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Research Article

Effect of some heavy metals on accumulation, histological and biochemical changes in local geese: A comparison between urban and rural regions

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Abstract
The lead is highly complex and constantly being released into the atmosphere, mainly from agricultural and industrial activity, which is toxic to animals. The present study was conducted to investigate the lead accumulation in the different organs (blood, liver, kidney, and small intestine), and the histological and biochemical changes in the local geese birds with a comparison between urban and rural regions of the Basrah city. The results the internal organs appeared the highest accumulation of lead, copper, cadmium and zinc in the urban regions. The biochemical parameters showed elevation in liver enzymes (AST, ALT and ALP) and glucose of the blood higher in the urban region compassion with the rural region. Bioaccumulation of heavy metals showed the highest values in the urban regions in the order of liver> small intestine > kidney > brain > blood. The mean of the heavy metals bioaccumulation in the different organs was recorded as Pb > Cu > Zn > Cd at the urban and rural regions. In histological examinations, there were differences in the structure of the liver, kidney and small intestine between urban and rural regions birds, represented by necrosis, degeneration and hypertrophy states. The geese birds in the urban regions showed a high lead accumulation and negative effect on biochemical and tissue values.

Keywords: Toxicity, Birds, Heavy metals, Histopathology.
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Introduction

Lead is one of the toxic heavy metals and its passive effects range from trivial physiological and biochemical disorders to serious acute pathological conditions in which some organs can be damaged or have their functions altered, according to the time and dose of exposure (Francisco et al. 2003). The gastrointestinal, renal, reproductive, and nervous systems and biosynthesis of haeme are all affected by inorganic lead (Verity 1997). The uptake of lead into an individual depends on its concentration in the environment, chemical form, exposure route, and exposed spices. However, species are different in absorption lead to a wide range of responses to lead exposure (Pattee & Pain 2003). Lead acts at the molecular level to inhibit many enzymes essential for normal biological function (Gwaltney-Brant 2002). Its accumulation in the body affects the digestive, cardiovascular, nervous systems (Patočka & Černý 2003). However, lead poisoning, the action of digestion in the digestive system, is disturbed and decreases body weight (El-Tantawy 2016).

Lead poisoning occurs via the oral pathway or less widely via the skin and respiratory pathway. It affects the blood vessels, liver, kidney, CNS, PNS, gastrointestinal tract, bone marrow, bone,

reproductive and endocrine systems, which appear as acute, sub-acute or chronic forms (Saleh & Meligy 2018). The Pb directly affects enzymes containing sulfhydryl groups; however, the thiol ingredient of RBCs, antioxidant protection, and mitochondria in many cells, which is observed in the medical condition. Aside from edema, cerebellar hemorrhage, and capillary injury, most non-essential heavy metals such as Pb and Cd are immune suppressant, nephrotoxic, teratogenic, gametotoxic, and noxious hematopoietic organs (Dewanjee et al. 2013; Matović et al. 2015). Based on the background mentioned above, this study aimed to investigate the lead exposure on geese birds, focusing on its accumulation, biochemical and histopathological changes.

Material and Methods:

Sixteen adult geese were collected from urban (n =8) (Ashar, Saraji, and AL Maqil) and rural (n = 8) regions of the Kibasi, Firuzyah and Qaryat Bbani Malik in Basra Governorate. According to the procedure (ACUP) of animal care, birds have euthanized by intravenous injection of an overdose of sodium pentobarbital in the wing (Pavek 2007). All birds were sacrificed and their blood samples were collected for measuring biochemical parameters (AST, ALT and ALP enzymes and glucose). The serum liver enzymes and glucose were determined by special kits using spectrophotometer. The bioaccumulation of the Pb, Cd, Cu, and Zn in the different organs viz. liver, kidney, small intestine, blood and brain were measured in the geese birds. A Shimadzu AA 680 flame atomic absorption spectrophotometer was used to determine the concentrations of heavy metals according to Mansouri et al. (2012). While the brain, liver, kidney and small intestine were removed and sampled to prepare histological sections. The samples were impregnated and blocked in paraffin wax, and after cutting by microtome, the sections were stained with hematoxylin and eosin (Luna 1968).

Statistical analysis: The data were analyzed using the statistical package SPSS (version 16.0). The mean±standard deviations of the parameters and heavy metals concentration in different tissues were calculated. Regarding the correlation coefficient level, if $P \geq 0.05$, it was evaluated as significantly different.

Results

The biochemical results showed high values to blood glucose and ALP, AST, and ALT in liver enzymes which consider as biomarker tools ($P \leq 0.05$) in urban regions (Table 1). The results of the heavy metals bioaccumulation (Pb, Cu, Cd, and Zinc) in Urban and Rural regions are shown in Tables 2, 3, 4, and 5. However, the heavy metals concentrations showed the highest values in the urban regions in order of liver> small intestine > kidney > brain > blood. The mean of the heavy metals bioaccumulation in the different organs in order of Zn > Pb > Cu > Cd at the urban and rural regions.

Histological examinations were revealed lesions more pronounced in birds of the urban regions, however, the alterations in the liver were higher such as loss of normal structure of hepatocytes. The hepatocytes showed degenerative and necrotic changes and acute inflammation. Varying degrees of degeneration were observed: cloudy swelling, vacuolar degeneration, fatty changes with peripherally arranged nuclei, and vessel congestion and hemorrhage. Multifocal areas of coagulation necrosis were consistent in the liver. Nucleolysis in the hepatocytes and aggregation of kupffer cells (Figs. 1, 2, 3, 4, 5) were higher in urban gees (Figs. 6, 7).

Histological changes were more pronounced in birds of the urban regions. However, the alterations in the kidney were as distorted and diminished glomeruli, edema exudate, necrotic foci, renal tubular shrinkage, and degeneration renal tubular. Also, kidneys showed asymmetry in glomerular size and shape, and glomeruli were decrease their size and atrophied. In addition, various degrees of

Fig.1. Location map of study area as the following: the urban region: (St.1 Ashar, St.2 Saraji and St. 3 AL Maqil) and the rural regions (St4. Kibasi, St.5 Firuzyah and St.6 Qaryat Bbani Malik).

Table 1. Shows the average (larvae, pupae, and adults) of food remaining for the rusty flour beetle treated with different concentrations of sunflower oil.

Biochemical parameters	Urban region	Rural region
AST	63.65±4.9*	19.5±2.22
ALT	88.99±7.9*	22.53±2.73
ALP	122.09±10.7*	78.54±5.97
Glucose	52.44±1.04*	22.31±2.99

* represents significant difference ($P \leq 0.05$).

Table 2. Mean concentrations of the lead (µg/g) in the internal organs of geese bird in the urban and rural regions.

Organs	Urban region	Rural region
Liver	15.21±1.3*	9.25±1.31
Kidney	9.33±0.53*	5.87±0.41
Blood	5.26± 0.97*	2.97±0.33
Small intestine	14.9±1.41*	6.11±0.96
Brain	6.51±0.92*	3.23±0.61

* represents significant difference ($P \leq 0.05$).

degeneration and apoptosis appeared as cystic dilatation and renal tubular atrophy in the urban regions samples (Figs. 8-13).

Discussion

The pollution of the living environment in urban and rural regions of the Basrah government is an ecological problem. The main recourses of pollution in the rural region, especially the lead, are many industrial and human activities, waste, and car exhaust. This study aimed to evaluate the harmful effects of heavy metals toxicity on the geese by

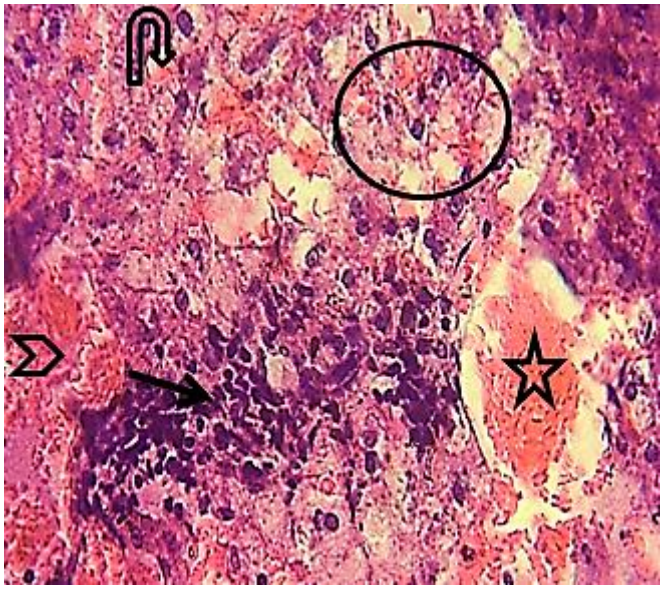

Fig.2. Liver section of geese bird in the urban regions showed inflammation cells (black arrow), hemorrhage (head arrow), necrosis (circle), degeneration vessel and sinuses Congestion (star), H&E 400x.

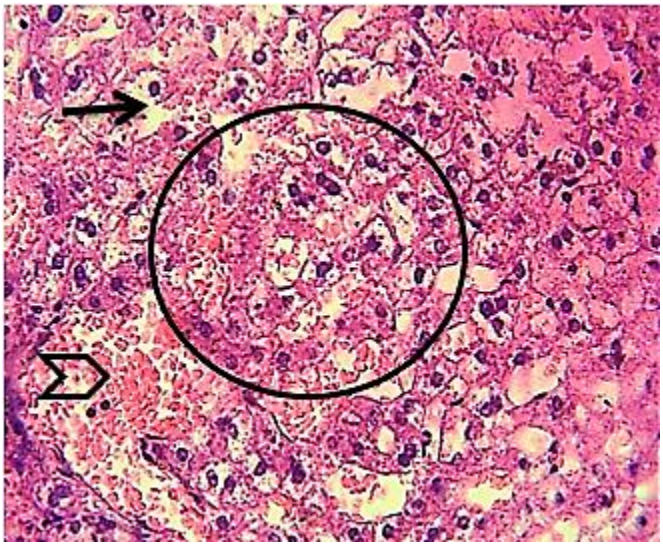

Fig.3. Liver section of geese bird in the urban regions showed hemorrhage (head arrow), cloudy swelling (black arrow), vacuolar degeneration and necrosis (circle) (H&E, 400X).

Table 3. Mean concentrations of the copper (µg/g) in the internal organs of geese bird in the urban and rural regions.

Organs	Urban region	Rural region
Liver	3.88±0.035*	2.21±0.066
Kidney	3.11±0.015*	1.38±0.035
Blood	1.88±0.029*	0.80±0.037
Small intestine	3.73±0.013*	1.94±0.190
Brain	1.23±0.006*	0.46±0.003

* represents significant difference ($P \leq 0.05$)

measuring tAST, ALT, AP and total glucose, and histological structure of liver and kidney, which was higher in the urban region showing its high pollution.

The liver plays a significant role in detoxification processes and its functions are affected by heavy metals toxicity. Measuring AST, ALT, and ALP

Fig.4. Liver section of geese bird in the urban regions showed Congestion of sinuses (black arrow), Loss of normal architecture of hepatocytes was evident (Star), aggregation of Kopffer cells (head arrow), acute hemorrhage and necrosis (circle) (H&E, 400X).

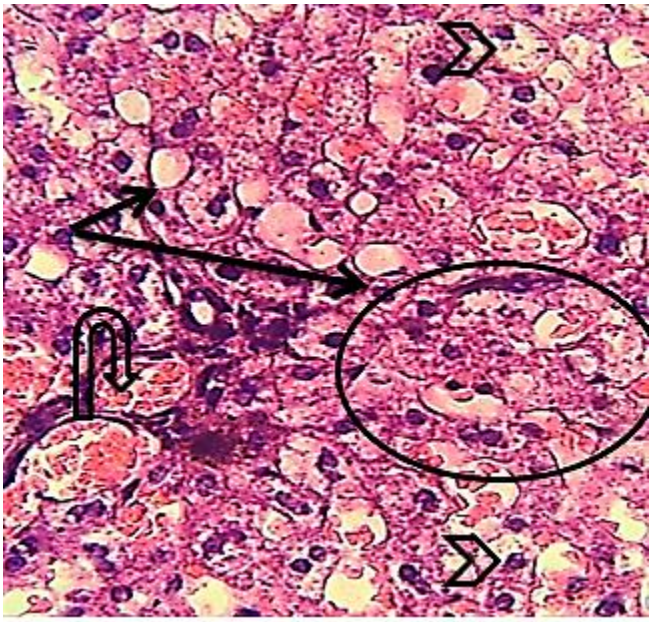

Fig.5. Liver section of geese bird in the urban regions showed hepatocellular fat vacuoles (black arrow), Multifocal areas of coagulation necrosis (circle), fatty changes with peripherally arranged nuclei were evident (head arrow) as well as showed vessel congestion and hemorrhage (curved arrow) (H&E, 400X).

levels is a well-established and useful diagnostic procedure for detecting liver dysfunction and hepatocellular damage (Lin et al. 2010). However, an increase of AST activity in geese may result from hepatic injury, which allows penetration of AST enzymes from intracellular into the blood. However, the increased AST activity provides information about the hepatic function (Lumeij 1994). ALT activity has been reported in the liver, skeletal muscle, and many other birds' tissues and leaks into the blood when such tissues are injured. Plasma ALT activity is neither a specific but a sensitive test for hepatocellular injury in birds. Plasma ALT activity in most species of normal birds ranges from 19 to 50 U/L and may be more useful for detecting hepatic disease in birds. Plasma ALT activity increases with

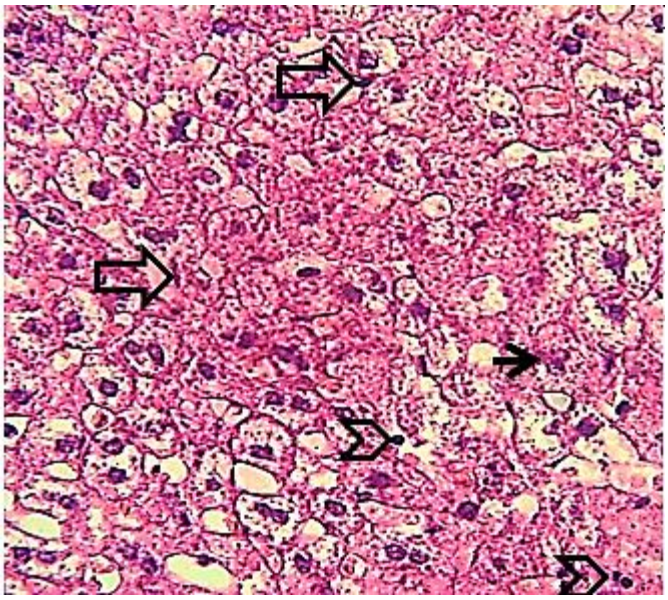

Fig.6. Liver section of geese bird in the urban regions showed Pyknotic (head arrow), nucleolus's (black arrow), cytoplasmic vacillation (curved arrow), necrosis and aggregation of Kopffer cells (thick arrow) (H&E, 400X).

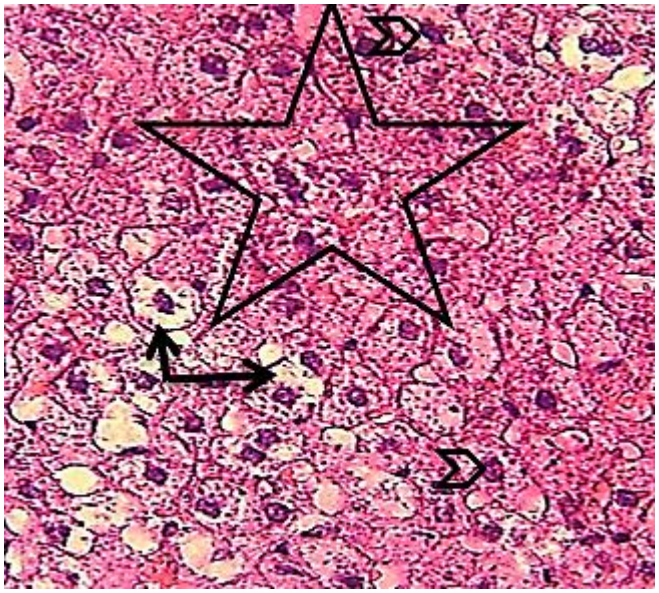

Fig.7. Liver section of geese bird in the rural regions showed that normal architecture of hepatocytes was evident (Star), except few of cytoplasmic vacoulation (black arrow), and hypertrophy of hepatocytes nuclear (head arrow) (H&E, 400X).

significant liver or muscle injury in birds (Lumeij 1987). The different tissue is responsible for alkaline phosphatase activity such as bone and intestines; however, increased plasma ALP concentrations resulted from increased cellular production. The osteoplastic activity caused the primary activity (Lumeij et al. 1998).

Other researchers showed that heavy metals such as lead have toxic effects on rats' brains, inducing vascular damage in addition to parenchymal necrotic lesions and vacuolation in the hypothalamus (Bokara et al. 2008; Hirano & Kochen 1975). Most heavy metals have a biological half-life and risk the liver and kidney (Sarkar et al. 2013). A harmful effect of heavy metals on histology and cellular structure of the small intestine epithelium in Japanese quail has been reported (Cigankova et al. 2010). The kidney and liver are the first organs where Cd is accumulated (McFarland et al. 2002). Heavy metals (non-essential) such as Cd, Cu and Pb are caused different

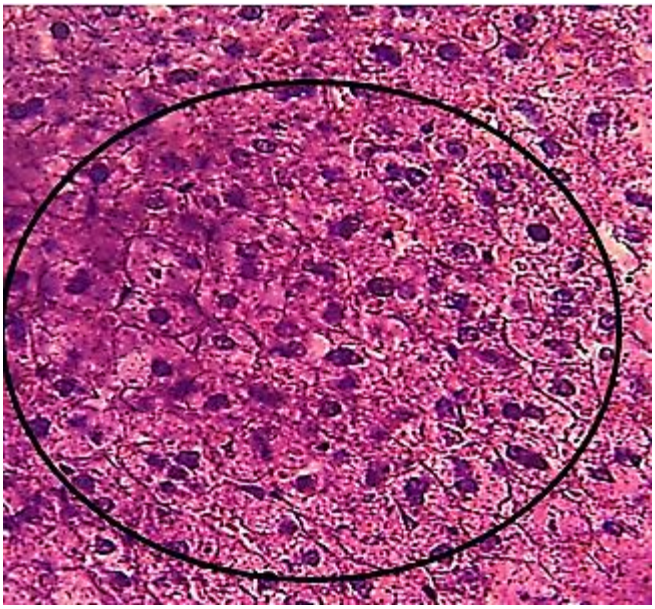

Fig.8. Liver section of the geese bird in the rural regions showed that normal architecture of hepatocytes was evident (circle) (H&E, 400X).

Table 4. Mean concentrations of the cadmium (µg/g) in the internal organs of geese bird in the urban and rural regions.

Organs	Urban region	Rural region
Liver	0.071±0.0043*	0.0098±0.00061
Kidney	0.038±0.0027*	0.0045±0.00078
Blood	0.005±0.0049*	N.S.
Small intestine	0.058±0.0033*	0.0031±0.00031
Brain	0.013±0.0075*	N.S.

* represents significant difference ($P \leq 0.05$)

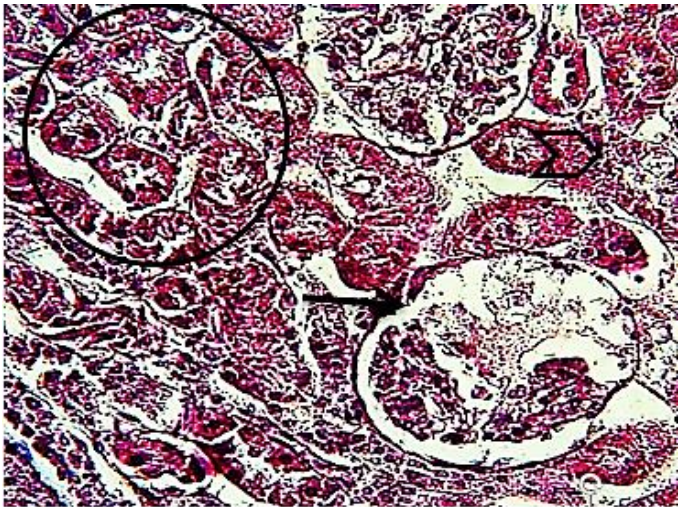

Fig.9. Kidney section of geese bird in the urban region showed distorted and diminished glomeruli (black arrow), edema exudate (head arrow), and necrotic foci (circle) (H&E, 400X).

histological changes, such as the dilation of sinusoids, inflammatory cells, necrosis in the liver (Holovska et al. 2013), and swelling of epithelium cells and necrosis in the renal tubules (Hesaraki et al. 2008). The heavy metals accumulation in the liver, kidney, small intestine, blood and brain of the geese birds living in the urbane regions than those in the rural regions. The urbane regions receive large

Fig.10. Kidney section of geese bird in the urban region showed hemorrhage (head arrow) and edema exudate (thick arrow), shrinkage and degeneration renal tubular (circle), apoptosis and Loss of normal shape of renal (thin arrow) tubules (H&E, 400X).

Table 5. Mean concentrations of the zinc (µg/g) in the internal organs of geese bird in the urban and rural regions.

Organs	Urban region	Rural region
Liver	16.76±0.88*	6.21±0.81
Kidney	25.73±0.61*	14.91±0.039
Blood	0.99±0.21*	0.42±0.052*
Small intestine	3.12±0.98*	1.77±0.049*
Brain	1.09±0.092*	0.89±0.055

* represents significant difference ($P \leq 0.05$)

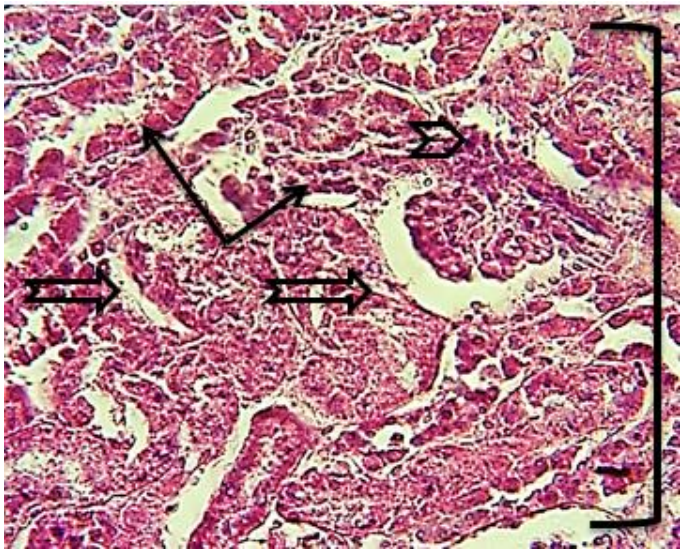

Fig.11. Kidney section of geese bird in the urban region showed Loss of normal shape of renal tubules (Arch), shrinkage and necrosis glomeruli (head arrow), various degrees of degeneration and apoptosis (thin arrow) and appeared as cystic dilatation and renal tubular atrophy (thick arrow) (H&E, 400X).

amount of wastewater causing major pressure on the natural condition of the wetland and its fauna. Clark & Scheuhammer (2003) consider the threshold toxic level of Pb exposure in liver and kidney and small intestine of birds as > 6µg/g d.w. and its threshold in the blood and brain as 5ng/g d.w. (Kalisinska 2000). In our study, the Pb level in the studied organs were higher than 5ng/g d.w. Cu and Cd concentration as toxic range have been reported 3-8ug/g dw in liver, kidney and small intestine (Scheuhammer 1987). The

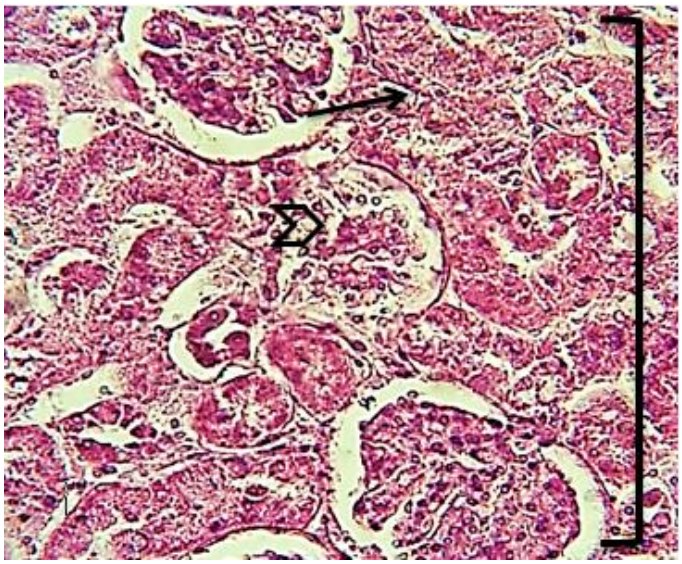

Fig.12. Kidney section of geese bird in the rural regions showed normal histological structure (arch) , slightly alteration represented by degeneration of glomerulus (head arrow) and renal tubules (black arrow) (H&E, 400X).

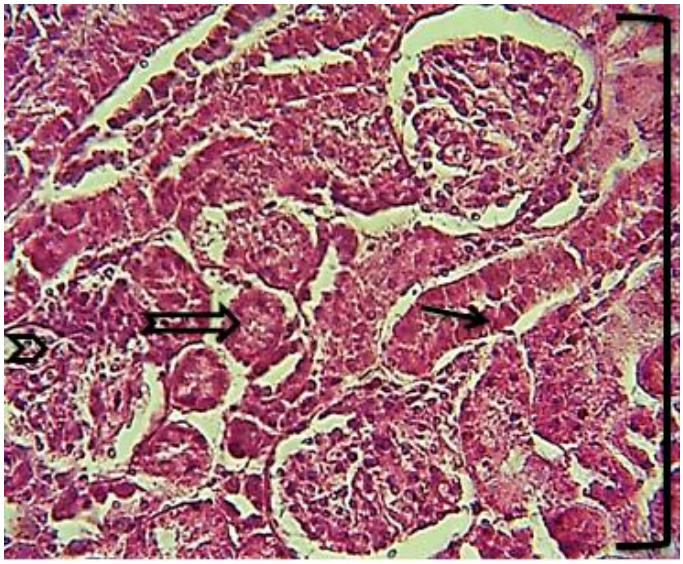

Fig.13. Kidney section of geese bird in the rural regions showed normal histological structure (arch), slightly alteration represented by degeneration of glomerulus (head arrow), renal tubules epithelium (black arrow) and congestion (thick arrow) (H&E, 400X).

Cu and Cd bio-accumulation in our study were lower than this reported level, except the Cu in the liver in urban region. Zn level in our study were lower than the Zn adverse threshold for wild birds (>122 ug/g d.w.) (Beyer et al. 2004; Gomez et al. 2004). The normal level of Zn in the liver of mammals and birds usually does not exceed 525.0μg/g dw (Taggart et al. 2009).

The level of metal absorption and accumulation depends on exposure, the chemical form of an element, the interaction with other elements, and the physiological factors of the bird species (Burger & Gochfeld 2016). Heavy metals are widely distributed in the environment and some of them can cause histological disorders (Roy Chowdhury 2009). Therefore, heavy metals can damage organs and tissues from the surface membranes to genetic and molecular levels. Most heavy metals are absorbed in the liver > kidney> other organs of mature birds (Scheuhammer 1987). According to Hinton & Lauren (1990), the biomarker is any physiological, histological, and biochemical changes induced by

pollutants in a not-too-sensitive organism. Lead exposure could cause lipid accumulation in the liver, resulting in an imbalance in nutrition and fatty liver syndrome by changing the rate of hepatic lipogenesis in birds (Lee et al. 2010). Trivedi et al. (1998) showed that the low and high doses of the lead induce peroxidation in the liver lipids, while the kidney showed no significant alteration in lipid peroxidation at both doses.

Several reports have been on histological alterations in the hepatocytes in aquatic birds exposed to a wide range of organic and inorganic compounds (Bokori & Fekete 1995; Binkowski et al. 2013). The present work also demonstrates that birds in the uraban regions have caused acute histological alterations in the liver and kidney compared with the rural region. The histological changes, such as inflammation, hemorrhage, necrosis, degeneration of the vessels and sinuses, loss of normal structure of hepatocytes, aggregation of kupffer cells detected in the liver of birds in the present study, are similar to those reported in *Anas platyrhynchos* and *Fulica atra* (Binkowski et al. 2013). Heavy metals have affinities for the thyol groups in the protein and enzymes responsible for the defense mechanisms. The long exposure leads to apoptosis, which leads to necrosis (Flora et al. 2008; Sanchez-Chardi et al. 2009). Numerous studies have investigated the effects of Pb, Cu, Cd, and Zn on the histological changes in the kidney of feral pigeons population living near the "Trepça" mining in Kosovo, the changes of degeneration of epithelium of proximal tubule, expansion of sucapsular spaces, atrophy of renal tubules and desquamation, leukocyte infiltration due to the high bioaccumulation of Pb and Cd in the kidney have been reported (Elezaj et al. 2011; Plakiqi Milaimi et al. 2014; Plakiqi Milaimi et al. 2015).

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