

## Physiological changes in the cattle Egret, *Bubulcus ibis*, as a Bioindicator of air pollution in New Damietta City, Egypt

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### ABSTRACT

Different heavy metals accumulate in the environment due to intensive human activities, which adversely affect wildlife. Biota usually develops different biological responses to face such stress; analysis of these biological responses could be used as a biomarker of pollutants. The current study aimed to compare hematologic and biochemical parameters in cattle egret collected from industrial and rural sites in New Damietta City, Egypt, to assess the effect of heavy metal pollution. Selected heavy metals concentrations including copper (Cu), cadmium (Cd), zinc (Zn), lead (Pb), nickel (Ni), arsenic (As), and mercury (Hg) were measured for the two sampling sites in aerosols from ambient air, feathers, and blood samples. Some hematological, biochemical parameters and oxidative stress markers were measured in the blood using a non-invasive method. Sampling was done in summer and winter during 2015-2016. Heavy metal concentrations in aerosols from the industrial area were significantly higher than those from the rural site. The highest concentrations of all heavy metals in both sites were recorded in summer. Cattle egret from the industrial area showed a significantly decreased RBCs count and PCV level and increased WBCs count, total protein, albumin, triglycerides, uric acid, ammonia, creatinine, bilirubin, and cortisol levels. Moreover, birds from the industrial area suffered from environmental stress as indicated by elevations of MDA, GPx and catalase activity in industrial birds as compared to rural birds. In conclusion, birds from the industrial areas were adversely affected by the elevated concentrations of selected heavy metals, so it could be used as an effective bioindicator of environmental contamination.

**Key words:** Air Quality, resident birds, heavy metals, biomarkers, oxidative stress, Cattle Egret.

### INTRODUCTION

The ambient air quality has deteriorated to such an extent that it adversely affects the health and welfare of human beings (Das *et al.*, 2015). Air pollution in the last decades is continuously uprising and become a global problem due to different intensive anthropogenic activities including industrial discharges, vehicle emissions and other activities critically affected the atmospheric environment (Swaileh and Sansur, 2006). The exposure to different air pollutants affects all the environmental systems including human health and

wildlife inhabiting such contaminated habitat (Albayrak and Mor, 2011).

Bioindicators are organisms or a species or a group of species, whose function and/or population status can be used to monitor the environmental quality. Changes in the population status, behaviour and physiology of such organisms are used to predict the occurrence of any environmental problem within a given ecosystem. Resident wild birds have been proved to be useful bioindicators of heavy metals levels in the environment because they are widely distributed, spend their entire lives in the same area, have a wide feeding spectrum,

and are sensitive to different environmental toxins (Kekkonen *et al.*, 2012). Birds as a bioindicator can provide a good picture of the possible risks to human more than measuring the physical parameters of the environment itself (Adout *et al.*, 2007). Sonne *et al.* (2010) used the significant decline in raptor populations as an indicator of DDT toxicity.

Cattle egret, *Bubulcus ibis*, is a resident wild bird which could be used as a reliable ecological indicator of atmospheric pollutants because it is resident, widely distributed in different habitats and closely associated to human activities (Thongcharoen *et al.*, 2018).

Most airborne heavy metals are biologically reactive elements which interfere with different metabolic and biochemical processes in a living organism (Koivula *et al.*, 2011). They cause toxic effects by altering the activity of different enzymes, increasing free radicals and disrupting the antioxidant mechanism (Isaksson, 2010).

Many studies have been conducted to evaluate the accumulation of heavy metals in biological samples such as eggs, feathers, brain, kidney and liver (Brait and Antoniosi Filho, 2011; Kaur and Dhanju, 2013; Aloupi *et al.*, 2017). Measuring the accumulation of the heavy metals and their concentration is a not enough tool to estimate the potential risks for the wildlife status but it is important to assess the effect of such pollutants on different physiological functions rather than their concentrations. The current study was conducted in New Damietta region and aimed (1) to use the changes in physiological parameters of cattle egret to assess heavy metal pollution in two different area, industrial area and rural area and (2) to compare the selected heavy metals between the two areas and in biological samples (feather and blood) during summer and winter seasons of 2015-2016.

## MATERIALS AND METHODS

### Study Area

This study was conducted in New Damietta City, Damietta Governorate, Egypt (31° 41' 42" N and 31° 27' 52" E), which is 30 years old and located along the Mediterranean Sea at 4.5 Km from Damietta port. It is also surrounded by wide vegetation areas including palm trees and small villages. The City contains different activities including residential areas (housing), industrial sector, petroleum activities, and reconstruction areas. In the last few decades, this region has an exponential population growth along with extensive anthropogenic pressure due to industrial activities, electricity plants, fertilizer industries, pesticides, chemicals, steel and plastic factories and intensive petroleum activities which may cause severe environmental pollution and affect the biota inhabiting it. Two areas were selected for sampling, industrial area (IA) and rural area (RA).

### Sampling of Aerosols in Ambient Air

Sampling of aerosols was carried out during two seasons (summer and winter) from 2015 to 2016. Sampling was carried out for 8 hrs with a mean flow rate of 1.5 l/min. The sampling equipment was located on a building at about 5 m from the ground. Particles were collected on Whatman 47 mm membrane filters with 0.45 µm pores size. Filters were weighed in temperature and relative humidity control.

### Blood and Feather Sampling

During two seasons (summer and winter) from 2015 to 2016, blood samples and feathers were collected from 23 cattle egrets caught with trap cages in 2 sites of New Damietta city, representing industrial area (IA) and rural area (RA). Licenses for capture and blood sampling of birds were obtained from the Egyptian Environmental Affairs Agency (EEAA). All procedures were approved by the local Ethical

## **Physiological changes in the cattle Egret, *Bubulcus ibis*, as a Bioindicator of air pollution in New Damietta City, Egypt**

Committee and the State Office for Environment Protection.

Birds were caught, weighed, and blood samples were collected from the brachial vein, representing no more than 1% of the body weight (Lumeij, 1997) using a 3ml syringe. Sampling was done with less than three minutes, between capture and blood sampling, to minimize handling stress.

Each blood sample was divided into three aliquots, the 1<sup>st</sup> aliquot was used for heavy metal analysis, the 2<sup>nd</sup> aliquot was used for haematological measurements and the 3<sup>rd</sup> aliquot was left for 1 h and then centrifuged for 20 min; the isolated plasma samples were collected and stored at - 20 °C until assayed for biochemical parameters later in the same day.

Accumulation of heavy metals in feathers occurs during its development which differs across the plumage (Johnston and Janiga, 1995), so feathers from different body parts may have different concentration of the heavy metals (Dauwe *et al.*, 2003). To standardize the procedures, only the two innermost secondary feathers were collected, packed in metal-free polyethylene bags and stored in -20 °C until further analysis. After sampling, birds were released.

### **Heavy Metals Analysis**

Heavy metal concentrations in aerosols samples were determined. A definite section of the filter on which suspended particles were accumulated was cut and digested in 3 M nitric acid. Concentrations of some metals were measured following well-established techniques (Lodge and James, 1988).

Blood and feathers were used as biomarkers to show the immediate and long-term exposure, respectively. Collected feathers were washed with 0.25M NaOH (Scheifler *et al.*, 2006), followed by rinsing 3 times in distilled water, in order to remove any attached

exogenous heavy metals. The washed feathers were allowed to dry for 24 h at 80°C in the oven to a constant dry mass, crunched to powder and weighed to the nearest 0.1 mg using a digital balance (Dauwe *et al.*, 2000). For digestion, 0.5 g from each feather sample was digested in 3:1 nitric acid (70% HNO<sub>3</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), alternately, in a drying oven at 60 °C for 48 h (Adout *et al.*, 2007). The whole blood samples were digested using wet protocol described by Cid *et al.* (2018). Finally, 5 mL of deionized water was added to all samples. All samples were measured for Cu, Cd, Zn, Pb, Ni, As and Hg and determined using atomic absorption spectrophotometer, (analyst100 Spectrometer, Perkin Elmer, USA).

### **Haematological and Biochemical Analysis**

Haematological parameters including blood cells count (RBCs), packed cell volume (PCV), haemoglobin content (Hb), and white blood cells (WBCs) were measured within one hour of sampling using automated blood cell counter.

Plasma samples were analysed for biochemical parameters such as glucose (GLU), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin, urea, uric acid, total bilirubin (TB), creatinine, total lipids (TL), cholesterol, triglycerides and cortisol. Moreover oxidative stress markers including, catalase (CAT), malondialdehyde (MDA) and glutathione peroxidase (GPx) were also measured in plasma, while superoxide dismutase (SOD) activity was measured in RBCs lysate. All parameters were determined using available commercial kits, absorbance values of all samples and standards were measured by a UV spectrophotometer.

### Statistical Analysis

The current study results were presented as means  $\pm$  SE. All parameters were tested for normal distribution. Data were statistically analysed using one-way analysis of variance (ANOVA). Level of significance was considered at  $P < 0.05$ . All statistical analyses were performed using XLSTAT program.

## RESULTS

### Metal Concentrations in Ambient Air

Table (1) shows the atmospheric heavy metal concentrations in both industrial and rural areas measured in summer and winter seasons during 2015-2016. The current study results show that in both study sites, element concentrations during summer were significantly higher than their respective concentrations in winter ( $P < 0.01$ ). Moreover, metal concentrations in the industrial area in both seasons were significantly higher than those in the rural area ( $P < 0.03$ ). Zinc showed the highest concentration in both areas during the two seasons.

During summer the order of metal concentrations in IA area was ordered as follow: Zn > Cu > Pb > Hg > Ni > Cd > As with average concentration  $329 \pm 32.9$ ,  $229 \pm 23.1$ ,  $179 \pm 48.43$ ,  $59.3 \pm 4.4$ ,  $42 \pm 8.6$  and  $16.9 \pm 1.4$  ng/m<sup>3</sup>; respectively. The metal order in the RA was slightly different from their order in IA area, it was Zn > Cu > Pb > Hg > As > Ni > Cd with average concentration  $247 \pm 35.1$ ,  $112 \pm 24.2$ ,  $68 \pm 37.4$ ,  $5.3 \pm 1.45$ ,  $4.6 \pm 0.73$ ,  $3.8 \pm 1.2$  and  $0.87 \pm 0.5$  ng/m<sup>3</sup>; respectively.

Regarding winter samples, the metal order in the aerosols from IA was Zn > Pb > Cu > Ni > Hg > As > Cd with average concentration  $294 \pm 26.4$ ,  $132 \pm 27.3$ ,  $91 \pm 13.84$ ,  $36 \pm 2.5$ ,  $12.5 \pm 5.6$ ,  $9.1 \pm 1.6$  and  $7.7 \pm 0.59$  ng/m<sup>3</sup>; respectively. The metal order in the aerosols from RA was slightly different from their order in IA where Zn > Cu > Pb > Ni > Hg > As > Cd with average concentration  $231 \pm 37.4$ ,  $69 \pm 14.5$ ,  $47 \pm 25.3$ ,  $3.1 \pm 0.95$ ,  $2.6 \pm 0.42$ ,  $1.4 \pm 0.3$  and  $0.56 \pm 0.06$  ng/m<sup>3</sup>, respectively.

**Table (1):** Seasonal atmospheric heavy metals concentrations (ng/m<sup>3</sup>) in the industrial area (IA) and rural area (RA) during summer and winter 2015-2016.

Heavy metal	Areas			
	IA		RA	
	Summer	Winter	Summer	Winter
Cu	229 $\pm$ 23.1*	91 $\pm$ 13.84*	112 $\pm$ 24.2	69 $\pm$ 14.5
Cd	16.9 $\pm$ 1.4*	7.7 $\pm$ 0.59*	0.87 $\pm$ 0.5	0.56 $\pm$ 0.06
Zn	329 $\pm$ 32.9*	294 $\pm$ 26.4*	247 $\pm$ 35.1	231 $\pm$ 37.4
Pb	179 $\pm$ 48.43*	132 $\pm$ 27.3*	68 $\pm$ 37.4	47 $\pm$ 25.3
Ni	42 $\pm$ 8.6*	36 $\pm$ 2.5*	3.8 $\pm$ 1.2	3.1 $\pm$ 0.95
As	13.4 $\pm$ 4.03*	9.1 $\pm$ 1.6*	4.6 $\pm$ 0.73	1.4 $\pm$ 0.3
Hg	59.3 $\pm$ 4.4*	12.5 $\pm$ 5.6*	5.3 $\pm$ 1.45	2.6 $\pm$ 0.42

\* Significantly different in the same season in both sampling localities. Data represented in means  $\pm$ SE.

### Metals Concentrations in Feathers

Table (2) shows element concentrations in feathers of cattle egret from the industrial and rural area. All measured elements in feathers showed significantly higher concentrations in industrial birds during summer than their concentration in rural birds during the

same season ( $P \leq 0.05$ ). Levels of studied elements in feathers of cattle egret during summer in IA are arranged in the following order: Zn > Pb > Cu > Ni > As > Hg > Cd with mean concentrations  $206.3 \pm 9.77$ ,  $8.87 \pm 1.98$ ,  $7.19 \pm 1.68$ ,  $2.46 \pm 0.39$ ,  $0.89 \pm 0.16$ ,  $0.89 \pm 0.15$  and  $0.30 \pm 0.02$  ppm, respectively. Heavy metals order in

### Physiological changes in the cattle Egret, *Bubulcus ibis*, as a Bioindicator of air pollution in New Damietta City, Egypt

RA was Zn > Cu > Pb > Ni > Hg > As > Cd where their mean concentrations were 114.3± 13.75, 5.63± 0.92, 2.35± 0.89, 1.87± 0.13, 0.55± 0.34, 0.42± 0.21, 0.21 ± 0.01 ppm, respectively.

Regarding winter samples, no significant changes were found between industrial and rural birds for all elements except Zn, Ni and Hg which were significantly higher in industrial birds during winter than rural birds ( $P= 0.002$ ,

0.02 and 0.04; respectively). The element order in the feather from IA was slightly different from their order during summer where Zn > Cu > Pb > Ni > Hg > As > Cd with average concentration 148.85± 7.3, 5.36± 0.51, 2.53± 0.92, 1.9± 0.48, 0.51± 0.2, 0.29 ± 0.13 and 0.15± 0.06 ppm, respectively. Rural birds in winter had the same element order in the feather as that of summer samples.

**Table (2):** Seasonal heavy metal concentrations (ppm) in feathers samples of cattle egret, *Bubulcus ibis*, from industrial area (IA, n = 11) and rural area (RA, n= 12) during summer and winter 2015-2016. (n) Represents sample size.

Heavy metals	Areas			
	IA		RA	
	Summer (n=6)	Winter (n=5)	Summer (n=7)	Winter (n=5)
Cu	7.19±1.68*	5.36± 0.51	5.63± 0.92	4.68±0.69
Cd	0.30± 0.02*	0.15± 0.06	0.21 ± 0.01	0.13± 0.03
Zn	206.3± 9.77*	148.85± 7.3*	114.3± 13.75	99.14± 8.27
Pb	8.87± 1.98*	2.53± 0.92	2.35± 0.89	1.21 ± 0.53
Ni	2.46± 0.39*	1.9± 0.48*	1.87± 0.13	0.68± 0.02
As	0.89± 0.16*	0.29 ± 0.13	0.42± 0.21	0.14 ± 0.01
Hg	0.89± 0.15*	0.51± 0.2*	0.55± 0.34	0.32± 0.08

\* Significantly different in the same season in both sampling localities. Data represented in means ±SE.

#### Metals Concentrations in Blood

Table (3) shows metal concentrations in blood samples of cattle egret collected from the industrial and rural area. All analysed elements were detected in the cattle egret blood samples. Blood Cu concentration in cattle egret (mean ±SE) was 1.5± 0.4 and 0.89± 1.4 ppm during summer and winter, respectively in industrial birds, those levels were significantly higher than those in rural birds ( $P < 0.01$ ). Similarly, blood Pb concentration in industrial birds was 1.01± 0.02 and 0.87± 0.59 ppm during summer and winter, respectively which were significantly higher than those of rural birds ( $P < 0.03$ ).

Regarding the other elements, Zn, Ni, As, Hg and Cd, industrial birds contained a significantly higher concentration during summer in comparison to their respective concentration in rural birds ( $P \leq 0.05$ ), but no significant changes were recorded between industrial birds and rural birds during winter. The concentrations of heavy metals in blood samples from IA during summer showed a different order from feathers: Zn > Ni > Cu > Pb > Hg > As > Cd, the same order was found during winter but Cd preceded As. The blood concentrations from rural birds showed the following order: Zn > Ni > Cu > Pb > As > Hg > Cd during summer and Zn > Ni > Pb > Cu > Hg > Cd > As during winter.

**Table (3):** Seasonal heavy metals concentrations (ppm) in blood samples of cattle egret, *Bubulcus ibis*, from industrial area (IA, n= 11) and rural area (RA, n= 12) during summer and winter 2015-2016. (n) Represents sample size.

Blood heavy metal	Areas			
	IA		RA	
	Summer (n=6)	Winter (n=5)	Summer (n=7)	Winter (n=5)
Blood Cu (Cu-B)	1.5± 0.4*	0.89±1.4*	0.7± 0.3	0.23± 0.12
Blood Cd (Cd-B)	0.08±0.01*	0.03± 0.01	0.03± 0.01	0.03±0.001
Blood Zn (Zn-B)	14± 2.41*	2.89± 1.56	2.58± 0.72	2.44± 0.11
Blood Pb (Pb-B)	1.01± 0.02*	0.87± 0.59*	0.63± 0. 2	0.53± 0.12
Blood Ni (Ni-B)	2.71± 0.53*	0.93± 0.79	1.84± 0.70	0.87± 0.43
Blood As (As-B)	0.51±0.01*	0.01± 0.01	0.23± 0.01	0.03± 0.01
Blood Hg (Hg-B)	0.56± 0.21*	0.25± 0.1	0.21± 0.04	0.19± 0.05

\* Significantly different in the same season in both sampling localities. Data represented in means ±SE.

### Haematological Parameters

Table (4) shows the results of red blood corpuscles count (RBCs), white blood cells count (WBCs), packed cell volume value (PCV) and haemoglobin content (Hb) in the blood of cattle egret (*Bubulcus ibis*) caught in the industrial area (IA) and rural area (RA).

In the current study, the season within the same area did not affect

haematological parameters, while habitat did. The count of RBCs and PCV value in blood were significantly lower in cattle egrets collected from the industrial area than those collected from the rural area in both seasons ( $P<0.001$ , Table 4). On the other hand, the number of WBCs in samples collected from the industrial site were significantly higher than those collected from the rural area in both seasons ( $P<0.01$ , Table 4).

**Table (4):** Haematological parameters of cattle egret, *Bubulcus ibis*, from industrial area (IA, n= 11) and rural area (RA, n= 12) during summer and winter 2015-2017. (n) Represents sample size.

Blood parameters	Areas			
	IA		RA	
	Summer (n=6)	Winter (n=5)	Summer (n=7)	Winter (n=5)
RBCs ( $10^6/\mu\text{l}$ )	3.06± 0.6*	3.9±0.41*	4.45± 0.7	4.7±0.32
Hb (g/dl)	12.8± 0.29	11.9± 0.49	11.7± 0.16	10.9± 0.51
PCV (%)	31.52± 5.40*	29±2.07*	55.12± 4.35	45.3± 2.12
WBCs ( $10^3/\mu\text{l}$ )	5.45± 0.95*	4.6± 0.91*	3.35± 1.45	3.9± 0.51

\* Significantly different in the same season in both sampling localities. Data represented in means ±SE.

### Plasma Enzymes and Metabolites:

As shown in Table (5), all plasma biochemical indices were influenced by the habitat. Aspartate aminotransferase (AST) activity was significantly higher in industrial birds than in rural birds during summer ( $P= 0.003$ ) and winter ( $P= 0.002$ ). AST mean activity during summer was  $55\pm 1.92$  U/ml and  $25\pm 3.29$  U/ml in

industrial and rural birds, respectively, while in was  $46\pm 3.41$  and  $30\pm 3.81$  U/ml in IA and RA birds during winter; respectively.

Similarly, alanine aminotransferase (ALT) activity was significantly higher birds collected from the industrial area than those from the rural area during summer ( $P= 0.009$ ) and winter ( $P= 0.003$ ).

**Physiological changes in the cattle Egret, *Bubulcus ibis*, as a Bioindicator of air pollution in New Damietta City, Egypt**

The summer activity of ALT in industrial birds was  $47 \pm 2.84$  U/ml and  $23 \pm 3.21$  U/ml in rural birds; similarly, it was  $30 \pm 3.65$  U/ml in cattle egret from the industrial area and  $18 \pm 1.95$  U/ml in cattle egret from the rural area during winter.

During summer, cattle egret from industrial area had higher mean plasma concentrations of protein ( $11.39 \pm 0.62$  g/l,  $P= 0.002$ ), albumin ( $8.88 \pm 0.3$  g/l,  $P= 0.004$ ), ammonia ( $242.47 \pm 42.25$   $\mu$ mol/l,  $P= 0.001$ ), uric acid ( $17.04 \pm 1.80$  mg/dl,  $P= 0.031$ ), bilirubin ( $3.22 \pm 0.52$ ,  $P= 0.05$ ), creatinine ( $0.54 \pm 0.06$  mg/dl,  $P= 0.002$ ), triglycerides ( $445.16 \pm 53.91$  mg/dl,  $P= 0.02$ ), and cortisol ( $550 \pm 89.5$  ng/ml,  $P= 0.004$ ) than the respective values in rural birds. However, cholesterol was significantly lower in industrial birds than in rural birds ( $225.64 \pm 41.31$  mg/dl,  $P= 0.039$ ).

The same significant differences were found during winter where industrial birds showed higher biochemical indices in comparison to rural birds. In industrial birds, the mean levels of total protein ( $10.62 \pm 0.53$ g/l,  $P= 0.0001$ ), albumin ( $6.83 \pm 0.18$  g/l,  $P= 0.001$ ), ammonia ( $212.90 \pm 26.17$   $\mu$ mol/l,  $P= 0.001$ ), uric acid ( $13.19 \pm 1.52$  mg/dl,  $P= 0.031$ ), bilirubin ( $3.15 \pm 0.94$  mg/dl,  $P= 0.05$ ), creatinine ( $0.44 \pm 0.08$  mg/dl,  $P= 0.002$ ), triglycerides ( $421.51 \pm 55.16$  mg/dl,  $P= 0.02$ ), and cortisol ( $420 \pm 28.3$  ng/ml,  $P= 0.004$ ) had higher mean plasma concentrations than the respective rural birds while cholesterol was significantly lower in industrial birds than rural birds ( $191.54 \pm 32.87$  mg/dl,  $P= 0.039$ ). Finally, other parameters such as glucose and urea did not change significantly either between seasons nor habitat.

**Table (5):** Biochemical parameters of cattle egret, *Bubulcus ibis*, from industrial area (IA, n= 11) and rural area (RA, n= 12) during summer and winter 2015-2016. (n) Represents sample size.

Plasma parameters	Areas			
	IA		RA	
	Summer (n=6)	Winter (n=5)	Summer (n=7)	Winter (n=5)
AST (U/ml)	$55 \pm 1.92^*$	$46 \pm 3.41^*$	$25 \pm 3.29$	$30 \pm 3.81$
ALT (U/ml)	$47 \pm 2.84^*$	$30 \pm 3.65^*$	$23 \pm 3.21$	$18 \pm 1.95$
T. protein (g/dl)	$11.39 \pm 0.62^*$	$10.62 \pm 0.53^*$	$6.70 \pm 0.49$	$5.48 \pm 0.73$
Albumin (g/dl)	$8.88 \pm 0.3^*$	$6.83 \pm 0.18^*$	$2.53 \pm 0.1$	$2.68 \pm 0.17$
Ammonia ( $\mu$ mol/l)	$242.47 \pm 42.25^*$	$212.90 \pm 26.17^*$	$144.09 \pm 9.78$	$106.99 \pm 35.13$
Urea (g/dl)	$7.43 \pm 0.84$	$8.22 \pm 0.67$	$8.12 \pm 0.85$	$8.96 \pm 1.02$
Uric Acid (mg/dl)	$17.04 \pm 1.80^*$	$13.19 \pm 1.52^*$	$4.07 \pm 0.45$	$3.85 \pm 1.24$
Bilirubin (mg/dl)	$3.22 \pm 0.52^*$	$3.15 \pm 0.94^*$	$1.31 \pm 0.51$	$1.23 \pm 0.43$
Creatinine (mg/dl)	$0.54 \pm 0.06^*$	$0.44 \pm 0.08^*$	$0.15 \pm 0.03$	$0.15 \pm 0.06$
Cholesterol (mg/dl)	$225.64 \pm 41.31^*$	$191.54 \pm 32.8^*$	$474.36 \pm 13.88$	$464.10 \pm 24.08$
Triglycerides (mg/dl)	$445.16 \pm 53.91^*$	$421.51 \pm 55.2^*$	$212.9 \pm 17.38$	$208.60 \pm 55.16$
Cortisol (ng/ml)	$550 \pm 89.5^*$	$420 \pm 28.3^*$	$102 \pm 7.8$	$98 \pm 6.8$
Glucose (mg/dl)	$50.31 \pm 3.61$	$46.88 \pm 4.47$	$49.06 \pm 7.36$	$50.69 \pm 11.34$

\* Significantly different in the same season in both sampling localities. Data represented in means  $\pm$ SE.





### Oxidative Stress Biomarkers

It was obvious from Table (6) that season did not affect the oxidative stress biomarkers in cattle egret, while sampling site seemed to be the principal source of change. In relation to the oxidative stress, lipid peroxidation product (malondialdehyde, MDA) was significantly higher in industrial birds in both summer and winter than the rural birds ( $P= 0.004, 0.02, \text{ respectively}$ ). Moreover, the antioxidant defence activity

was also affected by the sampling site rather than the season, glutathione peroxidase activity increased significantly in industrial birds in both summer and winter as compared to rural birds ( $P= 0.01, 0.05; \text{ respectively}$ ). Similarly, catalase activity was significantly higher in industrial samples in both summer and winter than rural birds ( $P= 0.0001, 0.041, \text{ respectively}$ ). Superoxide dismutase (SOD), on the other hand, did not show any significant changes.

**Table (6):** Oxidative stress biomarkers of cattle egret, *Bubulcus ibis*, from industrial area (IA, n= 11) and rural area (RA, n= 12) during summer and winter 2015-2017. (n) Represents sample size.

Parameters	Concentration			
	IA		RA	
	Summer (n=6)	Winter (n=5)	Summer (n=7)	Winter (n= 5)
MDA (nmol/ml)	79.87±7.853*	59.8±9.06*	29.0±11.10	18.0±7.02
GPx (U/ml)	13.67±1.34*	12.53±1.166*	22.00 ±1.04	18.5± 0.95
CAT (U/l)	529.3±38.11*	496.3± 38.46*	135.6±34.40	120.4± 41.12
SOD (U/gm)	165± 32.8	162±21.2	157±19.3	153±6.6

\* Significantly different in the same season in both sampling localities. Data represented in means ±SE.

### DISCUSSION

Bio-indicators and the changes in their biomarkers could be used as an early warning of environmental contamination and to improve the processes of risk assessment for populations and their habitat (Martínez-Gómez *et al.*, 2010). New Damietta region is considered one of the most important habitats for about 26 resident wild bird species and 40 migratory bird species (Abd-Allah *et al.*, 2008). According to the available literature, the current work is the first study in Egypt using the resident wild birds (cattle egret) for bio-monitoring selected heavy metals in ambient air (Cu, Cd, Zn, Pb, Ni, As, and Hg).

#### Metal Concentrations in Ambient Air

Heavy metal concentrations in aerosols from the industrial area have significantly higher concentrations for all

metals compared to those from the rural siteduring both seasons. The highest concentrations of all heavy metals in both sites were recorded in summer may be due to the extensive anthropogenic activities as transportation, industrial facilities and open waste burning which lead to increased emission of particulate matter (Salam *et al.*, 2008). According to the heavy metal order, zinc had the highest concentration in both areas and both seasons followed by Cu and Pb, while As and Cd had the lowest concentrations.

Zinc present in the air naturally but its concentrations are expected to elevate abnormally due to different anthropogenic activities such as construction work, traffic-related and residential activities (Awan *et al.*, 2013). Copper can enter the environment through waste dumps, combustion of fossil fuels and wastes,

## **Physiological changes in the cattle Egret, *Bubulcus ibis*, as a Bioindicator of air pollution in New Damietta City, Egypt**

wood production and phosphate fertilizer production (ATSDR, 2004).

The concentration of Pb in aerosol samples were within the permissible limits ( $1.0 \mu\text{g}/\text{m}^3$ ) recommended by the Egyptian Environmental Affairs Agency (EEAA, 1995). Pb has been considered as a marker of vehicle traffic as constituents of leaded gasoline (Cheng *et al.*, 2014). The lead-free fuel in vehicles may be responsible for the lower concentrations of Pb at all sites.

The mean concentration of Ni in IA was found higher than the permissible limit ( $20 \text{ ng}/\text{m}^3$ ) of National Ambient Air Quality Standard (NAAQS, 2009) during both seasons. Both arsenic and cadmium are very toxic elements which present naturally in the environment with very low levels. The mean concentration of As in IA was found higher than the permissible limit ( $6 \text{ ng}/\text{m}^3$ ) (NAAQS, 2009) during both seasons, while Cd levels were found below the permissible limit ( $15 \text{ ng}/\text{m}^3$ ) (NAAQS, 1994) at all sites during both seasons except for IA in summer season.

As may be produced by copper producing industries or agricultural activities, while Cd mainly emitted from Industrial processes; burning of municipal wastes containing discarded Ni-Cd batteries and plastics containing Cd pigments combustion sources and vehicular emissions including tire abrasions (Pal *et al.*, 2014). Generally; heavy metal concentrations of IA of New Damietta City are lower than those reported for other industrial areas in Egypt (Zakey *et al.*, 2008; El-Batrawy *et al.*, 2017).

### **Metal Concentrations in Biological Samples**

Feather is an indicator of long time exposure such as bioaccumulation of blood metals during their formation or mobilisation from internal organs (Leonzio *et al.*, 2009), while blood reflects a limited or present time of exposure to heavy metals (Burger and Gochfeld, 2002).

Accumulation of heavy metals in the developing feather is considered to be an effective way of detoxification (Agusa *et al.*, 2005). The levels of heavy metals in the feathers and blood of adult cattle egrets have not been studied previously in Egypt, only one study reported their concentration in feathers of cattle egret chicks in Cairo (Burger *et al.*, 1992). The concentration of different heavy metals in the bird's blood are highly regulated, any changes in their baseline concentration are indicators of changes in the bird's diet, habitat, mobilisation from internal tissue or even exposure to anthropogenic contaminants by inhalation (Rattner *et al.*, 2008).

Heavy metal concentrations in feathers from industrial birds were greater than those in rural birds which indicate local sources for contamination such as the extensive marble industries, transportation activities, wood preservation, construction activities, petroleum refining activities and burning solid waste in New Damietta City.

Copper is very important for maintaining normal growth, metabolism and function (Pappas *et al.*, 2006), Cu level in feather in the current study (ranged from 4.68 to 7.19 ppm) was higher than those reported for cattle egret in Pakistan (Malik and Zeb, 2009) and grey heron from Korea (Kim and Koo, 2007).

Cadmium is a toxic, non-essential heavy metal which does not accumulate in the food chain (Burger and Gochfeld, 2004). The average Cd level measured in egret feathers in the current study (ranged from 0.13 to 0.3 ppm) was higher than those recorded in common eider (Burger and Gochfeld, 2009) but lower than the threshold concentration of  $2 \mu\text{g}/\text{g}$  which may adversely affect birds (Burger and Gochfeld, 2000).

Zinc is an essential heavy metal for feather development, normal body functions and reduces Cd-induced renal toxicity. However, the concentration of Zn in the current study (ranged from 99.14 to 206 ppm) is higher than those of black-

crowned herons (Golden *et al.*, 2003), tufted puffin (Burger and Gochfeld, 2009) and little egret (Zhang *et al.*, 2006), but still lower than the dose which cause severe kidney damage, 200 mg/g, (Hutton and Goodman, 1980).

Lead is a highly toxic, non-degradable heavy metal which cannot be regulated (Scheifler *et al.*, 2006). The concentrations obtained in this study for Pb feathers were ranged from 1.21 to 8.81 ppm which were lower than those of Korean shorebirds (Kim and Koo, 2008) and great cormorants from Japan (Nam *et al.*, 2005) but higher than those in Black-crowned night heron (Kim and Koo, 2007).

Nickel is associated to feather pigmentation, Ni level recorded in the current study ranged between 2.46 and 0.68 ppm which were lower than those reported by Malik and Zeb (2009) in cattle egret from Ravi river, Pakistan but higher than its concentration in great tits from Belgium (Dauwe *et al.*, 2004).

Mercury emissions from industrial and natural sources have the ability to bioaccumulate in different animals. Mercury level in the current study ranged between 0.51 and 0.89 ppm in IA which is higher than those estimated in Osprey (Hughes *et al.*, 1997) and similar to those estimated in black-crowned night-herons (Golden *et al.*, 2003).

Arsenic, on the other hand, is a serious toxin (Mudhoo *et al.*, 2011), in the current study, cattle egret feather had As level from 0.32 to 0.89 ppm which is lower than those recorded for cattle egret in Pakistan (Abdullah *et al.*, 2015).

It has been suggested that concentration of heavy metals in blood reflect their level in food (Evers *et al.*, 2005), this may explain the higher accumulation of metals in the blood of industrial birds because they have large quantities of food represented by insects and worms available in urban garbage and industrial wastes. In comparison to other studies, the cattle egret blood in the current

study showed higher concentration in As, Cd, Hg, Zn and Cu as compared to black-crowned night heron from highly contaminated Baltimore harbour, USA (Golden *et al.*, 2003) and higher Ni and Pb levels as compared to Ospreys from Chesapeake and Delaware Bays, USA (Rattner *et al.*, 2008).

### Haematological Parameters

Differences in erythrocytes count and packed cell volume in the blood of the cattle egret between the two study area may be due to intoxication with heavy metal especially lead and zinc; Pb, for example, suppresses aminolevulinic acid dehydratase and ferrochelatase which are important for synthesis of haeme causing decreased haemoglobin synthesis and resulting in anaemia, appearance of immature and abnormal red blood cells in the peripheral blood (Pattee *et al.*, 2006; Katavolos *et al.*, 2007).

Red blood cell count and hematocrit changes could be used as a predictor of poisoning with heavy metal in birds (Millaku *et al.*, 2015). Similar to the current study results, Katavolos *et al.* (2007) found a decreased RBCs count and PCV levels in Canada geese, this could be explained by heavy metals intoxication which is responsible for hemolysis which in turn increase osmotic fragility and anaemia especially Pb and Cd. Moreover, elevated concentrations of heavy metals caused increased hemolysis and destruction of RBCs (Leggett, 1993).

White blood cells in the current study increased significantly in industrial birds as compared to rural birds. Leukocytosis in birds is usually associated with infectious or non-infectious reasons leading to immune system impairments (Alagbe, 2015), this finding is in agreement with Ogwuegbu and Muhanga (2005) who reported that lead and copper trigger an increase in WBCs count.

## **Physiological changes in the cattle Egret, *Bubulcus ibis*, as a Bioindicator of air pollution in New Damietta City, Egypt**

### **Biochemical Parameters**

Biochemically, the current study results showed IA birds have a significant increase in the activity of ALT and AST and in the concentration of total protein, albumin, ammonia, uric acid, bilirubin, creatinine, triglycerides and cortisol while cholesterol levels were significantly lower in IA than RA.

In this study, both ALT and AST showed a significant increase in the IA birds compared with RA birds. The increases in ALT and AST above the normal values usually reflect liver damage (Rao *et al.*, 2006). Therefore, the increments in those enzymes in cattle egret in IA may be an indicator of histopathological damage to the liver, especially liver is the main organ involved in detoxification (Akan *et al.*, 2010). In line with the current results, AST and ALT activities in Japanese quail increased significantly when exposed to Cd and Pb, meaning severe liver damage (Hamidipour *et al.*, 2016). In contrast, ALT and AST decreased in broiler chicks after exposure to lead and arsenic (Alagbe, 2015).

The current study also concerned with whether total protein or albumin are affected by metals or not. Both total protein and albumin levels were significantly higher in IA birds than RA birds. Increased levels of total protein and albumin could be an indicator for high protein diet (Jenni and Jenni-Eiermann, 1998) rather than metal induced effect. Cattle egret usually feed on different insects such as crickets, flies, moths, grasshopper, spiders and earthworms (Seedikkoya *et al.*, 2007). The main waste dumping yard of new Damietta city is located within IA (the industrial sector of the city), solid and decaying wastes from factories, shops, residences, vegetable markets, hospitals and slaughterhouses are dumped every day. Based on this fact, this waste yard is a rich source for the favoured cattle egret food that is richer in proteins content as compared to the rural area

which contains mainly vegetation. Similar results were found also in cattle egret populations collected from different sites in India (Seedikkoya, 2003; Seedikkoya *et al.*, 2005)

In this study, creatinine and uric acid concentrations were significantly higher in IA birds than RA birds. Creatinine level is naturally constant in normal status and its increase in blood is an indicator of kidney dysfunction. Similar results were found in quails (Hamidipour *et al.*, 2016). This increment in creatinine may be caused by the toxic impact of some elements such as Pb and Cd on kidney function. Moreover, uric acid increase may indicate nephrotoxicity of some heavy metal especially Pb and Cd (Khaki *et al.*, 2011). Elevated uric acid levels may reflect present stress, poor status, or a compensation mechanism (Cohen *et al.*, 2007). In the same line, great tits *Parus major* showed elevated uric acid levels in the polluted area more than their respective level in less polluted area in Antwerp, Belgium (Geens *et al.*, 2009).

Based on the results of this study, triglyceride concentration was significantly higher in industrial birds than in rural birds. Elevated concentrations of triglyceride usually is indicator of fat absorption (Jenni and Jenni-Eiermann, 1998) which reflects the feeding status of cattle egret and abundance of its food in this study. Similar findings were reported in great tits (*Parus major*) nestlings where they showed high triglyceride levels in polluted area indicating an abundance of food and high feeding rate (Geens *et al.*, 2009). On contrary, triglyceride level in Japanese quail dropped significantly after exposure to lead indicating malabsorption (Hamidipour *et al.*, 2016).

The increase in cortisol is widely used as a biomarker of heavy metal-induced stress (Franceschini *et al.*, 2009), regarding the current results, IA birds had elevated cortisol levels more than those from RA. These results in agreement with

another study on birds where cortisol level increased due to metal pollution (Wayland *et al.*, 2003), similarly, increased cortisol concentration was reported in marine iguanas (*Amblyrhynchus cristatus*) following acute exposure 7 days after an oil spill (Wikelski *et al.*, 2001). In disagreement with our results, Franceschini *et al.* (2009) reported that the corticosterone level increased as a stress response induced by mercury in free-living tree swallows, *Tachycineta bicolor*. It appears cortisol behave without predictable patterns with different contaminants.

### Oxidative Stress Biomarkers

The present results show significant changes in oxidative stress biomarkers and some antioxidant defences parameters (MDA, CAT and GPx activities) except SOD in the blood of cattle egret from metal contaminated area (such as industrial sector in New Damietta city). These changes indicate that the birds are affected by oxidative stress in response to heavy metal contamination.

Cu and Zn are redox-active metals while Pb, Cd, As and Hg are redox-inactive elements which can produce oxidative stress by interfering with pro-oxidant/antioxidant balance (Koivula and Eeva, 2010). In general, these elements can produce reactive oxygen species (ROS) and may decrease the major antioxidants of cells (i.e. GPx) and change the levels of MDA, SOD and CAT (Espín *et al.*, 2016).

The significant increase in malondialdehyde level (MDA) in the polluted area (IA) could be used as a useful biomarker for oxidative stress and lipid peroxidation in cattle egret exposed to metal contamination. Similar results were reported in different birds such as white stork, *Ciconia ciconia*, chicks (Tkachenko and Kurhaluk, 2014) and forester's terns, *Sterna forsteri*, chicks (Hoffman *et al.*, 2011).

Glutathione peroxidase (GPx) is mainly involved in biotransformation mechanisms, thiol transfer, and free radicals destruction (Rana *et al.*, 2002), so changes in GPx activity could be considered as a valuable biomarker to assess increased oxidative stress induced by metal (Isaksson, 2010).

The current results show that GPx activity decreased significantly in IA birds as compared to RA birds, many heavy metals have the ability to inhibit antioxidant enzymes by interacting with sulfhydryl groups of them. These results are in agreement with Mateo *et al.* (2003) who found significantly low GPx activity in mallard, *Anas platyrhynchos*, and Canada goose, *Branta Canadensis*, after exposure to lead and also in Eurasian eagle owls (*Bubo bubo*) exposed to different heavy metals (Espín *et al.*, 2014b). In contrast to the current results, GPx activity showed a significant increment in white stork (Tkachenko and Kurhaluk, 2014) and house sparrow, *Passer domesticus* (Herrera-Duenas *et al.*, 2014).

Birds that live in metal-polluted habitats may have higher oxidative stress or changes in enzyme activity as a result of metal exposure. CAT is a liver enzyme which is related to oxidative stress, in the present study, cattle egret from IA showed a significantly higher catalase activity than those from RA. In the same line, pied flycatcher nestlings exhibited an increase in CAT activity in relation to Pb contamination in northern Sweden (Berglund *et al.*, 2007) and Griffon vulture (*Gyps fulvus*) during exposure to Cd and Cu in Valencia, Spain (Espín *et al.*, 2014a). On the contrary, house sparrows had a significant decline in CAT activity after 30 days exposure to Pb (Cid *et al.*, 2018), while exposure to some heavy metal did not alter the CAT activity in Japanese quail (Paskova *et al.*, 2011).

Generally, exposure to heavy metal contaminated area caused oxidative stress in cattle egret. Berglund *et al.* (2007) also found that pied flycatcher, *Ficedula*

## Physiological changes in the cattle Egret, *Bubulcus ibis*, as a Bioindicator of air pollution in New Damietta City, Egypt

*hypoleuca*, nestlings in industrial area were affected by oxidative damage. Moreover, Pied Flycatchers exposed to concentrations of arsenic, calcium, cadmium, copper, nickel, lead and zinc showed increased oxidative stress markers near Cu-Ni smelters (Berglund *et al.*, 2011).

The current results could be concluded that heavy metals induced deleterious effects on cattle egret. The decreased haematological parameters and changes in biochemical measurements along with increased oxidative damage and decreased antioxidant mechanisms could be used as a useful tool to assess the health condition of the birds and provides positive correlation with different environmental burdens. Cattle egret could be a potential tool for bio-monitoring local heavy metal pollution, but further studies should be conducted for several successive years on bioaccumulation of heavy metals in birds and plants as well to give a realistic picture on current environmental contamination in these areas.

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## Physiological changes in the cattle Egret, *Bubulcus ibis*, as a Bioindicator of air pollution in New Damietta City, Egypt

التغيرات الفسيولوجية في بلشون الماشية، *Bubulcus ibis*، كمؤشر حيوي لتلوث الهواء بمدينة دمياط الجديدة، مصر

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### المستخلص

تتراكم المعادن الثقيلة المختلفة في البيئة نتيجة للأنشطة البشرية المكثفة، والتي تؤثر سلباً على الحياة البرية. تطور الكائنات الحية استجابات بيولوجية مختلفة لمواجهة مثل هذه الضغوط، يمكن تحليل هذه الاستجابات البيولوجية واستخدامها كدلالات حيوية للملوثات. هدفت الدراسة الحالية لمقارنة بعض معاملات الدم وبعض المعايير البيوكيميائية في بلشون الماشية والتي تم صيدها من منطقة صناعية ومنطقة زراعية بمدينة دمياط الجديدة، مصر وذلك لتقييم أثر تلوث الهواء بالمعادن الثقيلة. وقد تم قياس تركيزات المعادن الثقيلة المختارة وهي النحاس (Cu) و الكاديوم (Cd) و الزنك (Zn) والرصاص (Pb) والنيكل (Ni) والزرنيخ (As) والزنبق (Hg) لكلا الموقعين في عينات من الهواء المحيط والريش والدم. تم قياس بعض معايير الدم وبعض الدلالات البيوكيميائية ومؤشرات جهد الأكسدة في الدم بطريقة غير عنيفة. تم تجميع العينات في شتاء وصيف عام 2015-2016. كانت تركيزات المعادن الثقيلة في عينات الهواء المجمعة من المنطقة الصناعية أعلى معنوياً من مثيلاتها في المنطقة الزراعية. وتم تسجيل أعلى التركيزات لجميع المعادن الثقيلة في فصل الصيف لكلا من المنطقة الصناعية والمنطقة الزراعية. أشارت القياسات الحيوية في بلشون الماشية إلى انخفاض معنوي في عدد كرات الدم الحمراء وحجم الخلايا المكسدة وارتفاع معنوي في عدد خلايا الدم البيضاء وتركيز كلا من البروتين الكلي والألبومين و الدهون الثلاثية وحمض اليوريك والأمونيا والكرياتينين والبليروبين ومستويات الكورتيزول. علاوة على ذلك، عانت طيور المنطقة الصناعية من إجهاد بيئي ملحوظ كما هو موضح بارتفاع معنوي في مستوى المألون داي الدهيد (MDA) وارتفاع نشاط الجلوتاثيون بيروكسيداز (GPx) والكاتاليز مقارنة بطيور المنطقة الزراعية. ويمكن القول بأن طيور المنطقة الصناعية تأثرت سلباً بوجود تركيزات عالية من المعادن الثقيلة في الهواء، وبالتالي يمكن استخدام بلشون الماشية كمؤشر حيوي فعال للتلوث البيئي.