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RESEARCH ARTICLE

PHYSIOLOGICAL AND ULTRASTRUCTURAL CHANGES IN THE CATTLE EGRET (*BUBULCUS IBIS*), AS BIO-INDICATORS OF SOIL HEAVY METAL POLLUTION IN SHARKIA PROVINCE, EGYPT

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ABSTRACT

Heavy metals pollution has major health consequences for wildlife. This study aimed to evaluate the concentration of heavy metals including arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), and selenium (Se) in soil samples, as well as kidney and blood samples of the cattle egret (*Bubulcus ibis*) at two sites in Sharkia Province, Egypt: urban site (I) and rural site (II) during summer and winter 2021-2022. Some hematological and biochemical parameters, and renal ultrastructural alternations of the birds were evaluated. The results showed that the highest metal concentrations were recorded in the soil of the both sites in the summer. Metal concentrations in the soil, and kidney and blood samples of site "I" were significantly higher than those of site "II", except for Cr that was found in low levels. Alarming concentrations for As, Pb, and Se levels were found in the kidney samples of egrets in both sites. Egrets collected from site "I" showed a significant decrease in the red blood cell count (RBCs), hematocrit (Hct) value, and hemoglobin content (Hb), and an increase in the white blood cell count (WBCs), and serum creatinine and urea levels when compared to those of site "II". Furthermore, severe damage and enlargement in the renal tissues were observed in the cattle egrets of site "I" when compared to those of site "II". Based on these findings the heavy metals contamination in the surrounding environment can be predicted by alterations in hematological parameters and kidney functions in cattle egret.

INTRODUCTION

In recent decades, urbanization, industrial and agricultural activities are continuously releasing pollutants into the environment. Heavy metal contamination is one of the most prominent results of both natural and anthropogenic activity, which poses a threat to the quality of the ecosystem and biodiversity[1]. Due to the high density of metals, they can accumulate in the body and transfer to higher level organisms[2]. In this way even the essential elements can be toxic and cause diseases in all organisms and humans if their levels exceeded permissible limits[3]. Several previous studies showed that the accumulation of metallic contaminants causes abnormalities in growth rate, molting, reproduction, endocrine and nervous function[4]. In addition, metallic contaminants caused inhibition of enzymatic activity, hematological changes, and pathological changes in the bird populations[5,6].

Bio-indicator species are used to monitor environmental status and estimate the hazardous effects of pollution. Changes in the behavior, physiology, and population status of such species are used to predict the environmental contamination[7]. Avian species have been recognized as important bio-indicators of environmental pollutants specifically heavy metals[8], because of their abundance, wide distribution, sensitivity to toxins than other wild animals, feeding on a wide range of prey and their vulnerability to bioaccumulation[9,10]. Cattle Egret *Bubulcus ibis* (family: Ardeidae) is a resident wild bird and act as a top predator. They feed on a wide range of invertebrates and vertebrates[11] and have the ability to accumulate contaminants through bioaccumulation and biomagnifications[12]. The contaminated soil is the main source for heavy metals, and they accumulate in egret species *via* food[9,13]. As a result, these birds could be used as effective ecological indicators of soil pollution.

The purpose of this study was to determine and compare the concentrations of heavy metals (As, Cd, Cr, Hg, Pb, and Se) in the renal tissues and blood of cattle egrets, and the surrounding surface soil at the two studied areas; site "I" (urban area) and site "II" (rural area) in Sharkia Province, Egypt, during summer and winter seasons of 2021-2022. Determining the heavy metal accumulation alone is insufficient for estimating potential wildlife risks. So, it is important to investigate the impact of such contaminants on biological functions in parallel with their concentrations. Therefore, hematological and serum biochemical parameters, as well as the ultrastructural changes of the kidneys of these birds were used to assess their health at the two different areas.

MATERIAL AND METHODS

Sampling site

Soil samples and adult cattle egrets (*Bubulcus ibis*) were collected during two seasons (summer and winter) from 2021 to 2022 in Sharkia Province, Egypt (Latitudes 30° 34′ 0″ N, Longitudes 31° 30′ 0″ E). Two study sites were selected site "I" (Qynayat city, as an urban area, a dumping site) and site "II" (Kafr El-Ashraf, as a rural area). Birds of site "I" were commonly found feeding on municipal waste (where garbage is usually dumped), while site "II" is surrounded by a large cultivation area, so birds found to feed on fields (where fertilizers are added in soil).

Collection of the samples

Soil samples were collected (n = 8 samples × 2 stations × 2 seasons = 32 samples) from 5-10 cm depth from the grazing regions. Samples were dried, grained, and sieved to obtain a fine powder. Forty cattle egrets were caught from two sites by using trap cages. Blood samples of each bird were taken using a 3 mL syringe from the brachial vein. Each sample was divided into three aliquots for hematological measurements (with anticoagulant), heavy metal analysis (with anticoagulant), and the 3rd part (without anticoagulant) was left for 1.0 hours and then centrifuged for 10 minutes to obtain serum for biochemical analysis. Birds were then decapitated and dissected; kidney specimens of each captured bird were collected. Kidney samples were stored at −80°C for heavy metals analyses, other kidney samples were used for histopathological examination. All of the procedures were approved (ZU-IACUC/1/F/152/2023) by the Animal Ethics Committee of Zagazig University.

Heavy metal analysis

One gram of soil sample and bird renal tissues, as well as 0.5 mL of blood samples, were digested with nitric acid (HNO_3) and perchloric acid ($HClO_3$) solution (1:1) according to the methods mentioned by Akubugwo *et al*.[14]. After complete digestion, the content was filtered and diluted with deionized water till 50 mL for soil and kidney samples and 25 mL of blood samples. Blank samples were carried out in the same way. The concentration of arsenic (As), cadmium (Cd) and chromium (Cr), lead (Pb), mercury (Hg), and selenium (Se) were determined by Atomic Absorption Spectrophotometer "Buck Scientific 210 VGP, Norwalk, CT, USA" at the central laboratory in the Faculty of Veterinary Medicine, Zagazig University, Egypt.

Hematological and biochemical analysis

The hematological parameters; hemoglobin content (Hb), red blood cell count (RBCs), mean cell volume (MCV), hematocrit (Hct), platelet (Plt), and white blood cell count (WBCs) were measured at once using an automated hematology analyzer (Celltac α/MEK-6420K, Nihon Kohden Europe Ltd, Rosbach vor der Höhe, Germany). Serum urea and creatinine concentrations were determined as described by Patton and Crouch[15] and Henry[16] by using Human Diagnostics Worldwide kits (Wiesbaden-Germany).

The examination by light and electron microscopes

Specimens of the kidney tissues (small pieces of about 1 × 1 mm) from the cattle egrets for the two studied seasons and the two studied areas were fixed in glutaraldehyde 4%. Semi-thin sections (1-2 µm) stained with toluidine blue were examined under light microscope. By using transmission electron microscope (JEOL 100CX; Akishima, Tokyo, Japan) at Zagazig University Electromicroscope unit, Semi-thin sections (50-60 nm) contrasted with uranyle acetate and lead citrate were examined and photographed.

Statistical Analysis

Results were analyzed using One Way ANOVA and subsequent post-*hoc* multiple comparison with Duncan’s multiple range test (software SPSS; version 16.0). Data were expressed as mean ± standard error (SE), the value of $P<0.05$ defined statistically significant.

RESULTS

Metal concentrations in the soil

Heavy metals accumulation in soil collected from site "I" (urban area) and site "II" (rural area) during summer and winter 2021-2022 were given in Table "1". The mean concentration of As in soil samples of site "I" was 10.08±0.48 and 14.52±0.77 µg/g during summer and winter, respectively, those concentrations were significantly higher ($P<0.05$) than those of site "II". Similarly, Hg concentration of site "I" was 2.28±0.5 and 1.29±0.01 µg/g, which were slightly higher ($P<0.05$) than those of site "II" during summer and winter, respectively. Cadmium and lead in soil samples of site "I" revealed a highly significant concentration during summer in comparison to their respective concentration of site "II" ($P<0.05$), but no significant changes were recorded between soil samples of both sampling sites during winter (Table 2). Moreover, there were no significant changes in the concentration of Cr among seasons ($P>0.05$).

Metal concentrations in the biological samples of cattle egrets

The heavy metal concentrations in kidney and blood of cattle egrets collected from studied sites during different seasons were given in Table "2". The results revealed that among the analyzed metals As, Pb, and Se were the highest metals, while Cr was the lowest metal, which was below the detection limit in summer. The average concentration of As was 8.07±0.01 ppm in the kidney samples of birds during summer in site "I", which were significantly higher than in birds of site "II". In addition, Se concentration in birds of site "I" was 4.63±0.11 and 3.6±0.29 ppm during summer and winter, respectively, which were significantly higher than those in birds of site "II". The current results demonstrated that As and Se levels varied depending on both the season within the same area and habitat affect. Pb and Cd in kidney tissues showed significantly higher concentrations in birds of site "I" during summer than their concentration in birds of site "II" during the same season ($P<0.05$). Meanwhile, Hg levels showed no significant differences among seasons or sampling sites ($P>0.05$). In kidneys the metal accumulation decreased in the following order: Pb>As>Se>Hg>Cd>Cr.

Table 1: Seasonal variations of heavy metal concentrations (µg/g) in soil samples collected from studied sites during summer and winter 2021-2022.

Heavy metals	Sites			
	Site "I"		Site "II"	
	Summer	Winter	Summer	Winter
Hg	$2.28±0.50^{b}$	$1.29±0.01^{ab}$	$1.72±0.24^{ab}$	$0.76±0.76^{a}$
As	$14.52±0.77^{c}$	$10.08±0.48^{b}$	$11.57±0.28^{b}$	$9.55±0.42^{a}$
Cr	$0.033±0.008^{a}$	$0.020±0.015^{a}$	$0.023±0.003^{a}$	$0.008±0.008^{a}$
Cd	$0.330±0.005^{c}$	$0.180±0.013^{a}$	$0.243±0.018^{b}$	$0.18±0.001^{a}$
Hg	$2.28±0.50^{b}$	$1.29±0.01^{ab}$	$1.72±0.24^{ab}$	$0.76±0.76^{a}$
Pb	$9.36±0.41^{b}$	$2.39±0.01^{a}$	$3.00±0.32^{a}$	$2.85±0.03^{a}$
Se	$8.69±1.06^{b}$	$4.81±0.04^{a}$	$6.97±0.35^{a}$	$5.94±0.58^{ab}$

Data are represented as means ± standard error (n = 8 soil samples per season). Dissimilar superscripts in the same row indicate significant differences at $P<0.05$ (One Way ANOVA and subsequent post-*hoc* multiple comparison with Duncan’s multiple range test).

Table 2: Seasonal concentration of heavy metals in tissues (ppm) of adult cattle egret (*Bubulcus ibis*) collected from studied sites during summer and winter 2021-2022.

Samples	Heavy metals	Sites			
		Site "I"		Site "II"	
		Summer	Winter	Summer	Winter
Kidney	As	$8.07±0.01^{c}$	$5.55±0.42^{ab}$	$6.53±0.42^{b}$	$4.71±0.07^{a}$
	Cr	$0.003±0.003^{a}$	$0.009±0.005^{a}$	BDL	$0.007±0.003^{a}$
	Cd	$0.36±0.02^{b}$	$0.27±0.01^{b}$	$0.18±0.02^{a}$	$0.12±0.01^{a}$
	Hg	$0.68±0.02^{a}$	$0.31±0.01^{a}$	$0.20±0.02^{a}$	$0.20±0.08^{a}$
	Pb	$13.06±1.31^{b}$	$10.52±0.36^{ab}$	$9.13±0.61^{a}$	$8.90±0.55^{a}$
	Se	$4.63±0.11^{d}$	$3.60±0.29^{c}$	$1.91±0.08^{b}$	$1.13±0.22^{a}$
Blood	As	$6.41±0.04^{c}$	$4.31±0.01^{b}$	$2.28±0.01^{a}$	$2.39±0.14^{a}$
	Cr	BDL	$0.003±0.003^{a}$	BDL	$0.002±0.001^{a}$
	Cd	$0.160±0.010^{b}$	$0.060±0.003^{a}$	$0.033±0.003^{a}$	$0.050±0.001^{a}$
	Hg	$0.750±0.060^{b}$	$0.190±0.003^{a}$	$0.100±0.100^{a}$	$0.100±0.001^{a}$
	Pb	$0.780±0.040^{c}$	$0.300±0.050^{b}$	$0.180±0.002^{ab}$	$0.110±0.050^{a}$
	Se	$2.10±0.28^{ab}$	$2.93±0.11^{b}$	$1.73±0.15^{a}$	$1.63±0.01^{a}$

Data are represented as means ± standard error (n = 10 birds per season). Dissimilar superscripts in the same row indicate significant differences at $P<0.05$ (One Way ANOVA and subsequent post-*hoc* multiple comparison with Duncan’s multiple range test). BDL: below the detection limit.

As and Pb concentrations in blood of birds in site "I" during summer and winter, were significantly higher than those of birds in site "II". Regarding Hg and Cd accumulation, birds of site "I" contained a significantly higher concentration during summer in comparison to their respective concentration in birds of site "II" (P<0.05), but no significant changes were recorded between birds of both sampling sites during winter (Table 2). The case was reversed with blood Se level in birds. In blood the metal accumulation decreased in the following order: As>Se>Pb>Hg>Cd>Cr.

Hematological and biochemical parameters of cattle egrets

The mean concentrations of Hb content, RBCs count, MCV, Hct value, and WBCs count in the blood of cattle egret (*Bubulcus ibis*) collected from sites "I and II" were shown in (Table 3). RBCs count, Hb content, MCV, and Hct value showed significant reduction (P<0.05) in cattle egrets collected from site "I" than those collected from site "II" in both seasons. Meanwhile, the level of WBCs was significantly higher in birds collected from site "I" (95.56±2.37 and 86.83±3.33 $\times 10^3/\mu L$) than those collected from site "II" (76.1±3.33 and 55.87±3.04 $\times 10^3/\mu L$) during summer and winter seasons; respectively. The current results demonstrated that both the season within the same area and habitat affect hematological parameters.

Table “3” also revealed alternations in urea and creatinine levels in the serum of cattle egret collected from studied areas. Creatinine level was significantly higher in birds of site "I" than birds of site "II" during summer and winter. Creatinine mean concentration during summer was 1.80± 0.03 mg/dL and 0.79±0.02 mg/dL in birds of sites "I and II", respectively, while it was 0.65±0.03 and 0.50±0.02 mg/dL in birds of sites "I and II" during winter; respectively. Moreover, creatinine showed significant change between seasons of the same site. Concerning urea level, cattle egret in site "I" had higher mean concentrations of urea during summer and winter (8.09±0.33 mg/dL and 6.43±0.82 mg/dL, respectively) than the respective values in birds of site "II" (P<0.05). Urea was changed significantly by the habitat, but not between seasons of the same site.

Table 3: Hematological and biochemical parameters of adult cattle egret (*Bubulcus ibis*) collected from studied sites during summer and winter 2021-2022.

Parameters	Sites			
	Site "I"		Site "II"	
	Summer	Winter	Summer	Winter
RBCs ($10^6/\mu L$)	$2.18{\pm}0.28^{a}$	$2.59{\pm}0.30^{ab}$	$3.03{\pm}0.40^{bc}$	$3.53{\pm}0.15^{c}$
WBCs ($10^3/\mu L$)	$95.56{\pm}2.37^{d}$	$86.83{\pm}3.33^{c}$	$76.10{\pm}3.33^{b}$	$55.87{\pm}3.04^{a}$
Hb (g/dL)	$17.90{\pm}0.23^{b}$	$16.60{\pm}0.06^{a}$	$24.70{\pm}0.12^{d}$	$22.20{\pm}0.17^{c}$
MCV (fL)	$200.34{\pm}6.29^{b}$	$166.14{\pm}4.50^{a}$	$275.76{\pm}13.47^{d}$	$232.75{\pm}5.90^{c}$
Hct (%)	$66.40{\pm}3.35^{b}$	$49.87{\pm}2.48^{a}$	$81.67{\pm}4.65^{c}$	$59.47{\pm}3.44^{b}$
Creatinine (mg/dL)	$1.80{\pm}0.03^{d}$	$0.65{\pm}0.03^{b}$	$0.79{\pm}0.02^{c}$	$0.50{\pm}0.02^{a}$
Urea (mg/dL)	$8.09{\pm}0.33^{b}$	$6.43{\pm}0.82^{b}$	$4.6{\pm}0.42^{a}$	$4.20{\pm}0.38^{a}$

Data are represented as means ± standard error (n = 10 birds per season). Dissimilar superscripts in the same row indicate significant differences at P<0.05 (One Way ANOVA and subsequent post-*hoc* multiple comparison with Duncan’s multiple range test).

Ultrastructure of the kidneys of cattle egrets

The electron microscopic studies showed the dangerous effects of the metal pollution on the kidneys of the examined cattle egrets (Figures 1 and 2) in site "I" (urban area) and site "II" (rural area). The severity of histopathological alterations in the kidney cortex was increased in the cattle egret exposed to heavy metals in site "I" compared to that in site "II". Severe damage and enlargement in the kidney tissues of egrets were observed in site "I" (Figures 1 and 2).

DISCUSSION

The concentrations of As, Cd, Pb, and Se in the soil samples from site "I" (urban site) have significantly higher levels compared to those from site "II" (rural site) during summer. The highest levels of metals in both sites were investigated in summer, this could be due to the different anthropogenic activities, transportation, atmospheric deposition, and application of fertilizers and pesticides[17]. Arsenic is a very toxic element that is widely distributed in the environment. The mean concentrations of As in sites "I and II" during both seasons were found exceeding the world soil content (5 µg/g)[18]. This could be due to solid wastes from animals and human beings in urban site and the deposition of various pesticides at rural site. Chromium and cadmium are the environmental toxic pollutants in the world. Their measured content in the soil samples at all sampling sites were found below the reported average world soil (54 µg/g for Cr and 0.5 µg/g for Cd). However, mercury levels were found below the allowable limit (0.03-2.00 µg/g)[19] at all sites during both seasons except for site "I" in summer season. Concerning Pb concentration, Pb content in soil samples ranged from 2.39 to 9.36 µg/g. All the examined samples contain Pb levels were below the maximum allowable concentration of (25 µg/g). This may be due to the use of lead-free fuel in vehicles. In the current study, heavy metal concentrations were lower than those reported in other areas in Egypt[20-22].

Wild birds accumulate considerable amounts of metals because of their extended life span and their position in the food chain. Assessing metal concentrations in different organs demonstrates the bioavailability of the metal in the environment. Accumulation of metals in internal organs is considered an indicator of long-term exposure, while in blood reflects present time of exposure[23]. The results of the current study showed that metal concentrations in both kidney and blood of the cattle egrets from site "I" (urban birds) were greater than those in birds of site "II" (rural birds). These variations could be due to the different foraging strategies of the birds. Dumping of wastes containing metals, transportation activities, as well as atmospheric depositions were considered sources for contamination in site "I". Malik and Zeb[24] stated that both diet and different contamination sources were contributing factors to the metal's accumulation in the cattle egrets from different localities.

Arsenic exposure at high levels promotes carcinogenicity and mortality[25]. According to Sanchez-Virosta *et al*.[26], arsenic is the least investigated element in terms of exposure in birds, and a full evaluation of avian toxicity is recommended. The current work serves as a link to further update the existing arsenic toxicity status in birds. The normal and toxic ranges of arsenic as 0.01-0.20 µg/g and 5.0-10.0 µg/g, respectively, were reported by Pannu and Kler[27]. In this study, As concentrations detected in egret kidneys from the two studied areas ranged from 4.71 to 8.07 ppm indicating alarming contamination. Chromium is a vital element, but excessive amount of Cr may cause deleterious health effects[28]. In this study, a very low concentration of Cr was seen in kidney tissues of cattle egrets from both sites; this could be attributed to lack of heavy industry in the studied sites. Several investigations have found higher concentrations of Cr in the kidney of avian species than in our study, and this was linked to food[29,30]. However, no bioaccumulation

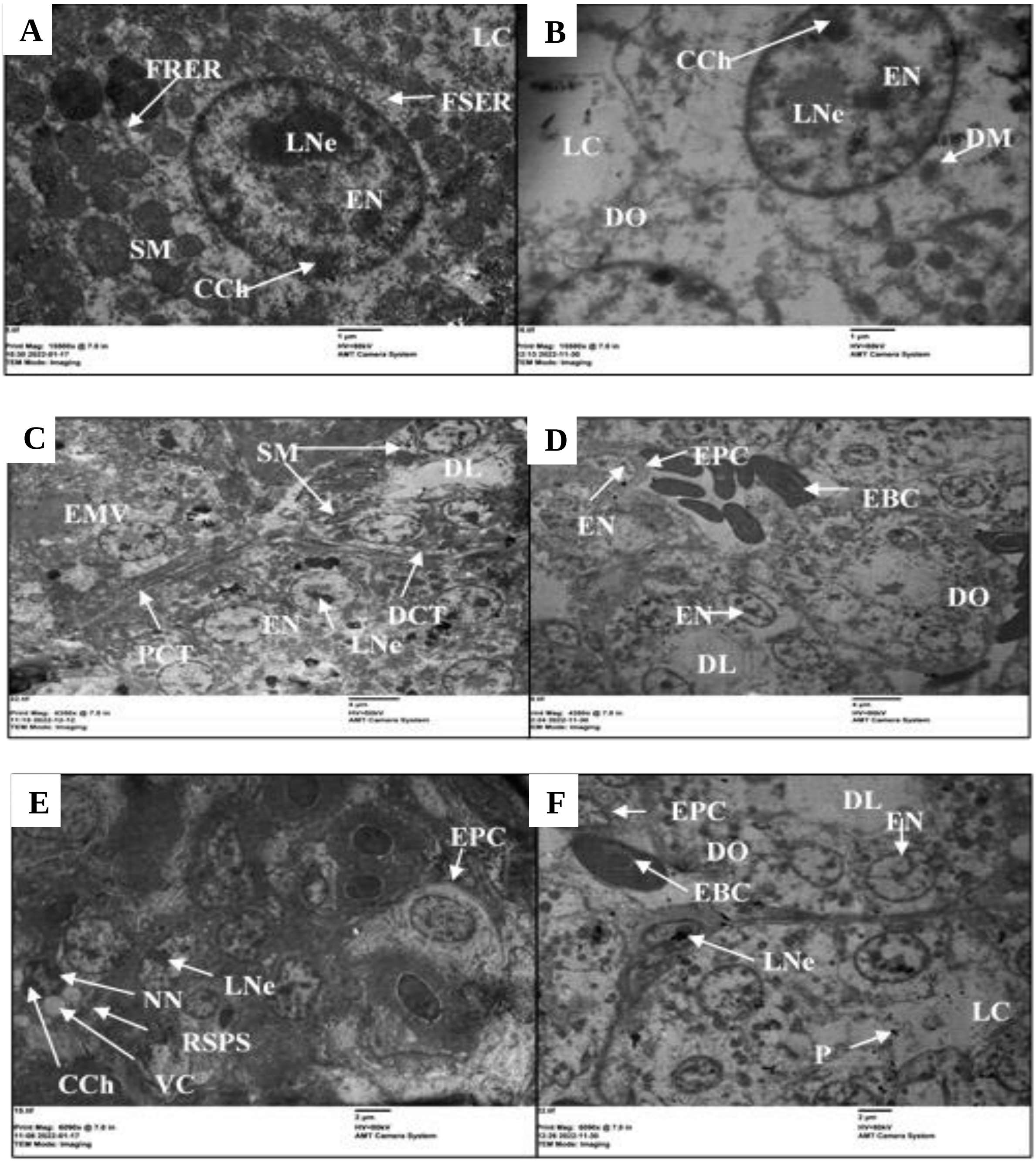

Figure 1: TEM photomicrograph of the kidney cortex components of cattle egret during summer 2021 showing elongated nucleus (EN), necrotic nucleus (NN), large nucleolus (LNe), condensed chromatine (CCh), lytic cytoplasm (LC), degeneration of the cytoplasmic organelles (DO), swollen mitochondria (SM), damaged mitochondria (DM), fragmented rough endoplasmic reticulum (FRER), fragmented smooth endoplasmic reticulum (FSER), vacuolation of the cytoplasm (VC), pigments (P), enlarged podocyte cell body (EPC), reduced subpodocyte space (RSPS), proximal convoluted tubule (PCT) with elongated microvilli (EMV), distal convoluted tubule (DCT) with dilated lumen (DL), and glomerular capillary has enlarged blood capillary (EBC). **(A** and **B)** High magnification of the nucleus at Kafr El-Ashraf village and Al-Kinayat city respectively (magnification = 15500×). **(B** and **C)** Kidney cortex components of site II and site "I"; respectively (magnification = 4350×). **(E** and **F)** General morphology of Malpighian corpuscle showing the general morphology of podocyte and glomerular capillary of site "II" and site "I"; respectively (magnification = 6090×).

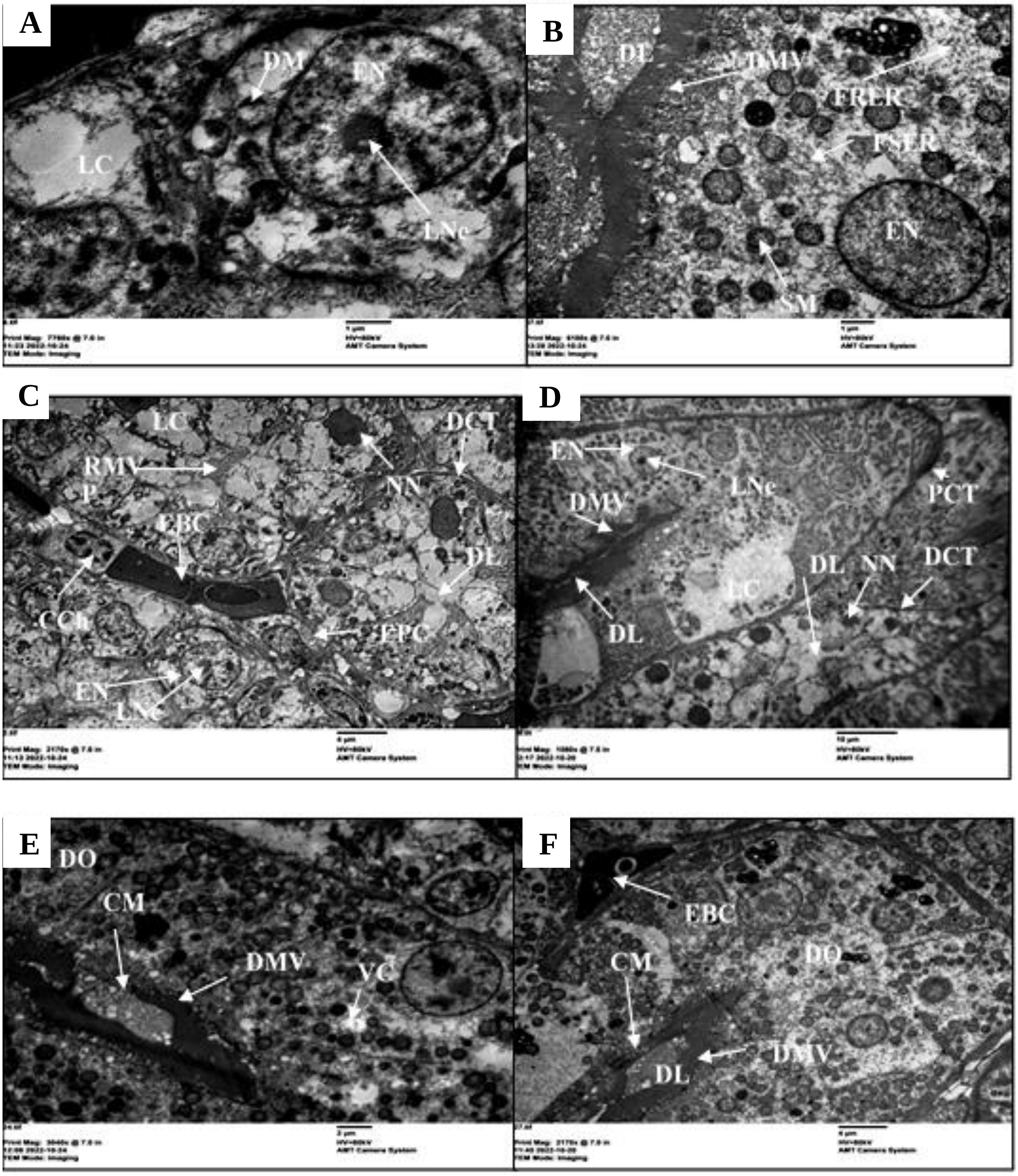

Figure 2: TEM photomicrograph of the kidney cortex components of cattle egret during winter 2022 showing elongated nucleus (EN), necrotic nucleus (NN), large nucleolus (LNe), condensed chromatine (CCh), lytic cytoplasm (LC), degeneration of the cytoplasmic organelles (DO), swollen mitochondria (SM), damaged mitochondria (DM), fragmented rough endoplasmic reticulum (FRER), fragmented smooth endoplasmic reticulum (FSER), vacuolation of the cytoplasm (VC), pigments (P), proximal convoluted tubule (PCT) with reduced microvilli (RMV), disordered microvilli (DMV), cutting in mucous membrane (CM), dilated lumen (DL), distal convoluted tubule (DCT) with dilated lumen (DL), glomerular capillary has enlarged podocyte cell body (EPC), and enlarged blood capillary (EBC). **(A**, **C** and **E)** Kidney cortex components of egrets in site "II" (magnification = 7760×, 2170× and 3040×, respectively). **(B**, **D** and **F)** Kidney cortex components of the cattle egrets in site "I" (magnification = 6180×, 1080× & 2170×, respectively).

of Cr was recorded in wetlands of Pakistan by Boncompagni *et al.*[9].

Selenium toxicity in aquatic birds has been reported by Spallholz and Hoffman[31]. In the current study, the average concentrations of Se detected in kidney samples of cattle egrets collected from the studied sites ranged from 3.60 to 4.63 ppm for site "I" and from 1.13 to 1.91 ppm for site II (Table 2). The concentration in kidneys of birds collected from site "1" in summer was higher than the toxic range for avian species[27], this indicating alarm contamination. Cadmium is a toxic and biopersistent heavy metal. The measured mean concentrations of Cd in kidney of egrets from the two studied sites ranged from 0.12 to 0.36 ppm. These levels were below the findings of Horai *et al.*[29]. Moreover, these levels were lower than the threshold level for bird species[32], this indicating that this metal has no threat to egrets' physiological processes in this study.

Mercury is a non-essential and toxic element. Pesticide, fertilizer, and industrial wastes are the most anthropogenic sources for Hg in the environment. In this study, the highest Hg level was found in egrets' kidney in urban area (ranged between 0.31 and 0.68 ppm). These levels were higher than those observed in egrets from Pakistan[33]. Lead is a highly toxic metal that can cause reproductive effects, and hematological and renal toxicity[34]. The levels of Pb in egret kidneys from both studied areas were ranged from 8.90 to 13.06 ppm, which were similar to those found in grey plover and little egrets[30], but exceeded the lead toxic limit>8[27]. These levels indicate serious pollution.

Metal contamination in blood can result from changes in the habitat, exposure to pollutants by inhalation, and to a greater extent *via* food chain transmission from soils[35]. This may explain the detected higher metal concentration in the blood of egrets at urban area, where they have access to large amounts of food, such as insects and worms in urban garbage. The results for metal concentration in the blood of cattle egret in this study, in comparison to other studies, revealed high levels of arsenic as compared to those reported by Shehzad *et al.*,[36] for avian species and Elarabany and El-Batrawy[37] for cattle egret. On the other hand, Pb, Cd, and Hg levels were comparable to those studies. The survival and productivity of wildlife are impacted by high levels of metal contamination[38]. Chronic metal exposure in birds can have a negative impact on their behavior, and other physiological processes[4]. Hematological parameters including RBCs count and Hct value are commonly used in toxicological and environmental monitoring studies as a potential indicator of physiological changes in birds under stress[39]. In this study, changes in the erythrocytes count and hematocrit values in the blood of the cattle egret between studied sites may be due to heavy metal poisoning particularly lead, mercury, and arsenic toxicity, which can result in red cell shrinkage, decreasing hemoglobin synthesis, and the generation of immature and aberrant erythrocytes in the peripheral blood[40]. According to Katavolos *et al.*[41] increased blood Pb concentrations in in Canada geese causes a decrease in RBCs count and Hct value. This could be a result from heavy metals intoxication, which caused hemolysis and in turn increase destruction of RBCs. In the present study, white blood cells (WBCs) increased significantly in birds of site "I" compared to birds of site "II", this may be attributed to metal toxicity. Leukocytosis in birds is typically associated with infectious or non-infectious causes[42]. This was consistent with the findings of Ogwuegbu and Muhanga[43], who stated that lead and copper cause an increase in WBC count.

The findings of this study revealed that egrets of site "I" have a significant increase in the concentration serum creatinine and urea when compared with birds of site "II". These results were in agreement with other studies on wild bird species such as cattle egret, laughing dove, and squacco heron where urea and creatinine levels increased due to metal pollution[37,44]. Creatinine levels in the blood are naturally constant in normal conditions and any increase in blood creatinine levels could be an indicator of kidney dysfunction. Elevated levels of creatinine could be caused by the toxic effect of metals on kidney in Japanese quail[45]. However, elevated concentrations of urea may reflect present stress or poor status and may indicate nephrotoxicity of some heavy metals such as Cd and Pb[46]. According to Geens *et al*.[47], great tits (*Parus major*) in Antwerp (Belgium) had higher uric acid levels in polluted areas than in less contaminated areas.

The kidney is a major organ for long-term metal accumulation because its primary function is to detoxify the blood of harmful metals. As a result, the accumulated metals may induce deleterious alterations in the kidney's structure. This was the first study to evaluate the effects of metals pollution on kidney of cattle egrets. Ultrastructural alternations including, marked glomerular, mitochondrial and tubular pathological changes were detected in the cortex of kidney of cattle egrets collected from both urban and rural areas in the present study (Figures 1 and 2). Swelling of the mitochondria, marked clumping of chromatin, rough endoplasmic reticulum dilatation and destruction of the proximal tubular epithelium have been reported in the kidney of migratory birds[6] and Japanese quail[48]. According to Geeth *et al*.[49] cadmium and lead are responsible for the pathological alternations of kidney, and these results were also confirmed by the results of heavy metals accumulation in this study.

The current study provided insight into the heavy metal contamination of cattle egrets found in Sharkia Province, Egypt, for the first time. Hematological and bio-chemical changes along with pathological alterations might be utilized to assess the health of birds and give a positive link with various environmental stresses. This study suggested using cattle egret as an efficient bio-indicator for metals pollution. Further, to overcome bio-accumulation and consequently the toxic effect on bird species, efforts should be made to limit the access to hazardous metals in the environment.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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التغيرات الفيزيولوجية والتركيبية الدقيقه في أبو قردان (*Bubulcus ibis*)، كمؤشر حيوي لتلوث التربة بالمعادن الثقيلة في محافظة الشرقية، مصر

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تلوث التربة بالمعادن الثقيلة له عواقب صحية كبيرة على الحياة البرية. هدفت هذه الدراسة إلى تقييم تركيز المعادن الثقيلة وتشمل ،الزرنيخ (As) ، والكادميوم (Cd) ، والكروم (Cr) ، والرصاص (Pb) ، والسيلينيوم (Se) ، والزئبق (Hg) في عينات التربة، وكذلك عينات الكُلى والدم في طيور أبو قردان (*Bubulcus ibis*) في موقعين بمحافظة الشرقية، مصر: الموقع الحضري "I" والموقع الريفي "II" خلال الصيف والشتاء 2021-2022م. وتم قياس بعض المتغيرات الدموية، والكيميائية الحيوية، والتركيبية الدقيقة الكُلوية للطيور. أظهرت النتائج أن أعلى تراكيز للمعادن قد سُجلت في التربة في الصيف في كلا الموقعين. كانت تركيزات المعادن في التربة وعينات الكُلى والدم في الموقع "I" أعلى إحصائيًا من تلك الموجودة في الموقع "II"، باستثناء الكروم الذي يوجد بمستويات منخفضة. كما توجد تركيزات مثيرة للقلق من "As و Pb و Se" في عينات الكُلى للطيور في كلا الموقعين. أظهرت الطيور التي تم جمعها من الموقع "I" انخفاضًا ملحوظًا إحصائيًا في عدد كريات الدم الحمراء، وقيمة الهيماتوكريت، ومحتوى الهيموجلوبين، وزيادة في عدد خلايا الدم البيضاء، ومستويات الكرياتينين واليوريا عند مقارنتها بمستويات الموقع "II". علاوة على ذلك، لوحظ تلف شديد وتضخم في أنسجة الكُلى في أبو قردان في الموقع "I" بالمقارنة مع تلك الموجودة في الموقع "II". بناءً على هذه النتائج، يمكن التنبؤ بالتلوث بالمعادن الثقيلة في البيئة المحيطة من خلال التغيرات في معايير الدم ووظائف الكُلى في طيور أبو قردان.