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Assessment of Heavy Metals Concentration in Water and Edible Tissues of Nile Tilapia (*Oreochromis niloticus*) from two Fish Farms Irrigated with Different Water Sources, Egypt

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ABSTRACT

Concentrations of some heavy metals namely; manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) were measured in water and dorsal muscle of Nile tilapia (*Oreochromis niloticus*) from two aquaculture fish farms irrigated with agriculture and sewage drainage water at Al-Abbassa and Shader Azzam, Egypt, respectively, during April 2013 – March 2014 to assess the contamination by metals in these farms. There was a significant ($P<0.05$) difference among seasons and farms for all studied metals concentrations in both water and fish muscle tissues. Iron (Fe) had the highest mean concentration in water and Cd exhibits the lowest one. The mean metals concentration in the fish muscle tissues decreased in the order: Zn > Fe > Cu > Mn > Pb > Cd. Mean concentrations of Cu, Cd and Pb in muscle tissues showed higher ($P<0.05$) concentrations in Shader Azzam fish farm. The concentrations of all metals in water (except Fe in both farms) and edible part of fish were found below the notified permissible limits. It is recommended that further studies should carry out for a continual assessment on the levels of pollutants in these areas.

Key words: Fish farms, heavy metals, *Oreochromis niloticus*,Tilapia, water, Egypt

Introduction

In aquatic ecosystems, heavy metals have received considerable attention due to their toxicity, accumulation in biota (Dural *et al.,* 2006) and biomagnification in the food chain (Erdogrul and Ates 2006). The main sources of heavy metal pollution are the sewage disposal, agriculture drainage water containing pesticides and fertilizers and industrial effluents (Santos *et al.,* 2005; Singh *et al.,* 2007). Heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystems and accumulated in aquatic organisms especially fish which situated at the top of the food chain and can accumulate large amounts of heavy metals (Yilmaz *et al.,* 2007).

Fish are considered as one of the most susceptible aquatic organisms to toxic substances present in water (Alibabić *et al.,* 2007), and the same time considered as the major part of the human diet due to high protein content, low saturated fat and sufficient omega fatty acids which are known to support good health therefore, various studies have been taken worldwide on the contamination of different fish species by heavy metals (Sivaperumal *et al.,* 2007; Bhattacharya *et al.,* 2010).

Heavy metals may accumulate in fish either through direct consumption of water or by uptake through epithelia like the gills, skin, and digestive tract (Burger *et al.,* 2002). Eventually, dietary intake of these metals poses risk to human health as fish occupied a significant part of human diet (Turkmen *et al.,* 2005). For these reasons, heavy metals load in fish has become an important worldwide concern, not only because of the threat to fish but also due to the health risks associated with fish consumption (Begum *et al.,* 2013).

The heavy metal accumulation in farmed fish is limited to a number of studies comparing tissue metal concentrations between farmed and wild caught fish (e.g. Calvi *et al.,* 2006; Yildiz, 2008; Nawaz *et al.,* 2010). So, this study was conducted to assess and compare heavy metals contamination in muscle tissues (edible part) of fish cultured in ponds received agriculture and sewage drainage water which could poses a threat to human health (consumers).

Materials and Methods

Study area

The present study was carried out at two fish farms irrigated with different water sources: Farm (A), a private fish farm located at Al-Abbassa, Abou Hammad, Sharkia governorate, which irrigated with agriculture

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drainage water from (El-Bahnasawy drain). Farm (B), a private fish farm laid at Shader Azzam, Port Said governorate and supplied with sewage water from Bahr El-Baqar Drain.

Sampling collection and preparation

Water

Three water samples from three fish ponds were collected monthly from each farm during the study period (April 2013 – March 2014). Water samples were collected by a PVC vertical water sampler from at least five spots in the pond between 9.00 am and 12.00 pm at a depth of 30 cm below the water surface and mixed together in a plastic container according to Boyd (1990). One liter of water samples at each pond was placed in polyethylene bottles previously washed with acid (0.01 N HNO_3) and rinsed by distilled water, then placed in a cooler at 4 °C and transferred to the lab for further analysis. The samples were digested by Nitric Acid Digestion method for total metals concentration according to APHA (2000).

Fish

A number of 30 fish samples, Nile tilapia (*Oreochromis niloticus*), of approximately similar size (average body weight 150 – 200 g) were collected monthly from each farm by a net during the study period. The fish samples were dissected and 25 g of dorsal muscle tissues were taken, placed in polyethylene bags and stored at -20 °C until analysis. About 1 g from previously oven dried muscle tissues was ignited and digested with concentrated HNO_3 and HCl according to procedures recommended by AOAC (2005).

Finally, all samples were analyzed using Flam Atomic Absorption Spectrophotometer (Thermo Electron Corporation S Series AA Spectrometer) for Manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and Lead (Pb) as mg/L for water and µg/g dry weight (dw) for muscle tissues.

Statistical analysis

Two-way ANOVA was employed to evaluate the variability of the concentration of each metal with respect to different seasons and farms, using the software CoStat ver. 6.4 (CoStat, CoHort software, USA). The analyzed data were expressed as mean ± standard error (SE). The inter-elemental relationships were performed through Pearson's correlation coefficient matrix. Significant differences are stated at $P<0.05$ (Bailey, 1981).

Results and Discussion

The contamination of water bodies in the Egyptian Nile Delta region is exposed to discharge of massive amounts of domestic sewage as well as agricultural and industrial effluents (Alne-na-ei, 2003). The present study assessed concentration of six heavy metals (Mn, Fe, Cu, Zn, Cd and Pb) in water collected from two fish farms. The results are shown in table (1). Manganese (Mn) revealed higher ($P<0.05$) annual mean at Shader Azzam (B) than Al-Abbassa (A), (0.38 & 0.27 mg/L), respectively. In general, Mn concentration revealed irregular variation among seasons, where the maximum concentration was recorded at winter and summer (0.36 & 0.45 mg/L) at farm (A) and (B), respectively.

These results are much lower than that obtained by Baidoo *et al.* (2012) at two different points in waste water-fed pond in Ghana (3.28 & 4.4 mg/L).

Iron (Fe) concentration ranged from 2.51 to 5.44 mg/L at farm (A), and from 2.77 to 5.59 mg/L at farm (B). The mean values for both farms tend to be so close to each other (3.45 & 3.53 mg/L) at farm (A) and (B), respectively. This mean concentration is near to that obtained by Badr *et al.,* 2014 (3.05 mg/L) in the Nile river at El-Tebeen district.

Copper (Cu) concentration in farm (A) ranged between 0.004 and 0.016 mg/L with an annual mean of 0.009 mg/L, while at farm (B) it ranged between 0.006 and 0.01 mg/L with an annual mean of 0.007 mg/L. Seasonally, the maximum concentrations of Cu observed at spring and winter at farm (A) and (B), respectively.

Zinc (Zn) showed similar pattern for minimum and maximum values at both farms in summer and winter, respectively. It had levels of 0.011 & 0.112 mg/L at farm (A), and 0.019 &0.053 mg/L for (B). In general, the annual mean was slightly higher (0.039 mg/L) at (A) than at (B) (0.033 mg/L).

Cadmium (Cd) was detected only at farm (A), whereas it was below the detection limits at farm (B). Cd concentration at farm (A) varies between 0.00022 mg/L in summer and 0.00072 mg/L in winter with the mean of 0.00047 mg/L. These values are lower than those obtained by Authman *et al.* (2013) in Sabal drainage canal water, where they found Cd concentration varies between 0.840 mg/L at summer and 1.82 mg/L at autumn with the mean of 1.37 mg/L. Abdel-Khalek (2015) recorded a lower range of 0.0072-0.0147 mg/L. As illustrated in table (1), cadmium concentration at farm (A), which irrigated with agriculture drainage water, was detected,

whereas it not detected at farm (B) which supplied with sewage water. This may be due to the fact that agriculture drainage water rich in phosphate fertilizers which is considered the main source of cadmium, as cadmium constitutes up to 35 mg /kg of phosphorous pentoxide, a component of phosphate-based fertilizers (IARC, 1993). Muntau and Baudo (1992) found that more cadmium was released from agricultural and urban run-off than from industrial and municipal sewage treatment plants (Muntau and Baudo, 1992).
The concentration of lead (Pb) is not detected at both farms during the study period.

Seasonally, the results in table (1) showed that the concentration of metals exhibited irregular pattern during different seasons. The order of metals concentrations in water at the two fish farms was as follows: Fe > Mn > Zn > Cu. Saeed (2013a) found the same pattern for heavy metals concentration in water at Al-Abbassa and Maruit fish farms. Metals concentration varied significantly ($P<0.05$) between the two fish farms through different seasons, except iron (Fe) concentration that showed no significant difference. These seasonal variations may be due to the fluctuation of the amount of agricultural drainage water, untreated domestic sewage and industrial wastes discharged into the canals and drains which feed ponds (Authman, 2008; Authman *et al.,* 2008). The seasonal variations of metals in water have been reported by different authors at different water bodies in Egypt: at Shanawan drainage canal (Khallaf *et al.,* 1998); at Lake Manzala (Bahnasawy *et al.,* 2011) and at Sabal drainage canal (Authman *et al.*, 2013). Generally all metals concentration in water at both farms except (Fe) are under chronic Criterion Continuous Concentration (USEPA, 2006) for protection of freshwater aquatic life, and the Egyptian Chemical Standards (ECS, 1994) for the maximum permissible limits of heavy metals in surface water.

Table 1: Seasonal mean (±SE) of heavy metals concentration in water (mg/L) of Al-Abbassa (A) and Shader Azzam (B) fish farms.

Season	Metals											
	Mn		Fe		Cu		Zn		Cd		Pb	
	A	B	A	B	A	B	A	B	A	B	A	B
Spring	0.29 ± 0.003	0.39 ± 0.01	5.44 ± 0.32	2.77 ± 0.09	0.016 ± 0.0009	0.008 ± 0.0005	0.021 ± 0.002	0.020 ± 0.001	0.00023± 0.00001	ND	ND	ND
Summer	0.15 ± 0.01	0.45 ± 0.04	2.51 ± 0.15	2.95 ± 0.11	0.004 ± 0.0001	0.006 ± 0.0002	0.011 ± 0.001	0.019 ± 0.001	0.00022± 0.00001	ND	ND	ND
Autumn	0.27 ± 0.01	0.33 ± 0.02	2.56 ± 0.17	2.79 ± 0.15	0.004 ± 0.0001	0.006 ± 0.0002	0.014 ± 0.001	0.042 ± 0.002	0.00071± 0.00004	ND	ND	ND
Winter	0.36 ± 0.02	0.34 ± 0.01	3.31 ± 0.20	5.59 ± 0.24	0.010 ± 0.0001	0.010 ± 0.0005	0.112 ± 0.001	0.053 ± 0.003	0.00072± 0.00003	ND	ND	ND
Mean	0.27 ± 0.02	0.38 ± 0.02	3.45 ± 0.37	3.53 ± 0.37	0.009 ± 0.001	0.007 ± 0.001	0.039 ± 0.013	0.033 ± 0.005	0.00047± 0.00007	ND	ND	ND
USEPA (2006)[a]	–		1		0.013		0.12		0.0025		0.0025	
ESC (1994)	-		1		1		5		0.01		0.05	
Two-way ANOVA (P-value)												
Site	<0.0001 ***		0.5479		<0.0036 **		0.0001 ***		<0.0001 ***		ND	
Season	0.0037 **		<0.0001 ***		<0.0001 ***		<0.0001 ***		<0.0001 ***		ND	

, ** and * are significant at (P<0.05), (P<0.01) and (P<0.001), respectively.*
a = Criterion Continuous Concentration (chronic) for protection of freshwater aquatic life
ND: Not detected

Heavy metals in fish muscle tissues

The study of heavy metal concentrations in fish tissues especially muscles are crucial where it's the main edible part of fish and can directly influence human health (Pintaeva *et al.,* 2011). This work studied the metal contamination in Nile tilapia (*Oreochromis niloticus*) as it's the most familiar and popular fish that represents about 80% of freshwater farmed fishes in Egypt. The seasonal variations in the concentrations of Mn, Fe, Cu, Zn, Cd and Pb in muscle tissues of fish samples collected from Al-Abbassa fish farm (A) and Shader Azzam fish farm (B) are shown in table (2). All metals concentration showed significant ($P<0.05$) variations with respect to seasons. These results are in agree with Authman *et al.* (2013), where they found that metals concentration in fish organs exhibited seasonal variations and they attribute these variations to the increase or decrease of drainage water discharged into the drainage canal. Tekin-Özan and Kir (2008) described that bioavailability of metals may influenced by physiological activities of fish during different seasons.

The results revealed that, the minimum concentration of all metals at both fish farms exhibit irregular pattern during different seasons. Among all metals, Zn showed the highest annual means (114.02 & 120.8 µg/g dry weight dw) and Cd was found to be the lowest mean value (0.25 & 0.29 µg/g dw) at farm A & B, respectively. The accumulation levels of metals in muscle tissues have been detected in the order: Zn > Fe > Cu > Mn > Pb > Cd, which agree with those obtained by Abdel-Khalek (2015) in muscle tissues of Nile tilapia at southern part of river Nile at Shoubra El-Khaema. This also complies with (Watanabe *et al.,* 2003; Masoud *et al.,* 2007) who mentioned that, bioaccumulation of metals in tissues varies from metal to metal. Moreover, Koca *et al.* (2005) postulated that the accumulation patterns of contaminants in fish and other aquatic organisms depend on both uptake and elimination rates of contaminants.

Table (2) showed that the annual mean of Mn concentration in fish muscles was the same (1.21 µg/g dw) for both fish farms and these levels are lower than (2.2 µg/g dw) obtained by Abdel-Khalek (2015).

Table 2: Seasonal mean (±SE) of heavy metals concentration in *O. niloticus* muscle tissues (µg/g dw) from Al-Abbassa (A) and Shader Azzam (B) fish farms.

Season	Metals											
	Mn		Fe		Cu		Zn		Cd		Pb	
	A	B	A	B	A	B	A	B	A	B	A	B
Spring	1.36 ± 0.1	1.00 ± 0.06	111.87 ± 7.21	96.66 ± 7.61	5.22 ± 0.55	4.12 ± 0.32	176.58 ± 7.29	137.20 ± 11.18	0.22 ± 0.02	0.28 ± 0.02	0.43 ± 0.06	0.61 ± 0.01
Summer	0.92 ± 0.04	0.92 ± 0.01	97.47 ± 6.54	94.99 ± 7.80	2.43 ± 0.28	6.95 ± 0.66	50.14 ± 1.18	52.14 ± 1.76	0.26 ± 0.01	0.23 ± 0.01	0.54 ± 0.06	0.49 ± 0.06
Autumn	1.08 ± 0.07	1.63 ± 0.16	119.70 ± 5.84	115.27 ± 2.85	3.55 ± 0.30	7.91 ± 0.62	61.70 ± 3.57	161.35 ± 15.71	0.33 ± 0.01	0.31 ± 0.01	0.68 ± 0.09	0.85 ± 0.09
Winter	1.47 ± 0.07	1.28 ± 0.08	107.58 ± 3.91	120.54 ± 5.88	6.05 ± 0.08	10.06 ± 0.57	167.67 ± 8.51	132.52 ± 10.08	0.20 ± 0.01	0.33 ± 0.02	0.51 ± 0.04	0.49 ± 0.09
Mean	1.21 ± 0.07	1.21 ± 0.09	109.15 ± 3.52	106.86 ± 11.71	4.31 ± 0.45	7.26 ± 0.69	114.02 ± 17.76	120.8 ± 13.24	0.25 ± 0.01	0.29 ± 0.01	0.54 ± 0.03	0.61 ± 0.05
*PL (mg/day) wet wt.	10		50		50		30		0.05		0.214	
Two-way ANOVA (P-value)												
Site	0.9826		0.6304		<0.0001 ***		0.2635		0.0041 **		0.0252 *	
Season	0.0006 ***		<0.0241*		<0.0001 ***		<0.0001 ***		0.0004 ***		<0.0001 ***	

, ** and * are significant at (P<0.05), (P<0.01) and (P<0.001), respectively.*

**Permissible limits (average daily intake in wet wt.), Fe, Cd and Pb according to guidelines in WHO, 2011. Cu as in IPCS (1998), Zn and Mn as in (SCF, 1993).To compare with PL: Wet weight conc . = dry wt. x (100 - % moisture) / 100.*

The highest iron (Fe) concentrations (119.7 & 120.54 µg/g dw) were obtained in autumn and winter at A & B farm, respectively. Fe annual mean concentration showed no significant (P<0.05) variation between the two farms, where it was (109.15 & 106.86 µg/g dw) at (A & B), respectively. Saeed (2013b) recorded Fe annual mean (61.94, 76.71 and 84.02 µg/g dw) for *O. niloticus*, *O. aureus*, *Tilapia zillii* collected from Lake Edku, Egypt, respectively. Wide seasonal variation in copper (Cu) concentrations was recorded, where it was varied between 2.43 – 6.05 µg/g dw at farm (A) and 4.12 – 10.06 µg/g dw at (B), with the highest concentrations during winter. This range was higher than those (1.73 and 3.06 µg/g dw) obtained by Al-Kahtani (2009), where the seasonal Cu concentration fluctuated between autumn and summer. Abdel-Khalek (2015) found a higher Cu mean value of 11.9 µg/g dw. Copper (Cu) annual mean concentration (4.31 & 7.26 µg/g dw at A & B farm, respectively) in fish muscles showed very high significant (P<0.05) difference between the two farms. Abdel-Khalek (2015) found a higher Cu mean value of 11.9 µg/g dw Zinc (Zn) concentration showed wide variation at both farms, it ranged between 50.14 – 176.58 µg/g dw in summer and spring, respectively at farm (A) and 52.14 – 161.35 µg/g dw in summer and autumn, respectively at (B). Taweel *et al.* (2013) recorded lower Zn values (20.85-26.13 µg/g dw) in muscle tissues of Nile tilapia at Langat River and Engineering Lake in Malaysia. The results showed that there's no significant (P<0.05) difference in the annual mean concentrations (114.02 & 120.8 µg/g dw) of Zn between at the two farms (A & B), respectively. Abdel-Khalek (2015) recorded a value of 63.7 µg/g dw for Zn.

Cadmium (Cd) and lead (Pb) are toxic at low concentrations, non-biodegradable, non-essential heavy metals and have no role in biological processes in living organisms. Thus, even in low concentration, it could be harmful to fish (Badr *et al.,* 2014). The obtained data illustrated that maximum (Cd) concentration was (0.33 µg/g dw) at farm (A) and (B) in autumn and winter, respectively. Saeed (2013b), recorded values of Cd between 0.13 and 0.49µg/g dw in autumn and winter in Nile tilapia. A significant (P<0.05) increase in Cd concentration in fish muscles at farm (B) (0.29 µg/g dw) was recorded compared with (0.25 µg/g dw) at farm

(A). Higher values (10.84 µg/g dw) were obtained by Authman *et al.* (2013). Maximum Pb concentrations (0.68 & 0.85 µg/g dw) were recorded in autumn at (A &B) farms, respectively. The results showed that there's a significant ($P<0.05$) difference in the annual mean concentration (0.54 & 0.61 µg/g dw) of Pb in fish muscles between the two farms. These concentrations are lower than that (10.20 µg/g dw) obtained by Authman *et al.* (2013).

It is obvious that, the levels of all heavy metals detected in *O. niloticus* muscle tissues were under the permissible levels (average daily intake for an adult) recommended by SCF (1993), IPCS (1998) and WHO (2011), as shown in table (2).

The essential metals, such as iron, zinc, copper and manganese are in higher concentrations, presumably due to their function as co-factors for the activation of a number of enzymes and regulated to maintain a certain homeostatic status in fish. On the other hand, the non-essential metals such as cadmium has no biological function or requirement and its concentrations in fishes are generally low (Kumar *et al.,* 2011).

Correlation of metals in fish muscle tissues

The Pearson's correlation coefficient matrix for the metals was done to determine the correlation between the metals pairs in muscle tissues. Table (3) showed various degrees of correlations between the elements. There were positive significant ($P<0.05$) correlations between (Mn and Fe; Mn and Cu; Mn and Zn; Fe and Cd; Cd and Pb). On the other hand, no significant ($P>0.05$) correlations were found between other paired of metals. The correlations between the different metals may result from the similar accumulation behavior of the metals in fish and their interactions (Rejomon *et al.,* 2010). Also, noted significant correlations among metals may reflect a common source of occurrence and indicative of similar biogeochemical pathways for subsequent accumulation in the muscle tissue of fishes (Kumar *et al.,* 2011).

Table 3: Correlation coefficient matrix (r) between concentrations of paired metals in *O. niloticus* muscle tissues.

Metal	Mn	Fe	Cu	Zn	Cd	Pb
Mn	1					
Fe	0.526**	1				
Cu	0.411*	0.354	1			
Zn	0.738**	0.266	0.359	1		
Cd	0.040	0.498*	0.228	-0.140	1	
Pb	0.340	0.225	0.011	0.030	0.481*	1

** and ** are significant at ($P<0.05$) and ($P<0.01$) respectively.*

Relationship of metals between water and Fish samples

Figure (1) illustrate the relationship of heavy metals levels between water and fish samples, it's clear that most metals had no significant ($P>0.05$) relationship between their concentration in water and muscle tissues, while Zn has a significant positive ($P<0.05$) correlation between its concentration in water and muscle tissues. The non significant correlation of metals concentration (except Zn) between water and muscle tissues may be due to higher concentration in muscle tissues than water and this indicate the metal accumulation in organs and biomagnification, also agree with the findings of Saleh *et al.* (1985), who reported that aquatic animals can accumulate high and ultimately lethal concentrations of metals over long periods from extremely low water concentrations. Fish accumulate toxic chemicals such as heavy metals directly from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediment and food (Goodwin *et al.,* 2003; Labonne *et al.,* 2001; Osman *et al.,* 2007).

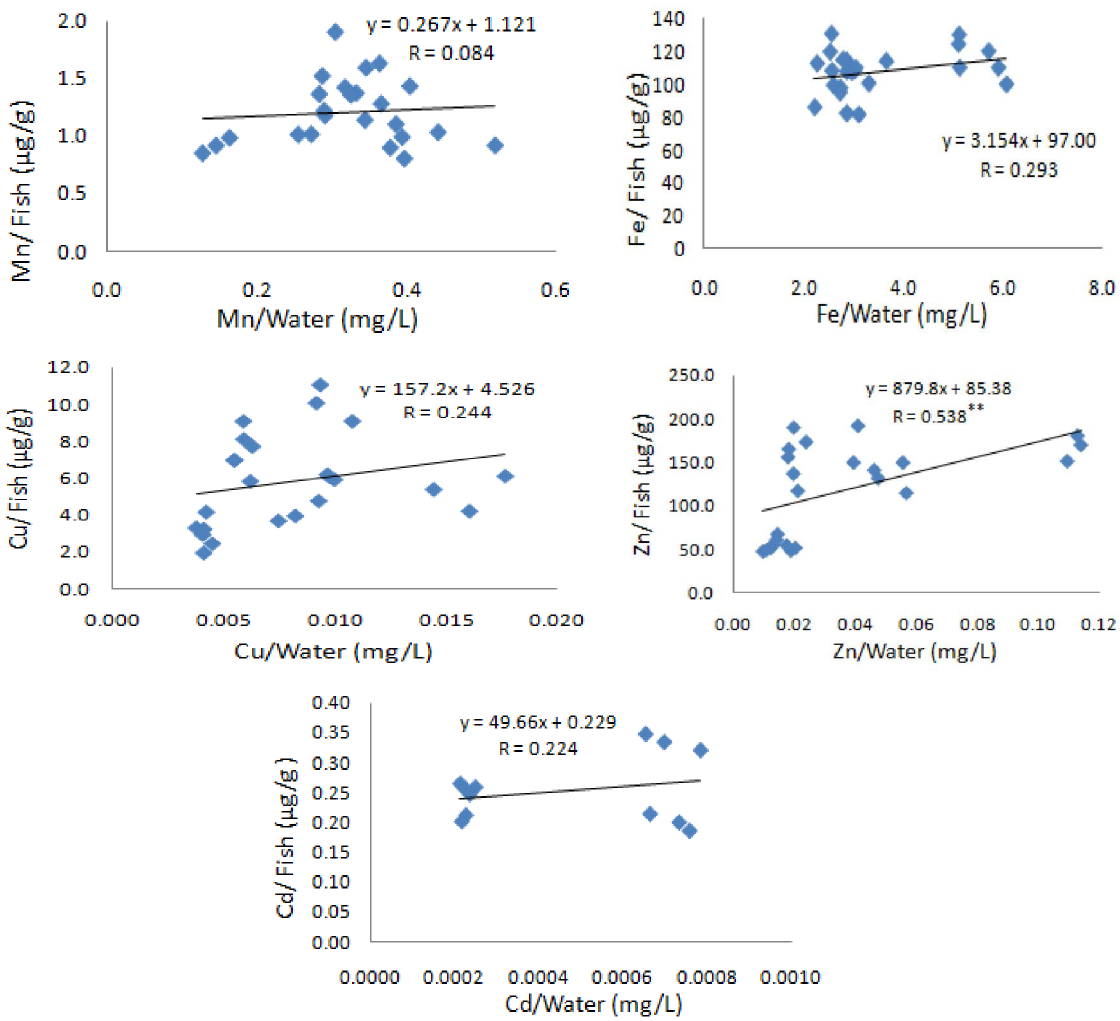

Fig. 1: The relationship of heavy metals levels between water and fish samples (** indicates the correlation is significant (P<0.01).

Conclusion

In this study, concentrations of all studied metals in both water and fish muscle tissues exhibited significant (P<0.05) seasonal variations. Only, mean concentrations of Cu, Cd and Pb in muscle tissues showed significant (P<0.05) differences between two farms with higher concentrations in Shader Azzam fish farm. It is obvious that, the levels of all heavy metals detected in *O. niloticus* muscle tissues were under the notified permissible limits. It is recommended that further studies should carry out for a continual assessment on the levels of pollutants in these areas.

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