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LEVELS OF SOME HEAVY METALS IN MUSCLES AND LIVER OF FRESHWATER FARMED FISH AT ABBASSA

Diaa A. Al-Kenawy* and Niema A.F. Aly**

**The WorldFish center, **Central Laboratory for Aquaculture Research, Abbassa, Abou-Hammad, Sharkia, 44662, Egypt.*

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Abstract

Fish samples were used to evaluate heavy metal pollution in some earthen fish ponds located at Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abu-Hammad, Sharkia Governorate, Egypt. Concentrations of some heavy metals (cadmium (Cd), lead (Pb), cupper (Cu), iron (Fe), manganese (Mn), and zinc (Zn)) and calcium (Ca), were analyzed in fish tissues (muscle and liver) using atomic absorption spectrophotometer. The levels of the tested metals in Nile tilapia (*Oreochromis niloticus)*, striped mullet (*Mugil cephalus*), African catfish (*Clarias gariepinus)*, common carp *(Cyprinus carpio)*, and silver *carp (Hypophthalmichthys molitix*) were consistently higher in liver than in muscle tissues of all the five fish species. Calcium concentration was anomalously higher in the muscle tissue than liver of Nile tilapia, common carp and silver carp but not in African catfish and striped mullet. Muscle-liver ratios (MLRs) of the respective heavy metals ranged between 0.04 (Cu) and 0.73 (Ca) for Striped mullet, 0.06 (Cu) and 0.79 (Ca) for African catfish, 0.03 (Cd) and 1.53 (Ca) for silver carp, 0.02 (Pb) and 1.33 (Ca) for Nile tilapia, 0.11 (Cu) and 2.15 (Zn) for common carp. Interestingly, copper indicated least MLR in almost all the investigated fishes except in Nile tilapia and silver carp. Calcium showed the highest MLR in all fish species except in common carp. On the other hand, the cadmium/zinc ratios were higher in the liver than muscles of all fishes analyzed. The threshold contamination value for human dietary risk was however, not exceeded.

Key words: Heavy metals, freshwater fish, fish ponds, muscle-liver ratio.

INTRODUCTION

Water quality is a critical limiting factor for fish life and fish flesh quality. Water physico-chemical parameters should be closely monitored and the optimal limit should at all time be provided to the cultured organism for optimum performance of the organism as well as profitability of fish production venture (Onada *et. al*., 2015). Nowadays, pollution of the aquatic environment is a serious and growing problem throughout the world. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment have led to various deleterious effects on the aquatic organisms, including fish. Heavy metals contamination of aquatic system has attracted the attention of several investigators in both the developed and developing countries of the world. The fact that heavy metals cannot be destroyed through biological degradation, and have the ability to accumulate in the environment makes these toxicants deleterious to the aquatic environment and consequently, to human. Metals can bioaccumulate in the body and in the food chain, and then it may become toxic.

Despite the natural sources of heavy/trace metals in the environment, anthropogenic supply to aquatic ecosystems from industrial effluents/wastes, agricultural and domestic wastewaters laden with metal toxicants, could be considered as extra sources. Heavy metal pollution is an important environmental problem (Ryams-Keller *et al*., 1998; Ho and Hui, 2001; Yilmaz, 2005), considering that some are hazardous substances and can bio-accumulate in the environment, plant and animal tissues (Zweig *et al*., 1999; van den Broek *et al*., 2002). Fishes are major faunal components of aquatic environments and are usually used as excellent environmental bio-indicator of the health of aquatic systems (Stiassny, 1996; Wildianarko *et al*., 2000). In recent times, there has been increasing interest in the utilization of fishes as indicative indicators of the integrity of aquatic environmental systems (Fausch *et al*., 1990; Whitefield, 1996; Toham and Teugels, 1999; King and Jonathan, 2003) because aquatic environments and the associated fish faunal assemblages are potentially affected by a wide array of anthropogenic perturbations (King and Jonathan, 2003). Many studies have indicated that aquatic organisms can accumulate and bio-concentrate heavy metals in their tissues many times above ambient levels (Voight, 2003; Kucuksezgin *et al*., 2006).

Various metals show different affinity to fish tissues. Most of these metals accumulate mainly in liver, kidney and gills. Fish muscles, compared to the other tissues; usually contain the lowest levels of metals, (Barbara, and Malgorzata, 2006). In aquatic ecosystems, heavy metals may enter fin/shell-fishes through direct absorption from the water through their gills and other exposed bio-membranes (Matagi *et al*., 1998). However, most pollutants in aquatic organisms arrive there through the food chain. First, phytoplankton, bacteria, fungi and other small organisms absorb these substances and are in turn eaten by fishes.

Since fish constitute an important human food, they are potentially an indirect source of heavy metals entering the body but they may in addition suffer wide range of ecological, physiological, metabolic and behavioral defects (Cucuk and Engun, 2005). Adeyeye *et al.* (1996) also showed that the concentration of metals was a function of fish species as it accumulates more in some fish species than others.

In fishes, it is observed that the external organs are affected due to the toxic chemicals (heavy metals for example), causing loss of equilibrium, increased opercular movement, irregular vertical movements, finally leading to death. Cadmium, lead, mercury and arsenic cause severe damage to the renal and nervous systems of fish as well as gill damage (severe destructive pathological changes, i.e. structural lesions), (Larse, 2003; Velcheya *et al*., 2010; Deore and Wagh, 2012; Mahino and Nazura, 2013).

The accumulation of metals in fish tissues depends on numerous factors, such as environmental concentrations, environmental conditions (i.e. pH, water temperature, hardness...etc), exposure duration and specie-specific living and feeding habits. Monitoring programs for bioaccumulation measurements serve as a biomarker for fish from contaminated places and provide information about the environmental conditions, (Zeitoun and Mehana, 2014).

Nile tilapia *(Oreochromis niloticus)*, African catfish *(Clarias gariepinus*), striped mullet (*Mugil cephalus*), common carp (*Cyprinus carpio*) and silver carp *(Hypophthalmichthys molitix*) are fish species that constitute an important source of protein to the local consumers and the entire country. Species of fishes used in this study differ in their habitats and feeding habits. Such fish species move in a certain depth such as surface area, subsurface, the middle or near pond's bottom. Striped mullet is a highly commercial species and usually feed from the middle layer of pond water depth by filtering phytoplankton, mainly diatoms. Silver carp is, however, similar to mullets in feeding behavior. Catfish, a major commercial fish species, inhabit in the bottom of ponds. It is an omnivorous species that feeds on fish seeds, insects, and detritus. Nile tilapia is a planktonivores fish that feed from the surface and subsurface of ponds. Common carp is an omnivore, which found at the mud bottoms of ponds. Most species are predators of fishes, while several are planktonivores. In general, Nile tilapia, striped mullet, catfish, common carp and Silver carp species are the most important in the artisanal/commercial fishery in fish farms.

In the present study, the levels of iron, zinc, cadmium, copper, lead, manganese, and calcium in muscle and liver tissues were investigated in different species of commercially available farmed fishes (Nile tilapia, striped mullet, African catfish, common carp, and silver carp) caught from some earthen ponds of the Abbassa fish farm as possible bioindicators of heavy metal pollution in water.

MATERIALS AND METHODS

Study area:

This study was carried out at Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou-Hammad, Sharkia governorate. The tested fish samples were collected from the ponds of the production fish farm that is supplied with water through El-Wady drain canal that receives agricultural drainage water from the surrounding agricultural lands.

Water quality parameters:

Monthly water samples were collected from each pond to be analyzed for different water physico-chemical parameters; Temperature, Secchi disk (water transparency), dissolved oxygen, pH, alkalinity, total hardness, unionized ammonia (NH_3), nitrate (NO_3) and phosphate (PO_4). Water samples were analyzed according to methods described by Boyd and Tucker (1992).

Fish samples collection:

Random **s**amples of fishes were collected from 5 earthen fish production ponds for analysis at the harvest time after a 6 months growing period. All the tested fish ponds were managed similarly throughout the growing period. The common fish species collected were Nile tilapia *(Oreochromis niloticus)*, African catfish *(Clarias gariepinus*), striped mullet (*Mugil cephalus*), common carp (*Cyprinus carpio*) and silver carp *(Hypophthalmichthys molitix*). The fishes collected were labeled with an identification number, the date and place of collection, before transporting in ice packed plastic coolers to the laboratory for analysis.

Heavy metal analysis:

For the determination of heavy metals, fish samples were carefully dissected to remove the liver and parts of the muscle. Then livers and pieces of edible muscle tissues were oven dried at 85 ^{o}C until constant weight (about 18 hours). A 1.0 g dry weight of tissue was ashed in muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) for 6 hours. Ash was digested with conc. nitric acids, and diluted with 2N HCL to a constant volume. The samples were filtered into acid-washed volumetric flasks and diluted to 50 mL for elemental analysis. Concentrations of cadmium, copper, iron, lead, manganese, zinc and Calcium were then determined according to APHA (1998) using atomic absorption spectrophotometer (Thermo 6600 Thermo Electron Corporation, Cambridge, UK).

RESULTS AND DISCUSSION

Data of the levels of water quality parameters are shown in table 1. Major water quality parameters measured during the experiment were within the favorable ranges (Table 1) for tilapia (Boyd, 1990), suggesting that tilapia growth performance was not limited by any of the water quality parameters. The dissolved oxygen level (mg/l) obtained from this study was in the range of 6.23 - 7.7 mg/l, these values agree with those of Saloom and Duncan (2005), they also pointed out that the minimum dissolved oxygen concentration should not be less than 5 mg/l for tropical fish. Slightly High rates of ammonia in the first three months of the study period could be attributed to the fertilization which resulted in high loads in the form of die-off plankton and other organic matter that mineralized by microbial activities to inorganic nutrients such as ammonia which stimulate algal growth in ponds (Boyd, 1990). The low levels of Secchi disk reading (9 – 11 cm) prove the high abundance of plankton in the water throughout the growing season.

Table 1. Levels of some water quality parameters in the fish ponds throughout the growing period.

Month	Tempe-rature°C	Secchi Disk (cm)	Dissolved Oxygen (mg/l)	pH	Alkalinity (mg/l)	Total Hardness (mg/l)	Ammonia NH_3 (mg/l)	Nitrate NO_3 (mg/l)	Phosphate PO_4 (mg/l)
August	30.00	9.83	6.23	8.80	128.00	168.00	0.41	0.32	0.42
September	28.47	9.00	6.60	8.73	130.33	172.67	0.39	0.44	0.74
October	27.13	10.50	7.70	8.77	131.67	191.33	0.50	0.43	0.70
November	23.33	11.00	7.57	8.83	132.67	200.00	0.30	0.30	0.75
December	18.40	11.00	7.33	8.90	134.67	204.33	0.27	0.58	0.22
Overall Average (±St. Dev*)	25.47 ± 4.66	10.27 ± 0.85	7.09 ± 0.64	8.81 ± 0.06	131.47 ± 2.5	187.27 ± 16.24	0.37 ± 0.09	0.42 ± 0.11	0.57 ± 0.24

** Standard deviation*

Concentrations of iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), cadmium (Cd), lead (Pb), and calcium (Ca) in the fish liver and muscle tissues and the calculated Muscle/Liver-Ratio (MLR) are shown in Table 2.

The results for Fe, Zn, Mn, Cu, Cd, Pb and Ca concentrations in the different fish species have revealed consistently higher levels in the liver than in the muscle tissues. This is in agreement with earlier study by Koli and Whitmore (1983). However, calcium demonstrated an anomalous trend in the fish samples. Calcium concentrations were comparatively higher in the muscle than in the liver of Nile tilapia, Common carp and Silver carp (Table 2).

Surprisingly however, this anomalous trend of calcium concentration was not found in striped mullet and catfish (Table 2); in these fish species, Ca levels were higher in the liver than in the muscle. The anomalous Ca bioaccumulation and storage potential in Nile tilapia, Common carp, and Silver carp could be attributed to the role of Ca in binding proteins of muscle tissue (Koli and Whitmore, 1983). Absorbed Ca cannot be distributed quickly to other target organs probably due to its complex form within the muscle tissues, thus it accumulate anomalously.

Table 2. Heavy metals concentrations (mg/kg^{-1}) and muscle-liver ratio (MLR) in the five tested fish species.

Fish Species		**Metal / Element**						
		Iron (Fe)	**Zinc (Zn)**	**Manganese (Mn)**	**Copper (Cu)**	**Cadmium (Cd)**	**Lead (Pb)**	**Calcium (Ca)**
Nile tilapia	Muscle	62.8	5.7	7.7	10.4	0.78	0.48	960
	Liver	136.5	22.9	42.46	169.7	7.9	21.09	720
	MLR	0.46	0.24	0.18	0.06	0.1	0.02	1.33
Striped mullet	Muscle	88.69	11.9	9.8	10.68	0.9	1.66	650
	Liver	231.18	26	44.3	278	10.64	24.7	890
	MLR	0.38	0.46	0.22	0.04	0.08	0.07	0.73
African catfish	Muscle	57.3	9.87	5.93	11.5	1.25	6.07	530
	Liver	172.7	21.7	28.4	176	8.25	18.7	666
	MLR	0.33	0.45	0.2	0.06	0.15	0.32	0.79
Common carp	Muscle	39.46	9.32	6.7	15.57	0.56	2.6	556
	Liver	51.8	4.33	4.1	138	2.28	6.07	450
	MLR	0.76	2.15	1.63	0.11	0.24	0.43	1.23
Silver carp	Muscle	70.38	8.84	4.25	16	0.16	1.74	690
	Liver	106.76	12.87	13.29	138.1	5.2	17.3	450
	MLR	0.65	0.68	0.32	0.11	0.03	0.1	1.53

The Muscle-Liver Ratios (MLRs) of the respective heavy metals accumulated by the fishes (Table 2) ranged between 0.02 and 1.33 for Pb and Ca in Nile tilapia; 0.04 and 0.73 for Cu and Ca in striped mullet; 0.06 and 0.79 for Cu and Ca in catfish; 0.11 and 2.15 for Cu and Zn in common carp; and 0.1 and 1.53 for Pb and Ca in silver carp. Essential trace elements (Fe, Zn and Cu) indicated preferential bioaccumulation in the liver than muscle tissue, while calcium showed similar trend in striped mullet and African catfish only. Interestingly, the values revealed that the least MLRs were generally recorded for copper element, except in Nile tilapia, where the lead ratio was the least.

The MLR for Calcium was the highest in most of the fish species except in Common carp (Fig 1), where zinc MLR was very high (2.15); which means that Zn concentration was 2.15 times higher in the common carp muscle than in its liver.

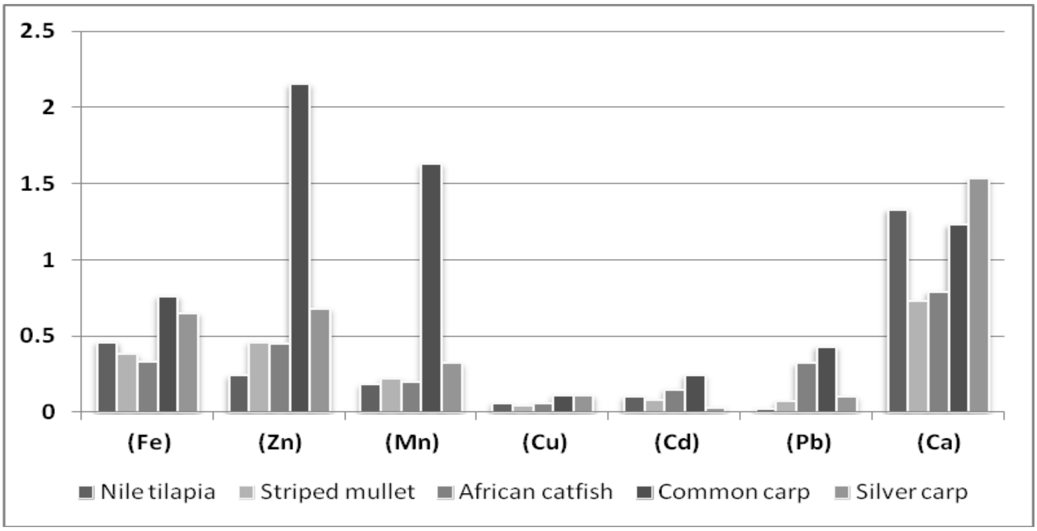

Figure 1. Metal Muscle/Liver-Ratio (MLR) for the five studied fish species.

The concentrations of cadmium in the liver exceeded the concentrations in the muscle tissue of all studied fish species (Table 2). The variation of the MLR for Cd (0.03-0.24), for all species considered in this study confirms earlier results that cadmium mainly accumulates in the liver than muscle tissues (Lemaire and Lemaire, 1992; Soengas *et al*., 1996; Voight, 2003; Cucuk and Engun, 2005). In fish, cadmium is absorbed both from the surrounding water by the gills and from the food by digestion and then transported through the blood, mainly to the liver and kidneys (Van Den Broek *et al*., 2002). Although it is a non-essential heavy metal, Cd has cumulative polluting effect in aquatic ecosystems and could lead to interruption of development and growth, skeletal deformations and pathological changes in aquatic organisms (Cucuk and Engun, 2005). Similarly, the concentrations of copper, iron and lead in the livers exceeded that of the muscle tissues of the fishes investigated. Moreover, the calculated MLRs for Cu, Fe and Pb varied for all species: Cu (0.04-0.11), Fe (0.33-0.76) and Pb (0.02-0.43). However, Cu though an essential element has limited range above which toxic effects is observed (Arellano *et al*., 1999) and possible sources to aquatic systems include domestic, agricultural use and industrial processes (Mason *et al*., 2002).

Muscle-liver ratio is an indicator of heavy metal cleansing mechanism in fish, owing to the rates of accumulation and removal from the fish body system (Koli and Whitmore, 1983). Thus, MLR could be a useful bio-monitoring indicator of heavy metal contamination in fishes within an aquatic system. Although, the muscle tissue of fishes is not known to accumulate enhanced levels of heavy metals, therefore heavy metals concentrations in the muscle is a reflection of their content in the transporting heam.

Cadmium is known to be among the most toxic heavy metals to aquatic organisms (Castano *et al*., 1998; Beauvails *et al*., 2001). Equally, zinc has been shown to be toxic to aquatic organisms arising mainly from domestic sources and natural occurring processes into aquatic systems (Zweig *et al*., 1999; van den Broek *et al*., 2002). Although, cadmium and zinc are chemically very similar (Scheyer, 2000), the difference, however, is that cadmium has a higher affinity for thiol (SH) groups in human physiology (Mengel and Kirby, 1982) which may cause more health problems when enhanced concentrations are taken in, through the consumption of fishes. Zinc is an essential biological mineral, which is regulated and maintained at certain concentrations in fish

(Widianarko *et al.*, 2000) due to physiological requirements for survival (homeostatic regulation) (Sorensen, 1991).

The WHO's limits for health (based on wet weight) are 30 ppm for Cu, 2.0 ppm for Pb and Cd, 50 ppm for Zn, while limits for Fe are not available (WHO, 1992). Fortunately, the obtained figures showed that concentrations of the studied metals in the muscle tissues of the fish samples were found to be within the safe limits; the illustrated levels in tables 2 – 6 are based on dry weight, which means that the levels in wet weight are far below these levels (moisture content in most tested freshwater fish is approximately 40%).

Cadmium and zinc levels were higher in the liver tissues of most fish species than in the muscle except zinc in common carp where was higher in muscle (9.32) than in liver (4.33) (Tables 2). However, estimation of Cd to Zn ratio summarized in Table 3, indicated that cadmium/zinc ratio in muscles were lower than those estimated for liver tissues in all species considered.

Table 3. Cadmium to zinc ratio in the examined fish species obtained from earthen fish ponds.

Fish species	Muscle	Liver
Tilapia	٠.١٤	٠.٣٤
Striped mullet	٠.٠٧	٠.٤١
Catfish	٠.١٢	٠.٣٨
Common carp	٠.٠٦	٠.٥٢
Silver carp	٠.٠٢	٠.٤

It can be deduced from the above findings that accumulation of heavy metals is more of species-related. In addition, it was noted that there is no relationship between the accumulation patterns of different metals in the different fishes. Thus the difference noticed in the levels of accumulation in different organs of a fish can be attributed to the differences in their physiological roles toward maintaining homeostasis, feeding habits, regulatory ability and behavior of each fish. This is in accordance with the findings of Cross *et al.* (1973). However, majorly muscle has the least concentration of the heavy metals compared with liver in the studied fish species. This is in agreement with previous study by Ishaq *et al.* (2011) which showed that muscle is not an active organ in the accumulation of heavy metals. Liver, on the other hand, has been reported as metabolically active site and can accumulate heavy metals in higher level.

In conclusion, levels of heavy metals and trace elements found in the edible tissues (muscles) of the examined fish samples were within the safe limits for human health (WHO, 1992), while levels of some metals in liver tissue were higher than the safe limits.

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مستويات بعض المعادن الثقيلة فى أنسجة العضلات والكبد لأسماك المياه العذبة بالمزارع السمكية بالعباسة

ضياء عبد الرحيم القناوي* و نعمة عبد الفتاح علي**

** المركز الدولي للأسماك، ** المعمل المركزي لبحوث الثروة السمكية، العباسة، أبو حماد، محافظة الشرقية، 44662 مصر.*

الملخص العربــى

تهدف هذه الدراسة الى تقييم مستويات بعض المعادن الثقيلة فى أنسجة الكبد والعضلات لخمس أنواع من أسماك المياه العذبة المستزرعة بالمزارع السمكية بالعباسة، محافظة الشرقية، جمهورية مصر العربية، ثم إيجاد نسبة تركيزات تلك العناصر في العضلات إلى نسبتها في الكبد (نسبة العضلات:الكبد). حيث تم تجميع عينات الأسماك من خمسة أحواض ترابية مستزرعة بالأسماك.

وتم قياس تركيزات عناصر الكادميوم والرصاص وعناصر والكالسيوم والنحاس والحديد والمنجنيز والزنك فى الكبد والعضلات لخمس أنواع من الأسماك: البلطي النيلي (*Oreochromis niloticus*)، البوري (Mugil cephalus)، القرموط الأفريقي(*Clarias gariepinus*)، المبروك العادي (*Cyprinus carpio*) والمبروك الفضي (*Hypophthalmichthys molitix*). وكانت تركيزات العناصر الثقيلة، غالباً، أعلى فى الكبد عنها في العضلات فيما عدا تركيز الكالسيوم حيث كان أعلى فى العضلات من الكبد لكل من سمك البلطي والمبروك العادي والمبروك الفضي، بينما كان أعلى فى الكبد عن العضلات فى كل من سمك القرموط الأفريقي والبوري. تراوحت نسب تركيز العناصرفي العضلات إلى الكبد (عضلات: كبد MLR) بين ٢ ٠.٠ للرصاص، ١.٣٣ للكالسيوم فى أنسجة البلطي النيلي، ٠.٣ للكادميوم ، ١.٥٣ للكالسيوم فى أنسجة المبروك الفضي، ٠.٤ للنحاس، ٠.٧٣ للكالسيوم فى أنسجة سمك البوري، ٠.٠٦ للنحاس، ٠.٧٩ للكالسيوم فى أنسجة القرموط الأفريقي، ٠.١١ للنحاس، ٢.١٥ للزنك فى أنسجة المبروك العادي. وسجل النحاس أقل مستوى MLR تقريبا ما عدا في البلطي النيلي والمبروك الفضي، بينما أظهر الكالسيوم أعلى نسبة MLR فى جميع أنواع الأسماك باستثناء المبروك العادي. من ناحية أخرى كانت نسب الزنك/الكادميوم أعلى فى أنسجة الكبد منها في الأنسجة العضلية بجميع الأسماك التى تم تحليلها. وكانت تركيزات العناصر في الأجزاء المأكولة (العضلات)، غالباً، في الحدود الآمنة لصحة الإنسان حسب معايير منظمة الصحة العالمية، في حين كانت تركيزات بعض العناصر في الكبد أعلى من الحدود الآمنة.