THE ENVIRONMENTAL INDICATIVE POTENTIAL OF METAL IN *CLARIAS GARIEPINUS* AND THEIR INFESTING *POLYONCHOBOTHRIUM CLARIAS* (CESTODES) *AND MACROGYRODACTYLUS CLARII* (MONOGENEA) PARASITES

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Abstract

To assess the bioindicator value of parasites, the concentrations of seven heavy metals (Cr, Cu, Pb Mn, Cd, Ni and Zn) were analyzed by atomic absorption spectrometry in some organs of catfish, *Clarias gariepinus* and tissues of their parasite were analyzed and subsequently compared with the data from sediments and water. Only Cu, Fe, Zn and Mn were detected in water; sediment; *Polyonchobothrium clarias; Macrogyrodactylus clarii* and fish specimens, while levels of Cr, Pb and Cd were below the detection limits. Four elements were found at higher concentrations in cestode and monogenea than in different fish tissues (musculature, liver and gills), being 1.6-37.4 times higher than that measured in musculature, liver and gills. Significant positive (for Cu) and negative (for Fe, Zn and Mn) correlations were found between the quantity of heavy metals in water and tissues of *P. clarias* while there were significant positive (for Cu and Zn) and negative (for Fe and Mn) correlations between the quantity of heavy metals in bottom sediment and tissues of *P. clarias* and *M. clarii* were found suitable to reflect the amount of heavy metals in sediments, providing more reliable information about the actual pollution of the reservoirs. Also the research assumed that the parasites were provide a vital services for the fish through their heavy metal accumulation role where, the research reflect actually, the possible parasite elimination role of metals from the environment of their host.

INTRODUCTION

Ecological risk assessment based on the diversity of biological communities provides a relevant and straightforward approach for examining stressed ecosystems .However, due to the complexity and variability of the data, the bioindication of detrimental changes at this level should always be coupled with optimized and powerful statistical treatment. Diversity analyses typically integrate a variety of species with different susceptibilities to stress which could make changes rather difficult to identify (Sures 2004).

In addition to the remarkable ecological and epizootiological importance of freshwater parasites there are many other reasons for their use as indicators of pollution. Fish parasites are a ubiquitous component of freshwater environments and appear to be sensitive to a wide array of environmental perturbations which may complicate the interpretation of in situ studies (Moller 1987 and Poulin 1992). The complexity of the system should also be considered with respect to trophic relations. Parasites move through the food web and are situated at the highest trophic levels of the ecosystem (Minchella and Scott 1991) Moreover the diversity of fish parasites in contaminated water probably reflects two effects disturbance of the entire parasite community and/or a reduction in the immunological response of fish that might facilitate infection by parasite species with a relatively low epizootiological potential. The degree of pathogenic activity exerted by ecto-and endoparasites living on the body surface and/or in internal organs of fish, can be influenced by water pollution (Khan and Thulin, 1991). Helminth parasites were proven to be useful sentinel species as indicators of heavy metal pollution in aquatic ecosystems (Sures, 2004). Parasite-host systems (helminth and fish) examined, acanthocephalans and some tapeworms have demonstrated the greatest capacity to accumulate heavy metals (Sures *et al.* 1997a and b, 1999 and Turčeková and Hanzelová 1999; Turčeková *et al.* 2002). Also Tenora *et al.* (2000), found that *Philometra ovata* which parasitizes fish from the genera *Abramis, Rutilus* and *Vimba* (*Cyprinidae*) accumulate high concentrations of heavy metals.

Riggs *et al.* (1987) reported elevation of selenium concentrations in the cestode *Bothriocephalus acheilognathi* in comparison to the tissues of its fish definitive host. Mean concentrations of lead and cadmium in *Monobothrium wageneri* from intestine of catfish were 75 and 40 times higher than in the musculature of the host (Sures *et al.* 1997a). Additionally, Tenora *et al.* (2000), Also he investigated Cr, Pb and Cd concentrations in the *Ligula intestinalis* and three of its hosts and found that Cestodes therefore show a high capacity of bioaccumulation of heavy metals located in the intestines of their respective final host. Dealing with the question as to whether environmental contamination could affect the composition of parasite communities in their final hosts (Halmetoja *et al.* 2000). Significantly higher quantity of heavy metals was concentrated in tissues of ecto and endoparasites than in their fish host (Sures 2003, 2004). And recently; Gheorghiu *et al.*, 2006 and 2007 proved that and recorded that zinc effects on reproduction, survival and morphometrics of *Gyrodactylus turnbulli* isolated from guppies. Thus, we examined the accumulation some of heavy metals in water, sediment, in musculature, liver and gills of catfish *Clarias gariepinus*; the tapeworm *Polyonchobothrium clarias* and the monogenetic trematode *Macrogyrodactylus clarii* .

The objective was to determine whether *P. clarias and M. clarii* are a useful bioindicator species as part of a parasite-host system with *C. gariepinus*, which is a bottom-dwelling benthivorous, which is highly likely to be exposed to water-borne contaminants and to assess the degree of correlation between these concentrations and pollution of the water reservoir.

MATERIALS AND METHODS

During the period of March 2006 to May 2007, thirty eight Catfish *Clarias gariepinus* (With body weights ranged from 350 to 665 g and lengths were 30.5-35 cm.) were caught. The sediments and water samples were also taken from Ismalia Canal and Abbassa fish farm ponds. The fish were killed by a blow on the head.

The samples were transferred to the laboratory immediately, the gills and intestine was removed from each fish, the length recorded and placed in a plastic Petri dish with saline solution (0.9% NaCl). The gills were examined for *Macrogyrodactylus clarii* Gussev, 1961 (Monogenea) and the intestine was opened, exposing the cestodes, *Polyonchobothrium clarias* Woodland. 1925 and the point of attachment was noted. The both worms from each fish were carefully removed and stored in a 25 ml glass bottle before the tissue was frozen until metal analysis. The liver and musculature were selected as reference tissues due to the high bioaccumulation shown in previous studies. These tissues were dissected out and placed in 25 ml glass bottles. Approximately 4 g of the expel musculature of the fish, the entire liver and two gills from each fish were dissected, washed with distilled water, dried with filter paper, weighed, packed in polyethylene bags and kept at -30°C until analysis.

Analytical procedures

Water samples were analyzed directly. The wet samples (tissues, sediment and both parasites) that were weighed (1 g) before, 5 ml nitric acid (65%) and 1 ml hydrogen peroxide were placed into the digestion bombs and digested in a microwave digestion system. After digestion, the samples were cooled to room temperature. The resulting solutions were made up to exactly 25 ml with high-quality deionized water and analyzed for Cu, Fe, Zn, Mn, Cr, Pb and Cd using a Perkin Elmer Atomic Absorption Spectrometer Analyst 800. The detection limit was 0.03 ppm for all elements. Element concentrations in the tissues of Catfish, its parasites and sediment of the Ismalia Canal were determined as mg kg-1 (wet weight) Soil samples were collected from the bottom of some pond and Ismailia canal for the layer of 0 – 20 cm depth. Samples were air dried, crushed sieved through a 2 mm sieve and kept in polyethylene bags for analysis. Micro elements were extracted by DTPA using developed method Lindsay and Norvell (1978) and their amounts were determined by the atomic absorption (Thermo ELECTRON CORPORATION S SERIES AA Spectrometer with Gravities furnace, UK, Made in

England), while water levels were determined as mg l/L. Spearman rank correlation coefficient was used to test for significant associations between the data obtained from Catfish tissues, its parasites, water and sediment. The level of significance was accepted with $P \leq 0.05$.

The ratio (R) of heavy metal concentrations in parasites (C_P) relative to their host (C_H) was computed as $R = C_P:C_H$ to evaluate heavy metal accumulation (Sures *et al.* 1999; Sures 2004). The bioconcentration factor was computed for water (C_W=mean value) as BFw=Cp/Cw and for sediments (C_S) as BFs=Cp/Cs according to Sures *et al.* (1999) with some modification (concentrations of heavy metals in water and sediments were used as aggregated mean value only). Owing to the low sample size and the non-adherence of data to the assumptions for parametric tests, comparisons of independent groups (e.g. fishes with and without parasites) were made using the Mann-Whitney *U* test and those of paired data (e.g. heavy metal content of parasites and hosts) using the Wilcoxon sign-rank test. Spearman rank correlation was used to test significant relationships between variables. The analyses were performed in Statistics for Windows 7.1 (Stat. Soft 2005: http://www.statsoft.com).

RESULTS

The concentrations of heavy metals in fish tissues (musculature, liver and gills are given in Table (1) and Figure(1) , only Cu, Fe, Zn and Mn were detected while other as Cr, Pb and Cd were below detection limit. Also Cu and Mn were undetectable in musculature and gills, while Cu was not found in gills. Also the table revealed that liver considered the ware house for heavy metals, where the highest levels were accumulated in liver followed by gills and then musculature of *Clarias gariepinus* Photo (1).

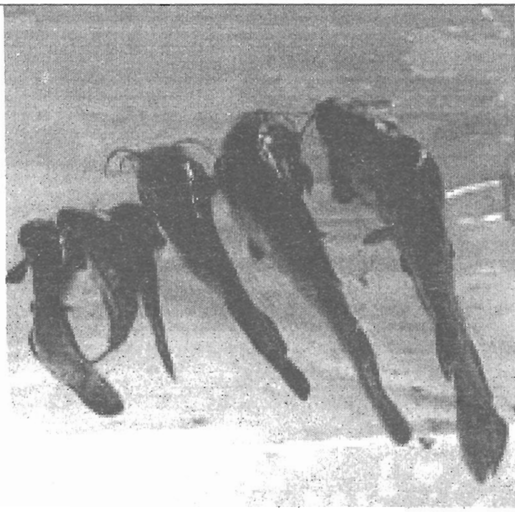


Photo 1. *Clarias gariepinus*

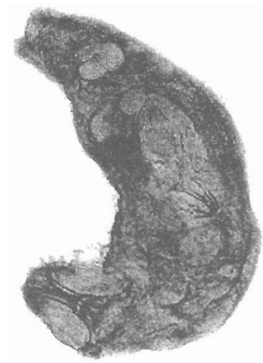


Photo 2. *Macrogyrodactylus clarii* x250

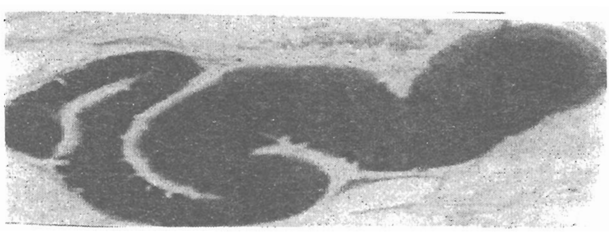
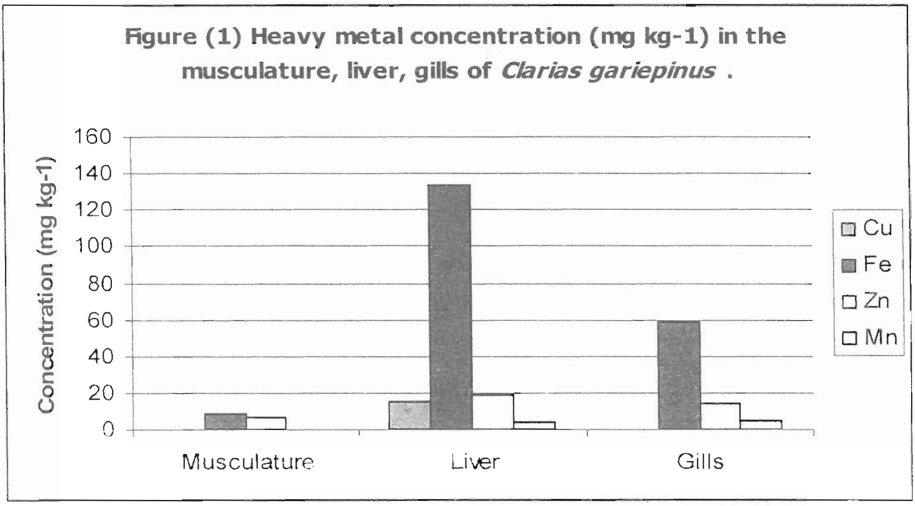


Photo 3. adult *Polyonchobotherium clarias* x250

Table 1. Heavy metal concentration (mg kg-1) in the musculature, liver, gills of *Clarias gariepinus*.

Metals	Musculature	Liver	Gills
Cu	BDL	15.39±10.83	BDL
Fe	8.67±4.57	133.82±93.38	58.62±16.88
Zn	7.01±3.09	19.31±3.17	14.17±1.93
Mn	BDL	3.44±0.26	4.98±2.45

BDL = below detection limits



Concerning the accumulation of heavy metals in parasites Table (2) and Figure (2) indicated that the cestode parasites, *P. clarias* Photo (2) accumulated heavy metals more higher than monogenetic trematode *M. clarii* where Fe level in *P. clarias* was 27 times higher than its concentrations in *M. clarii* also in the rest of elements the ratio were double times higher than *M. clarii* Photo (3) . Four elements (Cu, Fe, Zn and Mn) analyzed were higher in both parasites than in the liver, two (Fe and Zn) higher than in the musculature, three (Fe, Zn and Mn) higher than in the gills (Table 2). The copper level in *P. clarias* and *M. clarii* was 4.8 times and twice higher than in the liver of the catfish respectively. The iron level in *P. clarias* was 37.4 times and higher than that of the musculature and was 2.4 times higher than that of the liver and 5.6 times higher than that in the gills. The zinc level in *P. clarias* and *M. clarii* was 6.8 and 3.1 times higher than that in the musculature and 3.3 and 3.1 times higher than that in the gills respectively. The Mn level in *P. clarias* was 2.3 times higher than that in the liver and 1.6 times higher than that in the gills. Mean concentrations of copper, iron, zinc and manganese in *P. clarias* were, respectively, 175, 691 and 2.2 times higher than in the water. Iron was recorded in the highest amount in water and *P. clarias*. Cu and Zn concentrations in *P. clarias* differed significantly from the water ($P < 0.05$ and < 0.01). Mean concentrations of copper, iron, zinc and manganese in *M. clarii* were, respectively, 78, 25.5 and 66.6 times higher than in the water. Iron was recorded in little amount in water than *M. clarii*. Table (2) and Table (3)

Table 2. Heavy metal concentration (mg/ kg) in the *M. clarii* and *P. clarias*.

	M. clarii	*P. clarias*
Cu	32.01±10.16	72.01±11.37
Fe	12.08±833	325.08±52.34
Zn	22.00±2.06	48.00±8.03
Mn	3.08±1.39	8.08±2.35

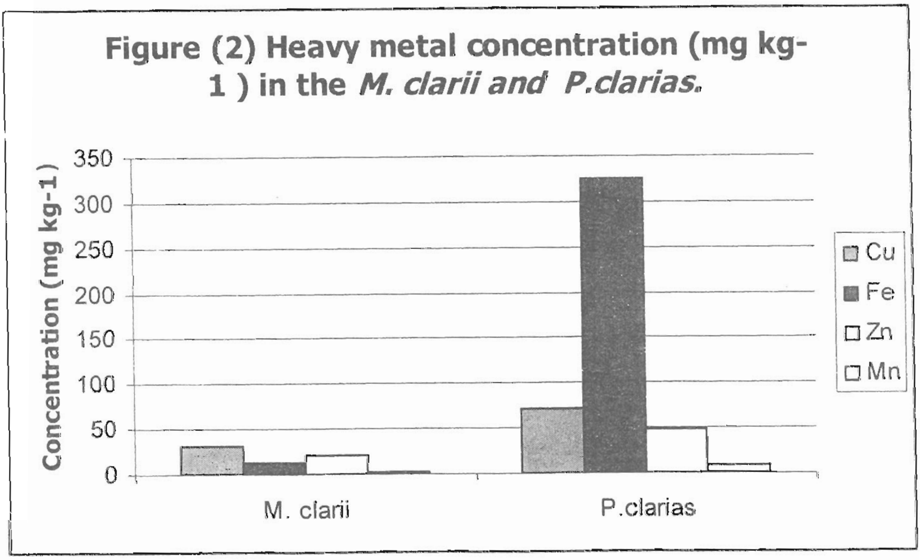
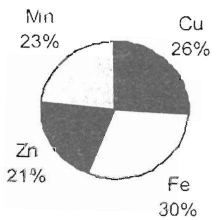


Table 3. Heavy metal concentration (mg/kg) in sediments and mg/L for water.

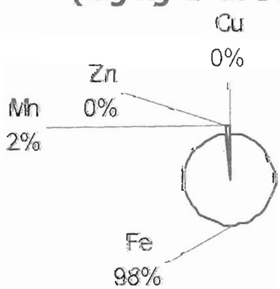
Metals	Sediment	Water
Cu	11.59±2.73	0.41±0.14
Fe	827.71±10.36	0.47±0.33
Zn	28.14±13.35	0.33±0.39
Mn	148.24±13.08	0.36±0.15

Figure (3) Heavy metal concentration mg l-1 for water.



To evaluate heavy metal accumulation, the ratio (R) of heavy metal concentrations in parasites (C_P) relative to their host (C_H) was computed as $R = C_P{:}C_H$ 0.41/72.01 =0.569 for Water- *P. clarias* and water- *M. clarii* r = 0. 569 for Cu in cestode parasite with respect to their contents in water Spearman correlation coefficient (r) and levels of significance determined for the relationship between the content of heavy metals in water and parasites

Figure(4) Heavy metal concentration (mg kg-1 in Sediment



No significant association were found for Fe and Mn concentrations of water and the metal levels of host tissues and *P. clarias* (Spearman, $P > 0.05$).

Positive (for Cu and Zn) and negative (for Fe and Mn) correlations were detected between the content in *P. clarias* and their quantity in bottom sediments of their reservoir. Iron, with maximum concentration in bottom sediments, was recorded in the highest amount also in *P. clarias* The Zn and Mn concentrations in *P. clarias* differed significantly from the sediment (<0.05) :

as $R = C_P : C_H$ 0.41/72.01 =0.569 for Sediment- *P. clarias*

Sediment- *M. clarii* where **r** = 0.569 for Zn in cestode and monogenea parasite with respect to their contents in sediments. Spearman correlation coefficient (*r*) and levels of significance determined for the relationship between the content of heavy metals in sediments and parasites.

DISCUSSION

Metal ions can be incorporated into food chains and concentrated in aquatic organisms to a level that affects their physiological state. In the Egyptian irrigation system, the main source of Cu and Pb are industrial wastes as well as algaecides (for Cu), while that of Cd is the phosphatic fertilizers used in crop farms (Saad and Emam, 1998).

Environmental health was included as one of the four main objectives of the strategic Healthy Egyptians 2010 Initiative. Specific objectives and plans for the initiative are presented by Wagida (2003).

In the present study, we measured Cu, Fe, Zn and Mn in *Clarias gariepinus* (musculature, liver and gills) and *Polyonchobothrium clarias* and *Macrogyrodactylus clarii*, water and sediment. Liver considered the ware house for heavy metals, where the highest levels were accumulated in liver followed by gills and then musculature of *Clarias gariepinus.*

These results are agreement with Shereif and Moaty (1995) found that heavy metals were significantly higher in fish viscera, including liver tissue than in the edible muscles. Mosua (2004) recorded concentration levels of iron, zinc, cadmium and lead as 12.2, 30.0, 0.5 and 5.2 µg/g respectively in Nile tilapia in Lake Burullos, and Shaker and Abdel Aal 2007 who reported that heavy metals was higher in Catfish than Mullet, which was higher than Tilapia, and liver is the highest organ for heavy metals accumulation as Catfish is carnivores' fish and feeding on some residual of organic.

Concerning the accumulation of heavy metals in parasites the result indicated that the cestode, *P. clarias* accumulated heavy metals more higher than monogenetic trematode *M. clarii* where Fe level in *P. clarias* was 27 times higher than its concentrations in *M. clarii* also in the rest of elements the ratio were double times higher than *M. clarii* .Four elements (Cu, Fe, Zn and Mn) analyzed were higher in both parasites than in the liver, two (Fe and Zn) higher than in the musculature, three (Fe, Zn and Mn) higher than in the gills . While *M. clarii* (Monogenea) accumulate heavy metals more than that of water but still lower than that in sediment as the water column the heavy metals were found in sediment and heavy metals almost entirely particulate and coupled with dissolved organic and inorganic compound in water(Rozan *et al.* 2000),it may still be available (Gagnon and Saulnier,2003). Manganese was present in a rather high amount (152.24 mg/kg) in sediment and 0.36 m/L in water of Ismalia Canal, but its accumulation in fish organs was not very high. The content of this element in catfish was rather low in organs (3.44 mg/ kg in the liver, 4.98 mg/ kg in the gills and < 0.03 mg kg^{-1} in the musculature) In his connection, Edwards (1992) stated that there may not be a substantial public health problem produced by the bioaccumulation of toxic elements because almost all organisms contained levels of contaminants, which are below Egyptian National Legal Limits. Thielen *et al.* (2004) reported that Mn was found to be significantly higher in the parasite (*Pomphorhynchus laevis*) compared to all fish host tissues.

Concerning essential elements, a relatively higher concentration of zinc has often been detected without any poisonous effect on the health of the organism. Moreover, zinc in interaction with particular toxic elements (e.g., Cd and Pb) may even reduce their toxicity. On the other hand, the higher concentrations of copper are usually toxic (Miller and Mackay 1980). In Ismalia Canal, bottom sediment and water contained low quantities of both copper and zinc. The content of Zn was higher in the sediment and its accumulation in fish organs was much higher compared with Cu. As Bireš *et al.* (1995) demonstrated, zinc can inhibit the accumulation of copper in animal tissues and hence, it affords certain protection against toxic effects of Cu. The concentrations of iron in sediments and water in Ismalia Canal were high (827.71mg/kg and 0.47 mg/L). Mean concentrations of iron in *Polyonchobothrium clarias* was 2.4 times higher than that in the liver of catfish, 37.4 times higher than that of the musculature and 5.6 times higher than that in the gills. The Fe occurs in proteins like hemoglobin or myoglobin and is also bound in proteins such as ferritin, which play important physiological roles and are found in high concentrations in the fish liver (Huebers and Finch 1984).

In this study, levels of Cr, Pb and Cd were below the detection limit. This may be related to their location in the body cavity of the host but other factors are also probably involved. The disparity in the accumulation capacity might be related to specific properties of the cestode and monogenea tegument, peculiarities of their life cycles as and other complex causes. For instance, distinct intermediate hosts and their different ecology might also have an impact on heavy metals accumulation. However, larval stages of helminths, especially cystacanths, show only a small tendency to accumulate metals despite close contact to their intermediate crustacean hosts with bottom pollutants (Siddall and Sures 1998). Differences in the incorporation various of heavy metals have also been found between different intestinal fish tapeworms, e.g., monozoic *M. wageneri* and *polyzoic Bothriocephalus scorpii* (Sures *et al.* 1997b) or among four tapeworms *L. intestinalis*, *Contluaria globitera* characterized by different biology and trophic preferences of their bird hosts (Baruš *et al.* 2000). Thus, these authors found out that not all species of helminths, especially that parasitizing terrestial hosts, reflect actual pollution in the environment, because their ability of heavy metal accumulation is not equal. Thus, it seems to be a characteristics feature of cestode to accumulate high amounts of some heavy metals. Due to this accumulation capacity and to their abundance in different aquatic ecosystems, cestode may serve as rather useful indicators of heavy metal contamination in the aquatic environment in addition to other indicator invertebrates. Concerning the role of fish-parasitizing cestode as indicators for heavy metals, other species dwelling in the intestine or the body cavity should be investigated.

Also the research assumed that the parasites were provide a vital services for the fish through their heavy metal accumulation role where ,the research reflect actually ,the possible parasite elimination role of metals from the environment of their host.

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التأثيرات البيئيه المحتملة للمعادن الثقيله على اعضاء القرموط الافريقى وطفيل البولى انكوبوثيريم كلارياس (ديدان شريطيه) والمكروجيرودكتيلاس كلارياس (احاديه العائل)

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٢. *قسم الامراض الباطنه والمعديه والاسماك كلية الطب البيطرى - جامعة المنصورة.*

لتقييم مدى صلاحية الطفيليات كمؤشر حيوى على تواجد التلوث بالعناصر الثقيله فى اسماك القرموط الافريقى والطفيليات الداخليه والخارجيه (طفيل البولى انكوبوثيريم كلارياس (ديدان شريطيه) والمكروجيرودكتيلاس كلارياس (احاديه العائل)) تم الاتى :

١-تم تحديد تركيزات سبعة من المعادن الثقيلة (الكروميوم و النحاس ، الرصاص ،المنغنيز ، الكادميوم ، الزنك والنيكل) فى المياة والتربه و بعض اعضاء سمكة القرموط الافريقى وانسجة طفيل البولى انكوبوثيريم كلارياس (ديدان شريطيه) والمكروجيرودكتيلاس كلارياس (احاديه العائل) باستخدام جهاز التحليل الطيفى الامتصاصى الذري.

٢- وبالتالى تم مقارنتها بالبيانات للعناصر الثقيله فى المياة والتربه فوجد ان فقط النحاس ، والحديد ، وعنصر الزنك والمنغنيز فى المياة والتربه و طفيل البولى انكوبوثيريم كلارياس) والمكروجيرودكتيلاس كلارياس وعينات من الأسماك فى حين ان مستويات الكروميوم والكادميوم والرصاص كانت اقل من الحدود االمسموح بها.

٣- كذلك وجد ان اربعة عناصر (النحاس والحديد والزنك والرصاص) بتركيزات عالية في طفيل البولى انكوبوثيريم كلارياس اكثر من تركيزها فى انسجه الأسماك المختلفه (العضلات والكبد والخياشيم) وفى انسجة طفيل والمكروجيرودكتيلاس كلارياس ، بنسب ١,٦ -١ ٢٧,١ مرات أعلى.

٤- يوجد ارتباط ايجابى بين عنصر النحاس وكمية العناصر الثقيله فى المياة والانسجه الطفيليه وسلبى مع باقى العناصر(الحديد والزنك والرصاص) فى حين يوجد ارتباط ايجابى عنصر النحاس والزنك وكمية العناصر الثقيله فى التربه والانسجه الطفيليه وسلبى مع باقى

العناصر(الحديد والرصاص) وذلك مما يعكس مقدار المعادن الثقيلة في الرواسب ، ومدى امكانيه استخدام تواجد طفيل البولى انكوبوثيريم كلارياس (ديدان شريطيه) والمكروجيرودكتيلاس كلارياس (احاديه العائل كمؤشرعلى مدى التلوث بالمعادن الثقيله وتوفير المزيد من المعلومات الموثوق بها عن واقع تلوث المخازان(الرواسب الطينيه فى قاع الاحواض).

٥-كذلك يطرح الباحثين ملاحظة تفسير الدور الايجابى لتواجد الطفيليات خارج وداخل الاسماك وامكانيتهم فى حماية الاسماك من التاثير السام للعناصر الثقيله وذلك بتركير تلك العناصر فى الانسجه الطفيليه .