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Assessment of heavy metals concentration in water, sediment and fish under different management systems in earthen ponds

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ABSTRACT

The study was conducted in two fish farming systems in earthen ponds located in North- Nile Delta, Egypt. The first system was the feed fish farm that depended on pelleted diet (25% crude protein) as feed input, while the second system was the fertilizer fish farm which used both organic fertilizer and crushed macaroni as supplementary feed. Each system was replicated in four ponds ($8400m^2$ each). The experimental period lasted from July 2014 to June 2015, including four months overwintering. Nile tilapia (*Oreochromis niloticus*), mullet (*Mugil cephalus*) and catfish (*Clarias gariepinus*) were cultured in each pond at initial weight of 2, 30 and 5 grams, respectively. Water, sediment and fish samples were collected to detect the heavy metals concentration. The results showed that heavy metals concentration in water, sediment and three organs of fish species were significantly ($P<0.05$) different between the feed and fertilizer fish farms. The pond management affected the content of heavy metals in water, sediments and fish. Accumulation of iron in water, sediment and three organs of fish were higher than other elements. Accumulation of cadmium and lead in water, sediment and three organs of fish were less than other elements. Accumulation of heavy metals in liver and gills were higher than that of muscles in all fish species. Metals concentrations in the edible part of farmed fish were within the permissible level for human consumption proposed by various international standard organizations.

INTRODUCTION

Water and soil pollution are thought to be one of the most perilous risks affecting the majority of world countries (Hamed *et al*., 2013). Pollution can directly and indirectly affect the aquatic ecosystem which consists of several segments (Kosygin *et al*., 2007). The contamination of freshwaters with extensive variety of toxins has turned into a matter of worry throughout the most recent couple of decades (Amanial and Adugna, 2016).

Heavy metals are considered natural trace components of the aquatic environment. Some metals are essential, and must be taken up from water, nourishment or sediment in trace amount for the ordinary metabolism of fish (Canlı and Atlı, 2003). For example, Zn, Cu and Fe play a biochemical role in the life processing of all aquatic organisms (Saeed and Shaker, 2008).

These essential metals can likewise deliver harmful impacts when the metal admission is unreasonably increased (Tekin-özan, 2008). Beside their natural existing, heavy metals levels have increased due to industrial, mining, agricultural and domestic activities in the drainage basin of a water system, with metal bioaccumulation through the food chain and related human wellbeing perils (Wright and Welbourn, 2002; Indrajith *et al.,* 2008; Agah *et al.*, 2009).

In most recent years, heavy metals have been considered as environmental pollutants and seriously analyzed in freshwater ecosystems due to the bioaccumulation and harmfulness of these metals (Wang *et al*., 2014). Contaminating components and mixes are transported by water and assembled in bottom and alluvial sediments (Ruiz-Fernández *et al.,* 2009). Organic fertilizers are considered as pollutants in aquatic ecosystems, due to the aimless and extensive application (Nath *et al*., 2016). The application of liquid and soil manure (or their derivates, compost, or sludge) or inorganic fertilizers can cause heavy metals accumulation in the soil (Martin *et al*., 2006; Atafar *et al.,* 2010).

Heavy metals, not like organic pollutants, cannot be chemically degraded or biodegraded by microorganisms, in this way, their substances have relentlessly expanded in soils and accordingly accumulated in plants, animals, and even in humans (Che *et al.*, 2006). Heavy metals at low concentration may be accumulated from water to higher level in fish tissue and become harmful to aquatic life (Sankar *et al*., 2006; Sivaperrumal *et al*., 2007). Liver and gills being considered as main organs for metabolism and respiration are target organs for pollutants accumulation as announced by many authors concerning pathological alternations to organs and tissues of fish (Khan, 2003). Evidently heavy metals accumulated frequently in fish flesh and in internal organs (Turkmen *et al*., 2005; Dural, *et al*., 2007). According to Teodorovic *et al*., (2000) the estimation of metals accumulation in fish muscle could be used as solid water quality marker than chemical analysis of water column and sediment. In general metal pollution in the aquatic environment can be detected by measuring the concentration of metals in water, sediments and biota (Monroy *et al*., 2014). Therefore, the present study was conducted to evaluate the effect of two different management systems which are commonly applied in the north of Nile-Delta region on heavy metals content in water, bottom sediments and fish in earthen ponds.

MATERIALS AND METHODS

Study area and experimental design:

The experiment was conducted in two fish farms (earthen ponds) at Kafr El-Sheikh Governorate in the north of Nile Delta (Egypt) and lasted for one year, starting July 2014 and ending June 2015. A detailed investigation of the fish farms area was required. The area between drain 7 and 8 is occupied by 30.000 feddan of fish ponds. The ponds were fed by water from agriculture drainage water and deep ground water wells. The ponds had 8400 m^2 as surface area and 1.25 m as an average water depth. The Ponds had four replicates for each farm (Fig. 1).

The two farms differed in management systems during the production cycle. The first system was the feed fish farm which depended on pelleted diet (25% crude protein) as feed input for the cultured fish, while the second system was the fertilizer fish farm which used both organic fertilizer and crushed macaroni as supplementary feed (Table 1).

Figure 1: A map showing the location of the two fish farms at Kafr El-Sheik Governorate in North of Nile Delta, Egypt.

Table 1: Chemical composition of the commercial diets and crushed macaroni (on dry matter basis).

Chemical analysis (%)	Commercial diet (25%)	crushed macaroni
Dry matter	91.25	90.40
Crude protein	25.04	14.32
Crude fat	5.94	3.50
Ash	5.73	2.9
Fiber	6.11	0.9
NFE	57.18	77.38
GE (kcal/kg)	4425	3946

NFE (nitrogen free extract) = 100 − (protein% + lipid% + ash% + fiber%), GE (gross energy): calculated after NRC (1993) as 5.64, 9.44, and 4.11 kcal/g for protein, lipid, and NFE, respectively.

The feed fish farm adopted feeding fish with pelleted diet at the rate of 25 kg/feddan/day, 6 days a week at the start of the production cycle (July 2014). Feeding rate was gradually increased to 50 kg/feddan/day by the end of October. Feeding was stopped during the overwintering period for four months (from November to the end of February). Feeding was resumed in March 2015 at 25 kg/feddan/day and gradually increased to 35 kg/feddan/day by the end of the production cycle in June 2015.

The fertilizer fish farm adopted supplementary feeding of crushed macaroni at 25 kg/feddan/day, six days a week at the start of the experiment, and was gradually increased to 50 kg/feddan/day by the end of October. Feeding with crushed macaroni was stopped during the overwintering period and resumed in spring 2015 at a rate of 25 Kg/feddan/day. Supplementary feeding rate was gradually increased to 40 Kg/feddan/day by the end of the production cycle in June 2015. Fertilization with chicken manure was applied at the rate of one ton/feddan/10 days, (equivalent to 100 kg/feddan/day) during the whole experiment including the overwintering period.

The earthen ponds were stocked with Nile tilapia (*Oreochromis niloticus*), mullet (*Mugil cephalus*) and catfish (*Clarias gariepinus*) for each pond. The feed fish farm was stocked with 18000 tilapia, 2000 mullet and 500 catfish per feddan, while 14000 tilapia, 2000 mullets and 500 catfish per feddan were stocked in the fertilizer fish farm. The average initial weights for both farms were 2g for tilapia, 30g for mullet and 5g for catfish, respectively.

Heavy metals in water:

Water samples were taken monthly from each pond during July 2014 to June 2015 with a water sampler from at least three spots at a depth of 30 cm below the water surface. Water samples were mixed together in a plastic container according to Boyd and Tucker (1992). One liter of each collected sample was put in plastic bottles and kept in icebox until transferred to the Central Laboratory for Aquaculture Research (CLAR) for analysis within 24 hour. Heavy metals concentrations in water were determined by atomic absorption spectrophotometer (THERMO ELECTRON CORPORATION S SERIES AA Spectrometer). The samples were prepared and analyzed in sequential for iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) according to APHA (2000). Water samples from both farms were taken once before the start of the study to detect the heavy metals concentration in the two fish farms water resources and the analysis determined approximate values for each metal. The heavy metals concentrations in the feed farm water were 0.42, 0.097, 0.077, 0.055, 0.009 and 0.005 mg/l for Fe, Mn, Zn, Cu, Pb and Cd, respectively while, recorded 0.44, 0.088, 0.088, 0.057, 0.009 and 0.005 mg/l for Fe, Mn, Zn, Cu, Pb and Cd, respectively in the fertilizer farm water. Secchi disk readings (SD) were recorded in the site and the average reading values were 12.6 cm in the fertilizer fish farm and 18.4 cm in the feed fish farm.

Heavy metals in sediments:

Sediments samples were taken twice, once at the beginning and the other one at the end of the study. Surface and subsurface samples (3 samples) from each pond were collected using a core sampler (surface layer 0-15 cm and the subsurface layer 15-30 cm) as described in Boyd and Tucker (1992) and kept in clean plastic bags. The three samples from each pond were combined together. In the laboratory, the samples were air dried, followed by grinding and sieving through a 2 mm sieve and kept in polyethylene bags for analysis. Heavy metals were extracted by Diethylenetriamine pentaacetic acid (DTPA) using developed method of Lindsay and Norvell (1978) and the amount of iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) were determined by the atomic absorption (THERMO ELECTRON CORPORATION S SERIES AA Spectrometer).

Heavy metals in fish:

Three fish of each species of Nile tilapia (*Oreochromis niloticus*), mullet (*Mugil cephalus*) and catfish (*Clarias gariepinus*) were collected during harvesting from each pond for heavy metals analysis. The collected fish were washed with distilled water, put in clean plastic bags and stored frozen until analysis. About 5 grams of wet organs (liver, gills and muscles) were dried, ignited and digested with concentrated HNO_3 and HCl according to procedures recommended by AOAC (1990). The digested samples were analyzed for iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) by atomic absorption spectrophotometer (THERMO ELECTRON CORPORATION S SERIES AA Spectrometer).

Statistical analysis:

Two or one-way ANOVA was used to evaluate the significant differences of the concentration of different elements studied with respect to sites and time. A probability level of 0.05 or less was considered significant. Standard errors were also estimated. All statistics were done using the SAS program (SAS, 2000).

RESULTS AND DISCUSSION

Heavy metals in water:

Average monthly values of heavy metals in water during the study are shown in Fig. (2). Fe concentrations ranged from 0.357 to 0.632 mg/l and 0.302 to 0.588 mg/l, Mn concentrations ranged from 0.023 to 0.107 mg/l and 0.020 to 0.089 mg/l, Zn concentrations ranged from 0.041 to 0.098 mg/l and 0.031 to 0.083 mg/l, Cu concentrations ranged from 0.016 to 0.074 mg/l and 0.014 to 0.062 mg/l, Pb concentrations ranged from 0.017 to 0.051 mg/l and 0.007 to 0.041 mg/l, and Cd concentrations ranged from 0.0020 to 0.0044 mg/l and 0.0011 to 0.0035 mg/l in the feed and fertilizer fish farms, respectively. The results showed that the concentrations of heavy metals in water decreased over time from the beginning to the end of the experimental period. Moreover, the feed fish farm had slightly significant higher concentrations of heavy metals compared to the fertilizer fish farm ($P<0.05$).

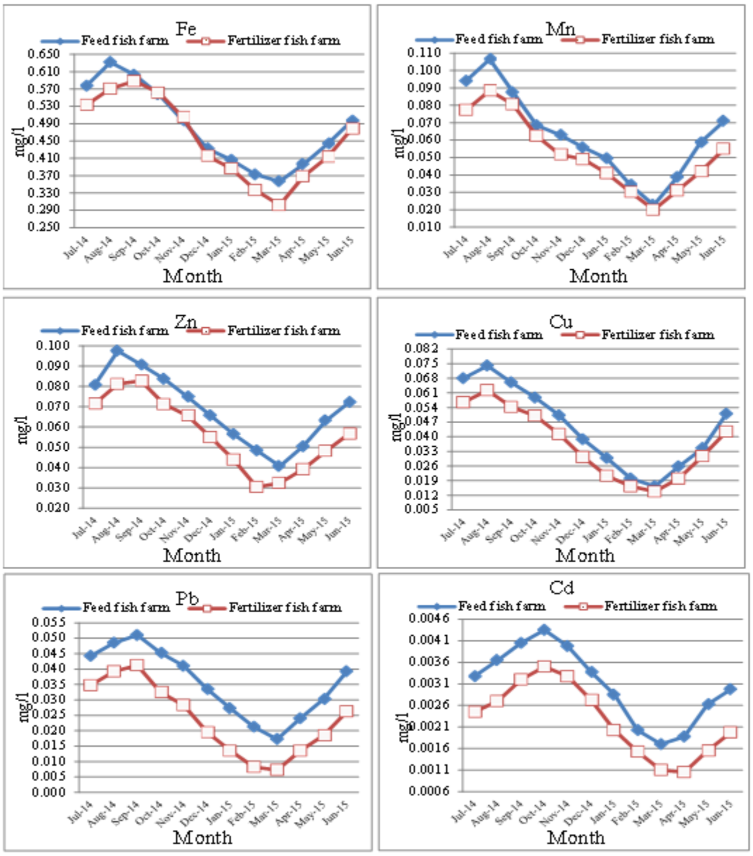

Fig. 2: Monthly Fluctuations of Fe, Mn, Zn, Cu, Pb and Cd (mg/l) in water of the feed and fertilizer fish farms during the experimental period from July, 2014 to June, 2015.

Seasonal and annual average values of heavy metals (Fe, Mn, Zn, Cu, Pb and Cd mg/l) in water had the same trend as shown in Figs. (3) and (4). All heavy metals concentrations in summer season were significantly ($P<0.05$) higher than in spring season, which may be due to the start of the experiment in summer along with increasing phytoplankton abundance over time. Phytoplankton bloom helped in decreasing heavy metals concentrations through the absorption of heavy metals from the water.

Higher average concentrations of heavy metals in water were found in the feed fish farm when compared to the fertilizer fish farm (P<0.05). This may be due to algal blooms in the fertilizer farm which absorbed heavy metals from water, reducing its concentrations in water column. Algae are known to absorb heavy metals from water. The high algal blooms had resulted from the high amount of organic manure (SD: 12.6 cm) applied in the fertilizer ponds compared to the feed fish farm which contained less amounts of phytoplankton abundance (SD: 18.4 cm).

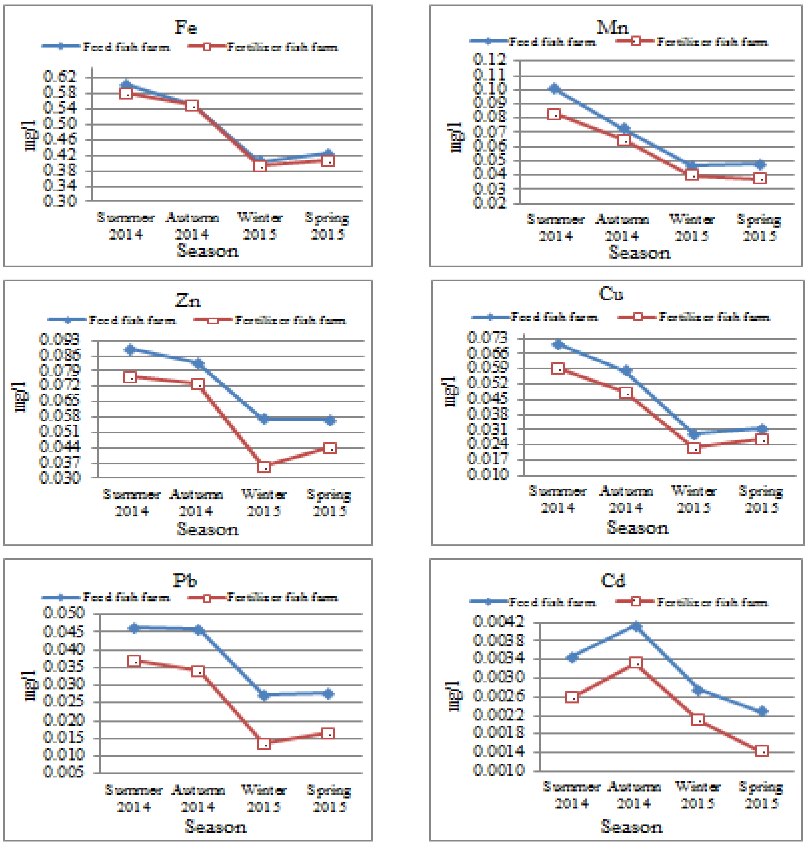

Fig. 3: Seasonal fluctuations of Fe, Mn, Zn, Cu, Pb and Cd (mg/l) in water of the feed and fertilizer fish farms during the experimental period from July, 2014 to June, 2015.

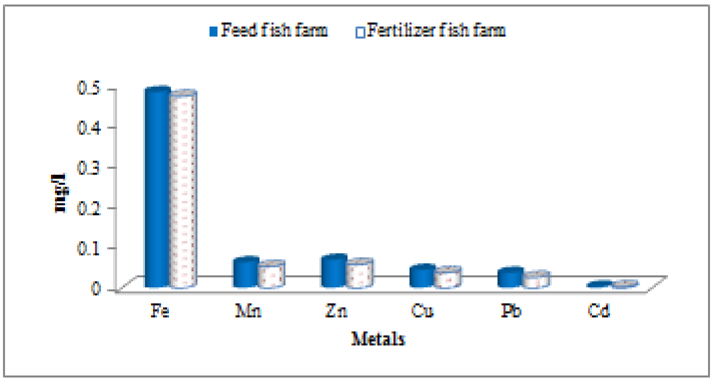

Fig. 4: Annual average of heavy metals (mg/l) in water of the feed and fertilizer farms during the experimental period from July, 2014 to June, 2015.

Overall, the observed annual average of iron (Fe) was the major element in the water, while cadmium (Cd) was the least. Shaker *et al*. (2015) showed that iron was the most metal found in water. The total heavy metal concentrations in the water samples from different locations ranked as follow: Fe > Zn > Mn > Cu > Pb > Cd. These results are in agreement with Shaker (2006) who indicated that the heavy metals from water samples showed monthly variation and attributed this to the consumption of these metals by phytoplankton, where the concentrations of heavy metals in plankton were 5000 - 10000 times higher than those in water. Moreover, El-Naggar *et al*. (2000) reported that the circulation of heavy metals in the aquatic environment relies upon the physico-chemical characteristics of the aqueous phase and the accessibility of both inorganic and organic complexing agents. Singh *et al*. (2017) stated that the physico-chemical properties of water conditions vary from season to season.

Heavy metals in sediments:

Heavy metals concentrations in soil are shown in Fig. (5). The Heavy metals content in different soil layers had increased slightly ($P<0.05$) from the beginning to the end of the experimental period in the feed and fertilizer fish farms, which may be due to the adsorption of different elements from water by the soil particles during the production cycle. Sediments are one of the conceivable media in aquatic observing which are besides water, responsible of nutrients and pollutants transportation in aquatic environment (Osman and Kloas, 2010).

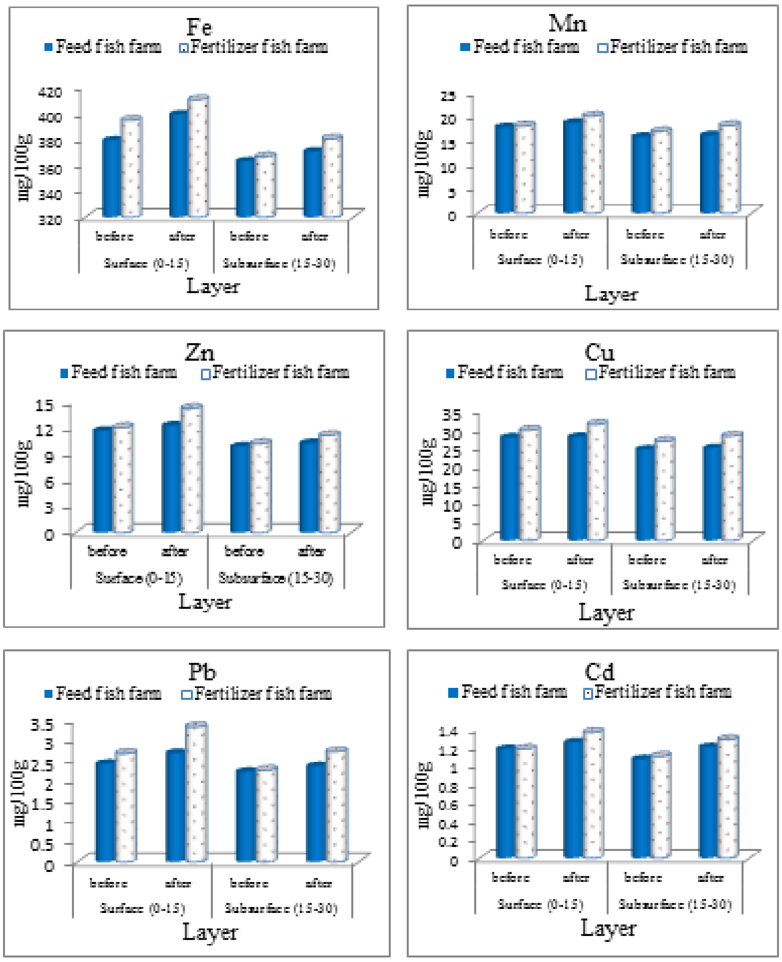

Fig. 5: Heavy metals concentrations (mg/100 g) in surface and subsurface soil layers before and after the study period in the feed and fertilizer farms.

Heavy metals content in sediment was significantly (P<0.05) increased in the fertilizer farm compared to the feed farm. These results can be explained by the sedimentation of the dead algae, which are rich in heavy metals in addition to the organic fertilizer on pond bottom. Aerobic bacteria decompose dead algae and organic manure resulting in the release of its heavy metals content and subsequently, their adsorption by sediment particles. High amount of chicken manure (100 kg/feddan/day) was used in fertilizer ponds in contrast to the lower amount of artificial diet (25-50 kg/feddan/day) which used in the feed ponds. These results support the findings of Tsai *et al.* (2003) who mentioned that heavy metals concentration in sediment increase by increasing the organic material.

The results showed that heavy metals concentrations were slightly higher (P<0.05) in the surface layer compared to the subsurface layer. This may be due to the high concentration of heavy metals in drainage water; which led to its uptake by phytoplankton and fixation on surface soil and consequently, their adsorption by soil particles. Hydrophobic chemical pollutants that enter water bodies are caught by sediments (McCready *et al*., 2006), and gradually discharge the contaminant once more into the water column (Chapman and Chapman 1996; McCready *et al*. 2006).

When comparing between the sediments and water in terms of the heavy metals accumulation, the results indicated that sediments accumulated higher amount of heavy metals than water. This may be because the sediments act as storage place for all pollutants and dead organic matter sedimenting from the ecosystem above. Hamed (1998), Nguyena *et al*., (2005) and Saeed & Shaker (2008) reported similar results.

These findings are also in agreement with Ferreira *et al*., (1996) who mentioned that the sediment quality is a decent indicator for pollution in water column, which tends to concentrate heavy metals and other organic pollutants. Sin *et al*., (1991) reported that pollutants concentration in sediments increased with diminishing particle size of sediments, moreover, sediment had restricted ability to absorb different ions from waters permeating through it. This capacity is lowest for carbonate-sandy fraction of sediments and highest for clayey organic matter rich sediments (Sin *et al.*, 1991). From a previous study by the authors on the same farms, it was observed that the surface soil layer texture was clay (Elnady *et al*., 2016).

Heavy metals in fish:

The average concentration of heavy metals in liver, gills and muscles of different fish species are present in Fig. (6). The results clarified that there were significantly (P<0.05) high differences between heavy metal elements concentration in different fish organs. Liver contained high levels of heavy metals in all fish species, followed by gills and lowest values of heavy metals were observed in the edible part of fish (muscles) in both the feed and fertilizer farms. Similar findings were observed in tilapia samples, which were taken from a natural lake in Ethiopia by Amanial and Adugna (2016). Abdel-Khalek *et al*. (2016) observed in a study of the metal accumulation in *Oreochromis niloticus* collected from Nile River in Egypt that the tissues of liver, kidneys and gills were strong bioaccumulators compared to muscular tissues.

Liver and gills had a high tendency to accumulate high concentrations of heavy metals, while muscle tissues tended to retain lower concentrations (Abdel-Baky and Zyadah, 1998; Hamed 1998; Osman and Klaos, 2010). The liver accumulates and stores heavy metal elements and other toxic materials in the body, consequently, the liver had high levels of heavy metals compared to other organs in fish body. Gills are the principal organ to be exposed to suspended sediments particles, so that they can be significant site for interaction with metal ions. Likewise, gills can uptake heavy metals at higher levels due to their specific role in metal exposure (Kraemer *et al*., 2005). Uluturhan and Kucuksezgin (2007) explained the lower tendency of the muscle to accumulate heavy metals by the high fat-content in muscle tissues with low proclivity to join with metals besides the low metabolic activity of muscle.

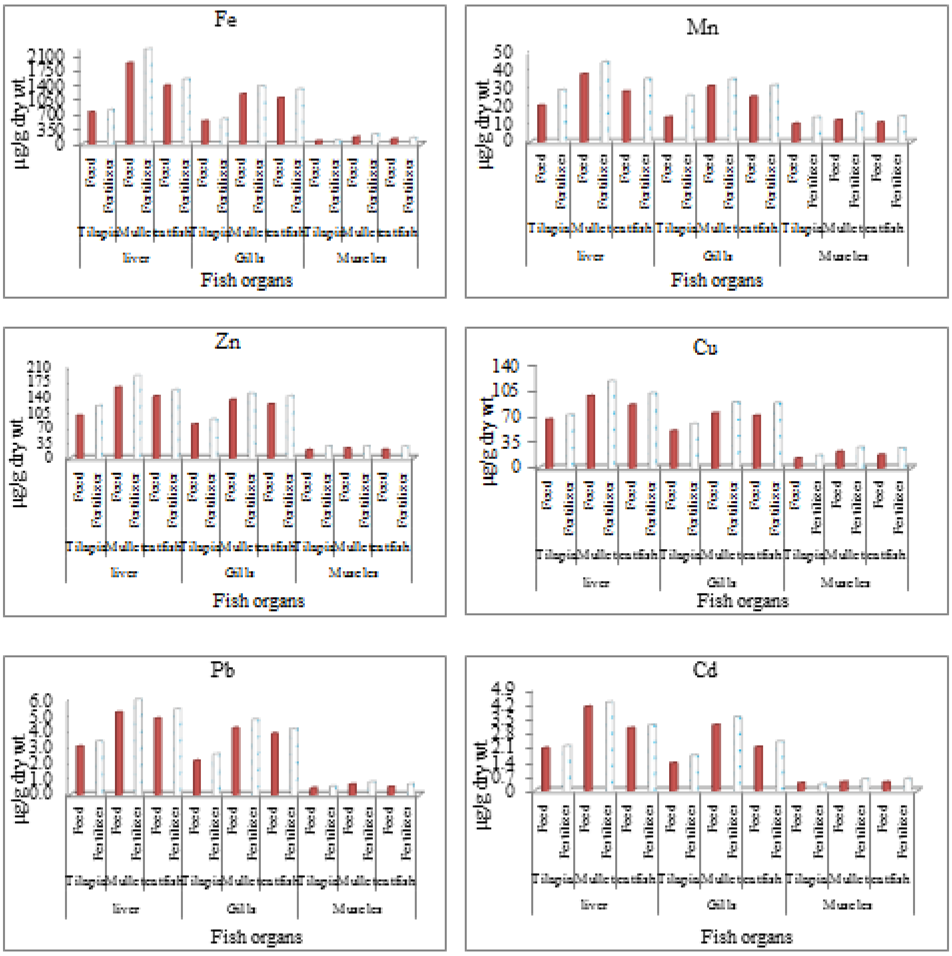

Fig. 6: Concentrations of Fe, Mn, Zn, Cu, Pb and Cd in different organs of different fish species in the feed and fertilizer fish farms after the experimental period (µg/g dry weight).

In addition, accumulation of iron in liver, gills and muscles was highest compared to other elements while, accumulation of cadmium and lead in the three organs of fish were least when compared to other elements. The heavy metals concentrations in different fish organs for the three fish species were detected in the following order: Fe > Zn > Cu > Mn > Pb > Cd. Similar findings were reported by Moussa (2004), Saeed and Shaker (2008). This may be attributed to the abundance of these metals in water and sediments by the approximate pattern. Fish may accumulate metals that enter their bodies either directly via water and sediment or indirectly through the food chain (Abdel- Khalek *et al*., 2016). Ibrahim *et al*. (2000) and Ibrahim and El-Naggar (2006) observed an obvious correlation between heavy metals concentrations in aquatic organisms and sediments. Moreover, Singh et al. (2017) indicated that heavy metals can fix in sediment for brief periods then, small amount of these fixed heavy metals will enter the overlying water body again and uptake by the aquatic biota.

The results showed that fish species in the fertilizer farm contained higher concentrations of heavy metals compared to those of the feed fish farm. It was also observed that mullet species contained highest levels of heavy metals in different organs, followed by the catfish species, while tilapia species contained the lowest values of different heavy metal elements in different organs ($P<0.05$). These results can be explained by the feeding habits effect of different fish species. Mullet is dependent completely on filtering phytoplankton from water column and detritus deposited on the bottom of the pond, whereas tilapia is a partial feeder on the phytoplankton through the production cycle in earthen pond, while catfish feed on artificial feed, decomposed organic matter and small fry. Zhong and Wong (2007) announced that one of the multiple factors that affecting heavy metal levels in fish is their feeding habits. Yilmaz *et al*. (2007) reported that the concentration of heavy metals (Fe, Mn, Zn, Cu, Pb and Cd) in fish varied relying upon tissues and species.

According to FAO (1992) the permissible limits of Mn, Zn, Cu, Pb and Cd as heavy metals content in fish flesh are 30, 40, 30, 2.0 and 2.0 µg/g (dry weight), respectively. The permissible level of Fe issued by WHO (1985) is 100 µg/g (dry weight). Our results showed that the concentrations of all heavy metals in the edible part of tilapia species were within the above permissible limits. It was also observed that the concentrations of Mn, Zn, Cu, Pb and Cd in catfish and mullet muscles were below the permissible limits, while Fe slightly exceeded the allowable level especially in the fertilizer farm. Moreover, the permissible limit of Fe in fish muscle which proposed by WHO (2004) is 50 µg/g (wet weight) accordingly; the concentrations of Fe in the current study were acceptable by the last standard for all fish species. However, many international standard organizations did not provide health-based guideline value for Fe as it is found naturally in fresh water.

CONCLUSION

The present study revealed some interactions between fish and their environment especially sediment and water. It could be stated that fish pond management plays a significant role and affects the amount of heavy metals accumulation in water, sediments and fish tissues. Organic fertilization is responsible for increasing the accumulation of heavy metals in sediments and fish tissues. Fish are more liable to be affected by environmental pollutants than land animals. Fish can accumulate heavy metals from their environment and act as bioindicators for these elements. Therefore, Fish can be considered as excellent organisms for the study of some long term change in heavy metals concentrations in their ecosystem.

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ARABIC SUMMARY

تقدير تركيز المعادن الثقيله في المياه و التربه و الأسماك تحت نظم رعايه مختلفه في الأحواض الترابيه

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تمت الدراسه الحاليه في مزرعتين ترابيتين واقعتين في شمال دلتا مصر بالاعتماد على استخدام نظامين مختلفين من نظم الرعايه. النظام الأول هو نظام التغذيه الصناعيه و اعتمد على استخدام العليقه الكامله التي تحتوي على ٢٥% بروتين و النظام الثاني هو نظام السماد و اعتمد على استخدام السماد العضوي مع التغذيه على المكرونه المجروشه. اتسم كل نظام بوجود اربعه تكرارات من الاحواض الترابيه و كانت مساحه كل حوض ٨٤٠٠م^{٢}، واستمرت التجربه لمده عام كامل حيث بدأت في يوليو ٢٠١٤ و انتهت في يونيو ٢٠١٥. تم استزراع أسماك البلطي النيلي و البوري الأصيل و القراميط الافريقيه معاً في كل حوض بأوزان ٢، ٣٠، ٥ جم على التوالي. تم اخذ عينات من المياه و التربه و الاسماك للكشف عن تركيز المعادن الثقيله في كل منها.

اظهرت النتائج وجود فروق معنوىه عند مستوى معنويه اقل من ٠.٠٥ بين مزرعه السماد و مزرعه العليقه بالنسبه لتركيز المعادن الثقيله في المياه و التربه وثلاثه اعضاء من الأسماك، وأن نظام الرعايه أثر على محتوى المياه و التربه والأسماك من المعادن الثقيله، كما ان تراكم الحديد في المياه و التربه و ثلاثه اعضاء من الأسماك كان اعلى من باقي العناصر بينما كان تراكم الكادميوم والرصاص في المياه و التربه و ثلاثه اعضاء من الأسماك اقل من باقي العناصر، كذلك اظهرت النتائج ان تراكم العناصر الثقيله كان اعلى في الكبد و الخياشيم عن العضلات في كل انواع الأسماك، وأن تراكم المعادن الثقيله في عضلات الأسماك المستزرعه كان في الحدود المسموح بها لإستهلاك الإنسان طبقاً للمعدلات العالميه.