diet and water sources (Taggart et al., 2009) .Some studies have suggested that differences in age and sex affect heavy metal levels while other studies show no difference based on sex and age (Battaglia et al., 2005; Sanchez-Chardi et al., 2007). Various physiological and biochemical factors, such as dietary habits, development, age, fertility, feather change, and migrations can affect concentrations and mineral redistribution in birds

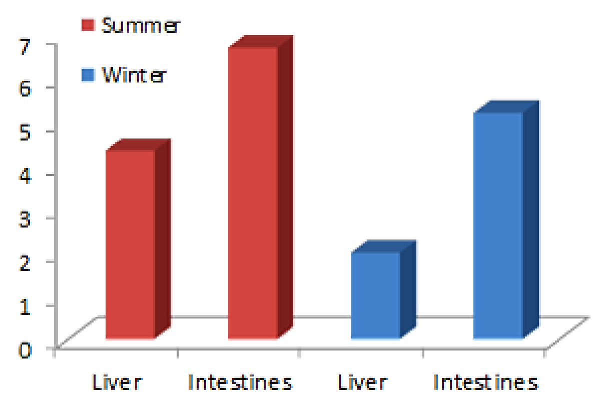

Figure 1. Comparison of Ni concentrations between liver and intestine tissues in both seasons.

(Kim et al., 2007). Although pollution in bird habitats is minimal but exposure to heavy metals may have a negative impact on their subsequent reproductive activity in their habitats or at laying sites (Kunisue et al., 2003). All sites in the studied marshes are polluted to varying degrees, affecting the aquatic life. The fertilizers and pesticides used in the agricultural field and household and industrial waste in the marshes are the main factors for water pollution with heavy metals. The concentrations of heavy metals in the waters of the Al-Dalmaj marsh are high and also vary according to the spatial distribution of minerals depending on the water depths (Faris Beg et al., 2021).

Cd concentration in the liver and intestine: The

Table 3. Pb concentrations of liver and small intestine tissues in both seasons.

	Constriction (ppm)	
Season	Liver	Small intestines
Summer	8.0715	7.566
Winter	3.4796	6.2952
Rates	5.78±0.89	6.93±0.55
LSD	2.194 *	1.362 NS
LSD value of Interaction	2.256 *	
*P≤0.05		

results showed significant differences in Cd levels in livers between the summer and winter (P≤0.05), and high concentrations in summer and winter as 1.1681 ppm and 0.9308 ppm in the intestine, respectively with no differences between its level in the two seasons (Table 2, Fig. 2). This is consistent with the findings of Jaleel (2020), in Cd accumulation in the Common moorhen, and it is consistent with the results of Salamat et al. (2014) in Cd values in Moorhen liver. Our study also showed that Cd accumulation was less compared to other elements in the studied tissues and this is consistent with the results of Carpene et al. (1995) who mentioned that Cd concentrations in tissues of the studied birds were relatively low compared to other assessed heavy metals. Cadmium is an essential element that can accumulate in the food chain in a lower concentration than mercury or lead, causing tissue damage and behavioral changes (Furness, 1996; Burger and Gochfeld, 2000). High levels of Cd in birds cause kidney and testicular damage, as well as malnutrition, slow growth, thinning of eggshells, and other problems (Çelik et al., 2021). Metabolic activity in living organisms requires some heavy metals such as Zn, Cu, Mg, and Fe, although they are toxic, but are necessary at a certain level, while Cd, Hg, Cr, and Pb are toxic elements even at low concentrations (Karadede-Akin, 2007).

Pb concentration in liver and intestine: Our results showed significant differences in Pb levels in the liver between summer and winter with a high percentage in summer (P≤0.05), while no significant differences were found in summer and winter in small intestines. The average Pb concentration (8.0715 ppm) in the liver during summer recorded the highest value of the elements evaluated in the tissues in the summer and winter seasons (Table 3, Fig. 3). The results are consistent with the findings of Kandoh (2021) that reported the highest concentration of lead in *Gallinula chloropus* liver in summer in the Al-Dalmaj marsh, and also consistent with the study of Danczak (1997) that found high concentrations of Pb in the liver of Anseriformes compared to other tissues. In Marbled Teal, changing patterns of metal levels concerning age have been observed, i.e. lead level increases with age

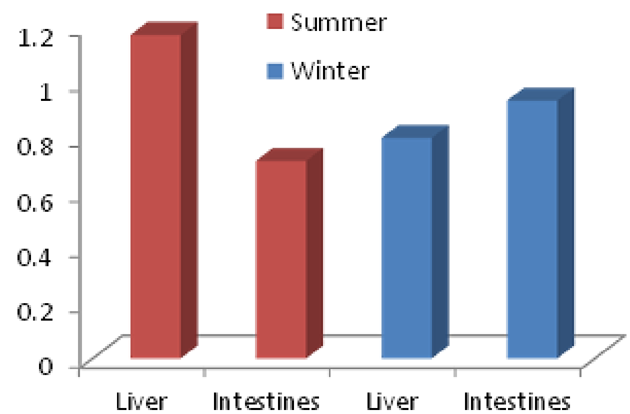

Figure 2. Comparison of Cd concentrations between liver and intestine tissues in both seasons.

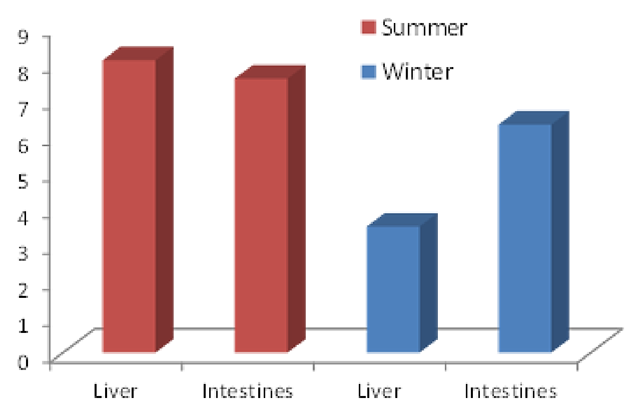

Figure 3. Comparison of Pb concentrations between liver and intestine tissues in both seasons.

in the liver and bones, and this increase is likely to reflect cumulative lead exposure due to long-term consumption (Svanberg et al., 2006).

Lead poisoning has been linked to mortality and growth retardation, and ecotoxicity studies are limited to little information about additional metal pollution or metal reactions (Herring et al., 2017), however, it can cause bird death (Svanberg et al., 2006). Heavy metals accumulate by consuming diet, the level of their absorption in the environment varies based on the physiology of living organisms and metal content and bioavailability, minerals accumulate in the body after ingestion and are excreted or stored in different body tissues or isolated in feathers (Furness et al., 1986). The threshold limit for lead is 2 ppm according to Burgat (1990) and Pain et al. (1995). Our study showed that the values of lead rates in the liver and small intestines were above this threshold, these values in the liver in particular may be unhealthy for consumption.

Conclusions

The results indicated a significantly higher concentration of heavy metals in the liver and small intestinal tissues of Marbled Teal during both studied seasons, due to the high proportions of these elements in the diet. The high rates of accumulation of heavy metals in the summer were recorded due to several factors, such as pollutants coming from agricultural land, physical factors, and the decline in water level ratios, that affect the diet of marbled teal. The high concentrations of metal elements in the intestines were caused by diet, and in the liver resulted from the long-term exposure to these elements from their habitats. This high percentage can significantly affect and threaten living organisms, especially since lead and cadmium are toxic even at low levels.

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