Accumulation of some heavy metals and its effect on hematological indices of fresh water fish, *Oreochromis niloticus*

El-Sayed El-Bastamy El-Sayed

Central Laboratory for Environmental Quality Monitoring, National Water Research Center, P. O. Bx 13621/6, El-Kanater, Egypt.

ABSTRACT

Accumulation of some heavy metals (copper, iron, manganese, nickel and zinc) and its effect on the hematological indices on the freshwater fish (Oreochromis niloticus) were studied. Fish Samples were collected from the River Nile near El-Kanater El-Khyria and contaminated water from Iron and Steel Basin in El-Safe Village near Helwan, south of Cairo. Results of this study showed that there was a significant difference between the water qualities indices among the two different locations. Meanwhile, fish samples that collected from polluted location showed high values for the hematological indices namely hemoglobin (Hb), red blood count (RBC), hematocrit (Hct), white blood cell count (WBC) and erythrocyte. Moreover, the concentrations of heavy metals were also high in the fish. However, the levels of the heavy metal accumulation were within the maximum permissible limit for human consumption (FAO, 1992). The order of bioaccumulation of such metals in the liver of fish were Fe > Zn > Cu > Mn > Ni. It was concluded for this study that the discharge of different types of wastes, especially heavy metals from washing process of Iron and Steel in Helwan Company deteriorated the water quality and consequently affecting the fish. It is recommended to treat the different wastes before discharging to Iron and Steel Basin in ElSafe Village.

Keywords: Heavy metals, fish, accumulation, hematology.

INTRODUCTION

Tilapia fish are likely to be the most important of all cultured fish in the 21st century. They are farmed in every manner from semi extensive to super-intensive farms (Fitzsimmons, 2000).

Most heavy metals which are released into the environment find their way into the aquatic environment as a result of direct input, atmospheric deposition and erosion due to rainwater, therefore aquatic animals may be exposed to elevated levels of heavy metals due to their wide use for anthropogenic purposes (Kalay and Canli, 2000). Those metals are non-biodegradable and once they enter the environment, bioaccumulation occurs in the tissue of fish tissue by means of metabolic and biosorption processes.

Contamination with such heavy metals has long been recognized as a serious pollution problem due to their persistence and accumulative nature. It may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al.*, 2007).

As the concentration of metal increases, the accumulation of metal and its damage effect increase (Buschiazzoa *et al.*, 2004).

The industrial drainage and sewage water revealed the highest concentrations of heavy metals followed by agriculture drainage water. The muscles, gills, kidney and liver tissues of fish samples collected from industrial drainage and sewage canals had the highest levels of heavy metal residues followed by agriculture drainage (El-Sayed *et al.*, 2011). In a polluted aquatic ecosystem, when the fish are exposed to high levels of heavy metals, they tend to take these metals that may accumulate in their tissues and may ultimately reach concentration hundreds or thousands of times above those measured in the water, sediment and food (Osman *et al.*, 2007 and Godwin *et al.*, 2003).

Toxic heavy metal contamination mostly occurs in aquaculture and frequently occurs in groundwater, rivers, estuaries, wetland and coastal areas. The toxicity of those elements is due to their ability to cause, oxidative damage to living tissues, while environmental changes can have significant effects on the immune system. Seasonal and diurnal changes in immune response and disease prevalence have been reported in wild fish; these factors being changes in temperature and light density (Bowden 2008).Also, it can cause dermatological diseases, skin cancer and internal cancers (liver, kidney, lung and bladder), cardiovascular disease, diabetes, and anaemia. Transport of metals in fish occurs through the blood where the ions are usually bound to proteins.

This work was aimed to determine copper, iron, manganese, nickel, and zinc in flesh, liver of fishes and its effect on some hematological indices in the fresh water fish (*O. niloticus*).

MATERIALS AND METHODS

Water samples

Water and fish samples were collected in summer during June 2014 from two different locations as shown in Table (1). The first location was River Nile (El-Kanater El-Khyria as control) which represented unpolluted water and the second location was Iron and Steel Basin in El Safe Village near Helwan which represented the polluted water.

Five water samples were collected from each location in Polyethylene containers of two-liter capacity. The water samples were preserved via adding concentrated nitric acid to reduce the pH below 2 to prevent the microbial reactions. Water analysis was achieved according to the standard methods for examination of water and wastewater (APHA, 2005).

	Location	No. of s colle	1	Site	Coordinates	
		water	fish			
	I 5 10 II 5 10		10	River Nile at El-Kanater El-Khyria (unpolluted, as control)	30° 11' 53" N 31° 07' 28" E	
			10	Iron and Steel Basin in ElSafe Village (polluted)	29° 43' 14" N 31° 19' 48" E	

Table 1: Locations of water and fish sampling

Fish samples

Ten fish, tilapia, *Oreochromis niloticus*, weighting 75-90 g, were collected from each location by using gill net and transferred in two separate aerated tanks to Central Laboratory for Environmental Quality Monitoring.

Samples preservation:

Water samples in the collecting bottles were kept directly in a refrigerator at + 4 °C. Whereas After catching the fish samples, fish were kept in plastic bags, tied well, transported directly to the laboratory and kept frozen in a deep freezer at-20°C.

Tested parameters:

Fish weight and total length were recorded individually for the nearest one gram and centimeter, respectively according to Abdelhamid (1996) for all fish. Five water samples collected from each location were analyzed physico-chemically and the analyses for the heavy metals (Cu, Fe, Mn, Ni and Zn). Analyses of physico-chemical parameters of water were carried out according to APHA (2005) including pH (WTW inolab 315i, Germany), temperature (temp.), electric conductivity (EC), and total dissolved solids (TDS) using WTW model 315i, Germany, dissolved oxygen (DO) using WTW model 310i, Germany, carbonate (CO₃), and bicarbonate (HCO₃) were volumetrically determined by titration method (0.02 N H₂SO₄ with phenol phethalein and methyl orange as indicators), ammonia (ORION model 9512 attached to Benchtop Ion Analyzer, ORION model EA 940 with built-in stirrer, U.S.A), major anions such as chloride (Cl⁻), sulfate (SO₄⁻⁻), and nitrate (NO₃⁻⁻) were determined in water samples using ion chromatography (IC) Model DX-600, USA. Major cations (Na⁺, K⁺, Ca⁺⁺ & Mg⁺⁺) and heavy metals (Cu, Fe, Mn, Ni, and Zn) were determined using the Inductivity Coupled Plasma (ICP-OES 5300 DV U.S.A). Biological oxygen demand (BOD) in the water samples was determined using an ORION BOD Fast Respirometry System (model 890, Germany) with a measuring range 0-4000 mg/l at 20°C incubation in a thermostatic incubator chamber (model WTW, Germany), While Chemical oxygen demand (COD) was measured by applying Closed Reflux colorimetric method, using a spectrophotometer at 600 nm.

About 10 fish were caught from each location, weight and total length for each fish were recorded, and then the fish was blotted carefully and the blood was collected from the heart anaerobically without narcosis. Part of the blood was collected in heparinized syringes for determination of blood parameters. The other part was collected via non heparinized syringes and directly transported into tubes. After one hour, the tubes were centrifuged at 3000 rpm for 15 min by Biofugu Centrifuge and serums were separated and frozen at -20°C for biochemical assays (Adakole, 2012).

Livers of fish from each location were removed and dried at 80°C till constant weight. Then, they were digested by using Microwave Labstation MLS- 1200 mega (Reagent used were HNO₃ 65% and H₂O₂ 30%). The concentration of heavy metals (residues) was estimated by using Inductivity Coupled Plasma ICP OES 5300 DV (Chandanshive *et al.*, 2012).

Statistical Analysis

The results obtained from both locations (water sample, and hematological indices and heavy metals content in the fish) were statistically analyzed to determine the significant differences by using independent t-test sample (SPSS version 7.0.1 copyright SPSS INC1997).

RESULTS AND DISCUSSION

The water quality parameters such as temperature, pH, electrical conductivity, total dissolved solids, alkalinity, dissolved oxygen, ammonia, biochemical oxygen demand, chemical oxygen demand and heavy metals (Cu, Fe, Mn, Ni, and Zn) were measured in both unpolluted and polluted locations as shown in Table (2). The present results showed that the water quality in the polluted location was significantly different compared to the unpolluted location in the Nile River. The mean values of both physical and chemical water parameters tended to be high in the polluted Iron and Steel Basin. The increase of water temperature was highly related to the outlets of Helwan Company that discharges directly the heated water to Iron and Steel Basin.

While, values of both electrical conductivity (EC) and total dissolved solid (TDS) were above the permissible limits of the law 48/1982, which reflect the strong effect of the effluents discharged from Helwan Company for Iron and Steel that leads to the increase of the total dissolved solids and ionic salts by using the water during the manufacturing process (cooling and wash the steel and iron rods).

property		Me	an	Std. Error		
		River Nile (El-Kanater El-Khyria)	Iron and Steel Basin	River Nile (El-Kanater El-Khyria)	Iron and Steel Basin	P-value (2-tailed)
Temperature (°C)	4	27.10	29.14	0.158	0.09	0,00
РН	4	8.09	8.05	0.005	0.003	0.003
$\mathbf{HCO_3}^{-1} \text{ mg } \mathbf{l}^{-1}$	4	155.80	178.40	2.31	1.43	0.00
EC dS m^{-1}	4	0.328	3.198	0.002	0.005	0.00
Ammonia mg l ⁻¹	4	2.00	8.26	0.13	0.15	0.00
Dissolved Oxygen mg l ⁻¹	4	6.70	2.60	0.38	0.24	0.00
Biological oxygen demand mg 1 ⁻¹	4	4.4	14.6	0.24	0.50	0.00
Chemical oxygen demand mg l ⁻¹	4	7.40	22.40	0.25	0.37	0.00
Ca^{2+} mg l ⁻¹	4	23.76	289.8	1.25	0.80	0.00
\mathbf{K}^+ mg l ⁻¹	4	3.80	11.4	0.48	0.60	0.001
Mg^{2+} mg l ⁻¹	4	12.82	38.6	0.48	0.50	0.00
Na^+ mg l ⁻¹	4	19.80	319.19	0.66	0.58	0.00
Cl^{-} mg l^{-1}	4	22.4	490	0.81	0.63	0.00
NO_3 mg l ⁻¹	4	0.21	7.20	0.042	0.96	0.00
$\mathbf{NH_4}^+$ mg l ⁻¹	4	2.00	5.26	0.15	0.13	0.00
$\mathbf{SO_4^{2-}} \operatorname{mg} l^{-1}$	4	15.40	739.60	0.92	0.74	0.00
Cu mg l ⁻¹	4	0.008	0.018	0.001	0.001	0.00
Fe mg l^{-1}	4	0.007	0.486	0.001	0.015	0.00
Mn mg l^{-1}	4	0.006	0.018	0.001	0.001	0.00
Ni mg l^{-1}	4	0.004	0.010	0.001	0.001	0.00
Zn mg l ⁻¹	4	0.004	0.0088	0.001	0.001	0.00

Table 2: Mean values of chemical properties in the surface water of River Nile and Iron and Steel Basin

• df = degree of freedom, Std. Error = standard error of the mean, P-value =attained level of significance

• Bold Value (P) >0.05 - *Italic Value* (P) <0.05

Also, the increase of ammonia within the polluted Iron and Steel Basin might be related to the domestic effluents from houses and accumulations of organic matter discharges from Helwan Company (Bolalak and Frankowaski, 2003). In addition, such increase might relate to excessive application rates of sewage discharge and industrial wastes as well as microorganism activities in decomposition of residues and fixation of atmospheric nitrogen (Galloway *et al.*, 2003). Exposure to ammonia may cause destruction of immune system, osmoregulatory disturbance, abnormalities structure of tissues and growth rate (Russo and Thurston, 1991). Furthermore, the present results showed that Helwan Company caused depletion of the dissolved oxygen which was far below standards of law 48/1982. This depletion may enhance the toxicity of ammonia on fish and other aquatic organisms, especially in the existence of high temperature and pH (Schwarzenbach, *et al.* 2003). It is also clear that aerobic bacteria can be replaced by anaerobic bacteria as the result of the depletion of the oxygen. These anaerobic conditions may cause disagreeable odors due to the production of gases, H_2S , NH_3 and CH_4 (El-Sherbini, *et al.* 1997).

Concerning the biological oxygen demand (BOD) and the chemical oxygen demand (COD), the present results showed that in the polluted water they increased to

the concentration of $14.6 \pm 0.50 \text{ mg l}^{-1}$ and $22.40 \pm 0.37 \text{ mg l}^{-1}$, respectively. These results coincide with the finding of Abdo (2005) who recorded high levels of BOD and COD in Abu Za'baal Ponds in Elqalyubia Governorate. The increase of the BOD and COD in polluted sector was higher than the permissible limits of law 48/1982. BOD and COD are useful indicator for organic pollution in the rivers (Loigu and Leisk, 1996). High BOD and COD levels in polluted sectors are related to the existence of high bacterial load and organic matters as well as relative high temperatures which enhance the enumeration of bacteria.

The concentration of copper, iron, manganese, nickel, and zinc recorded higher levels in the polluted sector than River Nile at El-Kanater El-Khyria. It was highly attributed to intense human activities and industrial effluents from Helwan Company for Iron and Steel and the increase of pH and mobilization of metals from sediment to water (Clare *et al.*, 2007).

Previous studies showed that the bioaccumulation of heavy metals does not only depend on the structure of the organ, but also on the interaction between metals and the target organs (Mersch et al., 1993). El-Naggar et al., (2009) observed that fish could accumulate trace metals and act as indicators of pollution. In the present results, the mean concentration of the heavy metals (Cu, Fe, Mn, Ni, and Zn) tended to be significantly high in the liver of fish which collected from the polluted water as shown in Table (3). The order of accumulation of such heavy metals was Fe > Zn > Cu >Mn > Ni. However, these concentrations were within the permissible limit for human consumption according to the standard mentioned. The results recorded for copper and iron in the control and polluted locations were $0.005 \pm 0.47 - 0.085 \pm 8.27$, 0.004 $\pm 0.5 - 0.735 \pm 7.05$ mg kg⁻¹ respectively. These results coincide with the finding of Ibrahim and Mahmoud (2005), Tayel et al., (2008), Yacoub (2007), Ibrahim and Tayel, (2005) and Carbonell et al., (1998) who detected high levels of copper and iron in fresh water fishes collected from different contaminated water. Also, the results match with the finding of Haggag et al., (1993) and Yacoub (2007) who observed the free metal iron concentration and thereby lead to accumulation of iron ligand protein (Hemosidrin) scattered in liver of fish which exposed to high iron concentration.

		Permissible	Mean		Std. E		
Property (mg/kg)	df	limit FAO (1983)	River Nile (El-Kanater El-Khyria)	Iron and Steel Basin	River Nile (El-Kanater El-Khyria)	Iron and Steel Basin	P-value (2-tailed)
Copper	9	30	0.005	0.085	0.47	8.27	0.002
Iron	9	30	0.0045	0.735	0.50	7.05	0.00
Manganese	9		0.0042	0.0207	0.37	3.51	0.009
Nickel	9		0.002	0.0021	0.001	0.34	0.002
Zinc	9	40	0.0066	0.179	0.10	1.06	0.00

 Table 3: Heavy metals concentration of the O. niloticus liver from water of River Nile and Iron and Steel Basin

• df = degree of freedom, Std. Error = standard error of the mean, P-value =attained level of significance

• Bold Value (P) >0.05 - *Italic Value* (P) <0.05

Manganese is considered as an essential constituent for bone structure, reproduction and normal functioning of the enzymes system, while it is toxic only when present in higher amount, but at low level is considered as micronutrient, (Doherty *et al.*, 2010). The results showed that manganese accumulated with high concentration in the liver of fish obtained from Iron and Steel Basin (0.0042 ± 0.37 –

 $0.0207 \pm 3.51 \text{ mg kg}^{-1}$ in control and polluted locations respectively. These results agree with those obtained by Yacoub, (2007) who determined high concentration of manganese in fresh water fishes in Cairo and Kalubia governorates.

Nickel is non-essential element and higher concentrations can occur in aquatic organisms close to anthropogenic sources. It is toxic even at low concentrations and has no known function in biochemical processes and it is known to inhibit active transport mechanisms, involving adenosine triphosphate (ATP), to depress cellular oxidation reduction reactions and to inhibit protein synthesis (Burden *et al.*, 1998). The study results showed that the concentration of nickel was high in the liver of fish that collected from the polluted basin ($0.0021 \pm 0.34 \text{ mg kg}^{-1}$). Nickel inhibits the impulse conductivity by inhibiting the activities of monoamine oxidase and acetylcholine esterase to cause pathological changes in tissue and organs (Rubio *et al.*, 1991) and impair the embryonic and larval development of fish species (Dave and Xiu, 1991).

Also, the results showed that zinc was highly accumulated in the liver $(0.179 \pm 1.06 \text{ mg kg}^{-1})$ of fish that collected from the polluted sector compared with control location $(0.0066 \pm 0.1 \text{ mg kg}^{-1})$. The fish can take up the zinc directly from water, especially by its mucous and gills (Hamed, 1998). The relatively higher zinc concentration in the liver of the different fish species may be due to the role of zinc as an activator of numerous enzymes present in the liver as recorded by Yacoub, (2007) and Cogun *et al.*, (2005).

Concerning the hematology, it is used as an index of fish health status in number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia (Duthie and Tort, 1985).

The results showed that in the freshwater Oreochromis niloticus which collected from the Iron Steel Basin, the mean values of hemoglobin, white blood cells (WBC), red blood cells (RBC), hematocrit (HCT), corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were significantly higher than that in the fish which collected from the Nile River as shown in Table (4). Also the results showed that in fish which collected from the contaminated water, the platelets were significantly higher than that in fish which collected from the Nile River (Table 4). Such increases were highly related to the pollutants especially heavy metals and reduction of dissolved oxygen in water. The reduction of oxygen generally expands synthesis of hemoglobin, releases of blood cells from storage sites, and enhances erythropoiesis. Similar results were recorded by (Mehibeen, and Nazura 2013) and Shah (2006), who reported significant increases in the red blood cells, hematocrit and platelets in Tinca tinca when it was exposed to heavy metals. The significant increases in the WBC may be as a result of intensive secretion of corticosteroid hormones (Dutta and Kaviraj, 1996). The secretion of these hormones is a nonspecific response to any environmental stressor which including the pollutants. Similarly, when the *Tilapia zillii* is exposed to high level of nickel, the fish reveals immunological defense by increasing the number of lymphocytes (Mourad et al., 1999). In addition, the increases in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in the present results may relate to a defensive mechanisms against the toxic effect of heavy metals which cause disturbances to the metabolic and the hemopoitic activities in fish (Javed and Usmani, 2013). Also the results of this study showed that when the fish was exposed to low dissolved oxygen, the number of MCV and WBC

increased as a result of (Praveena *et al.*, 2013) who reported an increase of MCV in *Labeo Rohita* as a result of hypoxic condition.

			Me	an	Std. E		
property		df	River Nile (El-Kanater El-Khyria)	Iron and Steel Basin	River Nile (El-Kanater El-Khyria)	Iron and Steel Basin	P-value (2-tailed)
Не	moglobin (g/dl)	9	5.13	5.88	0.07	0.16	0.008
Н	ematocrit (%)	9	14.91	19.54	0.24	0.38	0.00
WBCs (x10 ³ cells/mm ³)		9	178.60	215.6	3.77	3.076	0.00
RBCs (x10⁶ cells/mm³)		9	1.73	2.02	0.056	0.071	0.015
	MCV (μ^3)	9	90.18	96.78	0.18	0.06	0.019
Ses	MCH (pg)	9	30.48	29.26	0.51	1.51	0.021
Blood indices	MCHC (g/dl)	9	32.82	30.24	0.48	1.39	0.024
Pl	atelets(Count)	9	121.60	185.00	1.03	2.90	0.00

Table 4: Hematological parameters of the O. niloticus from water of River Nile and Iron and Steel Basin

df = degree of freedom, Std. Error = standard error of the mean, P-value =attained level of significance

• Bold Value (P) > 0.05 - *Italic Value* (P) < 0.05

The water pollution leads to damage the tissues and organs of fish, some specific cellular enzymes would leak into the blood (Zikic *et al.*, 1997). The most serum enzymes were significantly elevated in response to ambient water pollution (Sabae and Rabeh, 2006). The results (Table 5) showed that there were increases in serum AST & ALT concentration in fishes collected from Iron and Steel Basin in El-Safe Village, than recorded in River Nile at El-Kanater El-Khyria. These increases could by attributed to the environmental pollutants of heavy metal and organic substance in water (Matsuo *et al.*, 2004). Similar findings were reported by Hadi *et al.* (2009), who found that the increase in aluminum cause a significant increase in the activities of serum AST and ALT. The levels of ALT in *O. niloticus* seem to be elevated in response to the rise in ammonia-in ambient water (Sharsher, 2011).

		Mean		Std. E		
property	df	River Nile (El-Kanater El-Khyria)	Iron and Steel Basin	River Nile (El-Kanater El-Khyria) Iron and Steel Basin		P-value (2-tailed)
Serum ALT (IU/l)	9	143.40	209.60	2.36	0.68	0.00
Serum AST (IU/l)	9	222.98	616.80	0.74	1.07	0.00
Serum Urea (mg/dl)	9	16.87	21.40	0.16	0.51	0.001
Serum creatinine (mg/dl)	9	0.69	0.85	0.02	0.04	0.016

Table 5: Biochemical parameters of the O. niloticus from water of River Nile and Iron and Steel Basin

• df = degree of freedom, Std. Error = standard error of the mean, P-value = attained level of significance

• Bold Value (P) >0.05 - *Italic Value* (P) <0.05

Min and Kang (2008) reported that LDH and ALT play an important role in ammonia detoxification in fish, which increased due to exposure of high level of heavy metals. The activity of serum transaminases in freshwater fishes was significantly altered when water is polluted with different metals such as Zinc, copper and cadmium (Zikic *et al.*, 1997). Wieser and Hinterleitner (1980) reported increased activities of ALT in serum of rainbow trout in response to sewage loading in rivers.

Shakoori *et al.* (1990), found that hepatic AST and ALT were increased in freshwater fish, *Cirrhinus marigala*, toxicated with cadmium and copper for 7 days.

This study showed also that there is an increase in urea concentrations in serum of fish collected from polluted area (Iron and Steel Basin). They were generally higher than these from the control sample (River Nile at El-Kanater El-Khyria. The increases may be attributed to the heavy metal concentration in water especially copper and iron which cause a marked liver and renal diseases (El-Sabbagh, 1996). Serum creatinine was increased in fish collected from polluted sector compared to that in fish of unpolluted sector. Also, Hadi *et al.*, (2009) found that creatinine and uric acid was significantly high with the increase of aluminum concentration in water. The increase of urea and creatinine concentrations may be refered to kidney failure and increased muscular tissue catabolism (Pandey *et al.*, 2003). The rise in creatinine might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrate metabolism (Sevgiler *et al.*, 2004).

CONCLUSION AND RECOMMENDATIONS

Fish contaminated by heavy metals suffers pathological alterations, with consequent inhibition of metabolic processes, hematological changes, and decline in fertility and survival. It can be conclusively deduced from this study that the fresh water fish, *Oreochromis niloticus* has the tendency to bioaccumulate heavy metals in a polluted environment. Since virtually all metals investigated were found in higher concentration, so government should intact laws that will ensure that industries make use of standard waste treatment plants for the treatment of their wastes before they are being discharged into water bodies.

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ARABIC SUMMARY

دراسة تأثير تراكم المعادن الثقيلة على التغيرات الهيماتولوجية في سمكة البلطي النيلي

السيد البسطامي السيد الباحث بالمركز القومي لبحوث المياه

تتناول الدراسة الحالية تأثير تراكم المعادن الثقيلة (النحاس – الحديد – المنجنيز – النيكل – الزنك) على بعض القياسات الهيماتولوجية للأسماك المياه العذبة (البلطى النيلى)، والتى تم جمعها من نهر النيل عند منطقة القناطر الخيرية (كمنطقة ضابطة) والأخرى التى تم جمعها من حوض الحديد والصلب (كمنطقة ملوثة) بقرية الصف. أظهرت نتائج القياسات الفيزيوكميائية والمعادن الثقيلة فى عينات المياه اختلافاً معنوياً فى منطقة الدراسة، كما تبين من قياسات الفرزيوكميائية والمعادن الثقيلة فى عينات المياه اختلافاً معنوياً فى منطقة الدراسة، كما تبين من قياسات الفيزيوكميائية والمعادن الثقيلة فى عينات المياه اختلافاً معنوياً فى منطقة الدراسة، كما تبين من قياسات الدم أختلافاً معنوياً فى كلاً من الهيموجلوبين، عدد كرات الدم الحمراء وعدد كرات الدم البيضاء اذا ما قورنت بنتائج تلك القياسات فى عينات المجموعة الضابطة. وقد أوضحت نتائج تركيزات المعادن الثقلية فى عينات المجموعة الضابطة. وقد أوضحت نتائج تركيزات المعادن الثقلية من حوض الحديد إلى الحمراء وعدد كرات المعادن الثقلية فى عينات المجموعة الضابطة. وقد أوضحت نتائج تركيزات المعادن الثقلية من الميوجلوبين، عدد كرات الدم الحمراء وعدد كرات المعادن الثقلية فى عينات المجموعة الضابطة. وقد أوضحت نتائج تركيزات المعادن الثقلية من الموقعين أختلافاً معنوياً ، لكنها كانت ضمن الحدود المعادن الثقلية فى عينات المعادن الثقلية فى عينات المعادن الثقلية تركيزات المعادن الثقلية والزراعة والزامة (الفاو، ١٩٨٣)، وكانت تركيزات المعادن الثقلية كانتالى : الحديد > المعادن الثقلية من علي ألمام كانت ضمن الحدود المعادي الثقلية فى عينات المعادن الثقلية تؤدى الزنك > النحاس > المنظمة الأغذية والزراعة (الفاو، ١٩٨٣)، وكانت تركيزات المعادن الثقلية كانتالى : الحديد > الزنك > الندلس > المنظمة الأغذية والزراعة والفار المالي المالي في مختلفاً معنواع المعادن الثقلية تؤدى الزنك > النداس > المنظمة الأغذية والزراعة (الفاو، ١٩٨٣)، وكانت تركيزات المعادن الثقلية تؤدى الذيك > الندلس > المنظمة الأغذية والزراعة (الفاو، ١٩٩٨)، وكانت تركيز المالي المعادن الثقلية تؤدى الزنك > النداس > المنظمة الأغذية والزراعة (الفاو، ١٩٩٨)، وكانت تركيز على الأسان مع يمن المياه مالي مالي مالي ما علي من عمري المي، وما معد والملب والتى تحتوى على الأمالي مالي مالي مالي مالي ما