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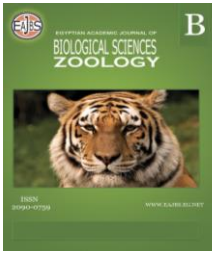

The Efficiency of *Sclerophrys regularis* (Anura: Bufonidae) as a Bioindicator For Habitat Destruction

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ABSTRACT

Many populations of amphibians are facing remarkable decline throughout the world. The reason for the decline is a direct response to habitat destruction and pollution including heavy metals. The blood of the toad is vascular and very plastic tissue. In fact, a variation of several hematological parameters in response to natural changes in the environment has been widely described previously. Since hematological parameters are influenced by a variety of environmental stressors, they have the potential to be used as biomarkers of aquatic pollution. Heavy metals are difficult to remove from the environment and cannot be degraded biologically. The current study assessed the impact of some heavy metals on hematological and serological parameters in the Egyptian toad (*Sclerophrys regularis*) from two different localities (North Giza and El Wahat regions). The current results have revealed a reduction in the means of RBCs, hemoglobin, and hematocrit values in addition to a remarkable increase in WBCs. In addition, the major serological parameters including protein, albumin, AST, and ALT were also altered in parallel with the deterioration that occurred between the two investigated regions. The present findings have revealed and confirmed that the Abu Rawash area is more impacted compared to El Bawiti.

INTRODUCTION

Amphibians play a pivotal role in properly functioning ecosystems, sharing in nutrient cycling, bio duration, energy flow, food webs, and other ecosystem dynamics (Hocking and Babbitt, 2014; Cortés-Gómez *et al.*, 2015). Indeed, these animals provide additional ecosystem services valuable to humans, such as regulating pests, serving as a food source, functioning as models for medical research, and giving enjoyment and intangible contributions that vary across cultures (Warkentin et al., 2009). Amphibians, a unique group of vertebrates containing over 7000 known species, are threatened worldwide and the number of extinct and threatened species will probably continue to rise around the world (Pounds *et al.*, 1997; Stuart *et al.*, 2004). There is little evidence for a single factor causing this decline, but multiple factors as increased ultraviolet radiation, fungal and bacterial epidemics, droughts, acid precipitation, climate changes, habitat destruction and fragmentation, exotic species, heavy metals, acid rain, pesticides, fertilizers can act together to cause mortality or

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sublethal effects. Severe environmental degradation is a logical reason responsible for declining (Blaustein and Johnson, 2003). Because of their central place in the food chain (being both prey and predator), amphibians often utilize both terrestrial and aquatic habitats and can have very different feeding ecologies at different stages of their life. Accordingly, they are considered excellent bioindicators of environmental health (Tejedo, 2003; Said, 2013). The most important family of the class Amphibia is the Bufonidae, which is distributed in all parts of the world except for Antarctica due to their wide distribution, bufonids are frequently used as model organisms in experimental biology studies. The genus Sclerophrys (previously part of the genus Bufo) is a widespread and well-known bufonid including the African Common Toad. Recently, the name *Sclerophrys regularis* (Reuss, 1833) was applied (Borkin and Litvinchuk, 2013; Ohler and Dubois, 2016).

The African common is distributed across a wide geographic range and is abundant, found in both moist and dry savanna, forest margins, Oases, montane grassland, and agricultural habitats. *Sclerophrys regularis* have moist, permeable skin and unshelled eggs that are directly exposed to soil, water and sunlight, and that can readily absorb toxic substances such as chemical pollution including heavy metals (Donnelly and Crump, 1998). Heavy metals represent a major environmental problem of increasing concern and their monitoring has received significant attention in the field and under laboratory conditions (Osman *et al.*, 2010). Because of their high degree of toxicity, some heavy metals are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure(Tchounwou *et al.*, 2012). Heavy metals can be taken up into toads either from ingestion of contaminated food via the alimentary tract or through the gills and skin (Fazio *et al.,* 2014). Effectively, after the absorption, metals in toads are then transported through the blood stream to the organs and tissues where they are accumulated (Krishnani *et al.*, 2003; Dupuy *et al.*, 2015). In general, metals can be categorized as biologically essential and non-essential. The nonessential metals (e.g., cadmium (Cd) and lead (Pb) have no proven biological function (also called xenobiotic or foreign elements), and their toxicity rises with increasing concentrations (Birungi *et al.*, 2007). Essential metals (e.g., chromium (Cr), and iron (Fe), manganese (MN)) on the other hand, have a known important biological role (Vinodhini and Narayanan, 2008) and toxicity occurs either at metabolic deficiencies or at high concentrations. The Impact of pollutants, particularly heavy metals on the blood parameters of many aquatic faunas including amphibian have received global attention. Recently, haematological variables have become promising biomarkers in measuring the effects of pollution, because blood parameters respond to low doses of pollutants. Blood parameters are important in diagnosing the structural and functional status of animals exposed to environmental pollutants. This study was conducted to investigate of the hemotoxic effect of some heavy metals using haemato-serological parameters of Egyptian toad *Sclerophrys regularis* as biomarkers responses for heavy metals impact.

MATERIALS AND METHODS

Study Area:

Two areas were selected (Fig. 1) for the current study (1) Abu Rawash area, located about 8 kilometers north of Giza, Egypt. This area receives a variety of pollutants from the point and non-point sources including municipal wastes, fertilizers, chemical wastes, in addition to the wastewater treatment plants. (2) El Bawiti area, northern Bahariya Oases, western desert, Egypt. The Bahariya Oases (El Wahat) located in the mid of the western desert at about 370 km to the southwest of Cairo. On the other hand, springs and wells are the main two-groundwater resources of water there. The Egyptian toad *Sclerophrys regularis*

(Reuss, 1833) a species of toad in the family: Bufonidae, Order: Anura was selected as study animals.

Fig. 1: Map showing the two studied regions, site 1 (Abu Rawash) and site 2 (El-Bawiti).

Sampling*:*

The sampling protocol starts during the breeding season of *Sclerophrus regularis.* Toads were collected around 2 km from each site. For metal analysis, water samples were collected by acid-washed polyvinyl chloride Van Dorn bottle. Total Fe, Pb, Cd and Cr were measured after digestion using Graphite Furnace AA (GFAA) spectroscopy. Soil samples were transported in clean plastic pages. For the digestion of soil samples, 1 gram sieved sediment is digested with repeated addition of nitric acid and hydrogen peroxide according to (Jackwerth and Würfels, 1994). For metal analysis in tissue, the liver was selected. One gram of toad liver is digested by means of a microwave after the addition of nitric acid and hydrogen peroxide as mentioned above. The metal concentration was calculated in µg/g wet weight for tissue, µg/g for sediment and µg/l for water. Some physicochemical parameters including water pH, electrical conductivity of the water (µS/cm), Total solids (TS) (mg/dl), Sulfate (SO_4) (mg/dl), phosphate (PO_4^{3-}) (mg/dl), ammonia (NH_3) (mg/dl), Nitrate (NO_3) (mg/dl), Alkalinity (mg/dl) and Soil pH were measured. Water pH and electrical conductivity of the water (µS/cm) were measured routinely using a water checker, soil pH according to (Alban and Kellogg, 1959) and the rest of the parameters were measured according to the traditional manual methods (APHA, 2005).

Hematological Analysis*:*

Blood samples were collected from the heart ventricle of individuals. Blood was collected using syringes in test tubes containing 1 mg of the anticoagulant for CBC count, for biochemical analysis blood was centrifuged. Red blood cells and white blood cells were counted manually using a hemocytometer (Shah and Altindağ, 2005). Red blood cells (RBCs) were counted in duplicate in an improved Neubauer chamber, after a 1:200 dilution of entire blood in Natt and Herrick’s Solution (Natt and Herrick, 1952). Hemoglobin (Hb) concentrations were determined by the cyanmethemoglobin method using a 1:200 dilution of entire blood in Drabkin’s solution (Drabkin and Austin, 1935).To estimate hematocrit or packed cell volume (PCV), blood was collected in heparinized capillary tubes that were centrifuged at 10,000 *g* for 5 min. Mean corpuscular volume (MCV), which represents an estimate of RBCs' volume, was calculated as:

MCV (fL)= (PCV (L/L)/RBC (1012cells/L)

Mean corpuscular haemoglobin (MCH), the average concentration of Hb per RBC, was calculated as: MCH (pg)= [Hb (g/dL) ×10]/RBC (1012cells/L).

Serological Analysis*:*

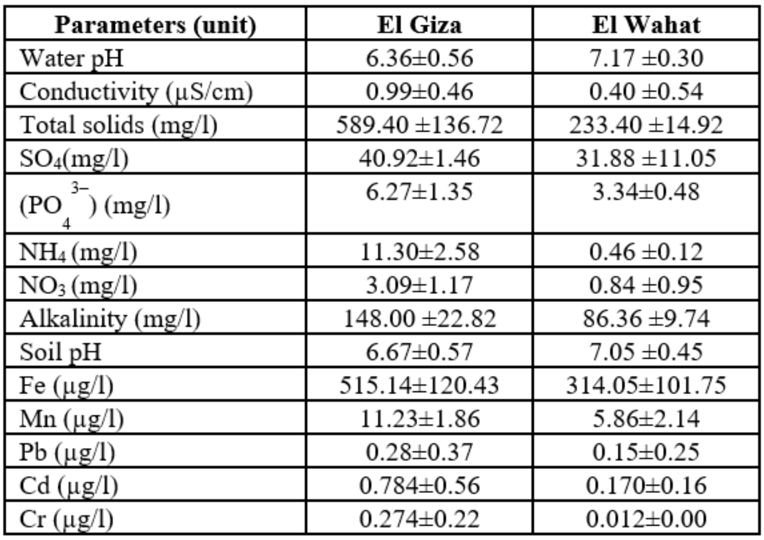
Biochemical analysis was done on serum samples in both Giza and El Wahat group toad by collecting the blood in small glass tubes and put in a centrifuge at 2000 rounds per minute to obtain the serum. Biochemical parameters were analyzed for total protein, albumin, bilirubin alanine aminotransferase (ALT), aspartic aminotransferase (AST) and creatinine. Colorimetric determinations of the selected biochemical parameters were performed using a spectrophotometer (Jasco-V530). The absorbency of the detected sample was examined at an appropriate wavelength ranging from 340 to 546 nm according to the parameter tested. Commercial diagnostic kits from Bio-Merieux chemicals were used. Serum total protein (g/dl), albumin(mg/dl), and bilirubin(mg/dl) was determined using a biuret test according to (Henry et al., 1974). Serum creatinine (mg/dl) was estimated according to the method of (Friedewald *et al.*, 1972). Activities of alanine aminotransferase (ALT, U/I) and aspartate aminotransferase (AST, U/I) were determined calorimetrically according to (Reitman and Frankel, 1957). All data were processed as five replicates for each variable and tabulated as Mean ± Standard Deviation. The statistical package SPSS (v20) software was used to process the current data. Probability values ≤ 0.05 and ≤ 0.01 were defined as significant throughout the current work. However, the values > 0.05 were considered non-significant. Probability values between 0.05 and 0.01 (both are included) were evaluated as significant.

RESULTS

Physicochemical Parameters and Heavy Metals Distribution:

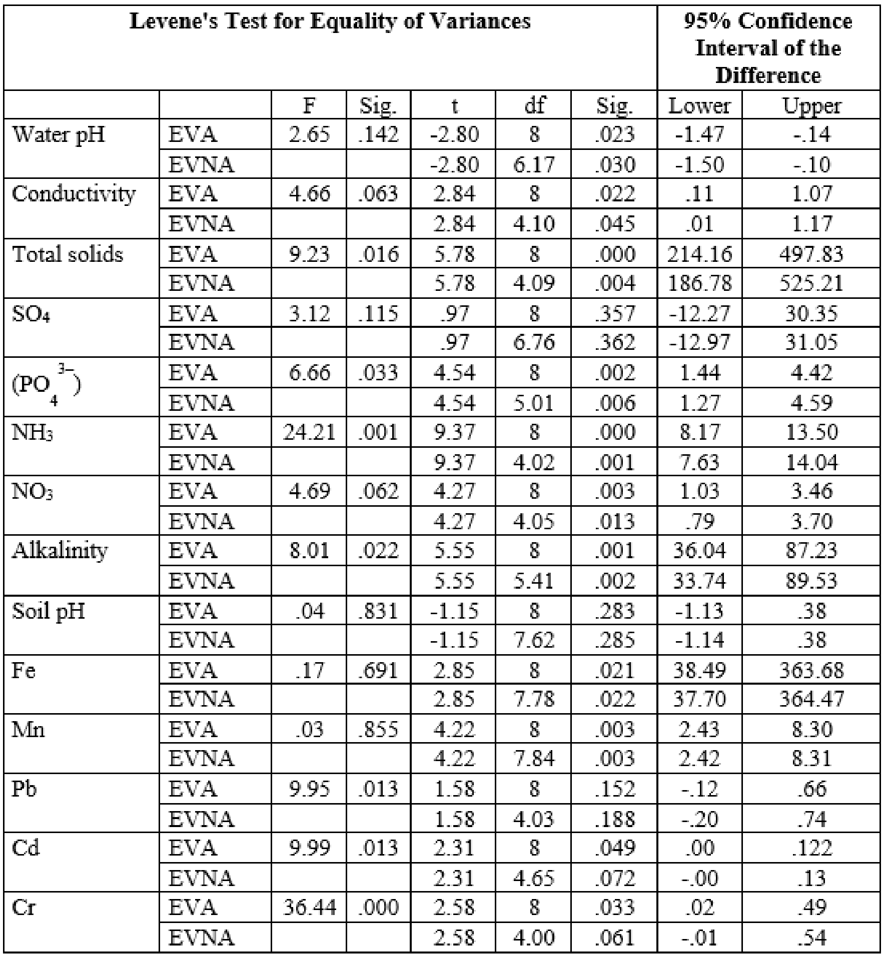
The selected physicochemical parameters of the two investigated sites are presented in (Table 1). The mean value of water and soil pH sampled from Abu Rawash (Giza) were slightly acidic than that of El Bawiti (El-Wahat Oases). Contrarily, the rest of the parameters, namely conductivity, total solids, sulfate, phosphate, ammonia, nitrate, and alkalinity are higher at Abu Rawash (Giza) than El Bawiti (El-Wahat Oases). Regarding the mean concentrations of heavy metals in water, Iron (Fe) exhibited tremendous concentration in water sampled from El-Wahat Oases (515.14±120.43) when compared to Giza (314.05±101.75). Also, the mean value of manganese of Abu Rawash (11.23±1.86) was twice that of El Bawiti (5.86±2.14). The rest of the heavy metals were higher incomparable values (Table 1). To verify the presence of significant differences (or not) between these means, the statistical T-test was applied (Table 2). The mean values of water pH, conductivity, total solid differed significantly between the two investigated regions ($p<0.05$). In addition, the mean values of phosphate, ammonia, nitrate and alkalinity of water gave a higher level of different significance ($p\leq 0.01$). The distribution of heavy metals in the water of two areas (Table 2) showed remarkable variations of differed means in the terms of Fe ($p=0.02$), Mn($p=0.003$), Cd($p=0.04$), and Cr($p=0.03$). The means of the concentration of the five heavy metals in soil are presented in (Table 3). As well as water, heavy metals concentration in soil was higher at site 1 in comparison to the second site. The highest mean was Fe (2122.81±722.63) at Abu Rawash and (2104.64±639.28) at El Bawiti. Other metals exhibited sharp differences between the two sites. The T-test (Table 4) has clarified these differences, whereas all means of heave metals differed significantly between the two sites ($0.05>p<0.01$). Also, the five metals were assessed in the liver (Table 3) and were in accordance with their concentrations in habitat (water – soil) and ranked as Fe> Mn >Pb> Cd> Cr at both sites. On the other hand, the T-test following Levene's Test for Equality of Variances has indicated the presence of different levels of significance (Table 4) between the means of liver-accumulated metals when the two sites compared each other ($0.05>p\leq0.01$).

Table 1: Means ±SD of water physicochemical parameters and soil pH at the investigated sites.



Parameters (unit)	El Giza	El Wahat
Water pH	6.36±0.56	7.17 ±0.30
Conductivity (µS/cm)	0.99±0.46	0.40 ±0.54
Total solids (mg/l)	589.40 ±136.72	233.40 ±14.92
SO_4(mg/l)	40.92±1.46	31.88 ±11.05
(PO_4^{3-}) (mg/l)	6.27±1.35	3.34±0.48
NH_4 (mg/l)	11.30±2.58	0.46 ±0.12
NO_3 (mg/l)	3.09±1.17	0.84 ±0.95
Alkalinity (mg/l)	148.00 ±22.82	86.36 ±9.74
Soil pH	6.67±0.57	7.05 ±0.45
Fe (µg/l)	515.14±120.43	314.05±101.75
Mn (µg/l)	11.23±1.86	5.86±2.14
Pb (µg/l)	0.28±0.37	0.15±0.25
Cd (µg/l)	0.784±0.56	0.170±0.16
Cr (µg/l)	0.274±0.22	0.012±0.00

Table 2: T-test showing for the means of water physicochemical parameters and soil pH between the two investigated sites.



Levene's Test for Equality of Variances							95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig.	Lower	Upper
Water pH	EVA	2.65	.142	-2.80	8	.023	-1.47	-.14
	EVNA			-2.80	6.17	.030	-1.50	-.10
Conductivity	EVA	4.66	.063	2.84	8	.022	.11	1.07
	EVNA			2.84	4.10	.045	.01	1.17
Total solids	EVA	9.23	.016	5.78	8	.000	214.16	497.83
	EVNA			5.78	4.09	.004	186.78	525.21
SO_4	EVA	3.12	.115	.97	8	.357	-12.27	30.35
	EVNA			.97	6.76	.362	-12.97	31.05
(PO_4^{3-})	EVA	6.66	.033	4.54	8	.002	1.44	4.42
	EVNA			4.54	5.01	.006	1.27	4.59
NH_3	EVA	24.21	.001	9.37	8	.000	8.17	13.50
	EVNA			9.37	4.02	.001	7.63	14.04
NO_3	EVA	4.69	.062	4.27	8	.003	1.03	3.46
	EVNA			4.27	4.05	.013	.79	3.70
Alkalinity	EVA	8.01	.022	5.55	8	.001	36.04	87.23
	EVNA			5.55	5.41	.002	33.74	89.53
Soil pH	EVA	.04	.831	-1.15	8	.283	-1.13	.38
	EVNA			-1.15	7.62	.285	-1.14	.38
Fe	EVA	.17	.691	2.85	8	.021	38.49	363.68
	EVNA			2.85	7.78	.022	37.70	364.47
Mn	EVA	.03	.855	4.22	8	.003	2.43	8.30
	EVNA			4.22	7.84	.003	2.42	8.31
Pb	EVA	9.95	.013	1.58	8	.152	-.12	.66
	EVNA			1.58	4.03	.188	-.20	.74
Cd	EVA	9.99	.013	2.31	8	.049	.00	.122
	EVNA			2.31	4.65	.072	-.00	.13
Cr	EVA	36.44	.000	2.58	8	.033	.02	.49
	EVNA			2.58	4.00	.061	-.01	.54

Table 3: Means ± SD of heavy metals concentrations of soil and liver of toads from the two investigated sites.

	Soil		Liver	
Heavy metals (unit)	El Giza	El Wahat	El Giza	El Wahat
Fe (µg/g)	6784.23±1285.21	505.14±151.91	2122.81±722.63	2104.64±639.28
Mn (µg/g)	93.01±34.86	12.62±2.83	66.02±17.05	3.66±8.23
Pb (µg/g)	7.67±1.28	0.12±0.08	9.13±1.12	1.43±0.25
Cd (µg/g)	1.02±0.49	0.27±0.35	4.54±1.42	1.20±0.39
Cr (µg/g)	6.11±0.80	0.59±0.31	3.02±1.52	0.97±0.42

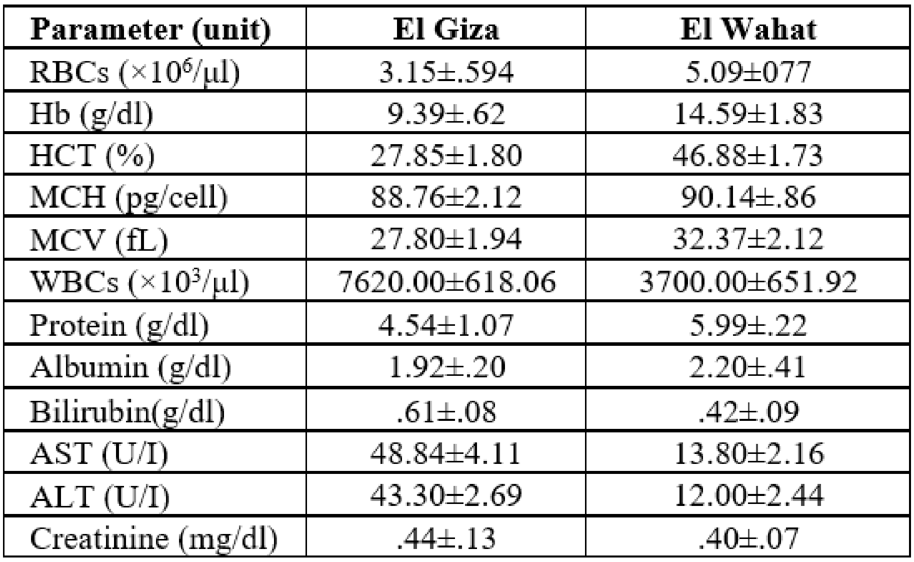
Table 4: T-test showing for the means of heavy metals concentrations in soil and liver of Sclerophrys *regularis* between the two investigated sites.

Levene's Test for Equality of Variances							**95% Confidence Interval of the Difference**	
		F	Sig.	t	df	Sig	Lower	Upper
Fe soil	EVA	4.91	.05	10.84	8	.000	4944.03	7614.14
	EVNA			10.84	4.11	.000	4688.75	7869.42
Mn soil	EVA	5.62	.04	5.13	8	.001	44.31	116.46
	EVNA			5.13	4.05	.007	37.17	123.60
Pb soil	EVA	16.88	.00	13.13	8	.000	6.22	8.88
	EVNA			13.13	4.03	.000	5.96	9.14
Cd soil	EVA	.36	.56	2.75	8	.025	.12	1.37
	EVNA			2.75	7.21	.028	.10	1.39
Cr soil	EVA	3.77	.08	14.28	8	.000	4.62	6.40
	EVNA			14.28	5.17	.000	4.53	6.49
Fe liver	EVA	.76	.40	.04	8	.967	-976.81	1013.17
	EVNA			.04	7.88	.967	-979.39	1015.75
Mn liver	EVA	15.30	.00	3.34	8	.010	8.82	47.89
	EVNA			3.34	5.76	.016	7.43	49.28
Pb liver	EVA	2.80	.13	14.97	8	.000	6.51	8.88
	EVNA			14.97	4.42	.000	6.32	9.07
Cd liver	EVA	14.23	.00	5.06	8	.001	1.82	4.86
	EVNA			5.06	4.60	.005	1.60	5.08
Cr liver	EVA	9.34	.01	2.89	8	.020	.41	3.67
	EVNA			2.89	4.62	.037	.18	3.91

Hemato–Serology Investigations:

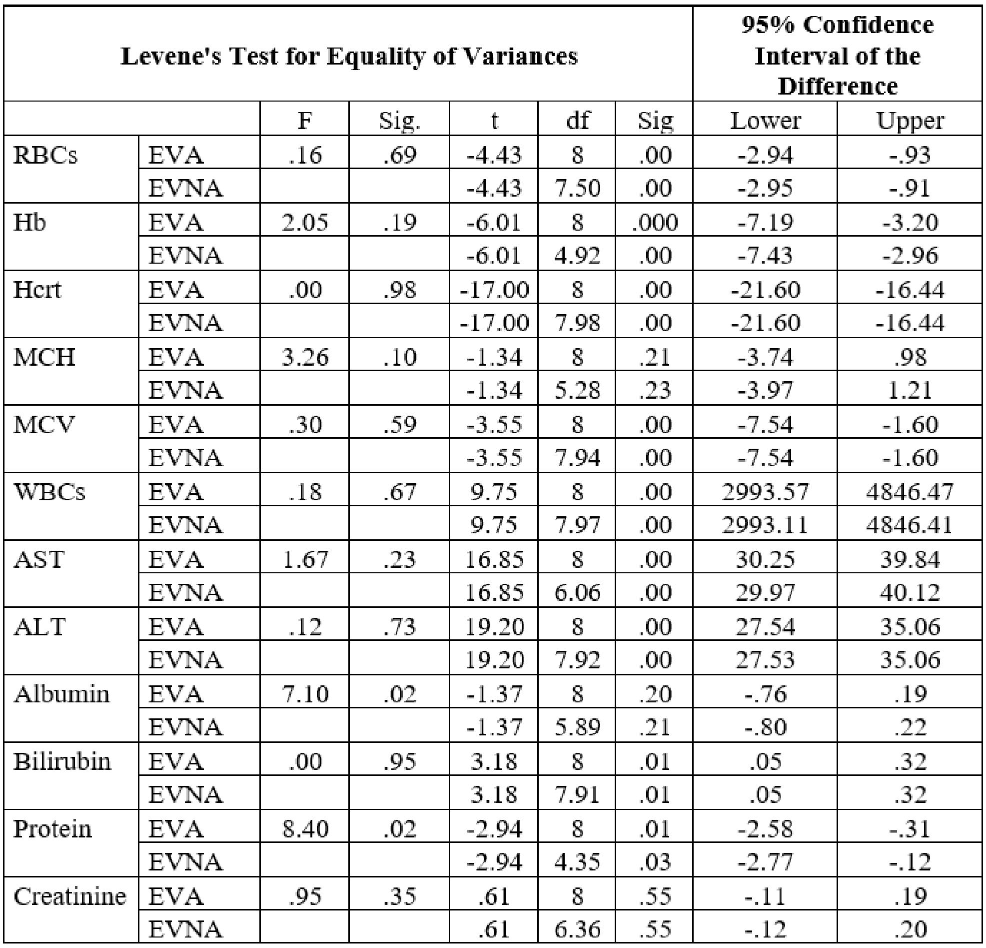
The means of some hematological and serological parameters were tabulated (Table 5). The scored mean of erythrocytes of the amphibian *Sclerophrys regularis* inhabiting El Wahat (5.09±077) $\times 10^6$/µl is almost twice their count at El Giza (3.15±.594) $\times 10^6$/µl. in parallel, the means of hemoglobin, hematocrit, mean cell hemoglobin and mean cell volume are higher in the blood of animals of El Wahat than those of El Giza. Furthermore, the immune indication, namely white blood cells WBCs increased dramatically in the blood of toads collected from Abu Rawash (7620.00±618.06) in contrast to the scored means (3700.00±651.92) from El Bawiti toads. In addition, the biochemical parameters including total protein, albumin, bilirubin, AST, ALT and creatinine were compared between the two sites (Table 5). Total protein was slightly lower at site 1 (4.54±1.07) compared to the second one (5.99±.22). AST and ALT recorded huge values at site 1 in contrast to the second site. Taking into account the tabulated results of the T-test (Table 6), except for MCH and creatine, the hematological and serological parameters differ significantly between the two assessed regions (0.05>p≤0.01).

Table 5: Means ± SD of haemato-serological parameters of *Sclerophrys regularis* collected from the investigated sites.



Parameter (unit)	**El Giza**	**El Wahat**
RBCs (×10^6/μl)	3.15±.594	5.09±077
Hb (g/dl)	9.39±.62	14.59±1.83
HCT (%)	27.85±1.80	46.88±1.73
MCH (pg/cell)	88.76±2.12	90.14±.86
MCV (fL)	27.80±1.94	32.37±2.12
WBCs (×10^3/μl)	7620.00±618.06	3700.00±651.92
Protein (g/dl)	4.54±1.07	5.99±.22
Albumin (g/dl)	1.92±.20	2.20±.41
Bilirubin(g/dl)	.61±.08	.42±.09
AST (U/I)	48.84±4.11	13.80±2.16
ALT (U/I)	43.30±2.69	12.00±2.44
Creatinine (mg/dl)	.44±.13	.40±.07

Table 6: T-test showing for the means of haemato-serological parameters of *Sclerophrys regularis* between the two investigated sites.



Levene's Test for Equality of Variances							**95% Confidence Interval of the Difference**	
		F	Sig.	t	df	Sig	Lower	Upper
RBCs	EVA	.16	.69	-4.43	8	.00	-2.94	-.93
	EVNA			-4.43	7.50	.00	-2.95	-.91
Hb	EVA	2.05	.19	-6.01	8	.000	-7.19	-3.20
	EVNA			-6.01	4.92	.00	-7.43	-2.96
Hcrt	EVA	.00	.98	-17.00	8	.00	-21.60	-16.44
	EVNA			-17.00	7.98	.00	-21.60	-16.44
MCH	EVA	3.26	.10	-1.34	8	.21	-3.74	.98
	EVNA			-1.34	5.28	.23	-3.97	1.21
MCV	EVA	.30	.59	-3.55	8	.00	-7.54	-1.60
	EVNA			-3.55	7.94	.00	-7.54	-1.60
WBCs	EVA	.18	.67	9.75	8	.00	2993.57	4846.47
	EVNA			9.75	7.97	.00	2993.11	4846.41
AST	EVA	1.67	.23	16.85	8	.00	30.25	39.84
	EVNA			16.85	6.06	.00	29.97	40.12
ALT	EVA	.12	.73	19.20	8	.00	27.54	35.06
	EVNA			19.20	7.92	.00	27.53	35.06
Albumin	EVA	7.10	.02	-1.37	8	.20	-.76	.19
	EVNA			-1.37	5.89	.21	-.80	.22
Bilirubin	EVA	.00	.95	3.18	8	.01	.05	.32
	EVNA			3.18	7.91	.01	.05	.32
Protein	EVA	8.40	.02	-2.94	8	.01	-2.58	-.31
	EVNA			-2.94	4.35	.03	-2.77	-.12
Creatinine	EVA	.95	.35	.61	8	.55	-.11	.19
	EVNA			.61	6.36	.55	-.12	.20

DISCUSSION

Amphibian declines may ultimately cause secondary impacts on ecosystems as amphibians are both predator and prey and therefore play a key role in energy flow and nutrient cycling (Campbell Grant *et al.*, 2016). Globally, biomonitoring constitutes a valuable assessment tool to provide critical up-to-date information on the status and health of amphibians worldwide(Brodeur *et al.*, 2020). Most environmental pollutants are threatening initially human and environmental health but also the integrity and function of ecosystems. As a sequence of increasing human activities, chemical pollution increased and adversely affected terrestrial and aquatic environments. Six major threats have been traditionally linked to amphibian declines: habitat loss and fragmentation, commercial overexploitation, introduced species, environmental contaminants, global climate change, and emerging infectious diseases (Bishop *et al.*, 2012). Recent evidence however suggests that the causes of the amphibian declines are probably more variable and locally-driven than previously assumed (Campbell Grant *et al.*, 2016). It is well known that many heavy metals pose toxic and carcinogenic effects in humans and animals (Sharma *et al.*, 2014). Most often highest concentrations of heavy metals are found in the site of detoxification liver (Javed and Usmani, 2011) and can potentially accumulate in higher amounts responding to their environmental distribution (Said, 2013; Said et al., 2015; Said et al., 2016; Said et al., 2017; Osman et al., 2018). Even at very low concentration heavy metals (Cu, Ni, Fe, Co, Mn, Cr, Zn, Hg, Cd, Pb) induce changes in morphology, physiological and biochemical indices in fish. Such effects besides including decrease in immunity (Mikrjakov *et al.*, 2001), affect carbohydrate metabolism and biochemical parameters (Bhatkar *et al.*, 2004), alteration in hematological indices(Javed and Usmani, 2015) . The capability of Abu Rawash wastewater treatment plant in Cairo is 1.2 million m^3 /day, which does not meet the legal requirements. consequently, excess sewage amounts are discharged without treatment causing water pollution in drains (El-fakharany, 2020). The groundwater resources in the Bahariya Oases are the dominant water resource for all purposes. The average TDS content determined in aquifer waters from Bahariya Oases was 286 mg/l (Sharaky and Abdoun, 2020). Also, they recorded the electric conductivity range of groundwater in the ranges between 0.21 and 2.24 dS/m with an average value of 0.48 dS/m. (Sharaky and Abdoun, 2020) attributed water acidity in El Wahat to the dissociation of H_2CO_3 formed from the dissolution of CO_2. Nitrate and phosphate are the major nutrients in ecosystems, but their higher amounts cause the growth of algal blooms (Eutrophication). The phenomenon of eutrophication alters aquatic ecosystems at all trophic levels. Moreover, acidity plays a direct role in the environment, where the chelated pollutants such as metals are release as water acidity (pH) increase. The current data of Fe and Mn in water (Table 1) of El Wahat are higher than those of (Sharaky and Abdoun, 2020) for the same region (1.34 ppm to 2.26ppm) and (0.27 ppm to – 0.71 ppm) respectively. It has been stated that the elevated iron may damage the irrigation system (FAO, 2017).The mean values of the electric conductivity, TDS, and water pH of El Wahat are still in the recommended ranges for drinking water by the World Health Organization in 2011(WHO, 2011). To some extent, it can be said that El Wahat area is still virgin with slight human impact in comparison with Abu Rawash.

Due to their toxicity and accumulation, heavy metals have gained great ecological consideration (Burridge, 1978). One of the presumptions concerning the bioaccumulation of metals in amphibians is that the only significant route of metal uptake for terrestrial stage individuals is through diet and gaseous absorption of volatilized metals (Linder and Grillitsch, 2000). In some respects this assumption makes sense, metals tend to bind tightly to soil constituents and the skin of every vertebrate is a specialized barrier that only selectively allows the transfer of elements and compounds from one side to the other (Bryer, 2008). On the other hand, (Gill and Epple, 1993) concluded that heavy metals potentially posed anemia due to impaired erythropoiesis as the alter hematopoietic centers (kidney /spleen). Also, the previous study (Said *et al.*, 2016) on *Amietophrynus regularis* exposed to lead has resulted in a significant decrease in hemoglobin, indicating that lead could cause anemia. The observed reduction in the RBCs count, hemoglobin and hematocrit values may be attributed to the decreased rate of production of red blood cells or an increased loss of these cells(Said *et al.*, 2016). On the other hand, the observed deterioration in biochemical parameters such as protein, albumin, bilirubin could be attributed to the change in the water quality particularly at Abu Rawash as a result of the discharged effluents from different sources. The decrease in total protein may be due to the inhibition of RNA synthesis that controls protein metabolism (Thoker, 2015).Protein, alanine aminotransferase (ALT) and aspartic aminotransferase (AST) were altered in the blood of fish exposed to copper nanoparticles and penconazole (Osman, 2019). Increased levels of ALT and AST indicate an adaptive response to the leakage of these enzymes into the bloodstream due to the tissue accumulated metals.

Conclusion

Based on the current findings, the patterns of alteration in hematological and serological parameters can be used as potential biomarkers for environmental biomonitoring. This approach helps us to compare the degrees of environmental impact on associated fauna from one extreme and to provide a database for decision-makers toward natural resources and their sustainable use on the other extreme.

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