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RESEARCH PAPER

Assessment of Metal Pollution around Sabal Drainage in River Nile and its Impacts on Bioaccumulation Level, Metals Correlation and Human Risk Hazard using *Oreochromis niloticus* as a Bioindicator.

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Abstract

This work aimed to assess the impact of Sabal drains (northern part of El-Minufiyah governorate, Egypt) on the metals load of river Nile. Four sites were selected to monitor the partitioning of metals in the aquatic components: reference site (site1); 20Km before Sabal drainage (site2); Sabal site (site3) and 20Km after Sabal drainage (site4). The maximum means of aqueous metals were observed at site3 in the following order (mg/l): Fe (0.76) >Mn (0.325) >Zn(0.07) >Pb (0.064) >Cu (0.041) >Cd (0.0035), while the trend is slightly different in sediments (mg/kg): Fe (527.9) >Mn (102.7) >Zn (17.65) >Cu (11.4) >Pb (1.26)>Cd (0.07). Tissue-specific patterns of metal accumulation in *Oreochromis niloticus* were observed. As indicated by the calculated metal pollution index and bioaccumulation factor, the tissues of liver, kidneys and gills were observed to be strong bioaccumulators compared to muscular tissues. Regarding the correlation study in site3, the bioaccumulated metals in most tissues showed homogenous-correlation with aqueous metals (r>0.5) and diverse-correlations with respect to sedimentary metals. The calculated hazard index (HI) indicated that each metal had low HI but, the cumulative impacts of all metals represented a real threat to fish consumers especially at site3. Metals elevation was highly correlated with Sabal discharges and regulations for water and fish consumption have to be applied.

Keywords: *Oreochromis niloticus*; Metals toxicity; Bioaccumulation; Human risk.

Introduction

In Egypt, releasing of untreated waste water into aquatic environments became a serious problem therefore the regression of water quality developed into major threatens to aquatic life. The deterioration of aquatic habitats results in a lot of malign effects on human health, water quality and fish activities (Bastami *et al*., 2015). In terms of aquatic pollutants, heavy metals represent an interesting group of elements due to their strong effects on the aquatic system equilibrium, bioaccumulation in living organisms, long term persistence and ability to accumulate in water and sediments (Monroy *et al.*, 2014). Metal pollution in the aquatic environment is growing at an alarming rate and has become an important worldwide problem (Aiman *et al.,* 2016). Metals can exist in the environment in different forms such as solid phase, free ions or absorbed to solid colloidal particles. They could invade aquatic habitats naturally via rock weathering, soil erosion and dissolution of water soluble salts or through the anthropogenic sources such as municipal wastewater, manufacturing industries, and agricultural activities (Elkady *et al.,* 2015). The trace amount of metals may play an important role in the biochemical life process of the aquatic organisms (Nakayama *et al.,* 2010). But, their high concentrations have lethal effects on fish and other aquatic biota especially in case of prolonged exposure (Abdel-Khalek, 2015). Among metals, some are potentially toxic (As, Cd, Pb, Hg), others are probably essential (Ni, V, Co) and many are essential (Cu, Zn, Fe, Mn) (Biswas *et al.,* 2012). These essential metals can produce toxic effects when the metals intake is excessively elevated (Tekin-özan, 2008). Bioaccumulation of metals may occur via direct contact through gills and skin and/or by ingestion of these metals in the digestive tract. Metal pollution in the water bodies is usually monitored by measuring metal concentrations in water, sediments and biota (Monroy *et al.*, 2014). Dependence only on water analyses is not considered as an accurate method to identify metals inputs in aquatic systems (Turkmen *et al.,* 2006). In addition to aqueous metals determination, metals concentration in sediments has an important role during water quality evaluation (Bastami *et al.,* 2015). Sediment is considered as the best indicator of trace metals because it has ability to absorb dissolved metals and act as a sink for metals that can consider as a non-point source of pollution by releasing it to water column under physicochemical changes (Pejman *et al.*, 2015). Aquatic organisms can bioaccumulate some environmental contaminants up to million times of the concentrations that detected in the water column (U.S.EPA, 2000). Among aquatic organisms, fish can be considered as one of the most significant bioindicator in aquatic habitats (Findik and

Cice, 2011). Fish are particularly vulnerable because they cannot escape from the detrimental effects of pollutants. Also, fish are considered as a key species in trophic levels that concentrate large amounts of metals (Mahboob *et al.*, 2014). Fish are widely consumed by humans due to its high protein content, low saturated fat and sufficient omega fatty acids which are known to support good health. Therefore, various studies have been done worldwide to assess the human risk status related to the consumption of fish tissues from highly polluted sites (Abdel-Khalek, 2015; Bastami *et al.*, 2015). In Egypt, Nile tilapia (*Oreochromis niloticus*) is well-known as the most important freshwater fish species that inhabit river Nile. The wide spreading of *O. niloticus* is mainly attributed to the ability of this species to feed on a variety of food items, high fecundity, grow to a large sizes and survive in a wide range of physical and chemical conditions (Öner *et al.*, 2008; Authman *et al.*, 2013). River Nile course receives about 78 main drains that discharging agricultural and industrial wastewater in it. The problems of disposing partially treated or untreated wastewater into river Nile have extremely increased in the past years (Authman *et al.*, 2013). So this work aimed to (1) investigate the impacts of Sabal drainage that releases its contents into the Rashid branch of the river Nile on water and sediment quality (2) characterize the pathway and partitioning of metals in different compartments of river Nile (water, sediment and fish) nearby and remote from Sabal discharges (3) assess the health risks of the consumers after consumption of studied fish species.

Materials and Methods

Characteristics of the Study Area

Sabal drain is 47 km in length and has 13 branch drains, which serves about 140000 Fadden of fertile lands. These branches carry wastewater from agricultural land to the main drain and spill about 290 million m^3/year to the Rosetta. The sewage water in Sabal drain comes from six towns which discharge sewage water in the drainage. The industrial wastewater comes from two existing factories. Sabal drainage and its branches were designed to carry a maximum total flow of 0.97 million m^3/day at its discharging point to Rosetta branch. The total discharges are 0.81 million m^3/d from agriculture, 0.16 million m^3/d from sewage and 0.0002 million m^3/d from industrial activities.

Sites of Collection as Represented in Figure 1

Site 1 (references site nearby El-kanater El-khyria): remote from any discharges and about 55 Km away from Sabal drainage.
GPS: 30°11'21.03" N and 31°6'34.08" E.
Site 2 (Upstream Sabal drain outlet): About 20 Km before Sabal drainage.
GPS: 30°31'57.94"N and 0°50'53.20"E.
Site 3 (Sabal drainage): lies in close proximity to Sabal drainage.
GPS: 30°32'13.47"N and 30°51'07.09"E.
Site 4 (Downstream Sabal drain outlet): About 20 Km after Sabal drainage.
GPS: 30°36'23.50"N and 30°47'51.89"E.

Samples Collection

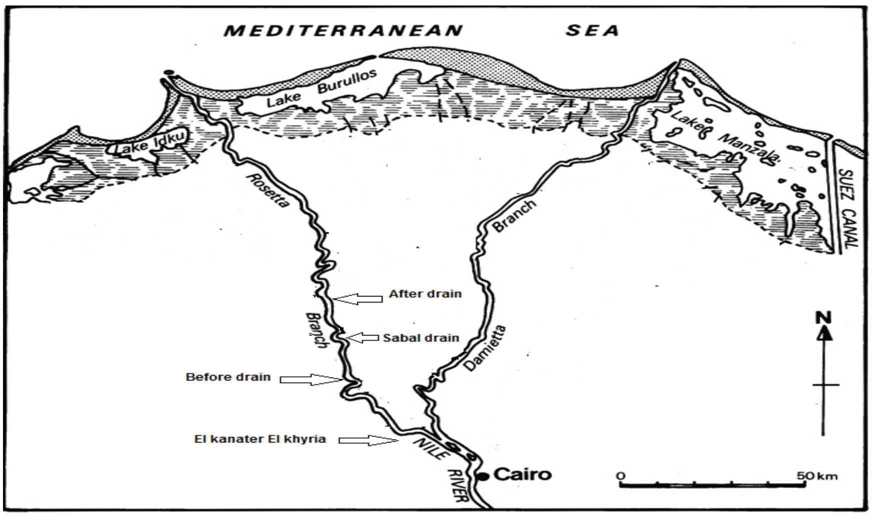

Figure 1. Map of the studied sites.

Water, sediment and fish samples were collected from four studied sites to study the impacts of metal pollution and water physicochemical characteristics on water quality, sediment contamination, inhabitant fish and human health.

Water Sampling

Water sampling was achieved according to standard methods for examination of water and wastewater (APHA, 2005). Water samples (n= 4) from each site were collected in various containers that specialized to suit the nature of each parameter. Polyethylene containers of two-liter capacity were used for most chemical analyses. Samples collected for metals analysis were preserved via adding concentrated nitric acid to reduce the pH below 2 to prevent any microbial reactions.

Sediment Sampling

Four core samples up to 20 cm in depth were taken from each sampling site using polyvinyl chloride (PVC) corers. The corers were immediately sealed and stored at 4ºC (Cabrera *et al.,* 1992).

Fish Sampling

Freshwater fish; *Oreochromis niloticus* at its commercial size were collected from the same areas where water and sediment samples were collected with the help of a professional fisherman. Fish were visually examined for the presence of any morphological body abnormalities and any abnormal fish were excluded.

Water Analyses

Field Parameters (pH, Electrical Conductivity and Dissolved Oxygen)

All these parameters were measured in the field using Corning Checkmate II multi-parameter meter.

Biochemical Oxygen Demand (BOD)

In order to determine biological oxygen demand in the collected water samples, an ORIN BOD fast respirometry system (model 890) with a measuring range of 0-400 mg/l with incubation in a thermostatic incubator chamber model WTW were used according to APHA (2005).

Ammonia (NH_3)

Colorimetric determination of ammonia was achieved using Nessler's solution as described by Sauter and Stoup (1990).

Nitrite and nitrate content

Nitrite and nitrate were determined in water by ion chromatography (IC) (model DX-600, USA) as described in APHA (2005).

Measurement of Metals in WATER, Sediment and Vital Tissues

Concentrations of six metals were determined in water, sediment and tissues by flam atomic absorption spectrophotometry, model PerkinElmer-2280, according to APHA (2005). The detection limits of the instrument in ppm were <0.002 for Cu, <0.005 for Zn & Pb, <0.01 for Fe & Mn and <0.0005 for Cd. Sediment samples and tissues named liver, gills, kidney, skin and muscles were acid-digested according to method that explained by Hseu (2004). The measuring accuracy was checked by standard reference material (Lake Superior fish 1946 NIST, National Institute of Standards and Technology, USA) and the recovery ranges was between 90% and 110%. The final metal concentrations were expressed as mg/l in water and as mg/kg dry weight in sediment and tissues.

Metal Pollution Index (MPI)

This index was assessed to estimate the total load of metals in the studied tissues of *O. niloticus* using the equation of Usero et al. (1997): MPI= $(M_1 \times M_2 \times M_3 \times \ldots.. M_n)^{1/n}$.

Where, M_n is the concentration of metal n (mg/kg dry wt.) in a certain tissue.

Bioaccumulation Factor (BAF) of Metals in the Different Tissues

It is the ratio of the contaminant in an organism to the concentration in the ambient environment at a steady state (U.S.EPA, 2010). Bioaccumulation of heavy metals was calculated in the different vital tissues (liver, gills, kidney, skin and muscles) of *O. niloticus* according to Arnot and Gobas (2006) using the following equation: BAF= Metal concentration in the organ (mg/kg)/Metal concentration in water (mg/1).

Risk assessment Estimated according to U.S.EPA (2000)

Average Daily Dose (ADD)

The level of exposure resulting from the consumption of a particular chemical in fish edible tissue (muscle and skin) can be expressed by estimation of daily intake levels in the following equation:

$$\text{Average Daily Dose (mg/kg/day)} = (C \times IR \times EF \times ED) / (BW \times AT)$$

Where, **C** is metal concentration in tissue (mg/kg), IR is mean ingestion rate which equal 0.0312 kg/day and 0.1424 kg/day for normal adult and habitual fish eaters respectively, EF (Exposure frequency) which is 365 days/year, ED represents the exposure duration in years over a lifetime (70 years), BW (Body weight) which is about 70 kg and AT is average time (70 years × 365 days/year).

Hazard Index (HI)

The hazard index is determined according to the following equation: Hazard Index = ADD/Oral RfD where, Oral RfD = Oral Reference dose of chemical (mg/kg/day) based on the upper level of intake mentioned earlier for each metal for an adult human with average body weight of 70 kg. The oral reference doses (RfD) for Cu, Zn, Pb, Fe, Mn and Cd suggested by FAO/WHO (2006) was 0.14, 0.214, 0.00357, 0.643, 0.157 and 0.001 mg/kg/day, respectively. HI<1.0 indicates that adverse health effects are not likely to occur. Meanwhile, if the HI was greater than or equal to 1.0, it is probably that adverse health effects will be observed.

Statistical Analyses:

The results were expressed as means ± S.E. Data were statistically analyzed using Duncan’s multiple range test to evaluate difference in means as indicated by different case letters at P<0.05. The correlation analysis and Pearson’s correlation coefficient (r) were used to show the association between the levels of metals in water, sediment, and fish tissues. The statistical analysis was done using SAS version 9.1 (SAS, 2006).

Results

Physicochemical Characteristics of Water

The physicochemical water analysis of the studied sites (Table 1) showed that, all measured parameters were elevated in Sabal site (site3) compared to that of reference site (site1), with the exception of DO. The present results exhibited that, the values of pH, EC, BOD, NH_3, NO_2 and NO_3 were directly proportional with the discharge activities while the values of DO were inversely proportional with it.

Heavy Metals in Water and Sediment

The levels of Cu, Zn, Pb, Fe, Mn and Cd in water and sediment samples collected from the studied sites are detailed in Table 2. The values of the analyzed metals in water and sediment samples showed various degrees of contamination with highly significant elevation in Sabal drainage site compared to the reference site. While, the decrease in their concentrations were observed in the other sites showing relative dilution of metals content with increasing distance from the discharge point.

Bioaccumulated Metals in Fish Tissues

The concentrations of almost all analyzed metals were significantly higher in fish tissues that collected from Sabal site (sites3) followed by downstream site (site4) compared with those collected from the reference site (Table 3). The bioaccumulation of metals in different fish tissues exhibited various levels of a tissue-specific pattern with elevated rate of accumulation in the highly metabolic tissues as liver, kidney and gills.

Metal Pollution Index (MPI)

The data that obtained from MPI (Table 4) showed that the active metabolic tissues (liver and kidney) in addition to gills had high bioaccumulation affinity to the all studied metals. Moreover, the collective accumulation of metals in tissues that collected from the studied sites (represented by the calculated MPI) was in the following order: site3> site4 >site2> site1.

Bioaccumulation Factor (BAF)

Our results indicated that the calculated BAFs of Cu, Pb & Cd were mainly higher in liver and kidneys while in case of Fe the highest values were obvious in gills, kidneys and liver tissues. Zn & Mn metals had favorable bioaccumulation pattern in gills and skin tissues. Meanwhile, the lowest bioaccumulation values of all studied metals were recorded in muscle tissues (Table 5).

The Correlation between Metals in Water-Tissues and in Sediment-tissues of the most polluted site (Sabal site).

Relationship between Metal Concentrations in Water-Tissues

The correlation between the aqueous and bioaccumulated metals was represented by correlation coefficient (r) in Table 6. The level of all studied aqueous metals in Sabal site showed positive correlations with Cu contents of all tissues except skin. Also, positive correlation between aqueous metals appeared with hepatic, dermal and muscular Cd contents as well as muscular Zn contents. In contrast, negative correlations were noted between most of aqueous metals and the rest of bioaccumulated metals in all tissues except gills content of Zn & Cd and renal Cd contents.

Relationship between Metal Concentrations in

Table 1. Physicochemical characteristics of water collected from the studied sites, mean ± SE, n= 4 for each site

Sites parameters	Site1 (reference site)	Site2 (upstream site)	Site3 (Sabal site)	Site4 (downstream site)
pH	7.73 ±0.17[b]	7.53 ±0.21[b]	8.13 ± 0.17[a]	7.8 ± 0.16[b]
Electric conductivity (EC)	375.48 ± 4.1[d]	480 ± 8.16[c]	678.95 ± 7.25[a]	547.3 ± 5.67[b]
Dissolved oxygen (DO mg/l)	7.47 ± 0.83[a]	4.45 ± 0.81[b]	1.69 ± 0.56[c]	4.81 ± 1.32[b]
Biological oxygen demand (BOD mg/l)	3.5 ± 0.51[c]	12.58 ± 1.7[b]	27.45 ± 1.36[a]	14.12 ± 1.51[b]
Ammonia (NH_3 mg/l)	0.58 ± 0.17[c]	6.02 ± 1.48[b]	9.74 ± 0.9[a]	9.18 ± 0.72[a]
Nitrite (µg/l)	13.6 8± 2.36 [d]	24.26 ± 3.39 [c]	43.37 ± 4.1 [a]	33.57 ± 4.16 [b]
Nitrate (µg/l)	31.50 ± 4.71[d]	50.2 ± 4.12 [c]	78.49 ± 6.58 [a]	61.57 ± 4.78 [b]

Means with the same letter in the same row for each parameter are not significantly (P<0.05) different, otherwise they do (Duncan's test).

Table 2. Concentrations of heavy metals in water (mg/l) and sediment (mg/kg dry weight) samples of the studied sites, mean ± SE, n= 4 for each site

	Site 1 (reference site)	Site 2 (upstream site)	Site 3 (Sabal site)	Site 4 (downstream site)
Copper Water	0.012 ± 0.002[d]	0.023 ± 0.002[c]	0.041 ± 0.004[a]	0.034 ± 0.003[b]
Sediments	0.76 ± 0.15[d]	3.09 ± 1.77[c]	11.4 ± 2.37[a]	6.27 ± 0.2[b]
Zinc Water	0.015 ± 0.003[d]	0.043 ± 0.004[c]	0.07 ± 0.005[a]	0.05 ± 0.004[b]
Sediments	4.75 ± 0.96[c]	8.41 ± 0.98[b]	17.65 ± 2.37[a]	15.96 ± 1.14[a]
Lead Water	0.015 ± 0.003[d]	0.034 ± 0.003[c]	0.064 ± 0.004[a]	0.048 ± 0.005[b]
Sediments	0.17 ± 0.08[d]	0.59 ± 0.153[c]	1.26 ± 0.04[a]	1.05 ± 0.12[b]
Iron Water	0.23 ± 0.006[d]	0.45 ± 0.007[c]	0.76 ± 0.008[a]	0.54 ± 0.005[b]
Sediments	285.31 ± 20.81[c]	367.31 ±21.49[bc]	527.9 ± 106.91[a]	414.93 ± 37.56[b]
Manganese Water	0.033 ± 0.004[d]	0.107 ± 0.005[c]	0.325 ± 0.004[a]	0.182 ± 0.006[b]
Sediments	34.16 ± 11.27[c]	52.93 ± 4.97[bc]	102.71 ± 21.26[a]	65.69 ± 1.33[b]
Cadmium Water	0 ± 0[c]	0.0013 ± 0.0005[b]	0.0035 ± 0.001[a]	0.0015 ± 0.001[b]
Sediments	0.02 ± 0.01[c]	0.03 ± 0.01[b]	0.07 ± 0.02[a]	0.04 ± 0.01[b]

Means with the same letter in the same row for each metal are not significantly (P<0.05) different, otherwise they do (Duncan's test).

Sediment-Tissues

The correlation between the concentrations of the examined metals in sediment with the bioaccumulated metals in different tissues that collected from the most polluted site (Sabal site) are represented by correlation coefficient (r) in Table 7. The relationship between sedimentary Cu was positively with Pb, Fe & Mn in all tissues and with hepatic, renal and dermal Zn contents along with Cu contents in skin tissue. On the other side, the sedimentary Cu was negatively related with Cu contents in all tissues except skin and with hepatic Cd contents in addition to muscular Zn & Cd contents. Zinc level in the sediment samples of Sabal site revealed strong positive relationships with Zn, Mn & Cd contents in the gills and with renal Zn & Cd contents but the only negative correlation was with hepatic Cd contents. The concentration of Pb in the polluted sediment showed significant positive relationships with Zn contents of gills, kidney & muscle tissues and with Cd contents of gills, kidney & skin tissues. Inversely, negative correlations were found with the Fe contents of the all tissues and with Zn contents of the liver and skin tissues. The sediment Fe contents correlated positively with Zn, Mn & Cd contents in the gill and with renal Zn & Cd contents. Moreover, there were significant negative correlations with hepatic Zn & Cd contents. The sedimentary Mn was positively correlated with all hepatic and dermal metals content except Cu & Cd. Another positive correlation was observed with gill contents of Pb & Fe as well as renal and muscular Pb, Fe & Mn contents. Moreover, there were significant negative correlations between sedimentary Mn with Cu contents of all studied tissues except in skin and with Zn & Cd contents in gill and muscle tissues as well as Cd contents in skin tissues. The level of Cd in the polluted sediment showed positive associations with the Cu contents of the all studied tissues except skin and with the Cd contents in all organs except the gill and kidney tissues as well as Zn contents of the muscle. On the other hand, significant negative relationships were recorded between Cd contents of sediment with Pb, Fe & Mn of all tissues and with Zn contents of gill, kidney & skin tissues as well as with

Table 3. Bioaccumulated metals (mg/kg dry wt) in different tissues of *O. niloticus*, mean ± SE, n = 8 for each site

Sites Tissues	Site 1 (reference site)	Site 2 (upstream site)	Site 3 (Sabal site)	Site 4 (downstream site)
Copper				
Liver	1.83 ± 0.43[d]	8.7 ± 1.19[c]	13.28 ± 1.01[a]	10 ± 0.79[b]
Gills	3.05 ± 0.13[d]	6.08 ± 0.43[c]	11.11 ± 0.25[a]	8.86 ± 1.44[b]
Kidneys	2.09 ± 0.25[c]	6.8 ± 1[b]	8.98 ± 0.86[a]	6.49 ± 0.88[b]
Skin	2.02 ± 0.29[d]	3.92 ± 0.5[c]	5.83 ± 0.29[a]	5.28 ± 0.47[b]
Muscles	2.14 ± 0.08[d]	4.51 ± 0.28[c]	7.56 ± 0.37[a]	6.39 ± 1.43[b]
Zinc				
Liver	3.20 ± 1.10[c]	33.78 ± 4.93[b]	55.79 ± 12.32[a]	35.04 ± 8.12[b]
Gills	12.14 ± 4.87[c]	53.07 ± 12.61[b]	112.18 ± 15.43[a]	55.08 ± 15.47[b]
Kidneys	6.78 ± 1.93[d]	26.25 ±8.55[c]	88.76 ± 21.18[a]	64.57 ± 10.20[b]
Skin	32.10 ± 8.62[b]	43.57 ± 8.48[b]	59.12 ± 14.31[a]	59.11 ± 13.52[a]
Muscles	5.49 ± 1.26[b]	13.88 ± 3.39[b]	67.41 ± 24.54[a]	13.39 ± 0.94[b]
Lead				
Liver	0.32 ± 0.13[d]	3.28 ± 0.57[c]	7.56 ± 0.98[a]	5.10 ± 1.35[b]
Gills	0.59 ±0.11[c]	1.72 ± 0.72[b]	3.07 ± 0.54[a]	1.48 ± 0.36[b]
Kidneys	2.50 ± 0.5[b]	1.55 ± 0.59[c]	4.10 ± 1.1[a]	2.23 ± 0.33[bc]
Skin	0.29 ± 0.05[c]	0.82 ± 0.16[b]	1.17 ± 0.20[a]	0.83 ± 0.08[b]
Muscles	0.20 ± 0.14[b]	0.96 ± 0.49[a]	1.18 ± 0.41[a]	1.02 ± 0..35[a]
Iron				
Liver	74.71 ± 12.34[d]	236.65 ± 32.91[c]	514.07 ± 34.61[a]	288.04 ± 32.79[b]
Gills	95.02 ± 24.16[c]	280.48 ± 19.72[b]	367.98 ± 29.08[a]	278.11 ± 30.92[b]
Kidneys	81.7 ±5.91[c]	270.00 ± 62.06[b]	446.49 ± 33.84[a]	261.00 ± 44.74[b]
Skin	38.41 ± 6.24[c]	133.3 ± 43.69[b]	200.86 ± 37.36[a]	54.58 ± 9.83[c]
Muscles	7.47 ± 1.53[c]	67.41 ± 18.55[b]	103.24 ± 21.67[a]	68.04 ± 6.89[b]
Manganese				
Liver	1.13 ± 0.22[d]	6.49 ± 0.51[c]	59.80 ± 9.49[a]	17.74 ± 3.01[b]
Gills	14.29 ± 4.68[b]	24.17 ± 3.8[b]	29.74± 17.01[a]	27.33 ± 5.83[a]
Kidneys	3.48 ± 0.83[c]	11.15 ± 1.72[c]	20.15 ± 2.91[a]	9.61 ± 1.64[b]
Skin	12.72 ± 2.76[c]	18.8 ± 2.76[b]	28.15 ± 1.71[a]	19.23 ± 3.05[b]
Muscles	0.55 ± 0.2[c]	0.42 ± 0.07[c]	2.64 ± 0.60[a]	1.52 ± 0.26[b]
Cadmium				
Liver	0.09 ± 0.02[c]	0.22 ± 0.05[c]	2.58 ± 0.31[a]	0.57 ± 0.25[b]
Gills	0.16 ± 0.02[c]	0.45 ± 0.04[b]	0.82 ± 0.27[a]	0.46 ± 0.28[b]
Kidneys	0.043 ± 0.02[b]	0.41 ± 0.05[b]	2.28 ± 0.67[a]	1.98 ± 0.45[a]
Skin	0.12 ± 0.05[c]	0.26 ± 0.11[b]	0.44 ± 0.08[a]	0.26 ± 0.16[b]
Muscles	0.03 ± 0.02[c]	0.25 ± 0.07[b]	0.32 ± 0.07[a]	0.35 ± 0.09[a]

Means with the same letter in the same row for each parameter are not significantly ($P<0.05$) different, otherwise they do (Duncan's test).

Table 4. Metal pollution index (MPI) of the studied metals in vital tissues of *O. niloticus* in the studied sites

Organs Sites	Liver	Kidneys	Gills	Skin	Muscles
Site 1 (Reference site)	1.56	2.75	4.10	3.21	0.81
Site 2 (Upstream site)	8.29	8.36	10.92	6.71	2.74
Site 3 (Sabal site)	27.62	20.16	18.03	10.01	6.12
Site 4 (Downstream site)	13.16	12.48	11.67	6.43	3.83

dermal Cu content.

Human Risk Assessment

Based on the values of HI at mean ingestion rate for normal adult and habitual fish eaters, there was no adverse health effect is likely to occur (all values of HI<1). But, some HI values (ex. cadmium in case of habitual fish eaters at Sabal site) were higher than other values along the studied sites showing alarming values (Table 8). In general, HI values for each examined metal do not pose unacceptable threats at mean ingestion rate for muscle and skin tissues. The cumulative values of all metals (in muscle and skin) represented a real health threat to fish consumers as the values of HI exceeded the safe limit and the risk may significantly increase when these tissues are collectively consumed.

Discussion

Physicochemical Characteristics of Water

The high level of all studied physicochemical parameters in addition to the sharp decrease in DO values represented the deterioration of water quality

Table 5. Bioaccumulation factor (BAF) of the analyzed metals (l/kg) in different tissues of *O. niloticus* in the studied sites

Sites Tissues	Site 1 (reference site)	Site 2 (upstream site)	Site 3 (Sabal site)	Site 4 (downstream site)
Copper				
Liver	152.5	378.26	323.9	294.12
Gills	254.17	264.35	270.96	260.59
Kidneys	174.17	295.65	219.02	190.88
Skin	168.33	170.43	142.20	155.29
Muscles	178.33	196.09	184.39	187.94
Zinc				
Liver	213.33	785.58	797	700.8
Gills	809.33	1234.19	1602.57	1101.6
Kidneys	452	610.47	1268	1291.4
Skin	2140	1013.26	844.57	1182.2
Muscles	366	322.79	963	267.8
Lead				
Liver	21.33	96.47	118.13	106.25
Gills	39.33	50.59	47.97	30.83
Kidneys	166.67	45.59	64.06	46.46
Skin	19.33	24.12	18.28	17.29
Muscles	13.33	28.24	18.44	21.25
Iron				
Liver	324.83	525.89	676.41	533.41
Gills	413.13	623.29	484.18	515.02
Kidneys	355.22	600	587.49	482.33
Skin	167	296.22	264.25	101.07
Muscles	32.48	149.8	135.84	126
Manganese				
Liver	34.24	60.65	184	97.47
Gills	433.03	225.89	91.51	150.16
Kidneys	105.45	104.21	62	52.80
Skin	385.45	175.70	86.62	105.66
Muscles	16.67	3.93	8.12	8.35
Cadmium				
Liver	0	169.23	737.14	380
Gills	0	346.15	234.28	306.67
Kidneys	0	315.38	651.43	1320
Skin	0	200	125.71	173.33
Muscles	0	192.31	91.43	233.33

in the vicinity of Sabal drainage. The relatively high pH might be attributed to the sewage discharges from Sabal drainage that might cause bad effects on some beneficial bacteria which necessitate slightly acidic pH to decompose toxic elements into less harmful forms (Malik *et al.*, 2010). Electric conductivity is the ability of the water to conduct an electrical current, and is an indirect measure of the ion concentration (Sarwar and Wazir, 1988). The increased EC recorded nearby Sabal drain being affected by the amount and quality of discharges, as well as the anthropogenic impact. The BOD is an empirical analysis that determines the amount of oxygen needed for microorganisms in the water samples to oxidize any biodegradable organic matter, as well as the quantity of oxygen used in its respiration (APHA, 2005). High BOD values reflect the elevated biological activities, the excessive growth of microorganisms and sever biological contamination of water. The biological activities of water were confirmed by high concentrations of nitrate and nitrite in water. These high concentrations may induce excessive algal growth and sever O_2 depletion in water (Osman *et al.*, 2010). Fathi and Flower (2005) showed that overflow of organic matter may cause a depletion of DO due to the decomposition of this suspended organic matter. Persistent exposure to low oxygen level will increase fish vulnerability to other environmental stress (Osman *et al.*, 2010) because fish have to increase the respiratory rate to compensate the low O_2 level and consequently increase the pollutants accumulation via gill membranes. The increased NH_3 in water indicates the existence of highly active pollutants that comes from sewage overflows, industrial discharge and agriculture runoff as well as due to the decomposition of organic matters. NH_3 can easily penetrate the membranes of the gills, causing nervous system disorders and even death in high concentrations (Osman and Kloas, 2010).

Aqueous and Sedimentary Metal Contents

Table 6. Pearson's correlation coefficient (r) between metals level in water (mg/l) and tissues (mg/kg dry wt.) samples collected from the most polluted site (site 3)

Water Organs	Cu	Zn	Pb	Fe	Mn	Cd
Liver						
Cu	+0.99*	+0.99*	+0.99*	+0.99*	+0.99*	+0.86*
Zn	-0.74*	-0.77*	-0.70*	-0.61*	-0.61*	-0.25
Pb	-0.99*	-0.99*	-0.99*	-0.99*	-0.99*	-0.86*
Fe	-0.89*	-0.91*	-0.87*	-0.80*	-0.80*	-0.50*
Mn	-0.99*	-0.99*	-0.99*	-0.99*	-0.99*	-0.87*
Cd	+0.81*	+0.78*	+0.84*	+0.90*	+0.90*	+0.99*
Gills						
Cu	+0.99*	+0.99*	+0.99*	+0.99*	+0.99*	+0.86*
Zn	-0.08	-0.03	-0.13	-0.24	-0.24	-0.61*
Pb	-0.99*	-0.99*	-0.99*	-0.99*	-0.99*	-0.86*
Fe	-0.89*	-0.91*	-0.87*	-0.80*	-0.80*	-0.50*
Mn	-0.89*	-0.87*	-0.92*	-0.96*	-0.96*	-0.99*
Cd	+0.14	+0.18	+0.09	-0.03	-0.03	-0.42
Kidneys						
Cu	+0.99*	+0.99*	+0.99*	+0.99*	+0.99*	+0.86*
Zn	-0.54*	-0.51*	-0.59*	-0.68*	-0.68*	-0.91*
Pb	-0.99*	-0.99*	-0.99*	-0.99*	-0.99*	-0.86*
Fe	-0.89*	-0.91*	-0.87*	-0.80*	-0.80*	-0.50*
Mn	-0.99*	-0.99*	-0.99*	-0.99*	-0.99*	-0.87*
Cd	+0.32	+0.36	+0.27	+0.16	+0.16	-0.25
Skin						
Cu	-0.99*	-0.99*	-0.99*	-0.98*	-0.98*	-0.82*
Zn	-0.93*	-0.95*	-0.91*	-0.86*	-0.86*	-0.58*
Pb	-0.9*	-0.9*	-0.99*	-0.9*	-0.9*	-0.86*
Fe	-0.89*	-0.91*	-0.87*	-0.80*	-0.80*	-0.50*
Mn	-0.99*	-0.99*	-0.99*	-0.99*	-0.99*	-0.87*
Cd	+0.89*	+0.91*	+0.87*	+0.80*	+0.80*	+0.50*
Muscle						
Cu	+0.99*	+0.99*	+0.99*	+0.99*	+0.99*	+0.86*
Zn	+0.9*	+0.91*	+0.87*	+0.81*	+0.81*	+0.51*
Pb	-0.9*	-0.9*	-0.99*	-0.9*	-0.9*	-0.86*
Fe	-0.89*	-0.91*	-0.87*	-0.80*	-0.80*	-0.50*
Mn	-0.99*	-0.99*	-0.99*	-0.99*	-0.99*	-0.87*
Cd	+0.98*	+0.99*	+0.97*	+0.94*	+0.94*	+0.73*

*Significant correlation ($P<0.05$)

The liberation of agriculture, industrial and sewage discharges has associated with metal accumulation in aquatic components that may harmfully affect the aquatic health in river Nile. As demonstrated by Abdel-Khalek (2015), combinations of metals in the aquatic habitats have much additive toxicological impacts on the aquatic biota compared to their single effects. In this study, aqueous metals were accumulated to the highest extent at Sabal site which receive different discharge types. In addition to water, sediments can be considered as a sensitive indicator during habitat quality monitoring (Bastami *et al.,* 2015). Metal concentrations in sediment samples were always higher than those of the overlying water. This may be due to the high metals quantity that are adsorbed by particulate matter or precipitated from water column (Gupta *et al.,* 2009). The non-residual fraction of the sediment is considered to be mobile and therefore, is likely to become available to aquatic organisms via re-suspension process (Bramha *et al.,* 2014). Thus, metals in water and sediment have to be taken in consideration because metals may undergo rapid alterations affecting the rate of uptake or release through water-sediment interaction. In comparison with the reference site, metal concentrations in some cases at Sabal site were exceeded by greater than 9 times for water and 15 times for sediments. Therefore, water quality nearby Sabal drainage posed an augmented threat to aquatic life.

Bioaccumulation of Metals in the Key Body Tissues

The accumulation of metals in the vital tissues reflects their concentrations in the surrounding milieu where the fish species lives and also achieves perfect image of fish-external environment interaction (Monroy *et al.,* 2014). Fish may accumulate metals that enter their bodies either directly via water and sediment or indirectly through the food chain. Fish then accumulate these metals in their tissues in significant quantities that exceed those found in their surroundings, eliciting a lot of damaging effects

Table 7. Pearson's correlation coefficient (r) between metals level in sediment (mg/kg dry wt) and tissues (mg/kg dry wt.) samples collected from the most polluted site (site 3)

Sediment Organs	Cu	Zn	Pb	Fe	Mn	Cd
Liver						
Cu	-0.99*	-0.37	+0.13	-0.16	-0.51*	+0.87*
Zn	+0.73*	-0.41	-0.80*	-0.59*	+0.97*	-0.25
Pb	+0.99*	+0.37	-0.13	+0.16	+0.51*	-0.87*
Fe	+0.89*	-0.15	-0.61*	-0.36	+0.87*	-0.50*
Mn	+0.99*	+0.37	-0.13	+0.16	+0.51*	-0.87*
Cd	-0.82*	-0.81*	-0.43	-0.67*	+0.04	+0.99*
Gills						
Cu	-0.99*	-0.37	+0.13	-0.16	-0.51*	+0.87*
Zn	+0.09	+0.97*	+0.97*	+0.99*	-0.79*	-0.61*
Pb	+0.99*	+0.37	-0.13	+0.16	+0.51*	-0.87*
Fe	+0.89*	-0.15	-0.61*	-0.36	+0.87*	-0.50*
Mn	+0.90*	+0.71*	+0.28	+0.54*	+0.12	-0.99*
Cd	-0.13	+0.89*	+0.99*	+0.97*	-0.90*	-0.42
Kidneys						
Cu	-0.99*	-0.37	+0.13	-0.16	-0.51*	+0.87*
Zn	+0.55*	+0.97*	+0.73*	+0.89*	-0.40	-0.91*
Pb	+0.99*	+0.37	-0.13	+0.16	+0.51*	-0.87*
Fe	+0.89*	-0.15	-0.61*	-0.36	+0.87*	-0.50*
Mn	+0.99*	+0.37	-0.13	+0.16	+0.51*	-0.87*
Cd	-0.31	+0.80*	+0.99*	+0.91*	-0.97*	-0.25
Skin						
Cu	+0.99*	+0.29	+0.29	+0.08	+0.57*	-0.82*
Zn	+0.93*	-0.05	-0.53*	-0.26	+0.82*	-0.58*
Pb	+0.99*	+0.37	-0.13	+0.16	+0.51*	-0.87*
Fe	+0.89*	-0.15	-0.61*	-0.36	+0.87*	-0.50*
Mn	+0.99*	+0.37	-0.13	+0.16	+0.51*	-0.87*
Cd	-0.89*	+0.15	+0.61*	+0.36	-0.87*	+0.50*
Muscle						
Cu	-0.99*	-0.37	+0.13	-0.16	-0.51*	+0.87*
Zn	-0.89*	+0.14	+0.60*	+0.35	-0.87*	+0.51*
Pb	+0.99*	+0.37	-0.13	+0.16	+0.51*	-0.87*
Fe	+0.89*	-0.15	-0.61*	-0.36	+0.87*	-0.50*
Mn	+0.99*	+0.37	-0.13	+0.16	+0.51*	-0.87*
Cd	-0.98*	-0.14	+0.36	+0.07	-0.69*	+0.73*

*Significant correlation (P<0.05)

(Agah *et al.,* 2009). Moreover, fish that exist in low-oxygenated aquatic environments should increase their respiratory rate to compensate O_2 deficiency and consequently increase metal accumulation (Abdel-Khalek, 2015). The excessive metals' accumulation in the studied fish at Sabal site is likely to be due to the high discharging activities that associated with a high influx of metals as a result of different surrounding sources of discharges. The dispersion of metals content with increasing distance from discharge point as in site4 may decrease metal concentrations to some extent but it still higher than that of the site2 where less discharge activities were observed. The low metal accumulation in fish that collected from site2 indicated that moderate amount of metal load can be regulated without massive bioaccumulation in tissues. The divergent metals accumulation among tissues in the same fish species depends on the mode and the duration of exposure to the surrounding metals (Maheswari *et al.,* 2006). The results of this study indicated that *O. niloticus* have a selective maintenance of metals in their tissues. Koca et al. (2005) reported that the bioaccumulation patterns of a certain pollutant in the aquatic biota depend mainly on the uptake and removal rates of this pollutant. Bioavailability of the studied metals was observed to be tissue specific with different accumulation patterns. The external tissues of gills and skin showed high accumulation pattern in case of some metals and this may be attributed to the anatomical location of these tissues which allow their direct and continuous contact with the external pollutants. While, the excessive metal accumulation in liver tissues are associated with the detoxification, transformation and excretion processes that occur in hepatic tissues (Zauke *et al.*, 1999). Also, the haemopoietic functions of kidney with abundant blood supply explain their excessive accumulation of metals. As indicated by MPI, the bioaccumulated metals were differentially distributed in the studied tissues with tendency to concentrate in the active metabolic organs either for long-term storage or excretion. This observation was in agreement with Omar *et al.* (2014) who reported that the metabolically active tissues as liver and

Table 8. Hazard index for muscle (HI_m) and skin (HI_S) consumption of *O. niloticus* calculated at mean ingestion rate (0.0312 kg/day) and subsistence ingestion rate (0.1424 kg/day)

		Site1 (reference site)		Site2 (upstream site)		Site3 (Sabal site)		Site4 (downstream site)	
		HIm	HIs	HIm	HIs	HIm	HIs	HIm	HIs
Copper	Normal eaters	0.07	0.06	0.14	0.12	0.24	0.64	0.20	0.17
	Habitual eaters	0.03	0.03	0.07	0.06	0.11	0.29	0.09	0.08
Zinc	Normal eaters	0.01	0.05	0.03	0.09	0.14	0.14	0.03	0.14
	Habitual eaters	0.05	0.33	0.14	0.42	0.65	0.56	0.14	0.56
Lead	Normal eaters	0.0004	0.0006	0.0019	0.0017	0.0024	0.0024	0.0021	0.0017
	Habitual eaters	0.0019	0.0028	0.0091	0.0078	0.011	0.011	0.0096	0.0079
Iron	Normal eaters	0.005	0.03	0.05	0.09	0.07	0.14	0.03	0.04
	Habitual eaters	0.02	0.12	0.21	0.42	0.33	0.64	0.21	0.17
Manganese	Normal eaters	0.002	0.04	0.001	0.05	0.007	0.08	0.004	0.05
	Habitual eaters	0.007	0.16	0.005	0.24	0.03	0.36	0.02	0.25
Cadmium	Normal eaters	0.01	0.05	0.11	0.12	0.14	0.2	0.16	0.12
	Habitual eaters	0.06	0.24	0.51	0.53	0.65	0.9	0.71	0.53
Cumulative values	Normal eaters	0.0974	0.2306	0.3329	0.4717	0.5994	1.2024	0.4261	0.5217
	Habitual eaters	0.1689	0.8828	0.9441	1.6778	1.781	2.761	1.1796	1.5979

kidneys had high affinity to concentrate the greatest amount of most metals in their tissues. In contrast, the lowest metals bioaccumulation were observed in muscles (lowest MPI) and this may be related to the high fat-content in muscle tissues with low affinity to combine with metals in addition to the low metabolic activity of muscle (Uluturhan and Kucuksezgin, 2007).

Bioaccumulation Factor (BAF)

The bioaccumulation factor is evaluated in relation to the concentration of the aqueous metal at which the studied fish inhabits. Accumulation of metals in aquatic biota includes complicated relation between exogenous and endogenous factors such as bioavailability of metal, physicochemical characteristics of surrounding water, species, age and physiological status (Moiseenko and Kudryavtseva, 2001). In general, the nonessential metals are bioaccumulated with less efficiency compared to the high bioaccumulation efficiency of essential metals in the various tissues. The relatively higher BAF of essential elements may be due to their role as an activator of numerous enzymes present in fish (Uluturhan and Kucuksezgin, 2007). Gills, liver and kidney tissues were witnessed to be active bioaccumulators for most metals since these tissues have a considerable mass in which the accumulated metals may be detoxified, regulated or excreted (Reinfelder *et al.*, 1998). These results were coincided with that observed by Jayaprakash *et al.* (2015) as they showed that the highest BAF values of Ni, Pb, Mn, Co, Cr, Fe, Cu and Zn were observed in the gills and liver tissues.

Correlation Between Metals

The correlations between metals in the different compartments depend on physical, chemical and biological activities that always occurring in aquatic habitats. Moreover, releasing of pollutants as well as other anthropogenic processes has strong impacts on the metals distribution and behavior in the aquatic environment (Baeyens *et al.*, 1998). During interpretation of correlation coefficient, one should keep in mind that correlation cannot conclusively prove causation it gives only a degree of relationship (Vasić *et al.*, 2012). Correlation analysis was done for the studied concentrations of metals in water & sediment with respect to the bioaccumulated metals in the different tissues. The main impact on the bioaccumulated metals in the different tissues was determined to be tissue specifically affected by both aqueous and sedimentary metal contents. The correlation-based study gives an indication about the potential relationships between metals: common source, related dependence and similar behaviors (Diop *et al.*, 2015). The aqueous metals showed positive correlation with Cu and negative correlation with Pb, Fe & Mn in almost all studied tissues which suggested that these metals had mutual sources, related dependence, uniform distribution and same performance in the aquatic environment. This present observation was supported by previous authors who endorsed that the bioaccumulated metals in fish are greatly affected by the concentration of metals in water they lived (Dsikowitzky *et al.*, 2013, PuYang *et al.*, 2015). While, the relationships between the accumulated metals in sediment and tissues was extremely diverse according to metal and tissue types suggested that metals in sediments does not share the above specific metal traits. In addition, sedimentary metals are easily re-suspended and modifying the distribution pattern between aqueous and particulate phases (Monferrán *et al.*, 2016). The bioaccumulated metals in the different tissues had different level of correlation with respect to metals concentrations in their surroundings, which showed that more intricate activities are involved during the bioaccumulation process including metal absorption efficiencies and efflux rates along the different tissues (Reis *et al.,* 2012).

HI

One of the main aims of this study was to recognize and quantify the potentially harmful metals in the edible tissues of fish that facing different pollution levels along river Nile. By this means, a risk assessment study has been done to establish the possible human health hazard around the studied sites. Bioaccumulation of metals in the edible tissues is a useful tool for examining the biological responsibility of those metals that present at elevated levels other than assessment of public health risk (Bastami *et al.,* 2015). In agreement with Omar *et al.* (2014), although the HI values do not pose any health hazards for the normal consumers, the levels are likely to be greater for fish-dependent consumers in addition the risk may significantly increase when these tissues are collectively consumed. Risk avoidance should then focus on diminishing the amount of discharged metals, especially those with long half-life time, into the hot spot sites of river Nile.

Conclusions

This study suggests that Nile tilapia was able to bioaccumulate several metals in its tissues with varying concentrations, and so it could be used as significant bioindicator to monitor metal pollution in many aquatic hot spots. Our studied metals showed different accumulation degrees in all components of the studied sites (water, sediment and fish) and accumulated to the highest extent at site3 in the vicinity of Sabal discharges. The spreading of metals with increasing distance from discharge point decreases metal concentrations to some extent. Some bioaccumulated metals in fish edible tissues were about to exceed safety threshold values which revealed alarming signs to consumer health.

Conflict of Interest

The authors declare that they have no conflict of interest.

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