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The Egyptian Toad (*Bufo regularis Reuss*) as bioindicator to environmental deterioration with heavy metal in El-Behera governorate

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ABSTRACT

There is a notable global drop in the number of amphibian populations. The loss can be directly attributed to pollution, particularly heavy metal contamination, and habitat damage. It has indeed been well documented in the past that several ecological parameters and hematological parameters might vary in response to environmental changes. There is a chance that hematological parameters could be employed as biomarkers of Terrestrial and aquatic pollution because they are susceptible to different environmental stresses. As difficult to eliminate from the environment and incapable of biological degradation, heavy metals are challenging to remove from the environment. The present study investigates the effects of various heavy metals in hematological and biochemical parameters of the Egyptian toad, *Bufo regularis Reuss*, in El-Behera governorate in three different locations-Damanhur (location 1), Kafr El Dawar (location 2), and Abu Homs (location 3)., The current results show increase in WBCs, a decrease in the means of hemoglobin, hematocrit, protein, albumin and red blood cells. Furthermore, changes were observed in the main biochemical markers, such as, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutathione (GSH), malondialdehyde (MDA) and Superoxide dismutase (SOD). Current research has demonstrated and validated that the Kafr El Dawar region is more affected than Damanhur and Abu Homs, So, this study calls for urgent management of heavy metals and reducing their release into the environment.

Key words: Egyptian Toad (*Bufo regularis Reuss*), heavy metal, El- Behera governorate.

1. Introduction

Amphibians have a pivotal role in properly functioning ecosystems, share in food webs, energy flow, bio duration, nutrient cycling, and other environmental processes (Hocking and Babbitt, 2014; Cortéz-Gómez et al., 2015; Khattab et al., 2021). In fact, Amphibians offer humans additional ecosystem services that are beneficial, like controlling pests, providing food, acting as model for medical studies, and offering pleasure and intangible benefits that differ throughout cultures (Warkentin et al., 2009; Khattab et al., 2021). With over 7000 recognized species, amphibians are a unique group of vertebrates that face threats on a global scale. According to Stuart et al. (2004), the number of extinct and threatened amphibian species is expected to climb further. Few factors appear to be responsible for the decline, but a number of them, including increased Ultraviolet (UV) radiation, bacterial and fungal infections, droughts, acid precipitation, alterations in climate, habitat destruction, heavy metals, exotic species, pesticides, acid rain, and fertilizers, can combine to have a fatal or semi-lethal effect. According to Blaustein and Johnson (2003), a logical explanation for the decline is severe environmental degradation. Because they occupy a pivotal position in the food chain as both predators and prey, amphibians can have quite distinct eating ecologies at different phases of their lives and frequently employ both terrestrial and aquatic habitats. As a result, said (2013) and Khattab (2021) consider them to be superior bioindicators of environmental health. Amphibians are susceptible to deadly impacts from contaminants, which could lower their survival rate. Many pollutants can suppress immunity, produce deformities, impair reproduction, and reduce growth and development when exposed to sub-lethal amounts (Taylor et al., 2005; Groner and Relyea, 2011; Gahl et al., 2011; Khattab et al ., 2021)The biphasic life cycle andsemi-permeable skin of amphibians are two characteristics that make them valuable bio indicator species (Venturino et al., 2003; Okeagu et al., 2022). Because of their exceptional abundance in a variety of ecosystems, toads and frogs also function as good indicator species, assessing many ecological changes within their surroundings. It is believed that pollutants, such as chemical fertilizers and heavy metal are a contributing factor in the recent worldwide drop in amphibian species abundance (Houlahan and Findlay 2003; Mann et al., 2009). According to Sparling et al. (2001), and Okeagu et al (2022) up to 90% of chemical fertilizers and heavy metal never reach their intended targets; amphibians are among the non-target groups most frequently impacted (Sparling et al., 2001). Nine species make up Egypt's very tiny amphibian biodiversity, whose current state and conservation needs are not well understood. Potential dangers to Egyptian amphibians' density, abundance, and vigor include excessive pesticide use (Ibrahim, 2013: Said et al., 2022).

Runoff from agriculture is the most frequent way fertilizers and heavy metal are exposed to frogs. Amphibians absorb chemicals by passive diffusion through their skin, gills, or digestive tracts through food. Tadpoles absorb through their gills and skin, but adult amphibian mostly absorb through their skin. One important method of absorption, particularly for adult frogs, is skin absorption through partial or total body immersion in chemical fertilizers and heavy metal containing water (Katagi and Ose, 2014). The Bufonide family is the most significant within the class Amphibia; endemic to all regions of the planet with the exception of Antarctica, bufonids are often employed as model animals in experimental biology research.

According to Osman et al. (2019) and Okeagu et al (2022), heavy metals are a serious environmental issue that is becoming more and more concerning. As a result, monitoring of these pollutants in the field and in laboratories has drawn a lot of attention. Owing to their extreme toxicity, several heavy metals are important for public health. Even in lower levels of exposure, these elements cause numerous organ damage and are classified as systemic toxicants (Tchounwou et al., 2012). Toads can absorb heavy metals through their skin, gills, or digestive tract if they consume contaminated food (Fazio et al., 2014). Metals in toads are essentially carried by the bloodstream to the organs and tissues where they accumulate following absorption (Dupuy et al., 2015). Metals are generally classified as non-essential and physiologically essential. Foreign substances, such as lead (Pb) and cadmium (Cd), have no known biological role and become more harmful at higher quantities (Okeagu et al., 2022). On the other hand, essential metals, manganese (MN), iron (Fe), and chromium (Cr), have a known critical biological role (Vinodhini and Narayanan, 2008). Toxicity can arise from either high quantities or metabolic deficits.

Global emphasis has been focused on the effects of contaminants, especially heavy metals, on blood parameters of numerous aquatic faunas, including amphibians. Due to the fact that blood parameters react to even low concentrations of pollutants, hematological variables have emerged as prospective biomarkers for assessing the impacts of pollution. When diagnosing the structural and functional state of animals exposed to environmental contaminants, blood parameters play a crucial role. Using Egyptian toad hematological parameters as biomarkers for heavy metal impact, this study looked into the hemotoxic effect of certain heavy metals (Said et al., 2016).

2. Materials and methods

Study areas

The Three cities in the El-Behera governorate were chosen: Damanhour (location 1), Kafr El Dawar (location 2), and Abu Homs (location 3). Ten Egyptian toads (*Bufo regularis Reuss*) were gathered from each location. The spawning ponds of both sexes were manually netted during the night in contaminated areas close to agricultural areas.

Experiment Design

We obtained 10 toads (*Bufo regularis Reuss*) as normal control toads of both sexes from the experimental animal house that were almost the same size. Selected toads weighing 50±10 g was placed in a glass container measuring 50 × 60 × 30 cm3, which was supplemented with mud, sand and a pool of 1.5 L of de-chlorinated water. This was done to give the frogs a natural habitat that offered them the choice of an aqueous and dry environment (Allran and Karasov, 2001). Every three days, water was replaced, and the container was completely cleaned. Earth worms were provided to the animals every two days. After being kept in a lab setting a 12-hour light/dark cycle and a temperature of 29–32°C for 7 days to allow the frogs to become acclimated. 30 toads were gathered from selected 3 cities. The spawning ponds of both sexes were manually netted during the night in contaminated areas close to agricultural areas. All animals (control and agricultural toad) that had been anaesthetized with ether and blood were drawn from toad in polyethylene tubes to measure hematological Parameters Additionally, liver samples were obtained and stored until biochemical analysis. Lastly, every experiment was conducted in compliance with university regulations and Egyptian legislation on the care of experimental animals.

Criteria for Water

Several ecological parameters were assessed using the water checker U-10 Horiba Ltd. Samples of water were collected in poly-ethylene bottles in order to calculate the following parameters: water temperature (ºC), pH, conductivity, dissolved oxygen, and, Nitrates, total solids (TS), and total organic carbon (TOC) using established techniques (APHA, 2005).

Heavy Metal Content

The following heavy metals—cadmium (Cd), manganese (Mn), lead (Pb), zinc (Zn), copper (Cu), iron (Fe) and chromium (Cr) were found in water, agricultural soil and in liver were measured. The concentration was calculated µg/g for both tissueand sediment and µg/l for water (Sekomo et al., 2011; Arserim et al.,2008).

Hematological and Biochemical Analysis

The blood samples were taken from animals collected in a tube which transported into ice container to the laboratory for chemical analysis. The counts of red blood cells, white blood cell, hemoglobin concentration and the hematocrit were measured (Shah, and Altindag., 2004) The measurement of total protein was done using the Armstrong and Carr (1964) method, while the measurement of albumin was done using the Doumas et al. (1971) method. Creatinine was measured according to Bartels and Bohmer (1972) The GSH content of the liver tissues using the Sedlak and Lindsay (1968) techniques. Sun and Zigma techniques in 1978 to measure superoxide dismutase activity (SOD). The Ohkawa et al. (1979) method was used to measure malondialdehyde (MDA).

Ethical approval

The ethical approval for this study from Faculty of Science, Damanhour University, Egypt (DMU-SCI-CSRE-24 09 05)

4. Statistical Analysis:

The obtained data in the current study are expressed as mean ± SE and the data were analyzed using the one-way ANOVA test of SPSS software program The significance of differences between the control animal group and collected animals' groups at Probability values $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$. However, the values > 0.05 were considered non-significant. Statistically non -significant, significant and highly significant outputs were accompanied by symbols NS, non–significant and *** highly significant at $p \leq 0.001$ comparing with the control group., **Significant at $p \leq 0.01$ comparing with the control group, and *Significant at $p \leq 0.05$ comparing with the control group. n=3.

3. Results

Distribution of Heavy Metals and Physicochemical Parameters

Criteria of Water

The three locations in the current study showed very slight variations in the water temperature. In locations 1, 2, and 3, it varied between 34.32 ºC, 36.65 ºC, and 31 ºC, in that order. Location 1 had the highest pH of 8.35, which was followed by locations 3 and 2's 8.10 and 7.10, respectively. Location 2 had the lowest amount of dissolved oxygen (2.31 mg/l), while locations 1and 3 had higher levels (6.15 and 4.94 mg/l, respectively). Total solids (TS) in locations 1, 2 and 3 equal 186.65 and 213.51 mg/l and 256.45 mg/l, respectively. In locations 1, 2, and 3, the concentration of Nitrates was 1.11, 2.8 and 2.40 mg/l, respectively. The samples from location 2 had the highest total organic carbon (TOC) content (7.33 mg/l), whereas samples from locations 3 and 1 showed the lowest TOC content (3.00 mg/l and 0.43 mg/l, respectively). The physico-chemical parameters at 3 locations were listed at table (1) and shown as mean ± standard deviation SD.

Heavy metals in water

In comparison to locations 1 and 3, location 2 displayed a higher level of water metals, with the exception of iron (820.33±7.12 µg/l in location 3, 615.42±5.70 µg/l in location 2, and 120.33±4.04 µg/l in location 1). The following order of metal concentrations in water was found by the results: In location 1, the order of elements is Fe > Zn > Cu > Mn > Pb > Cd >Cr, but in location 2, the order is Fe > Cu > Zn > Mn > Pb > Cr = Cd. Metals in location 3 were in the following order: Fe > Cu > Zn > Mn >Pb > Cr > Cd table (2).

Heavy metals in soil

The average cadmium content in the soil sampled from location 2 was 0.75 µg/g, then locations 3 and 1 having the concentrations is 0.66 µg/g and 0.53 µg/g, respectively. Lead levels in location 2 were twice as high as those in location 3 (5.01 µg/g), although location 1 had level (7 µg/g). Zinc reached its highest in location 2 (80.79 µg/g), fell in location 3 (75.61 µg/g), and in location 1 (64.90 µg/g). Location 2 had a greater concentration of copper (37.12 µg/g), while locations 1 and 3 had values (27.81 µg/g and 20.89 µg/g, respectively). Copper reached its highest in location 2 (37.12 µg/g), fell in location 3 (20.89 µg/g), and in location 1 (27.81 µg/g). Chromium, peaked in location 2 (6.67 µg/g), then locations 3and 1 (5.70 µg/g and 2.52 µg/g). Location 1 had the lowest mean iron concentration (105.36 µg/g), with difference between locations 2 (187.87 µg/g) and 3 (288.31 µg/g). Additionally, the mean for manganese was 33.92 µg/g in location 3 and 71.62 µg/g in location 2, with a peak of 50.19 µg/g in location 1 table (3).

Heavy metals in Liver

In location 2 the amphibian *Bufo regularis Reuss* liver has a highly significant increase (15.46 µg/g) for Pb, a significant increase in location 1(9.30 µg/g) and in location 3 (8.90 µg/g) when compared to control gp. Cd has highly significant increased compared to control gp location 2 (1.94 µg/g), the liver of toads taken from location 1 (0.31 µg/g) increased non-significantly, location 3 has significant increase (1.03 µg/g) compared to control gp. Location 2 and 3 have highly significant increase in Fe compared to control gp with the mean value 243.16, 300.33 µg/g respectively. location 1 has significant increase with 152.83 µg/g compared to control gp. Furthermore, a comparison was made between the three locations, which included a highly significant increase in Zn (90.27and 74.61 µg/g) in location 2 and 3, significant increase in location 1 (51.40 µg/g) compared to control gp. Cu has highly significant increased compared to control gp location 2 and 3 (171.07, 114.11 µg/g), location 1 (42.04 µg/g) increased non-significantly, compared to control gp. Our results showed significant increase of Cr in toads liver tissues in location 2 (3.41 µg/g**).** Significant increase in location 3 (2.42 µg/g**)** when compared to control gp The results showed non-significant increase in location 1 (mean = 1.50 µg/g) compared to control gp.

In addition, location 1 and 3 have a significant increase in Mn (4.26 and 3.94µg/g), Location 2 has highly significant increase in Mn (7.92 µg/g) when compared to control gp (table 4).

Hematological and Biochemical Analysis

In location 2 the amphibian *Bufo regularis Reuss* has a significant decrease (1.73×10^6 /µl) for its erythrocytes, non-significant decrease in location 1(2.04×10^6 ./µl) and in location 3 (2.15×10^6 ./µl) when compared to control gp. In addition, location 1 and Location 2 have a significant decrease of hemoglobin (8.48 and 7.75 g/dl), Location 3 has a non- significant decrease of hemoglobin (10.78 g/dl) when compared to control gp. Location 1 and Location 3 have non- significant decrease of Hematocrit values (25.12×10^3 /µl, 30.37 $\times10^3$ /µl). Furthermore, in contrast to the scored means (11.93×10^3 /µl) from location 2, it has a significant decrease when compared to control gp. The immunological indicator WBCs increased significantly compared to control gp location 2(11.93×10^3 /µl), the blood of toads taken from location 1 and3 (8.04×10^3 /µl) increased non-significantly compared to control gp (table 5).

Furthermore, a comparison was made of the parameter between the three locations, which included a significant increase in creatinine and bilirubin (0.52 mg/dland 0.90 g/dl) in location 2 only compared to control gp. A significant decrease in albumin, and total protein in location 2 only (1.74 g/dl and 1.72 g/dl) compared to control gp. Highly significant increase in AST and ALT in location 2 (53.03and47.44 U/I), Highly significant increase in ALT in location1 (39.55 U/I), significant increase in AST in location1 (43.79 U/I). Also, significant increase in ALT in location3 (32.31 U/I). Non-significant increase in AST in location3 (32.12 U/I) compared to control gp. Our results showed a highly significant decrease of GSH in toads liver tissues in location1and 2 (0.71and 0.62 U/mg protein) when compared to control gp. Significant decrease of SOD in location1and 2 (2.07 and 1.71 U/mg protein) when compared to control gp. The results showed non-significant promotes MDA in liver tissues of toads compared to control gp in three locations (table 6).

Table. 1. Physico-chemical parameters of water in the investigated locations.

Parameter	Location 1	Location 2	Location 3
Temperature	34.32±0.30	36.65±3.05	31.00±1.80
PH	8.35±0.15	7.10±0.05	8.10±0.2
Dissolved oxygen	6.15±0.12	2.31±0.44	4.94±0.06
Total solids	186.65±1.52	213.51±1.39	256.45±2.8
Total organic carbon	0.43±0.25	7.33±1.40	3.00±0.07
Nitrates	1.11±0.23	2.8±0.45	2.40±0.12

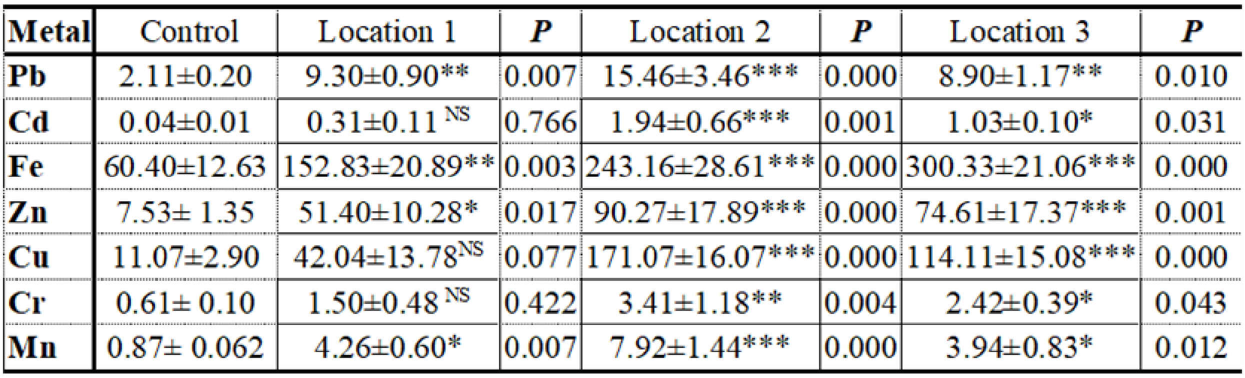
Table. 2. Metals concentrations (µg/l) in water at the investigated locations

Metal	Location 1	Location 2	Location 3
Pb	4.04±0.71	19.33±1.32	8.25±0.51
Cd	2.01±0.02	4.61±0.31	3.53±0.22
Fe	120.33±4.04	615.42±5.70	820.33±7.12
Zn	80.33±4.50	120.66±5.22	95.34±8.23
Cu	76.66±5.51	484.14±7.50	410.66±6.50
Cr	0.25±0.01	4.61±1.51	3.70±0.41
Mn	27±1.20	52.71±4.73	12±2.01

Table. 3. Soils metals concentrations (µg/g) in investigated locations

Metal	Location 1	Location 2	Location 3
Pb	7± 0. 07	10.03±0.6	5.01±1.30
Cd	0.53±0.26	0.75±0.19	0.66±0.11
Fe	105.36±0.91	187.87±5.53	288.30±2.66
Zn	64.90±4.80	80.79±8.27	75.61±1.02
Cu	27.81±0.67	37.12±5.34	20.89±2.76
Cr	2.52±0.45	6.67±3	5.70±0.88
Mn	50.19±1.34	71.62±3.4	33.92±1.02

Table. 4. Liver toads heavy metals concentrations (µg/g) collected from the investigated location



Metal	Control	Location 1	***P***	Location 2	***P***	Location 3	***P***
Pb	2.11±0.20	9.30±0.90**	0.007	15.46±3.46***	0.000	8.90±1.17**	0.010
Cd	0.04±0.01	0.31±0.11 ^{NS}	0.766	1.94±0.66***	0.001	1.03±0.10*	0.031
Fe	60.40±12.63	152.83±20.89**	0.003	243.16±28.61***	0.000	300.33±21.06***	0.000
Zn	7.53± 1.35	51.40±10.28*	0.017	90.27±17.89***	0.000	74.61±17.37***	0.001
Cu	11.07±2.90	42.04±13.78^{NS}	0.077	171.07±16.07***	0.000	114.11±15.08***	0.000
Cr	0.61± 0.10	1.50±0.48 ^{NS}	0.422	3.41±1.18**	0.004	2.42±0.39*	0.043
Mn	0.87± 0.062	4.26±0.60*	0.007	7.92±1.44***	0.000	3.94±0.83*	0.012

*** Highly Significant at $p \leq 0.001$ comparing with the control group, **Significant at $p \leq 0.01$ comparing with the control group, *Significant at $p \leq 0.05$ comparing with the control group. NS, non–significant ^{NS}

Table. 5. Hematological –biochemical parameters of toads collected from the investigated locations.

Metal	Control	Location 1	***P***	Location 2	***P***	Location 3	***P***
Pb	2.1±0.20	9.30±0.90**	0.007	15.4±3.4***	0.000	8.9±1.17**	0.010
Cd	0.04±0.01	0.31±0.11 ^{NS}	0.766	1.9±0.6***	0.001	1.03±0.10*	0.031
Fe	60.4±12.6	152.8±20.8**	0.003	243.1±28.6***	0.000	300.3±21.06***	0.000
Zn	7.5± 1.3	51.4±10.28*	0.017	90.2±17.8***	0.000	74.6±17.37***	0.001
Cu	11.0±2.9	42.04±13.78^{NS}	0.077	171.0±16.07***	0.000	114.1±15.08***	0.000
Cr	0.6± 0.10	1.50±0.48 ^{NS}	0.422	3.4±1.1**	0.004	2.4±0.39*	0.043
Mn	0.87± 0.06	4.2±0.60*	0.007	7.9±1.4***	0.000	3.9±0.83*	0.012

*** Highly Significant at $p \leq 0.001$ comparing with the control group, **Significant at $p \leq 0.01$ comparing with the control group, *Significant at $p \leq 0.05$ comparing with the control group. NS, non–significant ^{NS}

Table. 6. Hematological –biochemical parameters of toads collected from the investigated locations.

Parameter	Control	Location 1	*P*	Location 2	*P*	Location 2	*P*
Protein (g/dl)	3.4±0.94	2.6±0.11 ^{NS}	0.60	1.7±0.36**	0.01	2.9±0.15 ^{NS}	0.60
Albumin (g/dl)	2.7±0.61	2.0±0.10 ^{NS}	0.18	1.7±0.37*	0.04	2.06±0.06 ^{NS}	0.20
Bilirubin (g/dl)	0.6±0.07	0.79±0.08 ^{NS}	0.32	0.9±0.07*	0.02	0.71±0.06 ^{NS}	0.96
AST (U/I)	23.6±1.1	43.7±4.4**	0.006	53.0±5.1***	0.001	32.1±2.6 ^{NS}	0.26
ALT(U/I)	21.7±1.4	39.5±3.1***	0.001	47.44±3.2***	0.00	32.31±3.16*	0.02
Creatinine (mg/dl)	0.26±0.04	0.43±0.02**	0.002	0.52±0.05***	0.00	0.41±0.02**	0.004
GSH (U/mgprotein)	0.91±0.05	0.71±0.02***	0.001	0.62±0.04***	0.00	0.82±0.03 ^{NS}	0.07
SOD (U/mgprotein)	3.9±0.77	2.07±0.07**	0.006	1.7±0.09**	0.002	2.7±0.51 ^{NS}	0.06
MDA (U/mgprotein)	0.06±0.01	0.07±0.01 ^{NS}	0.96	0.08±0.011 ^{NS}	0.32	0.073±0.005 ^{NS}	0.88

*** Highly Significant at $p \leq 0.001$ comparing with the control group, **Significant at $p \leq 0.01$ comparing with the control group, *Significant at $p \leq 0.05$ comparing with the control group. NS, non–significant ^{NS}

4. Discussion

Egypt's natural standards are rapidly deteriorating as a result of the overuse of pesticides or fertilizers in agriculture, as well as the growing discharge of highly contaminated home & industrial effluents into rivers, including the Nile River (Abdel Wahaab and Badawy, 2004). The primary causes of Water contamination in El- beheira small private companies, and agro-industrial areas, together with numerous human activities along the Nile's shore. The water from several farm drains is likewise contaminated and flows into the Rasheed branch. At the Rasheed branch, fertilizer is thought to be the main point source of pollution (Abdel Wahaab and Badawy, 2004; Gideon, 2023). In general, life is threatened by rising pollution sources and declining environmental quality. While amphibians are both predators and prey and are therefore important for energy flow and nutrient cycling, their loss could eventually have secondary effects on ecosystems (Campbell Grant et al., 2016). According to Brodeur et al. (2020), bio monitoring is a useful evaluation method that offers vital, current information on the condition and health of amphibian's animals worldwide. The integrity and functionality of ecosystems are also at risk, in addition to the health of humans and the environment, from the majority of environmental pollutants. Chemical pollution escalated as human activity expanded, negatively impacting both land and marine environments.

According to Tsukada, et al. (2023) there are six main factors that have historically been connected to the reduction of amphibians: habitat loss and fragmentation, commercial overexploitation, imported species, environmental toxins, global climate change, and new infectious illnesses. However, recent indicates that the reasons for the decreases in amphibians are likely more diverse and locally driven than previously thought (Campbell Grant et al., 2016). Many heavy metals are recognized to have harmful and cancer-causing properties in both humans and animals (Sharma et al., 2014). Heavy metal concentrations are typically highest in detoxification liver location (Gideon, 2023). These concentrations may rise in response to the heavy metals' distribution in the environment (Said et al., 2013; 2015; 2016; 2017; Osman et al., 2019). Amphibian morphology, physiological, and biochemical parameters are altered by heavy metals (Zn, Cu, Ni, Hg, Fe, Co, Mn, Cr, Cd, and Pb) even at extremely low concentrations. In addition to lowering immunity, these impacts also change metabolic parameters and carbohydrate metabolism as well as hematological indices (Hocking and Babbitt, 2015; Soares, 2023). According to reference Rollins-Smith (2017)., fertilizers in agriculture have the potential to cause genotoxicity in the amphibian. The current nitrate values were greater than many of the earlier records, including (Rowinski, 2020)

In the current study, location 2 had the lowest dissolved oxygen. These logical conclusions are explained by the fact that municipal waste discharges need more oxygen in order for the organic materials in them to biodegrade. As a result, there will be less dissolved oxygen available. Agricultural fields of location 2 mostly rely on the different contaminants getting water when considering the irrigation method. From a morphological perspective, the crops at this location appear healthier, more abundant, and more vigorous; this is because the soils are rich in organic matter that comes from water. Due to the toads' habitation of the agricultural areas at this location, toxins from the water can come into touch with their skin. However, because toads breed in a variety of pools and aquatic areas, their progeny are also directly exposed to the damaged ecosystem in addition to the adults. The majority of vegetable and crop species that grow in soil contaminated with metals are unable to stop these metals from being absorbed (Gideon, 2023)

Although phosphate and nitrate are the two main nutrients in ecosystems, larger concentrations of these two substances lead to the development of algal blooms, or eutrophication. All trophic levels of aquatic environments are affected by the eutrophication phenomenon. Furthermore, acidity directly affects the ecosystem, as rising water acidity (pH) releases contaminants like metals. Nitrate can enter surface water & groundwater as a result of agricultural activities such as the over application of inorganic, nitrogenous fertilizers, wastewater treatment, oxidation of human & animal nitrogenous waste excreta in septic tanks. This information is attributed to global health organization (WHO, 2011) When nitrate-containing, oxygen-poor drinking water stagnates in galvanized steel pipes, or if chlor-aminat is used as a residual disinfectant and the process is not adequately well controlled, nitrite can also be chemically formed in distribution pipes by Nitrosomonas bacteria (El Bouraie et al, 2010). The physical-chemical characteristics showed statistically significant differences between the three locations (data not tabulated). Location 2 showed signs of eutrophication, or intense algal blooms. The increased water productivity brought about by nitrates and other nitrogenous chemicals from agricultural fertilizer may be the cause of this degree of eutrophication. Water moves slowly at second locations, which slows down the process of recovery and increases the bioavailability of contaminants, which causes buildup (Rumrill, 2018; Ngole-Jeme and Ndava, 2023).

Although El Bouraie (2010) and Ngole-Jeme and Ndava, (2023) observed higher quantities of Mn and Fe, the Pb, Zn, and Cu values in the current work the heavy metal concentrations are higher than those measured by El Bouraie (2010) and Ngole-Jeme and Ndava (2023). The results showed greater concentrations of Mn and Fe, which are consistent with El Bouraie (2010) and Katagi and Ose (2014). The recorded values of lead and cadmium values are consistent with Mann (2009) however the Zn, Cu, Cr, Fe, and Mn concentrations were lower than those found in the current data. The process of aquatic organisms absorbing metal from solution at their permeable surfaces is widely thought to be passive and energy-free (Yagi and Green., 2018). It may also be influenced by the affinity of certain metal ions for proteins and other components of the cell (Sánchez-Chardi et al .2009; Ngole-Jeme and Ndava, 2023).

This presumption states that the dissolved metal ions are attached to the transport protein from the external medium and that the element will be transported inside the cell through the permeable membrane before connecting with a high-affinity metal ligand internally (Katagi and Ose, 2014; Ngole-Jeme and Ndava,2023).

In terms of ecology, toads that consume heavy metals may be directly affected, leading to death, or they may accumulate in their tissue and subsequently spread to other creatures in the food web through predation (Williamson, 2019). The current study's mean copper and zinc concentrations are greater than those found in the tissue of the caudate amphibian, as reported by Loumbourdis (2007). They discovered that liver had mean copper concentrations ranging from 1.49 to 8.61 µg/g and mean zinc concentrations from 6.40 to 49.17 µg/g. A number of heavy metals cause DNA damage that results in clastogenic effects and can cause reactive oxygen species (ROS) to be produced. ROS can then cause biochemical, morphological, and histological changes, as well as eventual cell destruction or death (Rumrill, 2018; Gideon, 2023). Heavy metals have drawn a lot of attention from the ecological community because of their toxicity and buildup. One of the underlying assumptions regarding the bioaccumulation of metals in frogs is that food and gaseous absorption of volatilized metals constitute the sole meaningful pathway of metal uptake for individuals in terrestrial stages (Linder and Gillitsch, 2000). This notion is somewhat justified because metals have a tendency to bond securely to soil constituents and because each vertebrate's skin acts as a specialized barrier that only permits certain elements and compounds to pass from one side to the other (Tsukada et al., 2023).

According to Alimba (2024) concluded that heavy metals can cause anemia by impairing erythropoiesis as kidney and spleen, two hematopoietic centers. Furthermore, a major drop in hemoglobin was observed in the earlier study (Said et al., 2016) on amphibians exposed to lead, suggesting that lead may cause anemia. Red blood cell synthesis may have slowed down or there may have been an increase in the loss of red blood cells, which could account for the observed decline in hemoglobin, hematocrit, and RBC counts (Said et al., 2016; Soares, 2023).

In addition, the noted decline in biochemical indices including protein and albumin, may be linked to the alteration in water quality (Park et al.,2014), specifically at Kafr El Dawar (location 2), as a consequence of effluents being released from various sources. The suppression of RNA production, which regulates protein metabolism, may be the cause of the drop in total protein (Thoker, 2015; Tsukada et al., 2023).

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are found in red blood cells and the liver. Normally, low levels of AST and ALT are found in the blood. When body tissues or organs (such as the liver) are diseased or damaged, additional AST and ALT are released into the blood. The amount of AST &ALT in the blood is directly related to the degree of tissue damage. Free radicals cause lipid peroxidation in organisms. Malondialdehyde (MDA) is one of the final products of intracellular polyunsaturated fatty acid peroxidation. The increase in free radicals leads to excessive production of malondialdehyde (MDA). MDA levels are generally considered a marker of oxidative stress. Glutathione (GSH) is an important non-enzymatic antioxidant in cells. GSH can act directly as an antioxidant to protect cells from free radicals Superoxide dismutase (SOD) is a very important antioxidant that fights oxidative stress in the body and is a good therapeutic agent against diseases mediated by reactive oxygen species. (Samarghandian et al., 2014)

Amphibians subjected to copper nanoparticles showed changes in protein, ALT, and AST in their blood (Osman, 2019). Elevated ALT and AST levels an adaptive reaction to the bloodstream's leaking of these enzymes as a result of the metals accumulating in the tissue. The liver is the main organ that is responsible for detoxifying the body of pollutants since it is a highly metabolically active organ with high antioxidant and related enzyme activity. Van der Oost et al. (2003) and Soares, (2023) state that depending on the kind and amount of stressor, antioxidant enzyme activity may either increase or decrease. Reduction in SOD & GSH in the liver of a toad. According to Finkel and Holbrook

(2000), Păunescu (2012) and Soares, (2023) GSH is arguably the most significant ROS scavenger involved in regulating the intracellular redox status. The considerable decline in GSH content occurred under the current experimental condition may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from Heavy metals. Some previous studies concluded that the inhibition of antioxidant enzymes Cause decrease the ability of frogs to metabolize environmental contaminants, turning the animals more vulnerable for intoxication (Amphibia Web, 2022). An imbalance of free radicals in the body might impact essential biological processes, which are necessary for an animal to survive (Soares, 2023). The degradation of polyunsaturated fatty acids in cell membranes brought on by heavy metals, which in turn encouraged the production of free radicals, is most likely what caused the increase in MDA. A common reaction to toxicity brought on by many pollutants that upset the equilibrium between oxidants and antioxidants is called oxidative stress. This imbalance results from either an overabundance of reactive oxygen species (ROS) that depletes SOD or from the depletion of antioxidants such glutathione (GSH) (Scandalios, 2005; Tsukada et al., 2023).

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