RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Analysis And Pollution Assessment of Some Trace Heavy Metals in Freshwater, Drinking Water, Fish, and Sediments Samples in Suez Canal Region, Egypt by Flame Atomic Absorption Spectrometer

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Abstract

Monitoring of pollution levels of heavy metals in freshwater is significant to human health and environmental management. The objective of the current study was to monitor the freshwater quality in Suez Canal region, Egypt and to determine the efficacy of water treatment system in the removal of contaminates like heavy metals residues. The concentrations of some heavy metals (As, Cd, Cr, Pb, Hg, Ni, Cu, Mn) were determined in freshwater, treated water, in muscle of fish species (Tilapia nilotica) and sediment. The results of the study revealed that , all heavy metals concentrations in freshwater and treated water were within the permissible limits except, Lead concentration in treated water was higher than the permissible limits. In Port Said, all metals concentrations in both freshwater and treated water samples were within the permissible limits. Fish samples collected from the three governorates showed an increase in Cd and Pb levels. The heavy metals concentrations in sediment samples were remarkably high.

Keywords: Water; Fish; Sediment; Suez Canal region; Atomic Absorption; Heavy metals.

1. Introduction

Heavy metals normally occurring in nature are not harmful to the environment, because they play an essential role in tissue metabolism and growth of plants and animals (**Amundsen et al., 1997**). However one of the most crucial properties of these metals which differentiate them from other toxic pollutants is that they are not biodegradable in the environment (**Florence, 2007**). Their presence in the wastewater of several industrial processes, such as electroplating, metal finishing, metallurgical work, tanning, chemical manufacturing, mining and battery manufacturing, has brought about more environmental concerns due to their toxicity even at low concentrations (**Al-Rub, 2006**). In view of the human health impacts, each metal imparts different effects and symptoms. For instance, in the case of minor zinc (Zn) exposure, irritability, muscular stiffness, loss of appetite and nausea are common (**Bhattacharya et al., 2006**). Chromium (Cr) (**Martins et al., 2006**), on the other hand, can cause cancer in the digestive tract and lungs (**Kiran et al., 2007**), while manganese (Mn) triggers neurotoxicity, low hemoglobin levels, and gastrointestinal accumulation (**Parvathi et al., 2007**).As another example, copper (Cu) consumption in high doses brings about serious

toxicological concerns since it can be deposited in the brain, skin, liver, pancreas, and myocardium **(Vijayaraghavan etal., 2006),** and initiates intestinal distress, kidney damage and anemia **(Al-Rub et al., 2006)**Lead (Pb) has been cited as one of the three most toxic heavy metals that have latent long-term negative impacts on health, causing anemia, encephalopathy, hepatitis and nephritic syndrome **(Deng et al., 2006)** Cadmium (Cd) also exposes human health to severe risks, as it can provoke cancer, kidney damage, mucous membrane destruction, vomiting, diarrhea, bone damage, and itai-itai disease, as well as affect the production of progesterone and testosterone **(Johannes Godt et al., 2006)**. Moreover, the presence of nickel (Ni) exceeding its critical level might bring about serious lung and kidney problems aside from gastrointestinal distress, pulmonary fibrosis and skin dermatitis **(Borba et al., 2006).** Arsenic (As) is one of the most hazardous trace metals found in drinking waters is being both toxic and carcinogenic. Long term intake of arsenic may give rise to skin lesions **(WHO, 2001)** Finally, mercury (Hg) has been identified for its nervous system deterioration, including protoplasm poisoning **(Alluri et al., 2007).**

Therefore, the determination and monitoring of specific chemical forms of the heavy metals in environmental samples such as airborne particulates, waters, biological materials, soils or sediments are extremely important in the context of environmental protection, food and agricultural chemistry and high purity materials, and also for monitoring environmental pollution. Furthermore, it is now widely recognized that the total dissolved metal concentration is often a poor indicator of toxicity to aquatic organisms. In many instances, biological toxicity is related more closely to the concentrations of particular physico-chemical forms **(Das and Chakraborty, 1997).**

Sediments are the sink of metals in freshwater and marine environments. Total concentrations of most metals in sediments are several orders of magnitude higher than aqueous concentrations. However, total metals concentrations in sediments are not necessarily related to the biologically available metal concentrations. Many chemical extraction procedures have been proposed to estimate the concentration of metals in soils or sediments, which may be directly or indirectly available to organisms. Other studies report a good correlation between extractable metal concentrations and

metal content in some biota .The contamination of heavy metals is a serious threat because of their toxicity, long persistence, bioaccumulation and bio-magnification in the food chain. Fish samples can be considered as one of the most significant indicators in freshwater systems for the estimation of metal pollution level. In recent years, much attention has been directed to the concentrations of some inorganic elements in freshwater fish and other aquatic organisms. The commercial and edible species have been widely investigated in order to check for those hazardous to human health **(Meral Öztürk M et al., 2008).** It should be noted that this fish species are considered to be an essential part of the diet in the region.

Metals of major interest in bioavailability studies, as listed by the US Environmental Protection Agency (EPA) are aluminium, berylium, arsenic, cadmium, chromium, copper, mercury, nickel, lead, selenium, antimony. Other metals that are presently of lesser interest to the EPA are silver, barium, cobalt, manganese, molybdenum, sodium, thallium, iron and zinc. These metals were selected because of their potential for human exposure and increased health risk **(Greenberg et al., 2005).**

The literature survey reveals that heavy metals residues may be determined satisfactorily by a variety of methods, with the choice often depending on the precision and sensitivity required. Methods include colorimetric methods as well as instrumental methods, e.g. atomic absorption spectrometry (AAS) including flame (FAAS) **(Khajeh M, 2012),** electrothermal furnace (GFAAS) **(Escudero et al., 2012),** also heavy metals were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) **(Zhao et al., 2012)**, and anodic stripping voltammetry **(Fu et al., 2012)**

Flame atomic absorption methods generally are applicable at moderate (0.1- to 10-mgL^{-1}) concentrations in clean and complex-matrix samples. Electrothermal methods generally can increase sensitivity if matrix problems do not interfere. Inductively coupled plasma emission techniques are applicable over a broad linear range and are especially sensitive for refractory elements. Inductively coupled plasma mass spectrometry offers significantly increased sensitivity for some elements (as low as 0.01 $\mu g\ L^{-1}$) in a variety of environmental matrices **(Greenberg et al., 2005).**
The present study was carried out to assess the pollution effects with heavy metals on Suez Canal

region and to determine the bioaccumulation of these metals. The concentration of some heavy metals (As, Cd, Cr, Pb, Hg, Ni, Cu, Mn) were determined in freshwater, treated water, in muscle of fish species *(Tilapia nilotica)* and sediment from the three governorates of Suez Canal region (**Figure 1**) Ismailia, Port Said and Suez using atomic absorption spectrometer.

2. Experimental

2.1. Instrumentation

A Perkin-Elmer, *Series 711838 (v1.26)* atomic absorption spectrometer with deuterium background corrector was used in this study. With PerkinElmer single-element hollow cathode lamps were used in the AAS measurements. All measurements were carried out in guidelines of the manufacturers. A 10-cm long slot-burner head, a lamp and an air/acetylene flame were used. Each measurement was made in triplicate and the absorbance due to background was subtracted from the mean of the three values.

2.2. Reagents

All chemicals used in this work, were of analytical reagent grade and were used without further purification. Deionized water was used for the preparation of solutions. All the plastic and glassware were rinsed three times with tap water, once with chromic acid, three times with tap water, once with 1:1 nitric acid and then three times with double distilled water in that order and kept in the oven at 110 °C till need. Sampling bottles were further rinsed with a part of the sample water before sample collection.

The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg L^{-1} of the given element supplied by Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA). Stock solutions of diverse elements that were the matrix components of the natural water samples and/or have interfering effects in flame atomic absorption spectrometric determinations were prepared from their high purity compounds.

2.3. Sampling

The concentrations of the studied metals (Pb, Cr, Cd, Mn, Cu, Ni, Hg, and As) were determined in freshwater, treated water and sediment samples. Fish samples were tested for Hg, Cd, and Pb only. Samples were collected from Ismailia, Port Said and Suez in Suez Canal Region (Figure 1).
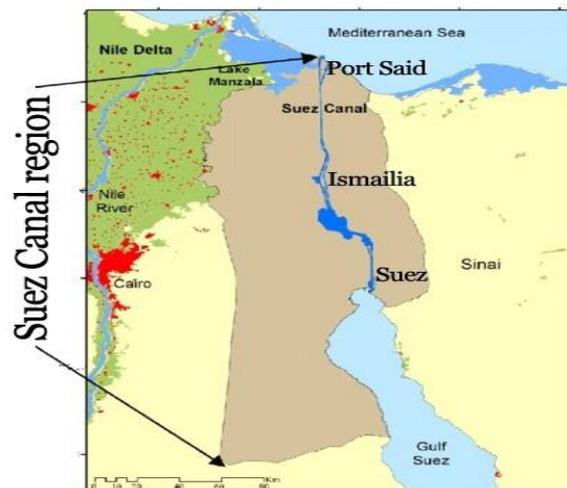

Figure 1: Map of Suez Canal region

2.3.1 Fresh water samples

The samples were collected bimonthly in 1 L polypropylene bottles 15-20 centimeter depth at the selected sites in Ismailia, Port Said and Suez. Sampling bottles were washed three times with the sample then samples collected and acidified immediately in the field with nitric acid and preserved in cool ice boxes throughout the field trip and during transportation to the laboratory. Seventeen samples were collected from each site during the research period.

2.3.2 Tap water samples

The samples were collected bimonthly in 1 L polypropylene bottles at the same times as freshwater sampling. The tap was turned on to a steady stream for at least 2-3 minutes to remove any stagnant water in the plumbing network water was allowed to flow until a constant temperature is reached (practically after 10 min flow) then the bottles were rinsed three times with this water then samples were collected. Samples were acidified immediately in the field with nitric acid and preserved in cool ice boxes.

2.3.3 Fish samples

Three fish samples were collected from each of the three described sites in Ismailia, Port Said and Suez. Samples were collected in monthly by fishermen using a cast net at the same times as freshwater and sediment sampling. Healthy and vigorous fish **Tilapia *nilotica species* (Bolti)** samples were chosen. Samples were placed in ice-cooled boxes and transported to the laboratory where they were washed with deionized water, dried in filter paper, the soft parts of fish samples were removed using a plastic knife and a muscle tissue sample (5 gm) was taken from the dorsal muscle and packed in polyethylene bags and stored below -20 °C until analysis.

2.3.4. Sediment Samples

The top 20 cm of the bottom sediment samples were collected using the Ekman bottom sampler and kept in glass bottles (1 kg). Three samples were collected from each of the three described sites in Ismailia, Port Said and Suez. Samples were collected at the same times as freshwater and fish sampling.

2.4. Sample preparation.

2.4.1 Freshwater and tap water sample preparation

According to APHA **(Aksu and Olsson, 2000)** Transfer a measured volume (500 mL) of well-mixed, acid-preserved sample to 2 L borosilicate glass beaker. In a hood add 3 mL conc. HNO_3 and cover with a ribbed watch glass. Place the beaker on a hot plate and cautiously evaporate to less than 20 mL, making certain that sample does not boil and that no area of the bottom of the container is allowed to go dry. Cool and rinse down walls of beaker and watch glass with a minimum of metal-free water and add 5 mL conc. HNO_3. Cover container with a non-ribbed watch glass and return to hot plate. Increase temperature of hot plate so that a gentle reflux action occurs. Continue heating, adding additional acid as necessary, until digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing). Cool. Add 10 mL 1 : 1 HCl and 15 mL water per 100 mL anticipated final volume. Heat for an additional 15 min to dissolve any precipitate or residue. Cool, wash down beaker walls and watch glass with water, filter to remove insoluble material and transfer filtrate to a 100-mL volumetric flask with rinsings.

2.4.2 For Fish samples

Fish flesh was minced and well mixed; the separated muscles were put in petri dishes to dry at 120 °C until reaching a constant weight then approximately 1.0 gm of the dried tissue was placed in a 150 mL beaker and adding 5 mL HNO_3 (65% nitric acid) and 0.5 mL H_2O_2 (30% hydrogen peroxide, Merck) and by heating on a hot plate **(Pettersson et al., 1998).** After cooling down, digestion vessels were vented and the digested mixture was then cooled and transferred to a 50 mL volumetric flask and brought to volume with deionized water.

2.4.3 For sediment sample

Sediment samples were allowed to defrost then air-dried in a circulating oven at 30 °C and sieved mechanically using a 2 mm sieve. One gram of dried sediment was digested using mixture of concentrated acids (HCl, $HClO_4$, and HNO_3) in ratio 1:1:1. The beakers were covered with a watch glass and set aside for 15 min in order to allow the initial reaction to subside. The beaker and its contents were heated at a temperature of the hot plate at 160° C. This boiling was done for 30 min and occurred with a reduction in volume. The contents of the beaker were transferred into a 50 ml volumetric flask and diluted to the mark with deionized water. The concentrations of the described heavy metal were determined using an atomic absorption spectrophotometer. Average concentrations were then calculated and converted from mg L^{-1} to mg kg^{-1} dry weight.

2.4.4 Metal analysis

The same volume of acid, aqua regia and digestion solution, which used in the digestion of water, sediment and fish samples were subjected to the same digestion, dilution and filtration procedures as previously described in the preparation of water, sediment and fish samples to detect any possible traces of the studies metals in the acids or distilled water.

Fish samples were tested for Pb, Hg, and Cd only. Quantitative determination of Pb, Cr, Cd, Mn, Cu and Ni was conducted by using flame atomic absorption spectrometer (FAAS) using Perkin-Elmer, *Series 711838 (v1.26)* atomic absorption spectrometer with deuterium background corrector. A PerkinElmer single-element hollow cathode lamps were used in the AAS measurements and a deuterium background corrector, at respective resonance line using an air-acetylene flame. Concentrations of Hg and As were determined using hydride generation atomic absorption. Recoveries were carried out by the addition of the standards of each element at different levels. All data were corrected according to the recovery percentage values.

Blanks were included in each batch of analysis. Calibration standards were regularly performed to evaluate the accuracy of the analytical method. Working calibration standards of Pb, Cr, Cd, Mn, Cu, Ni, Hg and As were prepared by serial dilution of concentrated stock solution (Merck, Germany) of 1,000 mg L^{-1}. These and blank solutions were also analyzed in the same way as for the digested samples.

3. Result and discussion

3.1. Freshwater and tap water sample preparation

The mean concentrations of different heavy metals in raw and tap water samples collected from Ismailia, Port Said and Suez samples are illustrated in tables [1- 3]. In samples collected from Ismailia freshwater the mean of metals concentrations were found in the following order: Cu > Pb > Cr > Mn > Ni > As > Cd > Hg, whereas they follow the order of Cr > Mn > Pb > Cu > Ni > As >Cd >Hg in Port Said freshwater, but in Suez freshwater, metals had the sequence of Mn > Cr > Ni > Pb > Cu > As > Cd > Hg.

In Ismailia and Suez all heavy metals concentration in both tap water and freshwater were within the acceptable limits except Lead concentration in tap water was higher than the standard limits of the Egyptian ministry of health. Whereas, in Port Said all metals concentrations in both tap water and freshwater were within the acceptable limits according to the standard limits of the Egyptian ministry of health. This is may be due to industrial wastes and water pipes. However, heavy metals in Port Said in both tap and untreated water were within acceptable limits.

3.2. Fish samples

Metal concentrations in fish samples collected from Port Said, Ismailia and Suez are illustrated in table 4. In Egypt the permissible limits of heavy metals in fish are regulated by the Egyptian standard specification (number 2360/2007) for fish products. The permissible limits of Pb, Hg and Cd in fish according to the Egyptian standard specification are 300, 500 and 100 µg kg^{-1} respectively and they are the same as those given by the WHO.

The mean of metals concentrations in fish samples collected from Ismailia and Port Said were found in the following order: Pb > Cd> Hg> while in Suez were in the following order Pb > Hg > Cd. The average concentration of lead was 277.66, 255.66 and 383.66 µg kg^{-1} for Port Said, Suez and Ismailia; respectively.

Table 1: Average concentration of different heavy metals (µg L^{-1}) in raw water and tap water samples collected from Ismailia

Metal	Concentration (µg L^{-1})		Egyptian limits of raw water	MCL in drinking water		
	Raw water	Tap water		WHO	EPA	EMOH
Pb	23.6	11.32	**50**	10	0	10
Cr	19.5	3.1	**50**	50	100	50
Cd	2.03	BDL	**10**	3	5	3
Mn	18.4	4.75	500	---	---	400
Cu	31.23	6.35	1000	2000	1300	2000
Ni	13.75	1.52	**--**	70	100	20
Hg	BDL	BDL	1	6	2	1
As	7.8	BDL	**50**	10	10	10

MCL: maximum contaminant level

BDL: below detection limit

WHO, Guidelines for Drinking-water Quality Fourth edition

EPA, 2012 Edition of the Drinking Water Standards and Health Advisories.

EMOH, Egyptian ministry of health.

Table 2 : Average concentration of different heavy metals (µg L^{-1}) in raw water and tap water samples collected from Port Said

Metal	Concentration (µg L^{-1})		Egyptian limits of raw water	MCL in drinking water		
	Raw water	Tap water		WHO	EPA	EMOH
Pb	26.8	4.2	**50**	10	0	10
Cr	29.2	BDL	**50**	50	100	50
Cd	1.8	BDL	**10**	3	5	3
Mn	28.5	4.75	500	---	---	400
Cu	24.23	6.35	1000	2000	1300	2000
Ni	19.22	1.52	--	70	100	20
Hg	BDL	BDL	1	6	2	1
As	13.3	BDL	**50**	10	10	10

MCL: maximum contaminant level
BDL: below detection limit
WHO, Guidelines for Drinking-water Quality Fourth edition
EPA, 2012 Edition of the Drinking Water Standards and Health Advisories.
EMOH, Egyptian ministry of health.

Table 3: Average concentration of different heavy metals (µg L^{-1}) in raw water and tap water samples collected from Suez

Metal	Concentration (µg L^{-1})		Egyptian limits of raw water	MCL in drinking water		
	Raw water	Tap water		WHO	EPA	EMOH
Pb	28.5	11.18	**50**	10	0	10
Cr	29.2	BDL	**50**	50	100	50
Cd	3.9	BDL	**10**	3	5	3
Mn	33.2	1.4	500	---	---	400
Cu	21.97	BDL	1000	2000	1300	2000
Ni	29.1	2.2	--	70	100	20
Hg	BDL	BDL	1	6	2	1
As	12.1	BDL	**50**	10	10	10

MCL: maximum contaminant level
BDL: below detection limit
WHO, Guidelines for Drinking-water Quality Fourth edition
EPA, 2012 Edition of the Drinking Water Standards and Health Advisories.
EMOH, Egyptian ministry of health.

Table 4: Concentration of Pb, Cd and Hg in fish samples collected from Port Said, Suez and Ismailia

Port Said					
Metal (µg/kg)	Sample 1	Sample 2	Sample 3	Average	WHO and Egyptian permissible limits
Pb	341	343	149	277.67	300
Cd	169	121	139	143.00	100
Hg	95	43	83	73.67	500
Suez					
	Sample 1	Sample 2	Sample 3	Average	WHO and Egyptian permissible limits
Pb	291	317	159	255.67	300
Cd	57	72	145	91.33	100
Hg	81	75	126	94.00	500
Ismailia					
	Sample 1	Sample 2	Sample 3	Average	WHO and Egyptian permissible limits
Pb	220	652	279	383.67	300
Cd	178	63	113	118.00	100
Hg	163	68	94	108.33	500

The average concentration of cadmium was 143, 91.33 and 118 µg kg^{-1} for Port Said, Suez and Ismailia; respectively and the average concentration of mercury was 73.66, 94 and 108.33 µg kg^{-1} for Port Said, Suez and Ismailia; respectively. The average cadmium levels of samples collected from Ismailia and Port Said are above the permissible limits.

The average lead levels in fish samples collected from Ismailia is above the permissible limits. The three samples collected in January-2009 showed an increase of lead levels than the permissible limits. All the samples collected from Port Said showed an increase in cadmium levels and two samples showed increase in lead levels.

3.3. Sediment samples

The mean of eight trace metals concentrations measured in sediment samples collected from Ismailia, Port Said and Suez are illustrated in table 5.

In Ismailia the metals concentrations were found in the following order: Mn > Cr > Ni > Cu > Pb > As > Cd > Hg, whereas in Port Said they followed the order of Mn > Cr > Cu > Ni > Pb > As > Cd > Hg, however in Suez, metals had the sequence of Mn > Ni >Cr > Cu > Pb > As > Cd > Hg. In view of the concentration levels of studied metals, the highest values were manganese at all samples from the three sites while mercury was the lowest; it was detected in one sample, which collected from Ismailia.

4. Conclusion

In Ismailia and Suez all heavy metals concentration in both tap water and freshwater were within the acceptable limits except Lead concentration in tap water was higher than the standard limits of the Egyptian ministry of health. Whereas, in Port Said all metals concentrations in both tap water and freshwater were within the acceptable limits according to the standard limits. Fish samples collected from the three governorates showed an increase in cadmium and lead levels. This may be due to industrial wastes, which needs more attention and monitoring.Sediment samples showed high levels of the studied heavy metals, so sediment act as heavy metal reservoir causing continuous water contamination especially during dredging processes which may cause dissolution of metals in water and rising the levels of heavy metals in water which suggest careful monitoring.

Table 5: Concentration of different heavy metals (mg kg^{-1}) in sediment samples collected from Port Said, Suez and Ismailia

Metal (mg kg^{-1})	Ismailia				Port Said				Suez			
	Sample 1	Sample2	Sample 3	Average	Sample 1	Sample2	Sample 3	Average	Sample 1	Sample2	Sample 3	Average
Pb	26.20	23.80	21.60	23.87	31.60	36.00	18.80	28.80	18.40	14.80	17.20	16.80
Cr	34.40	79.30	46.10	53.27	32.70	51.40	52.80	45.63	46.40	23.20	80.30	49.97
Cd	0.84	1.21	1.10	1.05	0.96	1.32	1.64	1.31	0.80	1.64	1.92	1.45
Mn	644.80	411.20	169.60	408.53	192.80	446.84	226.80	288.81	471.10	488.20	504.80	488.03
Cu	28.73	36.20	29.40	31.44	45.90	42.90	26.40	38.40	29.70	33.50	26.30	29.83
Ni	37.30	44.60	58.90	46.93	38.40	22.80	42.20	34.47	40.40	74.00	57.20	57.20
Hg	0.37	ND	ND	0.13	ND	ND	ND		ND	ND	ND	
As	ND	0.92	3.90	1.61	ND	6.70	8.40	5.03	3.10	8.40	6.30	5.93

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