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Some Heavy Metals Status in Ashtoum El-Gamil Protected Area

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ABSTRACT

A survey study was conducted on some heavy metals (Pb, Cd, Cu, Zn, and Fe) in water, sediment, and fish samples from Ashtoum El-Gamil protected area during May 2010 to January 2011. Data obtained revealed that there were significant ($P \leq 0.0001$) differences among sampling seasons and stations as well as their interactions concerning the levels of heavy metals tested in either water, sediment, or fish collected from this protected area. The elements level took the descending order Zn ≥ Cd ≥ Pb ≥ Fe ≥ Cu in the water, Pb ≥ Fe ≥ Cu ≥ Zn ≥ Cd in the sediment, and Fe ≥ Pb ≥ Zn ≥ Cu ≥ Cd in the fish body samples. Proximate analysis of the tested fish (mullet and tilapia) reflected also significant ($P \leq 0.0001$) effects due to sampling seasons and stations and their interactions besides fish species. Some significant correlations were calculated among heavy metals (in water, sediments, and fish) and chemical composition of the fish.

Keywords: Protected area, water, sediment, fish, heavy metals.

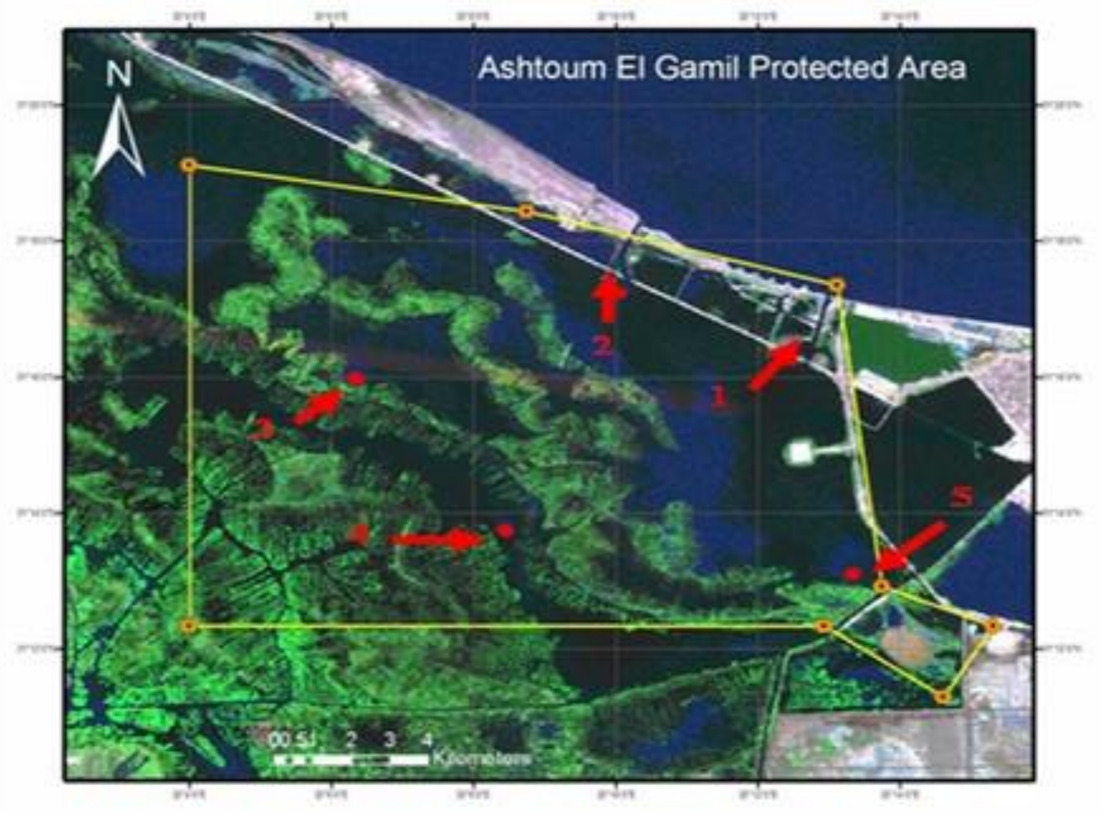
INTRODUCTION

Ashtoum El-Gamil and Tenis Island are declared as a protected area by Prime Minister's decree No. 459 / 1988 with an area of about 30 km^2 later was amended with decree No. 2780 /1998 to extend its area to be about 180 km^2. Ashtoum El-Gamil is located in the western north corner of Lake Manzalah, Egypt including new and old El-Gamil inlets; as well as the historical Tenis Island, with an area of about 8 km^2, that lies on the south west of Port Said city. The historical Tenis hill surrounded with water at a distance of about 300 m from all sides. Two types of water intrude with the lake: salty water from the Mediterranean, through the two inlets of El-Gamil in the North and fresh water from the South through number of agricultural drainages (such as El-Serw - Hados - Ramses) in addition to sanitary sewage drainages such as Bahr El-Baqar and El-Aninia drainages. Bahr El-Baqar drainage is one of the most important sources of pollution in the lake, as it discharge more than 1.5 million m^3 of wastewater daily, including more than 1.25 million m^3 from greater Cairo. All of these drainages located outside boundaries of Ashtoum El-Gamil protected area and can not be controlled by Ashtoum El-Gamil authority (Abd El-Karim, 2008). One of the important functions of Ashtoum El-Gamil protected area is the protection of gravid fish and fry during their passage to and from the Mediterranean Sea through El-Manzalah Lake. Ashtoum El-Gamil represents a modest example of a highly threatened and rapidly disappearing habitat in Egypt and the Mediterranean basin (Ahmed *et al*., 2000 and EEAA, 2003)**.**The objective of the present work was the tracing of the pollution status of this protected area, including water, fish (Nile tilapia and mullet) and sediment, concerning their heavy metals content (Cd, Pb, Cu, Zn and Fe).

MATERIALS AND METHODS

Sampling Locations

El-Manzalah Lake is the largest of the Nile Delta lakes. It is located in the northeastern part of Egypt, bounded on the east by the Suez Canal and on the west by Damietta branch of the Nile and separated from the Mediterranean Seaby a narrow sandy fringe at the north. The lake is connected to the Mediterranean Seathrough a narrow channel (Boughaz El-Gamil). The islands and reed beds divide the lake into well defined basins each is known as Bahr having more or less distinctive ecological conditions (Abdel-Baky *et al*., 1991). Samples were collected from five stations, being: station 1: The old inlet of El-Gamil in the north-east, station 2: The new inlet of El-Gamil in the north-west, station 3: Sea Kassab near to the middle, station 4: Sea Bashtier in the south-west and station 5: Sea Kur in the south-east, as illustrated in Fig. 1.



Collection of fish samples

Nile tilapia and mullet fishes were selected for the present study and collected from four sites in Ashtoum El-Gamil protectorate during the four seasons from May 2010 to January 2011, where the two species have a wide range of distribution in the aquatic habitat in Egypt. Fish samples were transported immediately to the laboratory in an ice-box for chemical analyses.

Collection of water samples

Water samples were collected in clean 1 liter polyethylene bottles from a depth of 50 cm of the sampling locations. Each sample was divided for the chemical and the bacteriological examinations. For heavy metal detection, samples were fixed with 10% formalin solution. Samples were kept for analysis in a refrigerator at + 4°C.

Collection of sediment samples

Samples of sediments were collected from the same locations using a dredge. Sediment samples were transported in polyethylene bags to the laboratory and kept at room temperature for analyses.

Samples preparation for heavy metals determination

1-Fish

Samples from each location for each season were homogenized. Three grams of each homogenized samples were transferred into acid washed screw - capped digestion tube with a polyethylene stopper. Ten ml of pure Analar concentrated nitric acid (99.9% -Merck) were added and thoroughly mixed then kept over night at room temperature. The mixture was heated in a shaking water bath at 65 - 70°C for 6 hours. The mixture was cooled at room temperature then few drops of 30% hydrogen peroxide were added. The tubes were reheated with the same previous manner but for 3 hours only. After cooling, 10 ml of deionized water were added, to dilute the digests. The dilute digests were filtered and the clear filtrates were kept in screw - capped tubes in a refrigerator, and then were used for detection of heavy metals.

2-Water

One-hundred ml of each collected water sample was transferred into clean glass bottles with 0.3 ml of pure concentrated nitric acid (99.9% - Merck) according to Polprasert (1982).

3-Sediment

The procedure outlined by Medina *et al.* (1986) was performed for preparing the sediment samples. The sediment samples were air dried at room temperature till complete dryness. After that, the samples were ground in a porcelain mortar. Five grams of each homogenized sample were transferred into a screw - capped digestion tube. Ten ml of pure concentrated nitric acid (99.9% - Merck) were added for digestion. The tubes were left over night at room temperature, and then transferred into a shaking water bath at 65 - 70°C for 6 hours. The tubes were left to cool to room temperature. Few drops of 30% hydrogen peroxide were added. The sediment digests were diluted with deionized water, and then filtered through Whattmann filter papers No. I, and kept in closed 30 ml - screw capped tubes in a refrigerator.

Elements determination

Perkin Elmer atomic absorption spectrophotometer (AAS) model 2380 equipped with MHS-10 hydride generation system was used for the quantitative determination of the studied elements (Fe, Cu, Pb, Cd, and Zn) according to Abdallah *et al*. (1993). Fe, Cu, Cd, Pb and Zn irons were analyzed using an air/acetylene (flow rate of 5.5/1.1 Vm) flame atomic absorption spectrophotometer. The burner height was 8 cm, fuel flow 30, oxidant flow 60, and slide width 0.7 nm. Blank samples from the used chemicals as well as specked samples (internal standards) were undertaken to correct the obtained data.

Chemical analysis of whole fish

A proximate analysis of whole fish samples from different sampling locations [mullet from sampling locations No. 1 and 2 (marine water fish) and Nile tilapia from sampling locations No. 3 and 4 (fresh water fish)], and seasons of sampling were analyzed according to A.O.A.C. (2000) for dry matter, crude protein, ether extract, and ash.

Statistical analysis

Using S.A.S. (2001) and Duncan (1955), numerical data collected were statistically analyzed for analysis of variance and least significant difference as well as Pearson correlation.

RESULTS AND DISCUSSION

Heavy metals in water of the protected area

Table 1 presents mean values for the effect of both collection seasons and collection stations of the fish rearing water as well as their interaction on some heavy metals (Pb, Cd, Cu, Zn and Fe). It reflects their significant ($P \leq 0.0001$) effects on these elements' contents in the water. The significant ($P \leq 0.0001$) high values were found in spring samples (for Zn), summer samples (for Pb and Cd), autumn samples (for Cu and Fe) and in station No. 2 (for Zn), station No.3 (for Cd), station No. 4 (for Pb), and station No.5 (for Cu and Fe). Samples of station No.4 during summer season gave the highest Pb and Cd values, being 1.0 and 1.4 ppm, respectively. The highest Cu level (0.160 ppm) was found in samples of station No.4 in autumn season. The highest Zn value (3.200 ppm) was given in water samples from station No.4 collected in spring season. Fe level was highest (0.300 ppm) in samples from station No.5 collected in autumn season. Generally, these studied elements took the following descending order: Zn ≥ Cd ≥ Pb ≥ Fe ≥ Cu in water. Therefore, assuming that average Zn level (1.805 ppm) is 100, then mean Pb level is 20.85%, Cd 28.95%, Cu 4.71%, and Fe 7.15% of Zn mean level. However, Pb, Cd, Cu, Zn, and Fe took the following ranges in the water: 0.08 – 1.00, 0.3 – 1.40, 0.04 – 0.16, 0.80 – 3.20, and 0.03 – 0.30 ppm, respectively.

Similar results, concerning seasonal and location effects were reported before by Abdelhamid *et al*. (2006b) who mentioned that Marsa Matroh water was the highest in Pb contents (in both seasons) among the different sampling locations. They added that other two elements (Fe and Cd) had variable trends from location to another. They found also, in summer season, Pb was > Fe > Cd; but in winter, Fe was > Cd > Pb. Also, Abdelhamid *et al.*(1997 and 2000) registered significant variations in heavy metals concentration in the natural fisheries water due to sampling locations. Heavy metals pollution of the Egyptian aquatic media was reviewed by Abdelhamid (2006). However, Pb content was significantly higher in feedstuffs, water and blood in winter than in summer (Abdelhamid and El-Ayouty, 1989). Lead causes hemorrhages and congestion of the gastrointestinal tract and kidneys of fish (Abdelhamid and El-Ayouty, 1991). The no effect level of Cd, Pb and Fe in water for growing aquatic life are 0.03, 0.10 and 1.00 ppm, respectively (Yokokawa, 2000). Comparing these standards with the levels obtained herein, it would be indicated that there is water pollution with heavy metals (Pb and Cd) in all tested locations and seasons.

Zyadah (1997) reported significant effects on water mineral contents (containing Cd and Pb) due to different locations and seasons. Also, he found high levels of heavy metals in the sediment and fish, exceeded the allowable limit. Yet, Aboul-Naga (2000) confirmed that sediment samples from Abu-Qir

Table 1*: Mean values for effect of collection season and station on some heavy metals content (ppm) in fish rearing water.*

HM	Pb	Cd	Cu	Zn	Fe
Season					
Spring	0.302^{b}	0.566^{b}	0.090^{b}	2.280^{a}	0.096^{d}
Summer	0.808^{a}	1.066^{a}	0.090^{b}	1.580^{c}	0.108^{c}
Autumn	0.132^{d}	0.402^{c}	0.104^{a}	1.680^{b}	0.160^{a}
Winter	0.264^{c}	0.056^{d}	0.056^{c}	1.680^{b}	0.152^{b}
± SE	0.000	0.000	0.000	0.000	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001
Station					
1	0.333^{e}	0.528^{c}	0.090^{b}	1.700^{c}	0.140^{c}
2	0.383^{c}	0.410^{e}	0.080^{d}	2.150^{a}	0.125^{d}
3	0.385^{b}	0.643^{a}	0.055^{e}	1.600^{d}	0.088^{e}
4	0.425^{a}	0.478^{d}	0.088^{c}	1.975^{b}	0.143^{b}
5	0.358^{d}	0.555^{b}	0.113^{a}	1.600^{d}	0.150^{a}
± SE	0.000	0.000	0.000	0.000	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001
Season* Station					
Sp*1	0.290	0.600	0.150	1.600	0.050
Sp*2	0.260	0.500	0.100	2.300	0.100
Sp*3	0.340	0.670	0.050	2.200	0.050
Sp*4	0.250	0.330	0.050	3.200	0.140
Sp*5	0.370	0.730	0.100	2.100	0.140
Su*1	0.760	0.950	0.050	1.500	0.160
Su*2	0.760	0.680	0.100	2.500	0.170
Su*3	0.720	1.300	0.050	1.600	0.110
Su*4	1.000	1.400	0.100	1.100	0.050
Su*5	0.800	1.000	0.150	1.200	0.050
A*1	0.080	0.500	0.120	1.400	0.130
A*2	0.150	0.400	0.040	1.200	0.070
A*3	0.200	0.570	0.080	1.500	0.030
A*4	0.130	0.110	0.160	2.000	0.270
A*5	0.100	0.430	0.120	2.300	0.300
W*1	0.200	0.060	0.040	2.300	0.220
W*2	0.360	0.060	0.080	2.600	0.160
W*3	0.280	0.030	0.040	1.100	0.160
W*4	0.320	0.070	0.040	1.600	0.110
W*5	0.160	0.060	0.080	0.800	0.110
± SE	0.000	0.000	0.000	0.000	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001

HM: heavy metal, Sp: spring, Su: summer, A: autumm, W: winter, SE: standard error, P: probability

Bay and in front of El-Maadiya channel indicate a non-polluted environment with trace elements including Cd, Fe and Pb. However, he reported high trace metal concentrations in front of El-Tabia Pumping Station. Iron was the dominant metal in all humic acids and sediments examined. Humic acids are trace metals holders in the sediments, therefore humic acids play a major role in the geochemical cycling of the elements in the aquatic environment. Moreover, Radwan (2000) reported average values of dissolved heavy metals (Cd, Fe and Pb) in Lake Burullos water as 1.93, 2.46 and 2.67 mg/l, respectively. He added that, levels of heavy metals are correlated with salinity changes due to the discharge of water. Hussein and Mekkawy (2001) revealed that fish reflect further mechanisms to avoid lead impacts such as the secretion of intestinal mucus that bind lead.

Cd in water negatively affected fish growth and feed and vitamin C utilization. Fe also is toxic for fish, since it damages fish gills and their function. Pb reduces hemoglobin content and red blood cells count of fish (Abdelhamid, 2003). However, any degree of poisoning will weaken the fish, making it vulnerable towards disease. Heavy metals can create problems and be concentrated in waterway organisms up to 9100 times more than the surrounding environment's levels, so may lead to acute or chronic effects (WRC, 2005). However, Abdelhakeem *et al.* (2002) cited the tolerance limits of Pb, Fe and Cd in fish water as 0.10, 0.35 and 0.10 ppm, respectively and in fish body as 2, 30 and 0.5 ppm, respectively. Heavy metal concentrations in fish varied significantly depending on the type of the tissue, fish species and sampling location. Yet, there was no significant seasonal variation in marine water metal (Cd and Pb) concentrations (Kucuksezgin *et al.*, 2006). However, heavy metal contamination of water is one of the environmental stressors affecting significantly and negatively lysozyme activity of fish serum, intestinal scrapping and skin mucus as well as serum hemolytic activity, leukocytes count, packed cell volume, hemoglobin concentration, plasma protein and glucose concentrations (Abdelhamid *et al.*,2006a).

Heavy metals in the protected area's sediments

Mean values of the heavy metals tested (Pb, Cd, Cu, Zn and Fe) in sediments of Ashtoum El-Gamil protected area are given in Table 2, which reflects significant ($P \leq 0.0001$) effects on these elements' contents in the sediment due to season and station of sampling as well as to their interaction (season X station of collecting samples). The significant ($P \leq 0.0001$) high values were found in autumn samples (for Pb and Cu), winter samples (for Cd), and spring samples (for Zn and Fe) and in station No. 4 (for the five elements). Samples of station No.4 during winter season (for Pb, Cd, and Cu) and the same station during spring (for Zn and Fe) gave the highest values, being 53.4, 0.69, 12.40, 5.80 and 28.40 ppm, respectively. Generally, these studied elements took the following descending order: Pb ≥ Fe ≥ Cu ≥ Zn ≥ Cd in the sediment. Therefore, assuming that mean Fe level (16.9 ppm) is 100, so Pb mean level is 91.36%, Cd 1.09%, Cu 22.57%, and Zn 11.73% of the average Fe level. However, the elements ranged 0.60 – 53.40, 0.05 – 0.69, 0.48 – 12.40, 0.30 – 5.80, and 11.00 – 26.60 ppm for Pb, Cd, Cu, Zn, and Fe, respectively. Similar results, concerning seasonal and location effects were reported before by Abdelhamid *et al.* (2006b), who found that sediments from the same sampling locations reflected higher concentrations in winter (0.049-0.284 and 5.879-500.0 ppm) than in summer (0.030-0.047 and 24.85-28.60 ppm) for Cd and Fe, respectively (except the 2^{nd} location in Fe) but the opposite was true for Pb (0.841-2.780 and 0.064-0.739 ppm in summer and winter, respectively) in all locations. Generally, they added that sediment samples from the four locations tested contained concentrations of Fe > Pb > Cd. There was no specific trend for these elements level in the sediments as affected by the sampling locations. However, Abdelhamid *et al*. (1997) registered significant variations in

Table 2: ***Mean values for effect of collection season and station on some heavy metals content in sediments (ppm, dry matter basis) of Ashtoum El-Gamil protected area***

HM	Pb	Cd	Cu	Zn	Fe
Season					
Spring	18.920^{b}	0.212^{b}	4.420^{b}	2.380^{a}	17.920^{a}
Summer	8.992^{d}	0.109^{d}	1.964^{d}	1.440^{d}	15.080^{d}
Autumn	19.428^{a}	0.188^{c}	4.836^{a}	1.992^{c}	17.760^{b}
Winter	14.412^{c}	0.225^{a}	4.040^{c}	2.116^{b}	16.840^{c}
± SE	0.000	0.000	0.000	0.000	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001
Station					
1	2.545^{c}	0.121^{c}	1.670^{c}	0.835^{d}	18.250^{c}
2	1.460^{e}	0.059^{e}	0.625^{e}	0.430^{e}	12.850^{d}
3	2.400^{d}	0.073^{d}	1.305^{d}	0.870^{c}	12.400^{e}
4	47.100^{a}	0.489^{a}	10.025^{a}	5.250^{a}	22.150^{a}
5	23.685^{b}	0.176^{b}	5.450^{b}	2.525^{b}	18.850^{b}
± SE	0.000	0.000	0.000	0.000	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001
Season* Station					
Sp*1	2.40	0.13	1.60	0.86	17.80
Sp*2	2.80	0.05	0.90	0.34	13.40
Sp*3	4.60	0.07	1.10	0.70	12.40
Sp*4	51.60	0.56	11.90	5.80	28.40
Sp*5	33.20	0.25	6.60	4.20	17.60
Su*1	1.66	0.05	0.68	0.68	20.80
Su*2	1.30	0.05	0.48	0.62	11.20
Su*3	1.00	0.09	1.46	0.68	11.00
Su*4	37.20	0.29	6.20	4.40	18.20
Su*5	3.80	0.07	1.00	0.82	14.20
A*1	1.80	0.14	2.80	1.10	18.80
A*2	1.14	0.07	0.52	0.46	13.80
A*3	2.60	0.07	1.06	0.80	12.80
A*4	46.20	0.42	9.60	5.00	16.80
A*5	45.40	0.25	10.20	2.60	26.60
W*1	4.32	0.17	1.60	0.70	15.60
W*2	0.60	0.06	0.60	0.30	13.00
W*3	1.40	0.06	1.60	1.30	13.40
W*4	53.40	0.69	12.40	5.80	25.20
W*5	12.34	0.14	4.00	2.48	17.00
± SE	0.000	0.000	0.000	0.000	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001

HM: heavy metal, Sp: spring, Su: summer, A: autumm, W: winter, SE: standard error, P: probability

Table 3*: Effect of collection season and station on some heavy metals content in fish carcass (ppm, dry matter basis)*

HM	Pb	Cd	Cu	Zn	Fe
Season					
Spring	64.25[c]	3.46[a]	21.27[a]	82.00[a]	1100[b]
Summer	36.75[d]	0.00[b]	9.00[c]	72.75[b]	868[c]
Autumn	131.75[b]	0.00[b]	11.00[b]	71.25[d]	594[d]
Winter	154.50[a]	0.00[b]	4.13[d]	71.50[c]	1303[a]
± SE	0.020	0.013	0.015	0.034	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001
Station					
1 (M)	72.75[d]	2.50[a]	18.01[a]	67.00[d]	1216.5[a]
2 (M)	86.00[c]	0.33[c]	13.51[b]	76.50[b]	1114.5[b]
3 (T)	136.00[a]	0.00[d]	7.88[c]	70.75[c]	854.0[c]
4 (T)	92.50[b]	0.63[b]	6.00[d]	83.25[a]	680.3[d]
± SE	0.020	0.013	0.015	0.034	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001
Season* Station					
Sp*1	166.00	10.00	51.03	81.00	1895.0
Sp*2	20.00	1.30	19.03	88.00	695.0
Sp*3	49.00	0.00	9.00	64.00	1011.0
Sp*4	22.00	2.53	6.00	95.00	799.0
Su*1	0.00	0.00	4.00	63.00	979.0
Su*2	0.00	0.00	13.00	70.00	1070.0
Su*3	147.00	0.00	14.00	77.00	758.0
Su*4	0.00	0.00	5.00	81.00	666.0
A*1	35.00	0.00	7.00	56.00	524.0
A*2	124.00	0.00	22.00	64.00	856.0
A*3	159.00	0.00	8.00	81.00	479.0
A*4	209.00	0.00	7.00	84.00	516.0
W*1	90.00	0.00	10.00	68.00	1468.0
W*2	200.00	0.00	0.00	84.00	1837.0
W*3	189.00	0.00	0.50	61.00	1168.0
W*4	139.00	0.00	6.00	73.00	740.0
± SE	0.040	0.026	0.031	0.069	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001

HM: heavy metal, Sp: spring, Su: summer, A: autumn, W: winter, SE: standard error, P: probability

heavy metals concentration in the natural fisheries sediments due to sampling locations.

Heavy metals in the protected area's fish

Data presented in Table 3 illustrate mean values of heavy metals (Pb, Cd, Cu, Zn and Fe) concentrations in fish (mullet and tilapia) body from different sampling seasons and stations (locations) in the Ashtoum El-Gamil protected area. Differing sampling seasons and stations (4 locations only for the similarity between locations No. 4 & 5, therefore No. 5 was escaped) as well as their interactions and fish species also were responsible for significant ($P \leq 0.0001$) effects on these elements' concentrations in fish carcass. The significant ($P \leq 0.0001$) high values were found in winter samples (for Pb and Fe) and spring samples (for Cd, Cu and Zn) and in station No. 3 (for Pb in tilapia fish), station No. 1 (for Cd, Cu and Fe in mullet fish) and station No. 4 (for Zn in tilapia fish). Samples of station No.4 during autumn season (for Pb), the same station during spring (for Zn) and station No. 1 during spring (for Cd, Cu and Fe) gave the highest values, being 209, 95, 10, 51.03 and 1895 ppm, respectively.

Generally, these studied elements took the following descending order: Fe ≥ Pb ≥ Zn ≥ Cu ≥ Cd in the fish carcass. Therefore, assuming that Fe mean concentration in fish body (966.25 ppm) is 100, so average Pb concentration is 10.02%, Cd 0.09%, Cu 1.17%, and Zn 7.69% of Fe level. However, the element's concentrations took the following ranges: 0.00 – 209.00, 0.00 – 10.00, 0.00 – 51.03, 56.00 – 95.00, and 479 – 1895 ppm, respectively. This reflects that the presence of a heavy metal in a fish body may not be followed its presence in the surroundings sediments or water. It may depend on its solubility, target medium, site of its deposition, as well as on different water quality criteria (salinity, pH, alkalinity, dissolved oxygen, microbial load….etc), sediment composition and fish species (differing in the metabolism). So, in the present study, tilapia fish tended to contain more Pb and Zn, but mullet fish contained more Cd, Cu and Fe than tilapia. Also, Cd was at least in sediments and fish, but Cu was at least in water. This may be interpretable by calculating the bio-accumulation factors (BAF, by dividing the element level in fish by the same element level in the water and multiplying by 100) of these elements which were 2.57×10^4, 1.66×10^2, 1.33×10^4, 4.12×10^3 and 7.49×10^5 for Pb, Cd, Cu, Zn and Fe, respectively, i.e. Fe was the heaviest element in the fish body, followed by Pb, Zn, Cu and at least Cd as mentioned before. In this respect, the bioaccumulation factors (BAF, of different heavy metals tested) in the *M. cephalus* studied from four sampling locations during two seasons showed significantly highest BAF in fish from Alexandria, Port Saied and El-Bardaweel for Pb, Fe and Cd, respectively. Winter Pb–BAF and summer Fe–BAF were significantly higher than those of the other season; yet, Cd – BAC did not influence by sampling seasons. These BAFs of heavy metals in fish did not influence by the level of these metals in the fish rearing waters, but were correlated with the level of Pb and Fe in winter sediment samples from Port Saied and Cd level in summer sediment samples from El-Bardaweel. The highest BAF of Fe in Port Saied fish samples was related also to the highest Fe contents in fish of this location. The same relation was confirmed for Cd in El-Bardaweel fish samples, but not for Pb (Abdelhamid *et al*., 2006b).

However, the commission regulation setting maximum Pb level for muscle meat of fish, released from the European communities, as 0.2 mg/Kg wet weight. Yet, the Egyptians' standards are 0.1 ppm Pb and Cd in food fish (ES, 1993). Comparing these standards with the levels obtained herein, it would be indicated that there is a water pollution with heavy metals in all tested locations, particularly with Pb in summer, Fe in winter (locations No. 2 and 4) and Cd in both seasons and all locations. Abdelhakeem *et al*. (2002) cited the tolerance limits of Pb, Fe and Cd in fish water as 0.10, 0.35 and 0.10 ppm, respectively and in fish body as 2, 30 and 0.5 ppm, respectively. Heavy metal concentrations in fish varied significantly depending on the type of the tissue, fish species and sampling location. Generally, *Mugil cephalus* L. showed higher levels of Fe and Pb concentrations than *Sparus aurata* L. (Yilmaz, 2005). Yet, there was no significant seasonal variation in marine water metal (Cd and Pb) concentrations (Kucuksezgin *et al.*, 2006). This may be due to the store tissue of each metal in/or on the fish, i.e. Pb was probably an external pollutant (Rashed and Awadallah, 1994), whereas Fe and Cd were internal pollutants. Therefore, Fe and Cd contents of fish affected positively their BAFs, but Pb was not. The same note is available for the effect of season, since BAF of Pb did not influence by its level in/or on the fish, whereas BAF of Fe was correlated with its level in fish, being the highest in summer season. Also, there were remarkable effects on microelements of fish muscles as well as their bioaccumulation factors due to sampling seasons and locations and fish species (Abdelhamid and El-Zareef, 1996).

Seasonal and location variations as well as fish species' effects were reported before by Abdelhamid *et al.* (2006b), who found that the highest ($P \leq 0.05$) levels of the tested heavy metals were found in fish collected from Marsa Matroh (0.851 ppm Pb), Port Saied (2.40 ppm Fe) and El-Bardaweel (0.081 ppm Cd). This may be related to the high content of Pb in water and sediments collected from location No. 1 during both seasons. Also, Fe level of the summer diet and winter collected sediments from Port Saied were the highest. Cd level in El-Bardaweel sediment collected in summer was also the highest. The Fe concentrations range (1.3 – 2.4 ppm) of fish tested was higher than that of Pb (0.172 – 0.851 ppm) than Cd (0.016 – 0.081 ppm), regardless to the sampling locations. However, Abdelhamid *et al.* (1997) registered significant variations in heavy metals concentrations due to different fish species from the natural fisheries and to sampling locations too. They found that the elements' concentrations in the sediments and fishes were much higher than the corresponding values in the water, particularly for iron. Lead and cadmium levels in fish muscles were concentrated more in fish, while iron was highest in sediments followed by fish tissues. *Mugil cephalus* samples were more frequently contaminated than*Liza ramada* and *Sparus aurata* (Abdelhamid *et al.*, 1997). The effects of varying sampling locations and fish parts on the heavy metal level or presence were reported also by Abdelhamid *et al.* (2000). Anyhow, Cd is known to be human carcinogen (Mandel *et al.*, 1995), Bahr El-Bakar drain water contained 0.910 and 0.0242 mg/l Pb and Cd, respectively, whereas its *M. cephalus* fish flesh contained 0.9376 and 0.0324 mg/Kg Pb and Cd, respectively (Galhoom *et al.*, 2000). Cd reduced fish growth, feed efficiency and mitotic index and led to abnormal chromosomal behavior (Magouz *et al.*, 1996). Additionally, Salem (2003) found that Cd and Pb caused significant reduction in fish performance, survival, and muscular area. Cd and Pb ions were able to induce metallothionein gene expression in fish tissues, e.g. liver and gills (Cheung *et al.*, 2004). Its residues in fish flesh increased by dose increase. The protein banding patterns fluctuated in numbers and intensities by Cd concentrations. Cd residues affected the DNA nucleotide sequences (El-Fadly *et al.*, 2006). Generally, *Mugil cephalus* L. showed higher levels of Fe and Pb concentrations than *Sparus aurata* L. (Yilmaz, 2005).

To interpret the collective death of fish in Domietta region, it was proved that the water of the studied area (El-Bostan village – Kafr El-Batiekh) has suffered from increase of iron concentrations. This picture is very harmful to fish life and production. Pollution of water was reflected in the form of heavy metal accumulation in different fish tissues. The lowest bioaccumulation factors were calculated in fish muscles; therefore, muscles only are suitable for human consumption. The bioconcentration of iron was higher than that of lead in fish muscles (Abdelhamid *et al*., 2000). Cd in water negatively affected fish growth and feed and vitamin C utilization. Fe also is toxic for fish, since it damages fish gills and their function. Pb reduces hemoglobin content and red blood cells count of fish (Abdelhamid, 2003). However, any degree of poisoning will weaken the fish, making it vulnerable towards disease. Heavy metals can create problems and be concentrated in waterway organisms up to 9100 times more than the surrounding environment's levels, so may lead to acute or chronic effects (WRC, 2005).

Hovanec (1998) mentioned that metals are involved in many aspects of fish keeping and aquarium water metals are acutely toxic while others are necessary for the life of the fish nitrifying bacteria. Still others are responsible for such basic water hardness. For a metal to be toxic, it almost always has ionized or free form. Water hardness can have a drastic effect on metal toxicity. Since the toxicity and biological activity of many metals and metalloids is profoundly influenced by their chemical form. The metabolism of ingested metals could significantly modify their toxicity. The micro-organisms in lakes, rivers and soil could biotransform metallic compounds (Rowland, 1981). Also, it is a fact that body adaptive balance mechanisms for lead impacts were evident in different organ tissues of fish. Yet, Mzimela *et al.* (2002) reported that lead negatively affected the blood hematology and acid-base balance of the groovy mullet, *Liza dumerili*. Usero *et al.* (2004) reported that heavy metal concentrations in the water, sediment and fish were variable from site to another. Significant correlations were obtained for the levels of numerous metals in water, sediment and fish. The results of Xie and Klerks (2004) suggest that reduced uptake and accumulation of Cd accounted for approximately two-third of the increased resistance in the Cd-adapted lines of fish. However, Cd has been found to accumulate in reproductive organs of fish and disrupt important endocrine processes.

Moreover, responses along the hypothalamus – pituitary – gonadal axis were more sensitive to Cd exposure (Tilton *et al.,* 2003). Metal concentrations in the sediment at each site depended more on the general characteristics of the sediment, such as the percentage of fine grained sediments and Fe content, than on whether or not there was replanted (Paulson *et al.,* 2001). Siam (2001) found high level of accumulation of Cd, Fe and Pb in the different organs (gills, liver, stomach and brain) of Alexandria coast fish, with respect to their corresponding in the muscle tissues. He added that the accumulation factors for these metals were higher in the herbivorous fish (*Siganus rivulatus*) than in the carnivorous ones (*Mugil capito).* Fe was the more pronounced one reflecting increase the trophic level of the fish. Cd level was generally lower than that of Pb in various organs while brain gained the highest values. Pb concentration ranged from 1.2 to 3.5 mg/kg in the stomach and brain while it ranged from 0.4 to 0.9 mg/kg in fish muscles. Most of the fish generally showed levels of Cd in the organs, which are close to that of the recommended standard (2.0 mg/kg) of the National Health and Medical Council in Australia. However, none of them contained Cd concentrations above 0.5 mg/kg in their muscle tissues. Total length, body weight and age are mostly correlated biometric parameters with metallothionein and soluble metal concentrations in striped red mullet and golden grey mullet (Filipovic and Raspor, 2003). Cadmium and lead were higher in muscular tissue from mullet (*Mugil sp.*) than snook (*Centropomus sp*.) and higher in summer than in winter (Joyeux *et al.,* 2004). Kirby *et al*. (2001) mentioned that mullet are directly exposed to trace metal concentrations as a result of feeding and the ingestion of contaminated sediment and detritus. Lower metal concentrations found in mullet tissues are attributed to the burial of highly contaminated sediment with material containing lower trace metal concentrations. Little of the variations in trace metal concentrations between mullet were explained by mass, gender, or age.

Geochemical controls of metal assimilation from contaminated sediment are relatively apparent for Cd. The influences of metal speciation on metal bioavailability can be confounded by the degree to which sediments are contaminated with metals (Fan *et al*., 2002). Heavy metals (Cd, Fe, and Pb) strongly inhibit the enzyme activity and the hexobarbital metabolism (Medline Repository, 2001). They alter the immune system and lead to increased susceptibility to autoimmune diseases and allergic manifestations (Bernier *et al.,* 2005). Suciu *et al.* (2005) reported that Cd-bioaccumulation factor was increased by age. Cd and Pb were accumulated more in males than female sturgeon. However, liver microsomal 7-ethoxyresorufin O-deethylase (EROD) activity of leaping mullet inhibits the toxicity of divalent metal ions through the inhibitory effect of the glutathione (GSH) on Cd inhibition of EROD activity indicating the protective action of GSH (Bozcaarmutlu and Arinc, 2004). Staniskiene *et al.* (2005) found high concentrations of Fe in 15 fish species as a direct result of water contamination with heavy metals. Metal concentrations were found to be influenced by fish type. Cadmium – binding protein level in the cells of the intestine was increased after exposure to Cd, so it appears that this protein is synthesized as a response to Cd exposure. This is a mechanism of the regulation of Cd levels (Demuynck *et al.,* 2004).

Chemical composition of fish from the protected area

Proximate analysis of Nile tilapia and mullet fish body as percent on dry matter basis is given in Table 4 which shows significant ($P \leq 0.0001$) effects of each of sampling seasons and stations (4 locations only for the similarity between locations No. 4 & 5, therefore No. 5 was escaped) in the Ashtoum El-Gamil protected area as well as their interactions and fish species also on the chemical analysis of the *tested fish. However, the highest Dm percent in fish carcass was in summer samples, whereas the highest CP and lowest EE and ash percentages were in autumn samples. Mullet fish contained higher DM and EE and lower ash than tilapia. Generally, the highest DM (31.8%) and lowest ash (13.3%) were found in summer mullet fish samples from location (station) No. 2; yet, the highest CP (67.5%) and lowest EE (9.4%) were recorded in autumn mullet fish samples collected from stations No. 1 and 2, respectively. Actually, sometimes there were positive relationships between DM on one side and each of CP, EE, and ash percentages on the other side. Also, there were negative relationships between CP on one hand and either EE or ash percentages on the other hand. These relationships were reported too by many authors (El-Ebiary and Zaki, 2003 and Abdelhamid et al., 2005a & b and 2006b). Abdelhamid et al. (2006b)* reported also significant effects of sampling locations and seasons on all proximate analysis of fish body. They found that Port Saied and Marsa Matroh fish reflected higher ($P \leq 0.05$) protein thanAlexandria and El-Bardaweel fish. Yet, the fat and ash contents differed also but not in a clear trend. However, winter fish contained more protein and less fat percentages ($P \leq 0.05$) than those of summer. They attributed the elevated protein content in winter to the lower ($P \leq 0.05$) heavy metals content (Pb and Fe) in fish flesh during this season than in summer. However, some significant correlations were calculated among heavy metals (in water, sediments, and fish) and chemical composition of the fish as shown from the following Table 5.

Table 4: ***Mean values for effect of collection season and station on chemical composition of fish carcass (% dry matter basis***

CC	Moisture	DM	CP	EE	Ash
Season					
Spring	74.83^{b}	25.18^{c}	55.69^{d}	19.48^{a}	24.83^{b}
Summer	72.42^{d}	27.58^{a}	56.85^{c}	16.45^{b}	26.70^{a}
Autumn	75.64^{a}	24.36^{d}	62.63^{a}	13.71^{d}	23.67^{c}
Winter	73.26^{c}	26.74^{b}	57.30^{b}	15.39^{c}	27.31^{a}
± SE	0.000	0.000	0.222	0.232	0.000
P- value	0.0001	0.0001	0.0001	0.0001	0.0001
Station					
1 (M)	72.84^{c}	27.17^{b}	57.60^{c}	17.91^{b}	24.49^{c}
2 (M)	71.68^{d}	28.33^{a}	61.20^{a}	19.17^{a}	19.63^{d}
3 (T)	75.51^{b}	24.49^{c}	52.93^{d}	14.36^{c}	32.72^{a}
4 (T)	76.13^{a}	23.88^{d}	60.74^{b}	13.60^{d}	25.66^{b}
± SE	0.000	0.000	0.096	0.222	0.232
P- value	0.0001	0.0001	0.0001	0.0001	0.0001
Season* Station					
Sp*1	71.7	28.3	53.0	25.7	21.3
Sp*2	72.1	27.9	51.8	25.2	23.0
Sp*3	76.9	23.2	55.6	14.6	29.8
Sp*4	78.7	21.4	62.4	12.4	25.3
Su*1	71.6	28.4	56.2	15.9	27.9
Su*2	68.2	31.8	63.7	23.0	13.3
Su*3	75.8	24.2	50.0	10.6	39.4
Su*4	74.1	25.9	57.5	16.4	26.1
A*1	74.4	25.6	67.5	16.1	16.4
A*2	75.4	24.6	66.2	9.4	24.4
A*3	74.8	25.3	54.3	19.1	26.6
A*4	78.0	22.0	62.5	10.2	27.3
W*1	73.6	26.4	53.7	13.9	32.4
W*2	71.1	28.9	63.1	19.1	17.8
W*3	74.6	25.4	51.8	13.2	35.0
W*4	73.7	26.3	60.6	15.4	24.0
± SE	0.000	0.000	0.192	0.444	0.464
P- value	0.0001	0.0001	0.0001	0.0001	0.0001

CC: chemical composition, Sp: Spring, Su: summer, A: autumm, W: winter, M: mullet, T: tilapia, SE: standard error, P: probability, DM: dry matter, CP: crude protein, EE: ether extract.

Table 5: ***Data of calculating Pearson correlation and probability (P) value among heavy metals in water (w), sediment (So), and fish (F)(and chemical composition of the fish***

	Pb_F	Cd_F	Cu_F	Zn_F	Fe_F	Pb_So	Cd_so	Cu_so	Zn_so	Fe_so	Pb_w	Cd_w	Cu_w
Cd_F	0.065												
	0.622												
Cu_F	0.103	0.823											
	0.431	0.000											
Zn_F	0.020	0.326	0.056										
	0.879	0.011	0.669										
Fe_F	0.198	0.504	0.404	-0.087									
	0.129	0.000	0.001	0.509									
Pb_So	0.090	-0.012	-0.254	0.513	-0.401								
	0.493	0.930	0.050	0.000	0.001								
Cd_so	0.083	0.049	-0.170	0.366	-0.267	0.912							
	0.529	0.709	0.195	0.004	0.039	0.000							
Cu_so	0.153	0.009	-0.239	0.450	-0.396	0.981	0.935						
	0.243	0.946	0.066	0.000	0.002	0.000	0.000						
Zn_so	0.011	0.023	-0.270	0.458	-0.376	0.949	0.929	0.936					
	0.936	0.863	0.037	0.000	0.003	0.000	0.000	0.000					
Fe_so	-0.012	0.161	-0.115	0.264	-0.226	0.770	0.749	0.820	0.695				
	0.925	0.221	0.382	0.041	0.082	0.000	0.000	0.000	0.000				
Pb_w	-0.537	-0.113	-0.119	0.026	0.022	-0.124	-0.171	-0.231	-0.072	-0.217			
	0.000	0.390	0.367	0.844	0.865	0.344	0.193	0.076	0.587	0.095			
Cd_w	-0.538	0.044	0.116	0.091	-0.240	-0.142	-0.262	-0.233	-0.141	-0.177	0.784		
	0.000	0.741	0.376	0.488	0.065	0.279	0.043	0.073	0.282	0.176	0.000		
Cu_w	0.019	0.352	0.280	0.367	-0.128	0.117	-0.040	0.087	0.063	0.005	0.019	0.167	
	0.888	0.006	0.030	0.004	0.329	0.374	0.763	0.508	0.634	0.968	0.885	0.204	
Zn_w	-0.095	0.106	-0.041	0.467	0.223	0.269	0.250	0.262	0.148	0.226	-0.164	-0.210	-0.041
	0.470	0.421	0.753	0.000	0.087	0.037	0.054	0.043	0.260	0.082	0.211	0.108	0.754
Fe_w	0.336	-0.240	-0.310	0.066	-0.027	0.368	0.234	0.397	0.222	0.336	-0.304	-0.428	0.106
	0.009	0.065	0.016	0.617	0.838	0.004	0.071	0.002	0.089	0.009	0.018	0.001	0.422
Mois	0.176	-0.075	-0.235	0.329	-0.486	0.574	0.434	0.579	0.534	0.384	-0.369	-0.034	-0.006
	0.178	0.570	0.071	0.010	0.000	0.000	0.001	0.000	0.000	0.002	0.004	0.797	0.961
DM	-0.176	0.075	0.235	-0.329	0.486	-0.574	-0.434	-0.579	-0.534	-0.384	0.369	0.034	0.006
	0.178	0.570	0.071	0.010	0.000	0.000	0.001	0.000	0.000	0.002	0.004	0.797	0.961
CP	-0.059	-0.204	-0.239	-0.012	-0.218	0.346	0.299	0.363	0.325	0.344	-0.258	-0.264	0.193
	0.655	0.117	0.066	0.930	0.094	0.007	0.020	0.004	0.011	0.007	0.047	0.042	0.139
EE	-0.236	0.454	0.460	0.084	0.396	-0.405	-0.312	-0.416	-0.374	-0.267	0.179	0.079	0.304
	0.070	0.000	0.000	0.522	0.002	0.001	0.015	0.001	0.003	0.039	0.171	0.550	0.018
Ash	0.237	-0.184	-0.159	-0.057	-0.127	0.024	-0.009	0.018	0.017	-0.084	0.079	0.164	-0.406
	0.068	0.159	0.226	0.667	0.335	0.857	0.943	0.889	0.895	0.525	0.547	0.210	0.001

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حالة بعض العناصر الثقيلة فى منطقة محمية أشتوم الجميل

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تم عمل دراسة مسحية على بعض العناصر الثقيلة (رصاص، كادميوم، نحاس، زنك، حديد) فى مياه ورسوبيات وأسماك منطقة محمية أشتوم الجميل خلال الفترة من مايو 2010م الى يناير 2011م. أكدت النتائج المتحصل عليها وجود اختلافات معنوية بين مواسم ومواقع جمع العينات وكذا للتداخل بين هذين العاملين بالنسبة لتركيزات هذه العناصر المدروسة فى كل من عينات المياه والرسوبيات والأسماك، وأخذت تركيزات العناصر الترتيب التنازلى: زنك ≤ كادميوم ≤ رصاص ≤ حديد ≤ نحاس فى المياه، رصاص ≤ حديد ≤ نحاس ≤ زنك ≤ كادميوم فى الرسوبيات، حديد ≤ رصاص ≤ زنك ≤ نحاس ≤ كادميوم فى أجسام الأسماك. وأظهر التحليل الكيماوى لأجسام الأسماك (بلطى و بورى) وجود تأثير معنوى لكل من مواسم ومواقع جمع العينات والتداخل بينهما بجانب نوع السمك على التركيب الكيماوى للأسماك. وثبت وجود ارتباط مابين تركيزات العناصر المقدرة فى كل من المياه والرسوبيات والأسماك والتركيب الكيماوى للأسماك.