# SNAKES: A POTENTIAL BIO-INDICATOR OF ENVIRONMENTAL HEAVY METAL POLLUTION IN EL-FAIYUM DESERT, EGYPT

**Mohamed A.M. Kadry[1]***

[1]Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

*Corresponding author E-mail: ecokadry@yahoo.com

## ABSTRACT

This study was investigated to compare between the ecotoxicological effects of Mg, Fe, Cu, Co, Mo, Mn, B, Al, Cd and Cr on the saw-scaled viper, *Echis pyramidum* and the Kenyan sand boa, *Eryx colubrinus* inhabiting Gabal El-Nagar and Kahk Qibliyyah respectively in El-Faiyum desert, Egypt. Accumulation varied significantly among the liver, kidney and muscle. The relationship between concentrations of heavy metals in snakes and those in the soil from the collected sites was performed by analyzing metal DPTA in soil. RBCs count, Hb content, PCV, MCV, MCH and MCHC showed a higher decrease in *E. c.* than in *E. p.*. Histopathological examination showed that the tissues of *E. c.* were more destructed than those of *E. p.*. The present study showed that the saw-scaled viper, *Echis pyramidum* can withstand hard conditions. Heavy metals had more deleterious effects on the health of the Kenyan sand boa, *Eryx colubrinus* than that of the saw-scaled viper, *Echis pyramidum*. It was found that Kenyan sand boa, *Eryx colubrinus* is rapidly decreasing in number and unable to withstand tough conditions unlike saw-scaled viper, *Echis pyramidum*. So this investigation proved that the Kenyan sand boa, *Eryx colubrinus* may be considered a useful bio-indicator in the bio-monitoring of heavy metals as inorganic environmental contaminants. As a measure of their conservation status in their contaminated habitat, a Red Spot in the IUCN Red List of saw-scaled viper, *Echis pyramidum* and Kenyan sand boa, *Eryx colubrinus* was suggested to categorize them as Near Threatened and Vulnerable, respectively.

## KEYWORDS

*Echis pyramidum*, *Eryx colubrinus*, heavy metals, DPTA, Bio-accumulation, Biological parameters.

## INTRODUCTION

Blood parameters and calculated blood indices are variable relying on sex, age, pregnancy, physical exercise, weather, stress, altitude and captivity (Santos et al. 2008). Many researchers have stated that there are great intra-specific and inter-specific variations of blood cell count in snakes (Arikan et al. 2009, Tosunoğlu et al. 2011).

The variations in the blood parameters and calculated blood indices could be explained by not only the differences of the normal behavior of each species but also, food and activities of each species (Vasaruchapong et al. 2013, Ozzetti et al.

2015) and they are considered as a useful tool in demonstrating the physiology of snakes after exposure to environmental contaminants (Wack et al. 2012). The difference in blood parameters among species was due to some physiological factors such as sex, age, pregnancy, physical exercise, weather, stress, altitude, captivity and diet (Parida et al. 2014, Gómez et al. 2016).

Gavrić et al. (2015) mentioned that the position of snakes in food chain makes them very suitable for the accumulation of contaminants and bio-monitoring, and also snakes play important ecological roles in controlling the flow of nutrients, energy and contaminants in food webs. Bio-accumulation of metals has been examined in tissues of snakes (Drewett et al. 2013, Serseshk and Bakhtiari 2015). Since the bioaccumulation of metals has been established, the present study focused on the soil as a media transmitting heavy metals into the snakes' tissues and the biological consequences as a result of heavy metals uptake into the snakes' tissues.

The relationships between the concentration of heavy metals in snakes and those in soil should be examined from their respective collection sites (Rainwater et al. 2005). In this report, the status of essential heavy metals (Mg, Fe, Cu, Co, Mo and Mn) and non-essential heavy metals (B, Al, Cd and Cr) in Gabal El-Nagar and Kahk Qibliyyah in El-Faiyum, Egypt was studied and associated with the bio-availability of heavy metals in the saw-scaled viper, *Echis pyramidum* and the Kenyan sand boa, *Eryx colubrinus*. El-Faiyum soils are alkaline in nature and rich in $CaCO_3$ (Abd Elgawad et al. 2007).

Snakes are considered to be an ideal species for monitoring contaminants in wildlife. All snakes are carnivores and are known to accumulate environmental contaminants (Wylie et al. 2009, Rezaie-Atagholipour et al. 2012). Most snake species are live in small home range; thus, it is possible to make comparison between contaminant data from multiple sites in a narrow geographical area (Campbell et al. 2005, Rezaie-Atagholipour et al. 2012).

Snakes need further investigation in the field of Ecotoxicology as they are mainly found in the top surface of the soil, which is mainly contaminated with more heavy metals than the bottom (Olafisoye et al. 2013).

The family, viperidae comprises approximately 270 species of venomous snakes. The saw-scaled viper, *Echis pyramidum* (Geoffroy 1827) belongs to the family viperidae and is found in Asia, Africa, India, Iraq, Iran and Afghanistan. It occurs on a range of different substrates, including sand, rock, and soft soil and in scrublands. Often found hiding under loose rocks (Kadry et al. 2015).

Boidae, is a family of non-venomous snakes that found in America, Africa, Europe, Asia, and some pacific islands. It includes relatively primitive snakes and comprises eight genera and 43 species. The kenyan sand boa, *Eryx colubrinus* (Linnaeus 1758) is found in northern Africa from Egypt as far west as Niger including Somalia, Ethiopia, Sudan, Kenya, and northern Tanzania. It occurs in semi-desert, scrub savannahs and rock outcroppings. Also it prefers sandy and friable soil (Kadry et al. 2015).

So, the aim of the presented study is to select two different species of common snakes inhabiting different habitats in El-Faiyum governorate to consider one of them as a good bio-indicator to the toxic potency of the contaminated habitat, measure blood parameters of the distinct populations of the snakes and to examine the effect of ecological contamination among populations, evaluate sites contaminated with compounds transferred via trophic mechanisms through heavy metal determination and demonstrate histopathological alterations occurred in some vital organs (liver, kidney and testes).

## MATERIALS AND METHODS

### Experimental snakes

**The two selected studied snakes are:**

**1)** The saw-scaled viper, *Echis pyramidum* (Geoffroy 1827) **(Fig. 1a)**

**Latin name:** *Echis pyramidum*

**Common name:** Saw-scaled viper; Haiya Ghariba Samra

It is a medium-sized snake, with a short, stocky body, tail is short, eye is moderate, dorsum gray, with a mid-dorsal series of dark-edged, whitish, narrow saddles, interspersed with large dark brown-gray blotches; a large series of smaller dark spots; dorsal side of head with a dark arrow like mark (often brown), indistinct dark & diagonal band below the eye, venter white.

**2)** The kenyan sand boa, *Eryx colubrinus* (Linnaeus 1758) **(Fig. 1b)**

**Latin name:** *Eryx Colubrinus*

**Common name:** The kenyan sand-boa: Dassas Saedi

It is a short and thick snake, A relatively short tail, head is covered with small scales, dorsum sandy, with large, irregularly shaped, dark brown blotches; venter plain & yellowish.

### Study area

Both species of selected snakes; *Echis pyramidum* and *Eryx colubrinus* were collected from two different sites in El- Faiyum desert, Egypt **(Fig. 2)**:

**Site 1 [Gabal El-Nagar] (Figs. 2-4)**

Specimens of saw-scaled viper; *Echis pyramidum* as well as soil samples were collected during the summer season from Gabal El-Nagar (Mahatet El-Rafa) [29° 22` N 30° 37`E]; a rocky area near water drainage and a cultivated area planted with wheat.

**Site 2 [Kahk Qibliyyah, Abshowy] (Fig. 2 & Figs. 5&6)**

Specimens of Kenyan sand boa; Eryx *colubrinus* as well as soil samples were collected during the summer season from Kahk Qibliyyah [29° 24` N 30° 38` E]; a planted area near a sandy road.

**Preparation of blood samples**

Eight adult male individuals of each population of the snakes; *Echis pyramidum and Eryx coloubrinus* were decapitated, soon after their arrival. Blood samples were withdrawn from the heart into heparinized eppendorfs and was used for the estimation of blood parameters [red blood corpuscles (RBCs) count, hemoglobin content (Hb) and packed cell volume (PCV)].

**Determination of hematological parameters**

**Determination of red blood corpuscles (RBCs) count**

Using improved neubauer haemocytometer.

**Determination of hemoglobin (Hb)**

Hemoglobin was estimated spectrophotometrically in whole blood collected in heparin according to the method of Drabkin and Austin 1932 using a kit of **Biodiagnostics™** Company.

**Determination of packed cell volume (PCV)**

We used hematocrit centrifuge.

**Blood indices**

Blood indices; Mean corpuscular volume **(MCV),** Mean corpuscular hemoglobin **(MCH)** and Mean corpuscular hemoglobin concentration **(MCHC)** were calculated according to Gupta 1977.

### Heavy metals bio-accumulation in different vital tissues

Snakes were dissected; livers, kidneys and muscles were removed. The method provided an acid digestion of the snakes' tissue samples in a closed vessel device using Temperature control microwave heating (Milestone ETHOS labstation with easyWAVE or easyCONTROL software HPR1000/10S high pressure segmented rotor- Microwave Digestion Labstation closed system, ETHOS Pro, Milestone, Italy) for the metal determination by spectroscopic methods.

### Inductively Coupled Argon Plasma (ICAP)

Inductively Coupled Argon Plasma, ICAP 6500 Duo, Thermo Scientific, England. 1000 mg/L multi-element certified standard solution, Merck, Germany was used as stock solution for instrument standardization.

### Determination of heavy metals in the snakes' vital tissues

The accumulation of essential heavy metals (Ca, Mg, Fe, Cu, Zn, Co, Mo and Mn) and non-essential heavy metals (B, Al, Sr, Pb, Ni, Cd and Cr) were determined livers, kidneys and muscles of both studied snakes' species; *Echis pyramidum* and *Eryx Colubrinus*.

### Calculation

Heavy metal concentration in tissue (ppm)

$$= \frac{\text{The reading from ICAP}}{\text{Wet weight of tissue}} \times 25(dilution\ factor)$$

### Determination of heavy metals in available soil

The determination of heavy metals in available soil was according to the method of Soltanpour and Schwab 1977.

### Calculation

Heavy metal concentration in ppm = Reading from ICAP $\times 2\ (dilution\ factor)$

### Histopathological examination:

Autopsy samples were taken from the liver, kidney and testes of the two selected studied snakes, and then they were fixed in 10% formol saline for twenty four hours. Washing was done in tape water followed by serial dilutions of alcohols (methyl, ethyl and absolute ethyl) for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for twenty four hours. Paraffin bees wax tissues blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxlin & eosin stains for routine examination through the light microscope (Bancroft and Gamble 2002).

**Statistical analysis**

Pairwise significance tests were made using Student's ***t-test*** to compare between Kenyan sand boa, *Eryx colubrinus* and saw-scaled viper, *Echis pyramidum* in all studied blood parameters and heavy metals bioaccumulation in their different tissues. A probability level at **(P<0.05)** was considered as significant, while at **(P<0.01)** was considered as highly significant.

All statistics were carried out using statistical analysis program; Predictive Analytics Software **(PASW statistics 18.0 Release 18.0.0)**.

## ETHICAL CONSIDERATIONS

We followed and performed our study protocol from the scientific ethical point of view in selection and handling of snakes according to Animal welfare Act of the Ministry of Agriculture in Egypt that enforces the humane treatment of animal.

## RESULTS

**Hematology:**

**Blood parameters:**

*Echis pyramidum* (*E. p.*) showed a very highly significant increase (P<0.001) in all studied blood parameters; Red blood corpuscles (RBCs) count, Hemoglobin content (Hb) and Packed cell volume (PCV) than in *Eryx colubrinus* (*E. c.*) **(Table 1)**.

**Calculated blood indicies**

*Echis pyramidum* (*E. p.*) showed a very highly significant increase (P<0.001) in all calculated blood indices; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) than in *Eryx colubrinus* (*E. c.*) **(Table 2)**.

## Heavy metals in soil

### Concentration of essential heavy metals in soil

While the essential heavy metals; Fe and Cu in soils collected from Kahk Qibliyyah showed a highly significant increase (P<0.001) than those collected from Gabal El-Nagar, Co and Mn recorded a significant increase (P<0.05) in soils of Kahk Qibliyyah than in the other site. Mg concentrations recorded a highly significant increase (P<0.01) in soils collected from Gabal El-Nagar compared to those collected from Kahk Qibliyyah, but Mo was significantly higher in Gabal El Nagar than in Kahk Qibliyyah (P<0.001) **(Table 3)**.

### Concentration of non-essential heavy metals in soil

While Cd concentration showed a highly significant increase (P<0.01) in soils collected from Kahk Qibliyyah compared to those collected from Gabal El-Nagar, the non-essential heavy metal; Cr revealed a highly significant increase (P<0.001) in soils of Kahk Qibliyyah than those collected from Gabal El-Nagar. B concentration recorded a highly significant increase (P<0.01) in soils collected from Gabal El-Nagar than in Kahk Qibliyyah, while Al concentration was higher in Gabal El-Nagar soils than in Kahk Qibliyyah but of non-significant difference **(Table 4)**.

## Heavy metals in snakes:

### Bio-accumulation of essential heavy metals in liver, kidney and muscle tissues

The Saw-scaled viper, *Echis pyramidum* bio-accumulated a very highly significant concentrations (P<0.001) of some essential heavy metals (Mg and Mn) in their studied tissues (liver, kidney and muscle) than in the Kenyan sand boa, *Eryx colubrinus*. Fe was bio-accumulated in the Kenyan sand boa, *Eryx colubrinus* in very highly significant concentartions in liver (P < 0.001), and highly significant concentrations in kidney (P < 0.01), and it was higher in muscle with insignificant difference compared to the saw-scaled viper, *Echis pyramidum*. While Mo was highly significant bio-accumulated (P<0.01) in liver and kidney tissues of the Kenyan sand boa, *Eryx colubrinus*, it revealed a significant increase (P<0.05) in muscle of the Kenyan sand boa, *Eryx colubrinus* than in the saw-scaled viper, *Echis pyramidum*. The bio-accumulation of Cu in kidney and muscle tissues of the Kenayn sand boa, *Eryx colubrinus* were very highly significant increase (P<0.001) and highly significant increase (P<0.01) respectively but it recorded a very highly significant increase (P<0.001) in liver of the saw-scaled viper, *Echis pyramidum* than in the Kenyan sand boa, *Eryx colubrinus*. Co was bioaccumulated in liver and kidney tissue of the Kenayn sand boa, *Eryx colubrinus* than in the saw-scaled viper, *Echis pyramidum* with very highly significant difference (P<0.001) while in muscle tissues, Co was detected only in the Kenyan sand boa, *Eryx Colubrinus* **(Table 5)**.

**Bio-accumulation of non-essential heavy metals in liver, kidney and muscle tissues**

The present data showed that the bio-accumulation of Cd in liver and kidney recorded a very highly significant concentrations (P<0.001) in the Kenyan sand boa, *Eryx colubrinus* than in the saw-scaled viper, *Echis pyramidum* while muscle recorded a very highly significant concentrations (P<0.001) in the saw-scaled viper, *Echis pyramidum* than in the Kenyan sand boa, *Eryx colubrinus*. Cr bio-accumulated in a very highly significantly concentrations (P<0.001) in kidney and muscle of the saw scaled viper, *Echis pyramdium*, while liver of the saw-scaled viper, *Echis pyramidum* revealed a very highly significantly concentrations (P<0.001) than the Kenyan sand boa, *Eryx colubrinus*. Al was very highly significantly bio-accumulated (P<0.001) in all studies tissues of the saw-scaled viper, *Echis pyramidum* than in the Kenyan sand boa, *Eryx colubrinus*. B bio-accumulated a very highly significantly concentrations (P<0.001) and highly significant concentrations (P<0.01) in [liver and muscle] and kidney respectively of the Kenyan sand boa, *Eryx colubrinus* than in the saw-scaled viper, *Echis pyramidum* **(Table 6)**.

**Histopathological examination:**

**I. Liver**

**I.1. Liver of saw-scaled viper, *Echis pyramidum*:**

Liver of the saw-scaled viper, *Echis pyramidum* showed congestion in central vein with intracytoplasmic vacuolization of hepatocytes **(Fig. 7a)**, and it also showed congestion in the portal vein surrounded by fibers and blood sinusoids that considered a sign of fibrosis **(Fig. 7b)**.

**I.2. Liver of Kenyan sand boa, *Eryx colubrinus*:**

Section of liver of the Kenyan sand boa, *Eryx colubrinus* showed severe dilations of central vein surrounded by fibers with severe degeneration in hepatocytes in the surrounding area **(Fig. 8a)**, it showed focal aggregation of melanin-pigmented cells in the hepatic parenchyma **(Fig. 8b)**, and **Fig. 8c** was a magnification of **Fig. 8b**.

## II. Kidney:

### II.1. Kidney of saw-scaled viper, *Echis pyramidum*:

Kidney of the saw-scaled viper, *Echis pyramidum* showed hypertrophy and vacuolization in the lining epithelium of distal convoluted tubules **(Fig. 9a)**, it showed focal extravasation of red blood cells between degenerated distal convoluted tubules **(Fig. 9b)**, it also showed hyalinization in the connective tissue stroma between the degenerated distal convoluted tubules **(Fig. 9c)**.

### II.2. Kidney of Kenyan sand boa, *Eryx colubrinus*:

kidney of the Kenyan sand boa, *Eryx colubrinus* showed hypertrophy and vacuolization in the lining epithelium of distal convoluted tubules **(Fig. 10a)**, it showed course granular eosinophilic cytoplasm in the tubular lining epithelium of distal convoluted tubules **(Fig. 10b)**.

**Fig. 10c** was the magnification of **Fig. 10b** to identify the granular eosinophilic cytoplasm of the lining epithelium of distal convoluted tubules; it also showed pyknosis deep bluish nuclei with deep eosinophilic cytoplasm of some lining tubular epithelium and distal convoluted tubules **(Fig. 10d)**.

## III. Testes:

### III.1. Testes of saw-scaled viper, *Echis pyramidum*:

Testes of the saw-scaled viper, *Echis pyramidum* showed degeneration in the seminiferous tubules and vacuolization in all the stages of sperm formation associated with the loss of spermiogenesis **(Fig. 11a)**, **Fig. 11b** was magnification of **Fig. 11a** and **Fig. 11c** was magnification of **Fig. 11b**.

### III.2. Testes of Kenyan sand boa, *Eryx colubrinus*:

Testes of the Kenyan sand boa, *Eryx colubrinus* showed degeneration of some seminiferous tubules **(Fig. 12a)**, **Fig. 12b** was a magnification of **Fig. 12a**, and **Fig. 12c** was a magnification of **Fig. 12b**.

## DISCUSSION

Hematology of snakes is considered as useful tools to evaluate the health conditions of individuals especially the adverse effects that are associated with pollution as a result of the exposure to environmental contaminants (Kadry 2011).
Results of the present study showed that a significant increase in the blood parameters and calculated blood indices of the saw-scaled viper, *Echis pyramidum*

while there is a significant decrease in the blood parameters and calculated blood indices of the Kenyan sand boa, *Eryx colubrinus*.

Results of Tosunoğlu et al. (2011) showed that the venomous snakes exhibited a significant increase in the blood parameters and calculated blood indices, while non-venomous snakes exhibited a significant decrease in the blood parameters and calculated blood indices, and this finding agreed with the present investigation.

Heavy metals are the most studied class of contaminants in toxicological reports of snakes (Sparling et al. 2010).

Magnesium is abundant in the living organisms. It is important in protein synthesis, muscle and nerve functions and regulation of blood pressure; elevated levels of Mg are associated with histological alteration in kidney (NIH 2016). hyalinization in the connective tissue stroma between the degenerated distal convoluted tubules could be attributed the significantly higher Mg levels in the soil of Gabal El-Nagar as well as their bio-accumulation in the kidney of *E. p.* than in *E. c.*.

Iron is is essential for erythropoiesis and a key component of hemoglobin, myoglobin heme enzymes, allofavoprotein enzymes and mitochondrial enzymes **(**IOM 2001, Papanikolaou and Pantopoulos 2005). Excess and insufficient Fe is toxic (Liu et al. 2008). Cobalt is an essential element required for the health of animals and it is considered a part of vitamin B12 and enhances the levels of red blood corpuscles, high levels of cobalt damage liver as well as kidney. The main toxic effect of cobalt is immunological disorder (Liu et al. 2008). Our findings revealed a significantly higher decrease in blood parameters as well as blood indices of *E. c.* than in *E. p.*. Also severe degeneration of hepatocytes and pyknosis of kidney with more hypertrophy and vacuolization of kidney of *E. c.* could be correlated with the higher Fe and Co levels in the soil of Kahk Qibliyyah and higher bio-accumulation of Fe & Co in their liver and kidney tissues.

Molybdenum is an important cofactor for some enzymes in animals (EC SCF, 2000) and has demonstrated reproductive toxicity in animals and reduces the fertility of male reproductive system in various animals (IOM 2001). Boron is widely found in earth crust and has no biological importance; it induces male reproductive toxicity and pulmonary disorders in animals (ATSDR 2010). Cd is considered one of the most toxic heavy metals and is associated with renal failure, and aberrant reproductive function (Rie et al. 2001). Degeneration of seminiferous tubules and vacuolization in all the stages of sperm formation associated with the loss of spermiogenesis in testes of *E. p.* might be attributed to the higher levels of B and Mo in the soil of Gabal El-Nagar, but the higher accumulation of Cd in the soil of Kahk Qibliyyah might be related to the degeneration of seminiferous tubules in testes of *E. c.*.
Manganese is an essential trace element (Liu et al. 2008, ATSDR 2012 a). High elevated level of Manganese damages liver (Mas 2006, Liu et al. 2008), damage of sperms which causes adverse changes in male reproductive system and

enhances renal disorders (ATSDR 2012 b). Aluminum is widely distributed in the earth's crust, it's reported in animal studies that Al induce respiratory disorders, nervous and testes toxicity (ATSDR 2008). Aluminum causes histopathological alterations in kidney (Liu et al. 2008). Chromium is abundant in air, soil, rocks, water and transmitted to plants and animals; Chromium causes sperm damage and destruction of male reproductive system (ATSDR 2012 a). Our results reported a higher bio-accumulation of Al, Mn and Cr in kidney of *E. p.* than in *E. c.* which could be related to the degeneration of distal convoluted tubules with hyalinization of connective tissue stroma between the tubules. Also these three heavy metals made a severe degeneration and vacuolization of seminiferous tubules in testes of *E. p.*.

Copper is essential for good health but very high intake can cause adverse health problems such as kidney and liver damage (Ikem and Egiebor 2005). Drinking water containing an excess amount of Cu is major threat to terrestrial and aquatic organisms (Liu et al. 2008). Because copper is an essential element, copper is a component of some enzymes and may play an important role in immune function (Yadollahvand et al. 2014). The higher Cu levels in soil of Kahk Qibliyyah as well as their higher bio-accumulation in the kidney of *E. c.* could be correlated with the severe kidney damage of *E. c.* compared to *E. p.*, but the higher bio-accumulation of Cu in liver of *E. p.* damaged the hepatocytes and might lead to fibrosis.

## CONCLUSION

In conclusion, the present study showed that the saw-scaled viper, *Echis pyramidum* can withstand hard conditions. Heavy metals had more deleterious effects on the health of the Kenyan sand boa, *Eryx colubrinus* than that of the saw-scaled viper, *Echis pyramidum*.

It was found that Kenyan sand boa, *Eryx colubrinus* is rapidly decreasing in number and unable to withstand tough conditions unlike saw-scaled viper, *Echis pyramidum*. So this investigation proved that the Kenyan sand boa, *Eryx colubrinus* may be considered a useful bio-indicator in the bio-monitoring of heavy metals as inorganic environmental contaminants.

In Egypt, both saw-scaled viper, *Echis pyramidum* and Kenyan sand boa, *Eryx colubrinus* were classified as Least Concern, but as a measure of their conservation status in their contaminated habitat, a Red Spot in the IUCN Red List of saw-scaled viper, *Echis pyramidum* and Kenyan sand boa, *Eryx colubrinus* was suggested to categorized them as **Near Threatened** and **Vulnerable**, respectively (IUCN 2005).

## CONFLICT OF INTEREST

Author stated that there is no conflict of interest.

## REFERENCES

Abd Elgawad, M.; Mohamed, H.A.A.; Shendi, M.M.; Ghabour, S.I. (2007) Status of some heavy metals in Fayoum district soils. The third Conference for Sustainable Agricultural Development, Faculty of Agriculture, Fayoum University, pp. 507-526.

Agency for Toxic Substance and Disease Registry (ATSDR) (2008) Toxicological profile for Aluminum (update). Agency for toxic substances and Disease Registery, Atlanta, Georgia, pp.1-310.

Agency for Toxic Substance and Disease Registry (ATSDR) (2010) Toxicological profile for Boron (update). Agency for toxic substances and Disease Registery, Atlanta, Georgia, pp.1-197.

Agency for Toxic Substance and Disease Registry (ATSDR) (2012 a) Toxicological profile for Chromium (update). Agency for toxic substances and Disease Registery, Atlanta, Georgia, pp.1-502.

Agency for Toxic Substance and Disease Registry (ATSDR) (2012 b) Toxicological profile for Manganese (update). Agency for toxic substances and Disease Registery, Atlanta, Georgia, pp.1-506.

Arikan, H.; Göçmen, B.; Atatür, M.K.; Kumlutaş, Y.; Çiçek, K. (2009) Morphology of peripheral blood cells from various Turkish snakes. *North-*Western Journal of Zoology 5(1): 61-73.

Bancroft, J.D.; Gamble, M. (2002) Theory and practice of histological techniques (edited by Jones, M. L.: lipids and Tolly, B. A.: mucins). 5th edition Churchill Livingstone, London, Edinburgh., New York, Philadelphia, St Louis Sydney, Toronto.

Campbell, K.R.; Campbell, T.S.; Burger, J. (2005) Heavy metal concentrations in northern water snakes (*Nerodia sipedon*) from East Fork Poplar Creek and the Little River, East Tennessee, USA. Archives of Environmental Contamination and Toxicology 49: 239-248.

Drabkin, D.L.; Austin, J.H. (1932) Spectrophotometric studies I. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. Journal of Biological Chemistry 98: 719-733.

Drewett, D.V.V.; Willson, J.D.; Cristol, D.A.; Chin, S.Y.; Hopkin, W.A. (2013) Inter- and intraspecfic variation in mercury bioaccumulation by snakes inhabiting a contaminated river floodplain. Environmental Toxicology and Chemistry 32(5): 1178-1186.

European Commission, Scientific Committee on Food (EC SCF) (2000) Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Molybdenum. European Commission, SCF/CS/NUT/UPPLEV/22 Final Report. Brussels.

Gavrić, J.P.; Prokić, M.D.; Anđelković, M.Z.; Despotović, S.G.; Gavrilović, B. R.; Borković-Mitić, S.S.; Radovanović, T.B.; Tomović, L.M.; Pavlović, S.Z.; Saičić, Z. S. (2015) Effects of metals on blood oxidative stress biomarkers and acetylcholinesterase activity in dice snakes (*Natrix tessellate*) from Serbia. Archives of Biological Sciences, Belgrade 67(1): 303-315.

Gómez, A.; Arroyo, C; Astorga, W.; Chacón, D; Rodríguez, S.; Jiménez, M. (2016) Hematological and biochemical reference intervals for *Bothrops asper* and *Crotalus simus* (Serepnates: Viperidae), maintained in captivity for venom extraction. Comparative Clinical Pathology 25(3): 615-623.

Gupta, P.K. (1977) Hematological techniques. 4th edition Syndicate, India, pp. 231.

Ikem, A.; Egiebor, N. O. (2005) Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). Journal of Food Composition and Analysis 18(8): 771-787.

IOM (2001) Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Institute of Medicine, Food and Nutrition Boards (eds.): Washington, edited by DC: National Academic Press, pp.1-769.

IUCN (2005) Global reptile assessment. Species account for Mediterranean species.

Kadry, M.A.M. (2011) Ecological and Biological studies in the lizard; *Laudakia stellio* inhabiting different geographical habitats. PhD Thesis. Zoology department Faculty of Science Cairo University.

Kadry, M.A.M.; Mohallal. E. M. E; Sleem, D. M. M.; Marie, M. A. S. (2015): Inter-specific biochemical diversity between *Echis pyramidum* and *Eryx colubrinus* inhabiting El-Faiyum, Egypt. Advances in Bioscience and Biotechnology 6: 495-500.

Liu, J.; Goyer, R.A.; Waalkes, M.P. (2008) Toxic effects of metals. In: Casarett&Doull's Toxicology The basic science of poisons, edited by Klassen, C. D. (Edn.), 7th edn. McGraw-Hill Medical publishing health, pp. 931-980.

Mas, A. (2006) Hepatic encephalopathy: From pathophysiology to treatment. *Digestion*, 73 (Suppl 1): 86-93.

NIH (2016) National Institute of Health, Office of dietary supplements. Magnesium. https://ods.od.nih.gov/factsheets/Magnesium-HealthProfessional/

Olafisoye, O.B.; Adefioye, T.; Osibote, O. A. (2013) Heavy metal concentration of water, soil and plants around an electronic waste dumpsite. Polish Journal of Environmental Studies 22(5): 1431-1439.

Ozzetti, P.A.; Cavlac, C.L.; Sano-Martins, I.S. (2015) Hematological reference values of the snakes *Oxyrhopus guibei* and *Xenodon neuwiedii* (Serpentes: Dipsadidae). Comparative Clinical Pathology 24(1): 101-108.

Papanikolaou, G.; Pantopoulos, K. (2005) Iron metabolism and toxicity. Toxicology and Applied Pharmacology 202(2): 199–211.

Parida, S.P.; Dutta, S.K.; Pal, A. (2014) Hematology and plasma biochemistry of wild-caught Indian cobra *Naja naja* (Linnaeus, 1758). Journal of venomous animal and toxins including Tropical Diseases 20: 14.

Rainwater, T.R.; Reynolds, K.D.; Cañas, J.E.; Cobb, G.P.; Anderson, T.A.; McMurry, S.T. and Smith, P.N. (2005) Organochlorine pesticides and mercury in cottonmouth (*Agkistrodon piscivorus*) from northeastern Texas, USA. Environmental Toxicology and Chemistry 24(3): 665-673.

Rezaie-Atagholipour, M.; Riyahi-Bakhtiari, A.; Sajjadi, M.; Yap, C.K.; Ghaffari, S.; Ebrahimi-Sirizi, Z.; Ghezellou, P. (2012) Metal concentrations in selected tissues and main prey species of the annulated sea snake (*Hydrophis cyanocinctus)* in the Hara protected Area, northeastern coast of the Persian Gulf, Iran. Marine Pollution Bulletin 64: 416-421.

Rie, M.; Lendas, K.; Callard, I.P. (2001) Cadmium: tissue distribution and binding protein induction in the painted turtle, *Chrysemys picta*. Comparative Biochemestiry and Physiology part C: Pharmacology Toxicology Endocrinology 130 (1): 41-45.

Santos, K.R.; Takahira, R.K.; Rall, V.L.M.; Calderón, C.; Sequeira, J.L. and Silva, R.J. (2008) Pulmonary, microbiological and hematological changes in *Crotalus durissus terrificus* (Serpentes, Viperidae) parasitized by nematodes of the genus Rhabdias (Nematoda, Rhabdiasidae). Arquivo Brasileiro de Medicina Veterinária e Zootecnia 60: 667-674.

Sereshk, Z.H.; Bakhtiari, A.R. (2015) Concentrations of trace elements in kidney, liver, muscle and skin of short sea snake (*Lapemis curtus*) from the Strait of Hormuz Persian Gulf. Environmental Science and Pollution Research 22: 15781-15787.

Soltanpour, P.N.; Schwab, A.P. (1977) A new soil test for simultaneous extraction of macro- and micro- nutrients in alkaline soils. Communications in Soil Science and Plant Analysis 8(3): 195-207.

Sparling, D.W.; Linder, G.; Bishop, C.A.; Krest, S.K. (2010) Recent advancement in amphibian and reptile ecotoxicology, In: Ecotoxicology of Amphibians and Reptiles, edited by D. W.; Linder, G.; Bishop, C. A. and Krest, S. (Eds.), Ecotoxicology of Amphibians and Reptiles, 2nd edn. Taylor &Francis, New York, NY, USA, pp. 1-12.

Tosunoğlu, M.; Gül, Ç.; Yilmaz, N.; Topyildiz, H. (2011) Hematological reference intervals of some snake species in Turkey. Turkish Journal of Zoology 35(2): 237-243.

Vasaruchapong, T.; Disarapong, P.; Chulasugandha, P.; Khow, O.; Chanhome, L.; Chiobamroongkiat, M.; Chaiyabutr, N.; Sitprija, V. (2013) Comparative studies on hematological and plasma biochemical parameters in different types of venomous snakes in Thailand. Comparative Clinical Pathology 23(4): 955-959.

Wack, R.F.; Hansen, E.; Small, M.; Poggenga, R.; Bunn, D.; Johnson, C. K. (2012) Hematology and plasma biochemistry values for the giant garter snake (*Thamnophis gigas*) and valley garter snake (*Thamnophis* References *sirtalis fitchi*) in the central valley of California. Journal of Wildlife diseases 48(2): 307-313.

Wylie, G.D.; Hothem, R.L.; Bergen, D.R.; Martin, L.L.; Taylor, R.J.; Brussee, B. E. (2009) Metal and trace elements in giant garter snakes (*Thamnophis gigas*) from the Sacramento Valley, California, USA. Archives of Environmental Contamination and Toxicology 56: 577–587.

Yadollahvand, R.; Kami, H. G.; Mashroofeh, A. and Bakhtiari, A. R. (2014): Assessment trace elements concentrations in tissues in Caspian Pond Turtle (*Mauremys caspica*) from Golestan Province, Iran. Ecotoxicology and Environmental Safety 101: 191-195.

## EXPLANATIONS OF FIGURES

**Fig. 1** Photos of *Echis pyramidum* (a) and *Eryx colubrinus* (b) inhabiting El-Faiyum desert, Egypt.

**Fig. 2** Satellite image showing the study sites of *Echis pyramidum* and *Eryx colubrinus* inhabiting El-Faiyum governorate, Egypt.

**Fig. 3** Gabal El-Nagar (Mahatet El-Rafa), El-Faiyum governorate, Egypt.

**Fig. 4**. Photomicrograph showing contaminated sites in Gabal El-Nagar
C.W: Contaminated water; T: Trash; W.L.M: Water lifting machine; W.L.S: Water lifting station; L.M: Lifting machine; W.P.M: Water pumping machine; C.S.S: Collected soil samples.

**Fig. 5** Kahk Qibilyyah, Abshowy, El-Faiyum governorate, Egypt.

**Fig. 6** Photomicrograph showing contaminated site in Kahk Qibliyyah
C.C: Contaminated coal; T.P: Tractor's print; C.S.S: Collected soil samples; R: Road; E.F: Exhausted fumes; W.P.M.: Water Pumping Machine and W.L.M.: Water Lifiting Machine.

**Fig. 7** Photomicrograph shows section of liver of saw-scaled viper, *Echis pyramidum*. C.V: Central vein, F: Fibers, H: Hepatocytes, P.V: Portal.

**Fig. 8** Photomicrograph shows section of liver of Kenyan sand boa, *Eryx colubrinus*. C.V: Central vein, F: Fibers, H: Hepatocytes, M.P: Melanin.

**Fig. 9** Photomicrograph shows section of kidney of saw-scaled viper, *Echis pyramidum*. G: Glomerulus D.C.T: Distal convoluted tubule B.Cs: Blood cells.

**Fig. 10** Photomicrograph shows section of kidney of Kenyan sand boa, *Eryx colubrinus*. G: Glomerulus D.C.T: Distal convoluted tubules.

**Fig. 11** Photomicrograph shows section of testes of saw-scaled viper, *Echis pyramidum*. S.T: Seminiferous tubule.

**Fig. 12** Photomicrograph shows section of testes of Kenyan sand boa, *Eryx Colubrinus*. S.T: Seminiferous tubule.

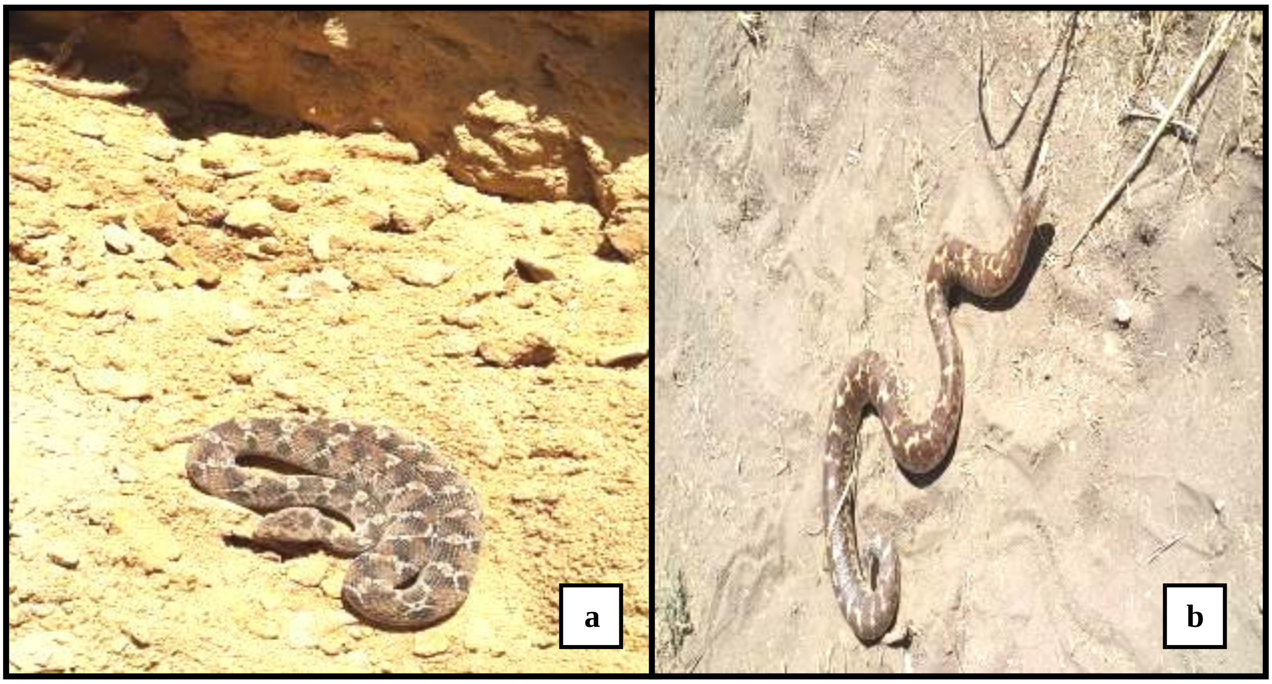


Fig. 1. Photos of *Echis pyramidum* (a) and *Eryx colubrinus* (b) inhabiting El-Faiyum desert, Egypt.

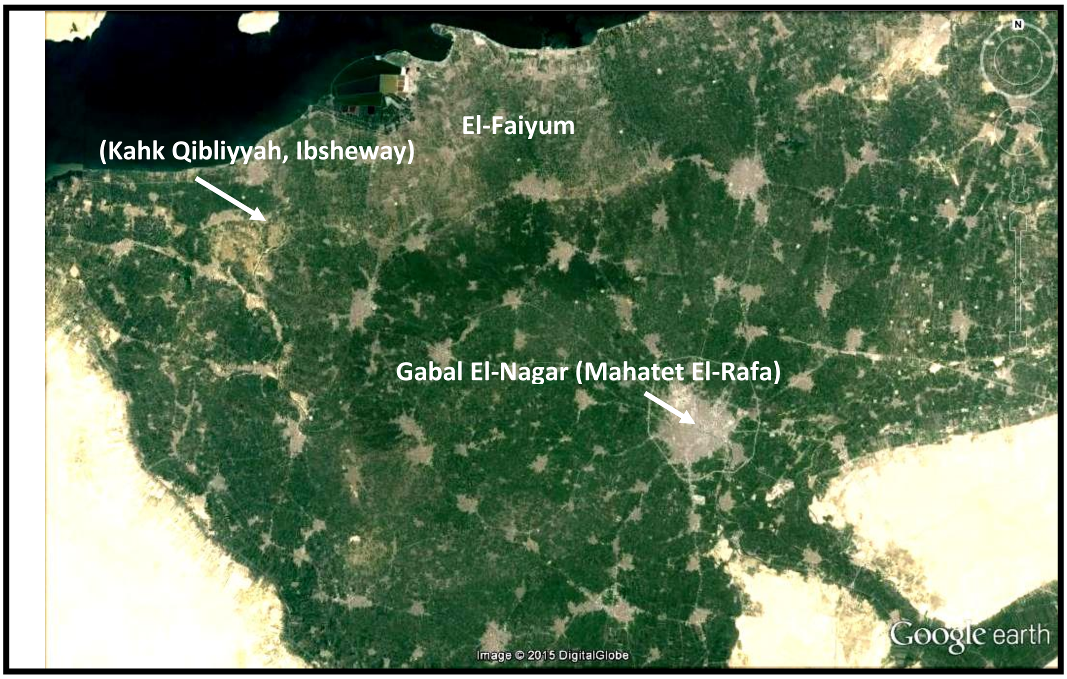


Fig. 2. Satellite image showing the study sites of *Echis pyramidum* and *Eryx colubrinus* inhabiting El-Faiyum governorate, Egypt.

Fig. 3. Gabal El-Nagar (Mahatet El-Rafa), El-Faiyum governorate, Egypt.


Fig. 4. Photomicrograph showing contaminated sites in Gabal El-Nagar
C.W: Contaminated water; T: Trash; W.L.M: Water lifting machine; W.L.S: Water lifting station; L.M: Lifting machine; W.P.M: Water pumping machine; C.S.S: Collected soil samples.

Fig. 5. Kahk Qibilyyah, Abshowy, El-Faiyum governorate, Egypt.


Fig. 6. Photomicrograph showing contaminated site in Kahk Qibliyyah

C.C: Contaminated coal; T.P: Tractor's print; C.S.S: Collected soil samples; R: Road; E.F: Exhausted fumes; W.P.M.: Water Pumping Machine and W.L.M.: Water Lifiting Machine.

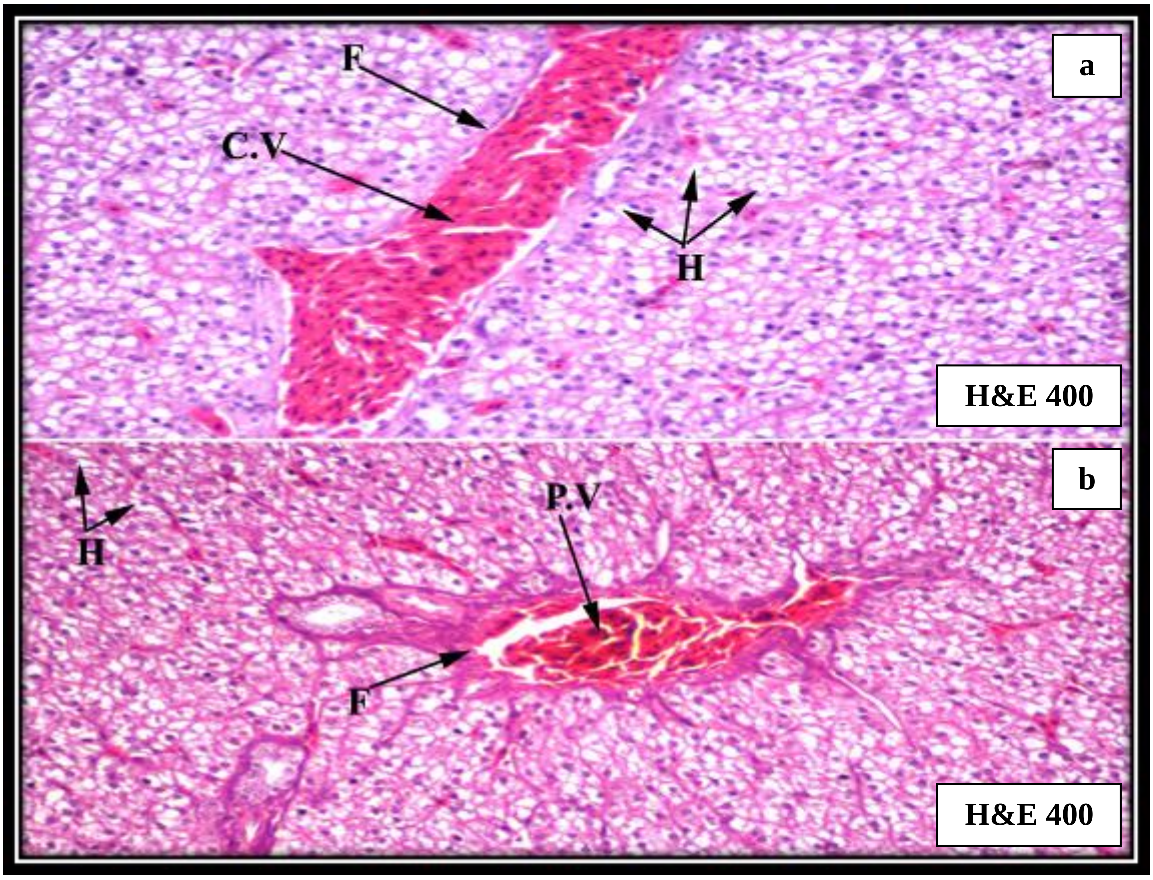


Fig .7. Photomicrograph shows section of liver of saw-scaled viper, *Echis pyramidum*.

C.V: Central vein, F: Fibers, H: Hepatocytes, P.V: Portal

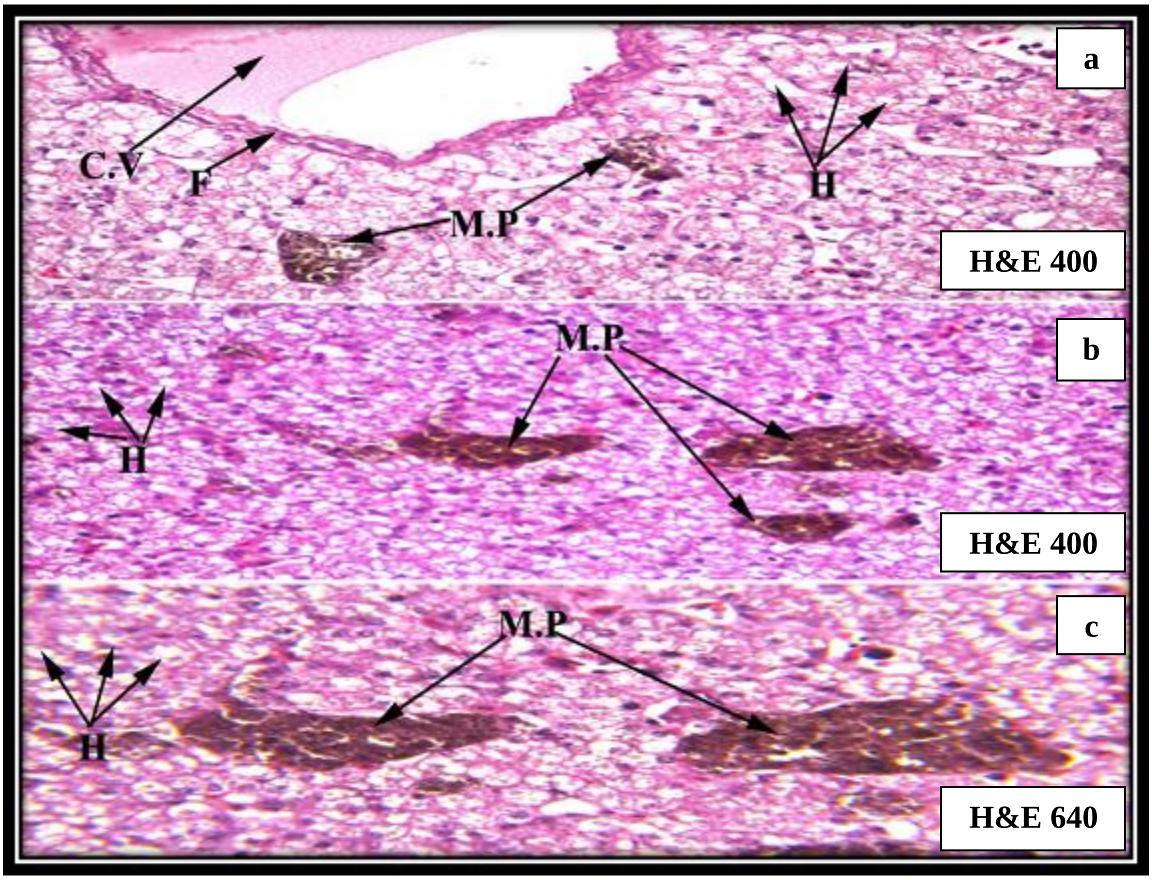


Fig. 8. Photomicrograph shows section of liver of Kenyan sand boa, *Eryx colubrinus*.

C.V: Central vein, F: Fibers, H: Hepatocytes, M.P: Melanin

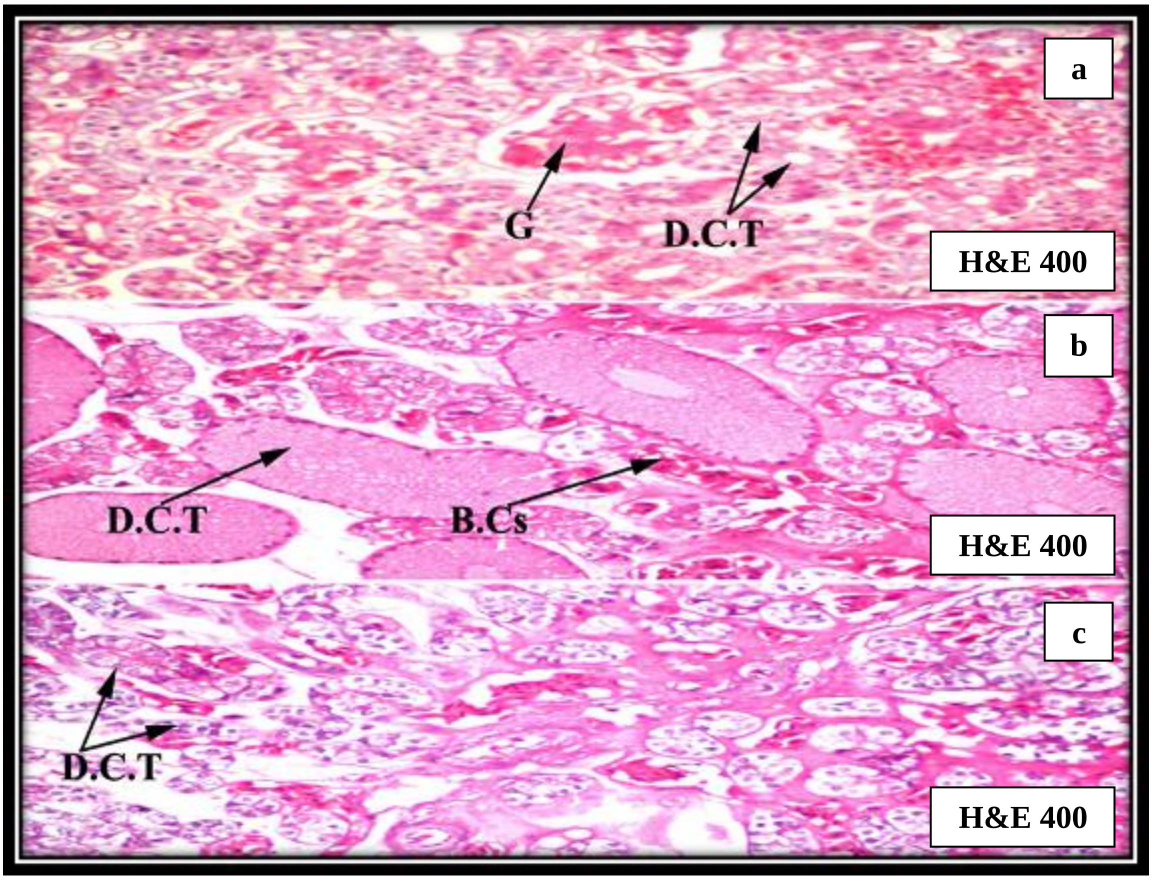


Fig. 9. Photomicrograph shows section of kidney of saw-scaled viper, *Echis pyramidum*.

G: Glomerulus, D.C.T: Distal convoluted tubule, B.Cs: Blood cells

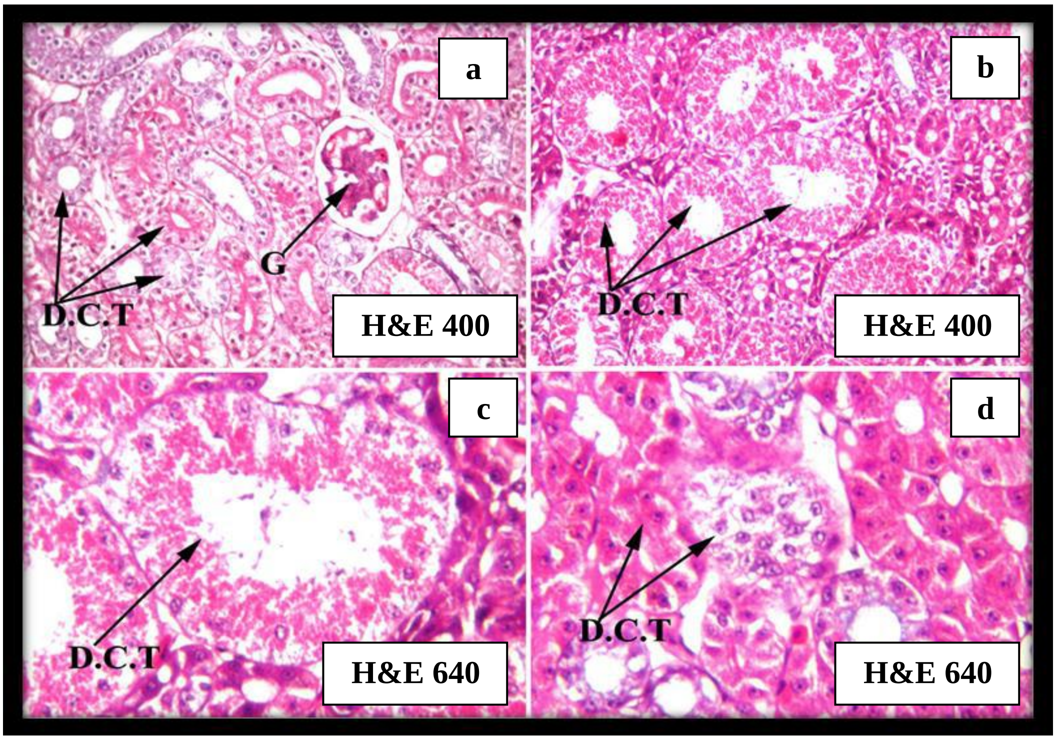


Fig. 10. Photomicrograph shows section of kidney of Kenyan sand boa, *Eryx colubrinus*.

G: Glomerulus, D.C.T: Distal convoluted tubules

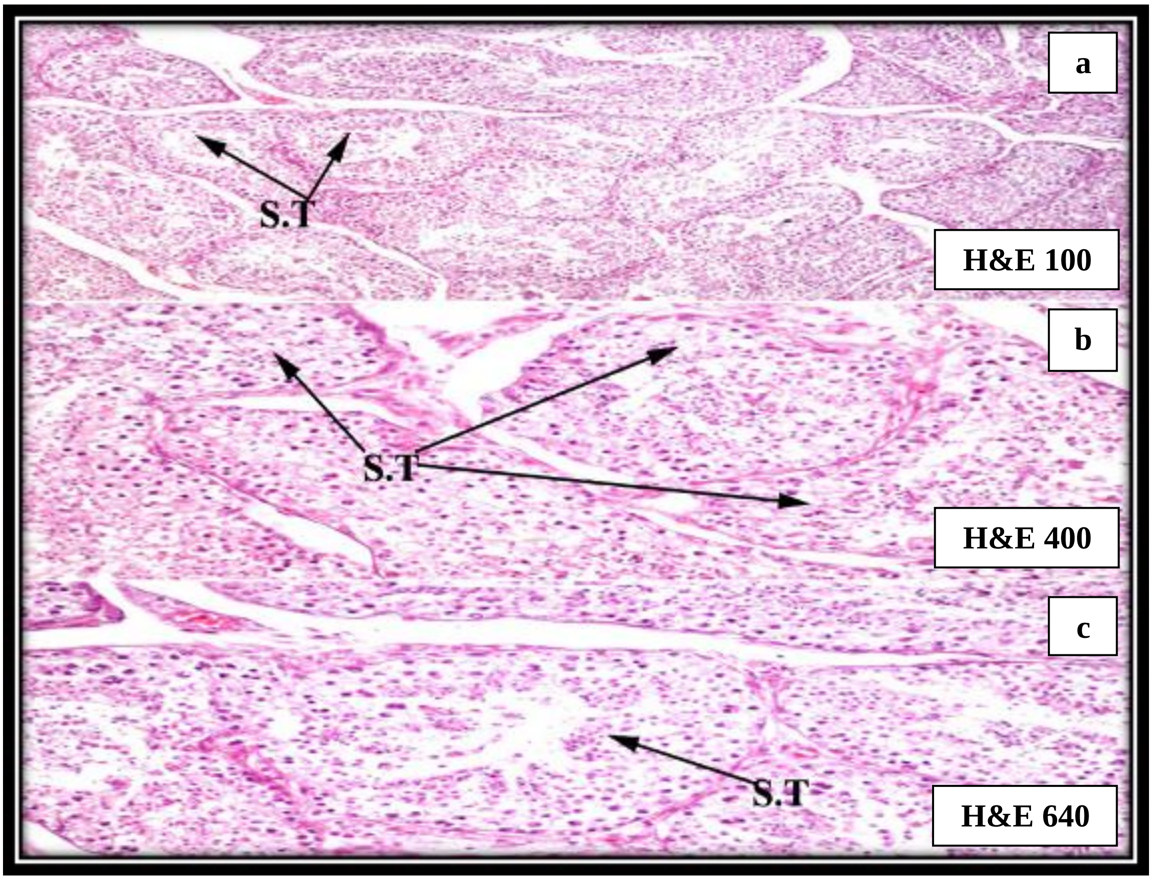


Fig. 11. Photomicrograph shows section of testes of saw-scaled viper, *Echis pyramidum*.

S.T: Seminiferous tubule

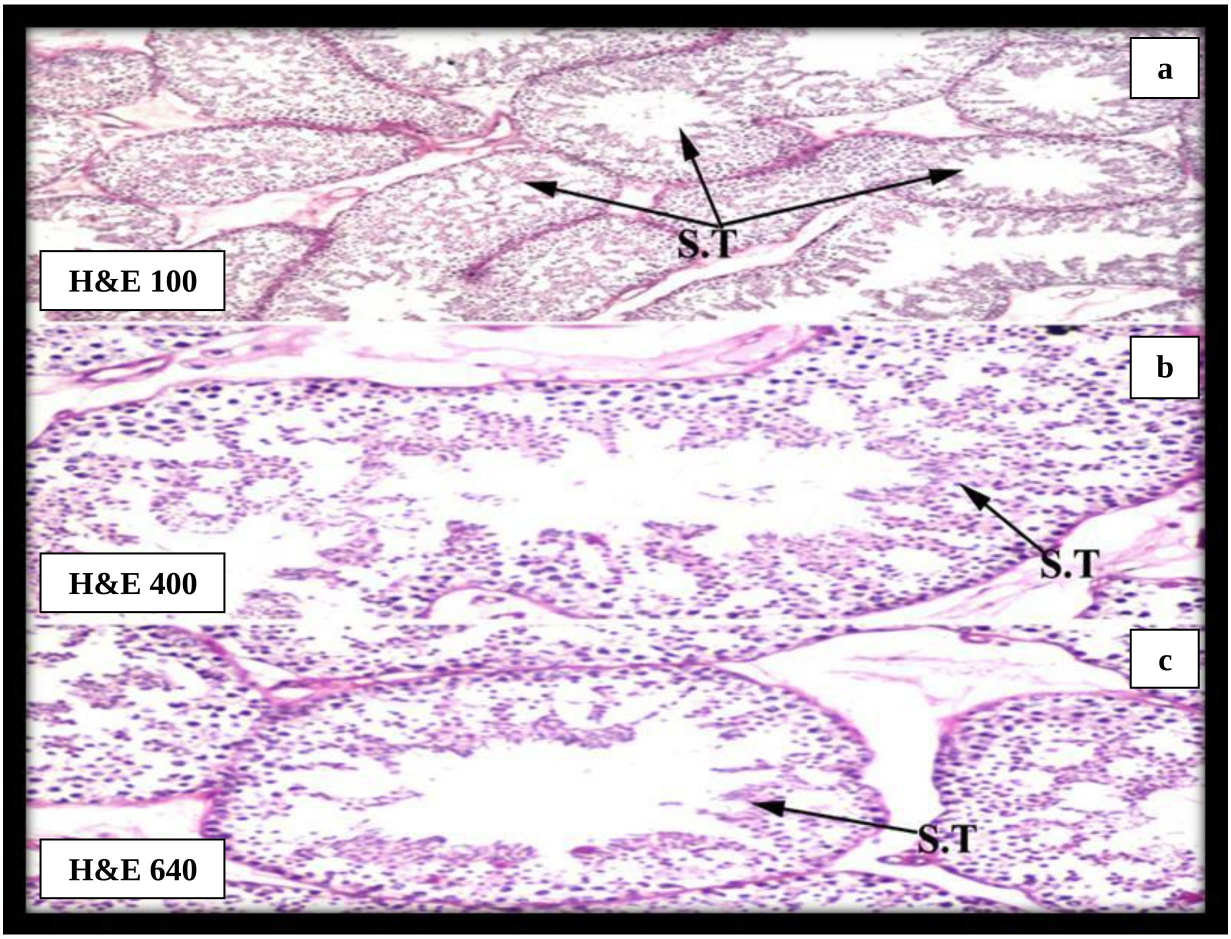


Fig. 12. Photomicrograph shows section of testes of Kenyan sand boa, *Eryx Colubrinus*.

S.T: Seminiferous tubule

Table (1): Blood parameters of saw-scaled viper, *Echis pyramidum* and Kenyan sand boa, *Eryx colubrinus* inhabiting El-Faiyum desert, Egypt.

| Parameters \ Species | *Echis pyramidum* | *Eryx colubrinus* | *t*-values |
|---|---|---|---|
| **RBCs ($\times 10^6$ /mm$^3$)** | **1.43±0.14** | **0.69±0.14** | **7.82***** |
| **Hb (g/100 ml)** | **33.61±4.55** | **7.27±1.07** | **5.01***** |
| **PCV (%)** | **28.26±1.13** | **27.88±5.14** | **11.04***** |

Data are represented as means of eight samples ± S.E.

***t-values*** = Student *t-test* between *Echis pyramidum* & *Eryx colubrinus* for each estimated blood parameter.

*** Very highly significant difference (P<0.001)

Table (2): Calculated blood indices of saw-scaled viper, *Echis pyramidum* and Kenyan sand boa, *Eryx colubrinus* inhabiting El-Faiyum desert, Egypt.

| Parameters \ Species | *Echis pyramidum* | *Eryx colubrinus* | *t*-values |
|---|---|---|---|
| **MCV ($\times 10^{-4}$ fl)** | **6.79±0.72** | **4.79±0.58** | **11.22***** |
| **MCH ($\times 10^{-4}$pg/cell)** | **6.18±0.52** | **3.66±0.99** | **7.79***** |
| **MCHC (g/100 ml)** | **44.20±2.57** | **34.46±8.62** | **8.69***** |

Data are represented as means of eight samples ± S.E.

***t-values*** = Student *t-test* between *Echis pyramidum* & *Eryx colubrinus* for each estimated blood index.

*** Very highly significant difference (P<0.001).

Table (3): Concentration of essential heavy metals (ppm) in the available soils collected from Gabal El-Nagar and Kahk Qibliyyah in El-Faiyum desert, Egypt.

| Soil samples / Heavy metals | El-Faiyum Desert | | *t*-values |
|---|---|---|---|
| | ***Site I*** **Gabal El-Nagar** | ***Site II*** **Kahk Qibliyyah** | |
| **Magnesium (Mg)** | **650.115±269.496 (62.66-1913.6)** | **509.267±57.606 (328.6-619.8)** | **4.356**** |
| **Iron (Fe)** | **9.333±2.702 (1.817-21.24)** | **21.747±2.868 (15.928-30.68)** | **5.861***** |
| **Copper (Cu)** | **1.582±0.375 (0.486-3.262)** | **5.137±0.475 (3.68-6.176)** | **5.521***** |
| **Cobalt (Co)** | **0.159±0.02 (0.066-0.205)** | **0.733±0.132 (0.347-1.064)** | **4.149*** |
| **Molybdenum (Mo)** | **0.085±0.005 (0.067-0.094)** | **0.038±0.009 (0.016-0.067)** | **6.919***** |
| **Manganese (Mn)** | **10.101±2.44 (4.956-21.3)** | **68.593±12.092 (40.4-105.06)** | **3.712*** |

Data are represented as means of six samples ± S.E. Lower and upper values between parentheses.
***t-values*** = Student *t-test* between the studied sites in El-Faiyum desert for each estimated heavy metal.
* Significant difference (P<0.05),
** Highly significant difference (P<0.01),
*** Very highly significant difference (P<0.001)

Table (4): Concentration of non-essential heavy metals (ppm) in the available soils collected from Gabal El-Nagar and Kahk Qibliyyah in El-Faiyum desert, Egypt.

| Species / Heavy metals | El-Faiyum Desert | | *t*-values |
|---|---|---|---|
| | ***Site I*** **Gabal El-Nagar** | ***Site II*** **Kahk Qibliyyah** | |
| **Boron (B)** | **2.571±0.774 (0.749-6.06)** | **1.616±0.51 (0.559-3.2)** | **4.505**** |
| **Aluminium (Al)** | **1.431±0.505 (0.048-2.814)** | **0.058±0.003 (0.048-0.064)** | **2.345** |
| **Cadmium (Cd)** | **0.031±0.006 (0.015-0.049)** | **0.053±0.016 (0.028-0.104)** | **4.809**** |
| **Chromium (Cr)** | **0.05±0.004 (0.043-0.07)** | **0.064±0.004 (0.05-0.071)** | **16.08***** |

Data are represented as means of six samples ± S.E. Lower and upper values between parentheses.
***t-values*** = Student *t-test* between the studied sites in El-Faiyum desert for each estimated heavy metal.
** Highly significant difference (P<0.01), *** Very highly significant difference (P<0.001).

Table (5): Bio-accumulation of essential heavy metals; Ca, Mg, Fe, Cu, Co, Mo and Mn (in ppm) in liver, kidney and muscle of saw-scaled viper, *Echis pyramidum* (*E. p.*) and Kenyan sand boa, *Eryx colubrinus* (*E. c.*) inhabiting El-Faiyum desert, Egypt.

| Tissues / Heavy metals | Liver | | | Kidney | | | Muscle | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***E. p.*** | ***E. c.*** | ***t-values*** | ***E. p.*** | ***E. c.*** | ***t-values*** | ***E. p.*** | ***E. c.*** | ***t-values*** |
| **Magnesium (Mg)** | **249.23 ±10.45** | **240.20 ±15.29** | **27.123***** | **294.68 ±26.58** | **257.31 ±6.33** | **19.64***** | **334.11 ±10.22** | **240.30 ±15.22** | **19.141***** |
| **Iron (Fe)** | **176.59 ±16.91** | **178.42 ±67.55** | **27.123***** | **128.4 ±16.6** | **148.1 ±63.71** | **4.333**** | **97.56 ±10.1** | **178.19 ±92.61** | **2.985** |
| **Copper (Cu)** | **3.54 ±0.32** | **3.36 ±0.61** | **10.341***** | **1.77 ±0.17** | **2.62 ±0.49** | **7.951***** | **0.8 ±0.06** | **2.7 ±0.67** | **4.274**** |
| **Cobalt (Co)** | **0.11 ±0.03** | **0.22 ±0.18** | **9.639***** | **0.11 ±0.01** | **0.15 ±0.01** | **11.476***** | **_** | **0.11 ±0.02** | **_** |
| **Molybdenum (Mo)** | **0.48 ±0.04** | **1.69 ±0.52** | **3.663**** | **0.19 ±0.01** | **0.53 ±0.15** | **4.220**** | **0.12 ±0.01** | **1.08 ±0.27** | **3.317*** |
| **Manganese (Mn)** | **3.08 ±0.106** | **2.87 ±0.35** | **13.204***** | **3.19 ±0.18** | **2.93 ±0.43** | **31.632***** | **3.54 ±0.34** | **2.16 ±0.42** | **9.03***** |

Data are represented as means of eight samples ± S.E

***t-values*** = Student *t-test* between *Echis pyramidum* and *Eryx colubrinus* in some selected organs for each estimated heavy metal.

* Significant difference (P<0.05),

** Highly significant difference (P<0.01),

*** Very highly significant difference (P<0.001).

Table (6): Bio-accumulation of non-essential heavy metals; B, Al, Cd and Cr (in ppm) in liver, kidney and muscle of saw-scaled viper, *Echis pyramidum* (*E. p.*) and Kenyan sand boa, *Eryx colubrinus* (*E. c.*) inhabiting El-Faiyum desert, Egypt.

| Tissues / Heavy metals | Liver | | | Kidney | | | Muscle | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***E. p.*** | ***E. c.*** | ***t-values*** | ***E. p.*** | ***E. c.*** | ***t-values*** | ***E. p.*** | ***E. c.*** | ***t-values*** |
| **Boron (B)** | **8.77 ±0.24** | **19.19 ±3.58** | **6.372*****| **17.08 ±3.11** | **22.83 ±15.55** | **2.592*** | **5.49 ±0.23** | **10.72 ±2.84** | **5.282***** |
| **Aluminium (Al)** | **108.75 ±8.34** | **48.06 ±9.00** | **7.98***** | **72.03 ±21.18** | **62.90 ±26.24** | **4.132**** | **132.8 ±17.94** | **44.25 ±9.87** | **5.856***** |
| **Cadmium (Cd)** | **0.11 ±0.01** | **0.13 ±0.02** | **12.778***** | **0.10 ±0.01** | **0.12 ±0.01** | **15.951***** | **0.11 ±0.01** | **0.10 ±0.01** | **18.085***** |
| **Chromium (Cr)** | **3.91 ±0.31** | **2.54 ±0.15** | **13.239***** | **1.68 ±0.18** | **1.89 ±0.22** | **12.556***** | **1.99 ±0.33** | **2.49 ±0.18** | **11.677***** |

Data are represented as means of eight samples ± S.E.
***t-values*** = Student *t-test* between *Echis pyramidum* and *Eryx colubrinus* in some selected organs for each estimated heavy metal.
* Significant difference (P<0.05),
** Highly significant difference (P<0.01),
*** Very highly significant difference (P<0.001).