Egypt. J. Aquat. Biol. & Fish., Vol. 20, No. 3: 103 - 112 (2016) ISSN 1110 – 6131

Comparison of heavy metals levels in muscles, liver and gills of three fish species collected from agricultural drainage water AT El- Abbassa fish farm, Sharkia, Egypt.

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ABSTRACT

The concentrations of heavy metals; iron, zinc, copper, lead and cadmium (Fe, Zn, Cu, Pb, and Cd) were determined in water and Nile tilapia, common carp and catfish caught from agricultural drainage water at El- Abbassa fish farm in the period from February to April 2016. Metals concentrations in the water were ranked in the following order Fe>Zn > Cu>Pb> Cd. The concentrations of Cu, Zn and Cd in water were below the permissible levels according to the Egyptian Standards. Fe and Pb in the water samples exceeded the permissible limits. The fish muscles, gills and liver were carefully dissected out for digestion and determination of heavy metals. The concentration of heavy metals was analysed in tissues of muscles, gills and liver. The results revealed that Fe and Zn concentrations were the highest in all tissues analyzed, followed by Cu, Pb, and Cd in almost all three fish species. The highest concentration of Iron (463.8±3. µg /g dry. wt) was detected in the gills of catfish. Whereas the lowest (255.1±7.3 µg /g dry. wt) in the muscles of common carp. The highest concentration of Zn (203.0±1. µg /g dry. wt) was found in the gills of cat fish. While, the lowest (133.8±5.0 µg /g dry. wt) was recorded in the muscles of common carp. The highest concentration of copper (39.2±3.8 µg /g dry. wt) was detected in the liver of the catfish. While the lowest value (24.5±1.2 µg /g dry. wt) in the muscles of the Nile tilapia. For lead and cadmium the highest were found in the liver of catfish (3.7± 0.4and 2.4±0.19 µg /g dry. wt), while, the lowest in the muscles of common carp (1.6±.6and1.1±0.12 µg /g dry. wt). The results of the present study revealed that the abundance of heavy metals in fish organs followed the order: Fe>Zn> Cu >Pb and Cd. The concentrations of heavy metals detected in samples of the three fish species, were lower than the recommended maximum level allowed in food by (E.O.S.Q.C.2005), (FAO/WHO1999), (EOS 1993) and (WHO 1989).

Keywords: Heavy metals, nile tilapia, common carp, catfish, muscles, liver, gills.

INTRODUCTION

Among environmental pollutants, metals are of particular concern, due to its potential toxic effect and ability to bio-accumulate in aquatic ecosystems (Censi *et al.*, 2006).

The pollution of the aquatic environment with heavy metals has become a worldwide problem in recent years, because they are indestructible and most of them have toxic effect on organisms (Mac Farlane and Burchett, 2000).

Heavy metals may have a significant impact on aquatic organisms, disturbing the ecological balance and potentially contaminating the aquatic food chain as well as human (Saeed and Mohamed, 2012). Thus, the concentrations of these compounds are important indicators for contamination level and also can be serious health problem for the population that feeds on them (Mancera *et al.*, 2006).

Many factors including season, physicochemical properties of water, habitat, age and physiological conditions of fish play a significant role in accumulation of these metals in fish (Kargin, 1996). Gills are directly in contact with water, therefore the concentration of metals in gills reflects metal concentration in water. Presence of high metal concentration in the liver represents storage of metals from water for detoxification (Romeo *et al.*, 1999).

The accumulation of heavy metals within the fish varies depending on route of metal uptake, type of heavy metal and fish species (Begum *et al.,* 2009). The presence of higher amount of heavy metals in any part of the body will induce changes in biochemical metabolisms, serum biochemical changes, histopathological changes and other induced stresses. Therefore the studies on heavy metals accumulation in various organs of the fish are important.

The present study has been conducted to determine iron, zinc, copper, lead and cadmium, concentrations in the gills, muscles and liver of three fish species, Nile tilapia *(Oreochromis niloticus*), Cat fish (*C. gariepinus)* and the common carp (*Cyprinus Carpio*) collected from a fish farm located at Al-Abbassa, Sharkia governorate during February to April 2016.

MATRIALS AND METHODS

Study area

The present study was carried out in Al-Abbassa fish farm at Abou Hammad, Sharkia Governorate through February to April 2016.

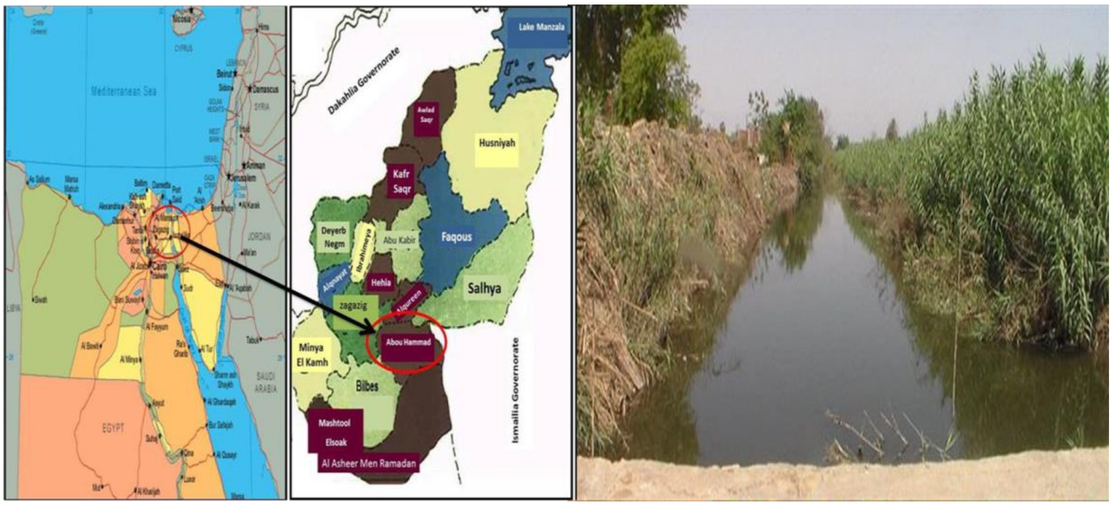

Fig. 1: Map of the Study Area in Egypt.

Fish Sample:

Three fish species (Seven individuals of each/month) namely the Nile tilapia *(Oreochromis niloticus*), the cat fish (*Claries gariepinus)* and common carp (*Cyprinus Carpio*) were used during this study. They were brought from El-Abbassa agricultural drainage water; close to El-Abbassa Fish farm, during February to April 2016. The tissues from 7 fish individuals per month of the same species were pooled to make 3 sub-samples.

Analysis of heavy metals:

In water:-

Unfiltered 500 ml water samples were digested according to the procedure reported by APHA (1995). The metals ions of Fe, Zn, Cu, Pb and Cd were analyzed by SHIMADZU Atomic Absorption Spectrophotometer Model (AA-6800).

In fish:

The selected pieces of muscles, gills and liver were dried at 105°Cfor 48 hours and then grounded to a fine powder. The dried samples were digested according to the method of Ghazaly (1988). One gram dry powder was digested in AR grade conc. nitric and perchloric acid (5ml+5ml). The samples were heated on a hot plat at 800 - 900 C^o until they become clear. After cooling the solutions were filtered and the filtrates were adjusted to 50 ml with de-ionized distilled water. The metallic ions were analyzed in water and fish muscle, gills and liver (Fe, Zn, Cu, Pb and Cd) using SHIMADZU Atomic Absorption Spectrophotometer Model AA-6800 equipped with flame unit and auto-sampler SHIMADZU ASC – 6100. Results were expressed in µg/g dry weight of the tissue.

Statistical analysis:

Statistical analysis was performed using the analysis of variance one way (ANOVA) and Duncan's multiple Range Test, to determine differences between means at significance rate of $P < 0.05$. The standard errors of means were also estimated. All statistics were carried out using Statistical Analysis program (SAS, 2000).

RESULTS AND DISCUSSION

Morphmetric data:

The biometric characteristics (standard length and weight) of the analyzed fish are listed in Table (1).

Table 1: Mean weights and lengths of examined fish species (mean ± SE).

Fish species	Number of fish	Body length (cm)	Body weight (g)
O. niloticus	7	17.41±1.2 ^{b}	120.5±3.05 ^{c}
C. carpio	7	31.76±1.00 ^{a}	461.7±11.53 ^{a}
C.gariepinus	7	28.98±0.87 ^{a}	214.3±8.4 ^{b}

Data shown with different letters are statistically significant at the $P < 0.05$ level.

Heavy metals concentrations in water:-

The concentrations of heavy metals in water from the agricultural drainage water are presented in Table (2). Heavy metals were found to be in the following increasing order of concentrations. Fe (345) >Zn (157.5) > Cu (40.95) > Pb (19.1) > Cd (3.65) (µg/l). The average concentration of Fe and Pb in the water samples exceeded the permissible limits prescribed by EOS (1993).

Table 2: The permissible limits, range and (mean ± SE) of concentration of heavy metals (µg/l) in water.

	Fe	Zn	Cu	Pb	Cd
Range	315 – 375	142 – 173	38.6 –41.7	16.7 –21.5	2.6 –4.7
Mean ± SE	345.0±14.5	157.5±4.9	40.95±3.4	19.1±0.6	3.65.±0.04
EOS (1993)	300	5000	1000	10	10

Heavy metal concentrations in fish sample:-

The results of heavy metals concentrations in the organs (liver, gill and muscle) of the three fish species (*O. niloticus, C. carpio and C. gariepinus*) are presented in Tables (3& 4) and Figs. (2 & 3). The concentration of heavy metals measured in the organs of the three fish species studied generally lower than the levels issued by E.O.S.Q.C. (2005), FAO/WHO (1999), EOS (1993) and WHO (1989) are presented in Table (5).

Table 3: Concentrations of heavy metals (Mean ± SE) (µg /g dry.wt) in different organs of fish species.

Fish species	Organ	Fe	Zn	Cu	Pb	Cd
O. niloticus	Muscle	299.3±7.2^b	165.9±6.4^a	24.5±1.2^b	1.84±.04^b	1.13±0.03 ^{b}
	Gills	332.9C±7.8^a	172.5±9.3^a	31.7±2.5 ^{a}	2.8±.08^a	1.6±0.07 ^{a}
	Liver	351.2±14.8^a	172.3±9.5 ^{a}	28.8±3.3 ^{b}	2. 3±0.3^a	1.4±0.15 ^{a}
C. carpio	Muscle	255.1± 7.3^c	133.8±5.0^a	28.2±3.4^a	1.6±0.6 ^{b}	1.1±0.12^b
	Gills	301.3± 8.3 ^{b}	144.5±7.3^a	26.1±0.9^a	2.6±0.21^a	1.9± 0.27^a
	Liver	336.5± 5.9 ^{a}	149.9±3.5^a	32.6±1.5^a	2.4± 0 .4^a	1.8 ±0.38^a
C.gariepinus	Muscle	370.5± 9.5 ^{b}	169.3±1.5^b	27.3±1.1^a	1.9±0.1^b	1.4±0.09 ^{b}
	Gills	463.8±3.8 ^{a}	203.0±1.0^a	35.1±1.3^a	2.5± 0.3 ^{a}	1.9±0.08 ^{a}
	Liver	393.2± 6.0 ^{b}	185.5±4.1^b	39.2±3.8^a	3.7± 0.4 ^{a}	2.4±0.19^a

*All results are expressed as Mean± Standard Error (SE) of Mean; n=21.
*Data shown with different letters are statistically significant at the P < 0.05 level.

Table 4: Mean concentrations of heavy metals (Mean ± SE) (µg /g dry.wt) of fish species.

Fish species	Fe	Zn	Cu	Pb	Cd
O. niloticus	327.8± 9.9 ^{b}	171.4± 8.4^c	28.3± 2.3^b	2.3±0.2 ^{a}	1.3± 1.08 ^{b}
C. carpio	297.6± 7.1^b	142.7 ± 5.2 ^{b}	28.9 1.9^b	2.0± 0.7^a	1.5± 0.25 ^{a}
C.gariepinus	409.2± 6.4^a	185.9± 2.1^a	33.8± 2.1^a	2.7±0.6^a	1.9± 0.15^a

*All results are expressed as mean± Standard Error (SE) of Mean; n=21.
*Data shown with different letters are statistically significant at the P < 0.05 level.

Table 5: Maximum Permissible Limit (MPL) of heavy metals in fish (mg/kg wet wt.) according to international standards.

	Fe	Zn	Cu	Pb	Cd
E.O.S.Q.C. (2005)	30	40	20	0.5	0.05
FAO/WHO(1999)	43.0	60.0	3.0	0.214	0.1
EOS (1993)	30	40	20	2.0	0.5
WHO (1989)	50	40	30	0.5	0.5

*Permissible limits according to guidelines in E.O.S.Q.C. (2005), FAO/WHO (1999), EOS (1993) and WHO (1989)

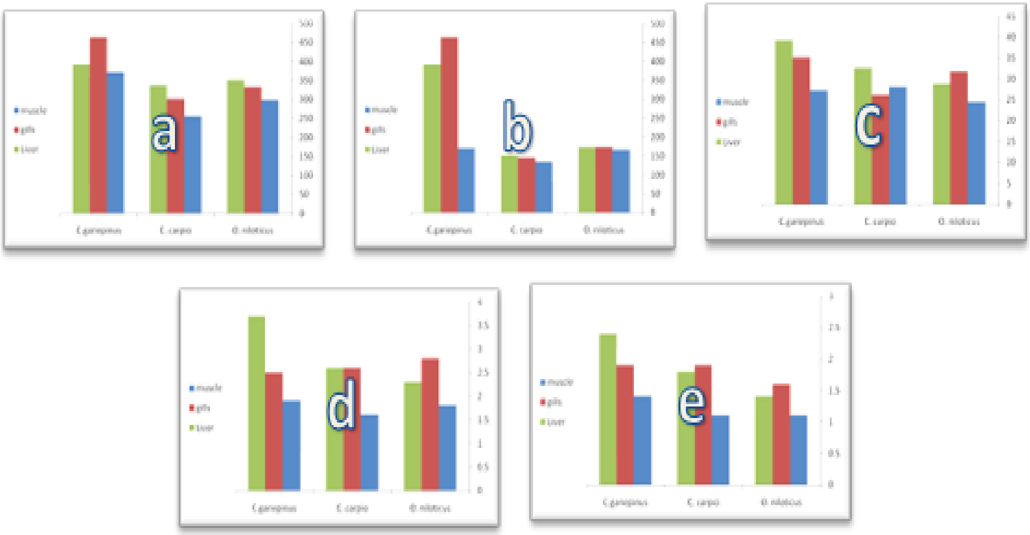

Fig. 2: Variation of heavy metals concentrations in different organs of fish species A: Iron (Fe), B: Zinc (Zn), C: Copper (Cu), D: Lead (Pb), E: Cadiumum (Cd).

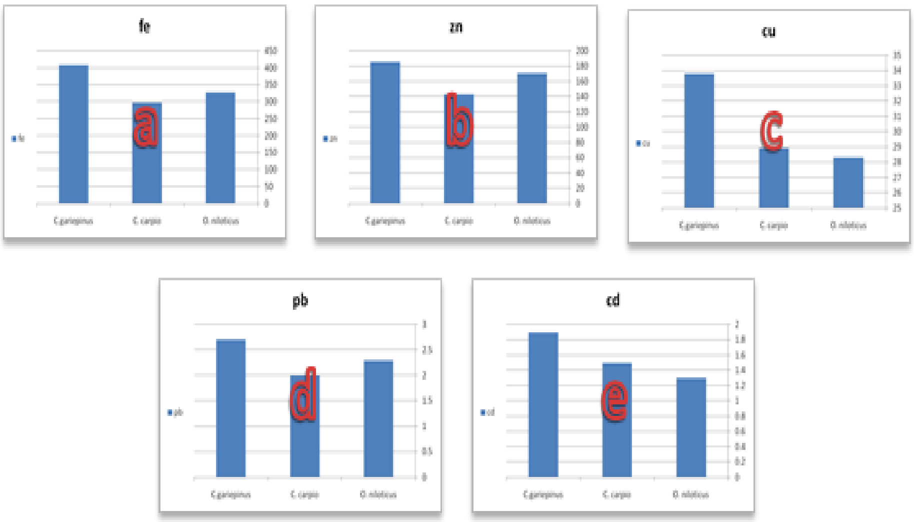

Fig. 3: Variation of heavy metals mean concentrations in different fish species A: Iron (Fe), B: Zinc (Zn), C: Copper (Cu), D: Lead (Pb), E: Cadiumum (Cd).

Iron (Fe):

The concentrations of Fe ranged from 255.1± 7.3 µg /g drywt in muscles of *C. carpio* to 463.8±3.8 µg /g dry.wt in gills of *C. gariepinu*s. It was found that the three fish spices had a significant difference between organs in Fe concentrations ($P< 0.05$). Its distribution in both *C. carpio* and *O. niloticu* was similar, it was in the increasing order (muscles< gills< liver) with a significant differences among all organs with each other except liver which showed no significant difference in each of the two fish species. In *C. gariepinu*s Fe was in the increasing order (muscles < liver < gills) with a significant differences among all organs in this fish. For Fe level in the three fish species, *C. gariepinu*s showed the highest level than *C. carpio* and *O. niloticu* with a significant difference ($P< 0.05$) among fish spices.

Iron (Fe) concentration in liver and gills were higher than that of muscles. This may be because liver and gills had a high tendency to accumulate high concentrations of heavy metals, while muscles tend to retain lower concentrations, these results agree with that recorded by Hamed (1998); Khalil (1997); Singh *et al.* (1990). Also, it was more than that recorded by El-Ghobashy *et al.* (2002) and Ali (2007) which ranged between 23.8- 167.3 ppm for muscles, gills and liver and 30.3-194.0ppm in muscles and gills of tilapia spp. Similarly, other studies reported in *C. gariepinus* (Osman *et al.* 2010) and *Tinca tinca* (Selda *et al.* 2005), also revealed the maximum accumulation of Fe is in liver. However, in other studies the highest accumulation was seen in organs such as gills in *O. niloticus*, *L. niloticus* (Mohamed 2008), *O. mossambicus* (Jenny and Avenant 2006), *Liza aurata*, *Mugil cephalus*, and *Liza ramada* (Uysal *et al.* 2008).

Zinc (Zn):

The lowest value in muscles of *C. carpio* was133.8±5.0 µg /g dry.wt and the highest was in gills of *C. gariepinu*s 203.0±1.0 µg /g dry.wt. For Zn concentration in different organs, *O. niloticus* and *C. carpio* showed no significant difference but *C. gariepinu*s had a significant difference (P< 0.05). Its distribution in both *C. carpio* and *O. niloticu* was similar, it was in the increasing order of muscles < gills< liver with no significant differences among all organs of the two fish species. While, it was in the increasing order of muscles < liver < gills in *C.gariepinu*s with a significant differences among organs *C. gariepinu*s showed the highest level of Zn than in *C. carpio* and *O. niloticu* with significant differences (P< 0.05) between fish species.

Highest accumulation of Zn in liver and gills has also been reported in *C.gariepinus* (Coetzee *et al.* 2002). In the present study, it is observed that Zn content was lower in muscles than liver or gills. Other workers also reported the highest concentration of Zn in liver of *C. punctatus* (Murugan *et al.* 2008). However highest accumulation was seen in other organs such as gills of *C. punctatus* (Vineeta *et al.* 2007), testis of *O. niloticus* and *L. niloticus* (Mohamed, 2008). Zinc was detected in all the fish samples and the highest concentrations were observed in gills tissues, followed by liver and muscles. Fish can accumulate zinc from both the surrounding water and from their diet (Eisler, 1993). Although zinc is an essential element, at high concentrations, it can be toxic to fish, causing mortality, growth retardation and reproductive impairment (Sorenson, 1991). Zinc is capable of interacting with other elements and producing antagonistic, additive or synergistic effects (Baumann and May, 1984).

Copper (Cu):

The lowest Cu concentration was found in muscles of *O. niloticus* 24.5±1.2 µg/g dry.wt and the highest was found in liver of *C. gariepinu*s 39.2±3.8 µg/g dry.wt. It was found that *C. gariepinu*s and *C. carpio* showed no significant difference between organs, but *O. niloticus* had a significant difference (P< 0.05) between organs. Its distribution in both *C. carpio* and *C. gariepinu*s was similar; it was in the increasing order of muscles< gills< liver with no significant differences among all organs of the two fish species. In *O. niloticus* it was in the increasing order of muscles < liver < gills with a significant differences among organs in this fish. It was found that *C. gariepinu*s showed the highest level and significant values for *C. carpio* and *O. niloticus* (P< 0.05). This agrees with the findings in other organs such as liver of *O. nilotica* (Abdel- Baki *et al.* 2011), *Onchorynchus mykiss, C. carpio* (De Boeck *et al*. 2004), *O. niloticus* (Mohamed, 2008) and gills of *C. punctatus* (Vineeta *et al.* 2005), *Lithognathus mormyrus* (Uysal *et al.* 2008). Muscles accumulated lesser copper than liver and gills, indicating that the food is the primary pathway for uptake of copper. Similar results were observed in different fish species from El Max Bay Alexandria

(Khaled, 2004).In the present study the lowest concentrations of Cu were observed in the muscles of studied fish, similar to results recorded by Yacoub (2007).

Lead (Pb):

The concentrations of lead in this study showed it was between the lowest value in muscles of *O. niloticus* 1.84±.04 µg/g dry w.t and the highest value in gills of *C. gariepinu*s 3.7± 0.04 µg/g dry w.t.

Lead (Pb) concentration showed no significant (P< 0.05) difference between organs of the three fish species in liver and gills, but the highest from muscles. Its distribution in both *C. carpio* and *O. niloticus* was similar; it was in the increasing order of muscles <liver < gills. While, its distribution in *C. gariepinu*s in the increasing order of muscles< gills < liver. In comparison of Pb level in the three fish species, it was found that *C. gariepinu*s showed the highest level than in *C. carpio* and *O. niloticu* (P< 0.05). Our recorded results were in parallel lines to those reported by many investigators (Hamouda, 1996; Othman *et al*. 2004; Perziosi *et al*. 2006). Lead is highly toxics to aquatic organisms, especially fish (Rompala *et al.* 1984). The biological effects of sublethal concentrations of lead include delayed embryonic development, suppressed reproduction and inhalation of growth, increased mucous formation, neurological problems, enzyme inhalation and kidney disfunction (Rompala *et al.* 1984).

Cadmium (Cd):

The concentrations of Cd in the tissues of fish varied from 1.1 µg/g dry w.t in muscles of *C. carpio* to 2.44 µg/g wet weight in liver of *C. gariepinu*s. Cd concentration in different organs showed no significant (P< 0.05) difference between organs of the three fish species in liver and gills but highest significant in muscles. Comparing Cd levels in the three fish species, it was found that *C. gariepinu*s showed the highest level and a significant from *C. carpio* and *O. niloticu* (P< 0.05). In the present study muscles showed no significant (P< 0.05) between the three fish species. These results agree with that recorded by Canli *et al.* (1998); Khaled (2004); rahman *et al.* (2012). The results of the present study showed that, liver and gills accumulate and concentrate highest concentrations of Cd. Jent *et al.* (1998) found that, Cd concentration increased in fish liver collected from water near the agricultural areas. Rashed (2001) found the same results in tilapia fish collected from Lake Nasser. The high accumulation of Cd in the Kidney corroborated the results obtained by Malik *et al.* (2010).

CONCLUSION

The results of the present study revealed that the abundance of heavy metals in fish organs followed the order of Fe>Zn> Cu >Pb and Cd. The concentrations of heavy metals detected in samples of the three fish species were lower than the recommended maximum level allowed in food by E. O. S. Q. C. (2005), FAO/WHO (1999), EOS (1993) and WHO (1989). The metals content in the livers and gills of fish was considerably higher than that of muscles. A comparison of the species analyzed showed that the *C. gariepinu*s accumulates the most of the metals in the liver and gills followed by *O. niloticus* and *C. carpio*. This study indicates that the heavy metals concentrations in muscles of the three fish species are lower than that in gills and liver and within the permissible limits recommended by guideline standards. Therefore these fishes are safe for human consumption.

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ARABIC SUMMARY

مقارنة بين مستويات بعض المعادن الثقيلة في عضلات وكبد وخياشيم ثلاثة أنواع من الأسماك التي تم جمعها من مياه الصرف الزراعي بالمزرعه السمكية، بالعباسه، الشرقية، مصر.

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استهدف هذا البحث دراسه بعض العناصر الثقيله فى مياه الصرف الزراعى بالمزرعه السمكيه بالعباسه (حديد- زنك – نحاس – رصاص- كاديميوم) فى المياة وعضلات وخياشيم وكبد البلطى النيلى والمبروك العادى وسمكه القرموط .واظهرت النتائج فى المياه ان تركيز كل من الحديد والرصاص كانت اعلى من الحد المسموح به اما النحاس والزنك والكاديميوم كان فى الحدود المسموح به. اما فى الاسماك فقد أوضحت الدراسة أن تراكيز المعادن الثقيلة كانت تتغير بتغير نوع النسيج ونوع السمك. فكانت نسبه الحديد والزنك عالية فى الانواع الثلاث مقارنه بالرصاص والنحاس والكاديميوم. وكانت اعلى قيمة للحديد والزنك فى خياشيم سمكه القرموط واقل قيمه كانت فى عضلات سمكه المبروك. اما بالنسبه لعنصر النحاس والرصاص والكاديميوم فكانت اعلى قيمة فى الكبد لسمكه القرموط واقل قيمة كانت فى عضلات سمكه البلطى النيلى بالنسبه لعنصر النحاس اما الرصاص والكاديميوم كانت اقل قيمة فى عضلات سمكه المبروك. واظهرت الدراسه ان أنسجة الاسماك فى كل من الكبد والخياشيم كانت لها قدرة كبيرة لإختزان العناصر بينما كانت العضلات أقل وكان مستوى جميع العناصر فى عضلات الأسماك أقل من الحد المسموح به دوليا وأنها لاتمثل خطورة على جمهور المستهلكين لهذه الأسماك فى تلك المنطقه.