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Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon

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Abstract

Two hundred fish samples were collected seasonally from November 2000 to December 2001 from the Tuzla Lagoon. Heavy metal (Cd, Pb, Cu, Zn, and Fe) concentrations were measured in the muscle, gill, liver and gonad of three fish species (*Sparus aurata*, *Dicentrarchus labrax* and *Mugil cephalus*). The concentrations of heavy metals were determined by using flame atomic absorption spectrophotometry (FLAAS) and graphite furnace atomic absorption spectrophotometry (GFAAS) after wet digestion method.

Our results indicated that all heavy metals were found the highest in muscle tissue in *S. aurata*. Although Cd and Zn were found in spring, Fe, Cu, and Pb were observed in winter. In addition, *D. labrax* and *M. cephalus* accumulated lowest heavy metals in muscle during this study. While the highest concentrations of Zn, Fe and Cu were measured in the liver of *M. cephalus,* the highest levels of Cd and Pb were determined in gill tissue of *M. cephalus.*

However, in some seasons Zn, Cd and Pb concentrations in the muscle were higher than the maximum levels set by law. Especially, for each species in spring high levels of Zn were measured; for *D. labrax* and *M. cephalus* in spring and for *S. aurata* in winter high levels of Pb were measured; for *S. aurata* in spring and for *M. cephalus* in winter high levels of Cd were measured for human consumption.

Keywords: Heavy metals; Bioaccumulation; Fish; Tuzla lagoon; Turkey

1. Introduction

Heavy metal pollution of the marine environment has long been recognized as a serious environmental concern. The presence of some heavy metals in aquatic environments and their accumulations in fish and in the other organisms has been investigated during recent years (Barbosa, Gutierrez-Galindo, & Flores-Munoz, 2000; Bassi & Sharma, 1993; Bei, Catsiki, & Papathanassiou, 1992; Freedman, 1989; Wolfe, 1974). Heavy metals are present in the aquatic environment where they bio accumulate along the food chain. Accumulation occurs in the tissues of aquatic animals and may become toxic for fish and also for people when it reaches a substantially high level. An early example of an environmental problem due to heavy metal occurred, starting in 1952, in the vicinity of the Japanese fishing harbor of Minimata. A hitherto unknown disease (Minimata disease) arose and grew rapidly into a real epidemic and was shown to be due to organomercury compounds (Vandecasteele & Block, 1991). Especially, since well-known instances where fishermen from Minimata Bay and villagers from Jintsu River died or became paralyzed from mercury and cadmium poisoning, respectively. For this reason, determination of chemical quality of aquatic organisms, particularly the contents of heavy metals is extremely important for human health (Cid, Boia, Pombo, & Rebelo, 2001; Ravera, 1979).

This research was conducted for the first time for Tuzla Lagoon which is located in the İskenderun Bay, eastern Mediterranean coast of Turkey. Along the coast of

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İskenderun Bay, there are agricultural lands and industrial plants (iron–steel plants, LPG plants, oil transfer docks, and cargo ship's ballasts water). In this region, there are several big lagoons and among these the third biggest lagoon is Tuzla. Due to heavy industrial and agricultural activities in the region, the bay has the polluted coastal waters of Turkey. Therefore, mainly untreated agricultural municipal and industrial wastes affect the lagoon direct or indirectly. This lagoon has a great importance for the local fisheries activities. According to the report of the Ministry of Agriculture and Rural Affairs of Turkey, 30 tons of fish was supplied from the lagoon, in 1995. Besides being an important area for local fisheries, Tuzla lagoon is also stated as the wild life protection area because of its biodiversity. Although, a few studies were completed on determination of pesticide pollution (Çetinkaya & Altan, 1998; Erbatur, Kuşvuran, & Erbatur, 1997; İzcankurtaran & Yılmaz, 2001), this research, on determination of heavy metal pollution, was carried out for the first time for Tuzla Lagoon.

Our specific objectives were to determine the distribution of heavy metals in economically important fish species such as *Dicentrarchus labrax*, *Sparus aurata* and *Mugil cephalus* from Tuzla Lagoon.

2. Materials and methods

The samples were caught by fishermen's nets seasonally from December 2000 to November 2001 from the Tuzla Lagoon (Fig. 1) and transported daily to the laboratory. Samples were stored in plastic bags at −20 °C until dissection. The total length (cm) and weight (g) of fishes were measured (Table 1). Each sample collected from the Tuzla lagoon seasonally was dissected for its muscle, gill, liver and gonad tissues. Sample preparation and analysis were carried out according to the procedure described by UNEP Reference Methods (1984). The tissues digested with concentrated nitric acid and perchloric acid (2:1 v/v) (Merck) at 60 °C for 3 days and all samples were diluted with double distilled water. Following acid digestion, all samples were analyzed for five elements by atomic absorption spectrometry (Phillips AAS with double beam and deuterium background corrector). Fe and Zn were determined in air–acetylene flame. Cd, Cu and Pb were analyzed in a graphite furnace (GBCGF 3000 with Zeeman background corrector) with an auto sampler. All digested samples were analyzed three times for each metal. The standard addition method was used to correct for matrix effects. The instrument was calibrated with standard solutions prepared from commercial materials. Analytical blanks were run in the same way as the samples and determined using standard solutions prepared in the same acid matrix. All chemicals and standard solutions used in the study were obtained from Merck and were of analytical grade. The quality of data was checked by the analysis of standard reference material (DORM-2 National Research Council, Canada) (Table 2).

Statistical analysis of data was carried out using SPSS statistical package program. One-way analysis of variance (ANOVA) and Duncan multiple range test were used to assess whether metal concentrations varied significantly among tissues (a, b and c) seasonally. (Tables 3–7). As the distribution of metals data was markedly skewed, logarithmic transformations of the data were applied. Data shown in different letters are significant at the 0.05 level. Summer data were not presented in the tables as the fish samples were not available during this period because of legal prohibition of fishing.

3. Results

The levels of zinc, iron, cadmium, copper and lead in various tissues of *D. labrax*, *S. aurata* and *M. cephalus* are given in Tables 2–6 for each metal, respectively. The tables show significant differences in the accumulation levels of metals in the tissues throughout the year in these species.

The highest Zn (99.8 μg/g dw) concentration was detected in *M. cephalus*'s liver in winter and Cd (1.59 μg/g dw) and Pb (6.75 μg/g dw) were highest in spring in *M. cephalus*'s gill. The highest level of Cu (12.03 μg/g dw) was measured in the *M. cephalus*'s liver in autumn, while maximum Fe (383.7 μg/g dw) was detected in *M. cephalus*'s liver in winter. Muscle, generally, accumulated the lowest levels of metals in every season. The results showed that the metal accumulation in spring and winter seasons was higher than in the other seasons. This high level accumulation could be due to heavy rainfall during these seasons which increases the metal content of water by washing down the agricultural wastes.

No significant differences were detected in *D. labrax*'s gonad and *S. aurata*'s liver (Table 2). Fe was occurred in different concentration levels in tissues (Table 3). Although there were no significant seasonal changes for Fe concentrations in muscle, gill and gonad tissue of *D. labrax*, yet there were some variations in other species. Similarly, Cd concentrations also did not vary significantly among seasons except in liver, gill and gonad of *M. cephalus* (Table 4). Generally, there were significant seasonal changes for Cu concentrations in all tissues of all fish species except gill and gonad of *M. cephalus* (Table 5). Table 6 indicates that no significant seasonal changes were observed in all tissues of all fish species except the gill of *D. labrax*, liver of *S. aurata* and gonad of *M. cephalus* ($p < 0.05$).

4. Discussion

A number of studies have shown that various factors such as season (Kargın, 1996), length and weight, physical and chemical status of water (Jezierska & Witeska, 2001) can play a role in the tissue accumulation of metals. Seasonal changes of metal concentrations in fish may result from intrinsic factors such as growth cycle and reproductive cycle and from changes in water temperature. Addi-

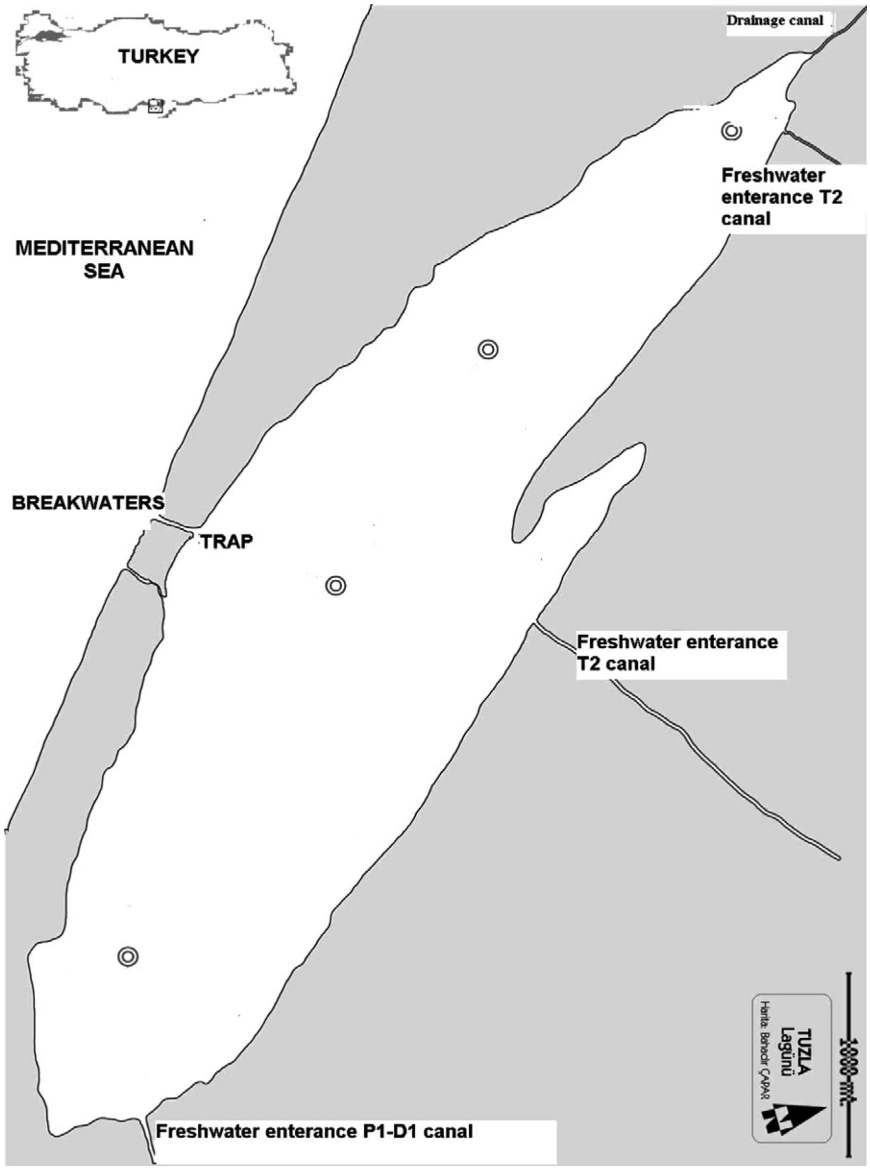

Fig. 1. A Map of Tuzla Lagoon, Turkey.

Table 1
Minimum, mean, maximum length weight of fish species used in this research

Species	Season			Weight (g) (Min–Max)	Length (cm) (Min–Max)
	Autumn	Winter	Spring		
Dicentrarchus labrax	20	15	22	112.8 ± 14.3 (51.81–223.8)	20.2 ± 1.06 (18.1–27.8)
Sparus aurata	20	22	25	54.10 ± 3.86 (23.24–127.5)	14.4 ± 0.90 (12.6–20.0)
Mugil cephalus	25	23	28	77.6 ± 5.01 (35.97–186.1)	22.1 ± 0.81 (14.5–27.1)

tionally, the differences noted in the metal concentrations in different tissues between seasons could have been the result of local pollution.

This investigation showed that different fish species contained different metal levels in their tissues. Due to variations in feeding habits, habitats and behavior of the three species, the levels of metals found in tissues of the *M. cephalus* were generally higher than those found in other species throughout the year. Our results show that generally metal accumulation is highest in liver and gills, while it is low in gonad and muscle in all species. This was the case in many fish species, although interspecies differences were shown on the accumulation of various metals in these tissues (Gey, 1983; Kargın, 1996; Usero, Izquierdo, Morillo, & Gracia, 2003; Yılmaz, 2003). The levels of cadmium, zinc, copper, iron and lead were determined in the muscle in each species because of its importance for human consumption and also the liver, gill, gonad were analyzed since

Table 2
Observed and certified values of elemental concentrations, as μg/g dry weight, in standard reference materials DORM-2 from the National Research Council, Canada ($n = 3$)

	Certified values (μg/g)	Measured values	Recovery (%)
DORM-2			
Zn	25.6 ± 2.3	24.9 ± 2.4	97
Fe	142 ± 10	137 ± 11	96
Cd	0.043 ± 0.008	0.045 ± 0.009	104
Cu	2.34 ± 0.16	2.26 ± 0.17	96
Pb	0.065± 0.007	0.069 ± 0.008	106

Table 3
Mean concentrations (μg metal/g d.w.) and standard deviations of Zn in the tissues of examined species and comparison of different seasons within the same species

Species	Tissues	Seasons		
		Autumn	Winter	Spring
Dicentrarchus labrax	Muscle	8.99 ± 1.36^b	72.28 ± 15.77^a	75.38 ± 40.37^a
	Liver	21.7 ± 2.50^a	90.3 ± 1.47^b	34.8 ± 0.57^c
	Gill	31.1 ± 0.92^a	53.1 ± 6.20^b	57.8 ± 16.40^b
	Gonad	41.5 ± 21.94	58.8 ± 6.45	43.5 ± 11.0
Sparus aurata	Muscle	25.21 ± 38.16^b	8.82 ± 2.74^b	76.98 ± 51.82^a
	Liver	37.7 ± 7.70	59.2 ± 15.31	53.5 ± 1.20
	Gill	35.2 ± 4.92^a	41.7 ± 12.13^a	12.2 ± 0.67^b
	Gonad	19.5 ± 3.48^a	38.1 ± 6.69^b	41.5 ± 5.30^b
Mugil cephalus	Muscle	8.27 ± 2.18^b	49.7 ± 14.71^a	60.86 ± 56.10^a
	Liver	26.7 ± 4.35^a	99.8 ± 1.27^b	28.3 ± 3.71^a
	Gill	24.1 ± 3.62^a	60.5 ± 6.57^b	45.6 ± 31.56^{ab}
	Gonad	25.7 ± 21.50^a	53.8 ± 12.20^{ab}	68.5 ± 14.30^b

[a,b,c] Show differences among seasons ($P < 0.05$).

Table 4
Mean concentrations (μg metal/g d.w.) and standard deviations of Fe in the tissues of examined species and comparison of different seasons within the same species

Species	Tissues	Seasons		
		Autumn	Winter	Spring
Dicentrarchus labrax	Muscle	7.75 ± 3.79	10.63 ± 2.65	11.13 ± 4.02
	Liver	62.3 ± 6.70^a	188.9 ± 2.40^b	88.3 ± 12.30^a
	Gill	341.3 ± 401.2	103.7 ± 7.70	127.3 ± 63.20
	Gonad	7.00 ± 4.2	13.1 ± 3.20	10.0 ± 0.20
Sparus aurata	Muscle	7.16 ± 2.08^a	16.5 ± 9.67^b	11.12 ± 5.51^{ab}
	Liver	109.30 ± 15.30^a	230.6 ± 82.70^b	48.10 ± 6.70^a
	Gill	67.50 ± 30.40^a	136.4 ± 41.70^a	60.50 ± 0.07^b
	Gonad	6.25 ± 0.70^a	13.5 ± 1.20^b	8.50 ± 1.20^a
Mugil cephalus	Muscle	7.15 ± 1.93^b	8.80 ± 3.05^{ab}	11.12 ± 4.13^a
	Liver	87.7 ± 58.70^a	383.7 ± 41.40^b	305.0 ± 179.60^b
	Gill	132.6 ± 64.70^a	246.8 ± 6.34^{ab}	344.8 ± 157.90^b
	Gonad	11.3 ± 2.90^a	23.3 ± 3.20^b	11.6 ± 0.80^a

[a,b,c] Show differences among seasons ($P < 0.05$).

these organs tend to accumulate metals (Marcovecchio, Moreno, & Perez, 1991). These organs are also good indicators of chronic exposure to heavy metals because they are the site of metal metabolism. It is well known that heavy metals accumulate in the tissues of aquatic animals, and therefore heavy metals measured in the tissues of aquatic animals can reflect the past exposure. The liver is often considered a good monitor of water pollution with metals since their concentrations are proportional to those present in the environment. No correlation was reported, however, between metal concentration in the water and fish muscles. According to Miller, Munkittrick, and Dixon (1992), muscle was a poor indicator of low level copper and zinc contamination. That is also true for most other metals, except for mercury which shows higher affinity to the muscles comparing to other metals (Jezierska & Witeska, 2001). The liver tissue is highly active in the uptake and storage of heavy metals. It is well known that large amount of metallothionein induction occurs in the liver tissue of fishes (Heath, 1987; Hodson, 1988; Langston, 1990; Marafante,

Table 5
Mean concentrations (μg metal/g d.w.) and standard deviations of Cd in the tissues of examined species and comparison of different seasons within the same species

Species	Tissues	Seasons		
		Autumn	Winter	Spring
Dicentrarchus labrax	Muscle	0.07 ± 0.002	0.08 ± 0.01	0.03 ± 0.03
	Liver	0.17 ± 0.09	0.19 ± 0.007	0.10 ± 0.02
	Gill	0.95 ± 0.06	1.12 ± 0.03	0.70 ± 0.57
	Gonad	0.14 ± 0.01	0.47 ± 0.54	0.11 ± 0.007
Sparus aurata	Muscle	0.08 ± 0.02	0.10 ± 0.03	0.12 ± 0.002
	Liver	0.15 ± 0.007	0.19 ± 0.06	0.14 ± 0.007
	Gill	1.18 ± 0.007	1.01 ± 0.24	1.02 ± 0.01
	Gonad	0.11 ± 0.007	0.25 ± 0.31	0.40 ± 0.43
Mugil cephalus	Muscle	0.09 ± 0.05	0.11 ± 0.004	0.08 ± 0.01
	Liver	0.02 ± 0.02^{a}	0.21 ± 0.005^{ab}	0.35 ± 0.17^{b}
	Gill	0.04 ± 0.05^{a}	1.27 ± 0.005^{b}	1.59 ± 0.42^{b}
	Gonad	0.17 ± 0.02^{a}	0.08 ± 0.001^{b}	0.21 ± 0.005^{c}

[a,b,c] Show differences among seasons ($P < 0.05$).

Table 6
Mean concentrations (μg metal/g d.w.) and standard deviations of Cu in the tissues of examined species and comparison of different seasons within the same species

Species	Tissues	Seasons		
		Autumn	Winter	Spring
Dicentrarchus labrax	Muscle	0.42 ± 0.11^{a}	0.26 ± 0.05^{b}	0.33 ± 0.07^{ab}
	Liver	0.35 ± 0.05^{a}	0.69 ± 0.02^{b}	0.68 ± 0.03^{b}
	Gill	1.75 ± 0.05^{a}	1.10 ± 0.01^{b}	2.51 ± 0.06^{c}
	Gonad	1.16 ± 0.21^{a}	3.38 ± 0.02^{b}	3.21 ± 0.01^{b}
Sparus aurata	Muscle	0.57 ± 0.13^{a}	0.82 ± 0.007^{b}	0.55 ± 0.002^{a}
	Liver	2.32 ± 0.21^{a}	2.63 ± 0.49^{ab}	2.88 ± 0.10^{b}
	Gill	3.70 ± 0.16^{a}	1.94 ± 0.55^{b}	0.83 ± 0.07^{c}
	Gonad	0.49 ± 0.02^{a}	0.65 ± 0.07^{b}	0.48 ± 0.03^{a}
Mugil cephalus	Muscle	0.49 ± 0.001^{b}	0.47 ± 0.12^{b}	0.62 ± 0.008^{a}
	Liver	12.03 ± 0.52^{a}	4.77 ± 0.03^{b}	5.74 ± 3.11^{b}
	Gill	4.09 ± 0.09	3.43 ± 0.02	7.82 ± 5.81
	Gonad	1.14 ± 0.02	1.08 ± 0.007	1.07 ± 0.04

[a,b,c] Show differences among seasons ($P < 0.05$).

Table 7
Mean concentrations (μg metal/g d.w.) and standard deviations of Pb in the tissues of examined species and comparison of different seasons within the same species

Species	Tissues	Seasons		
		Autumn	Winter	Spring
Dicentrarchus labrax	Muscle	0.47 ± 0.33	0.40 ± 0.06	1.58 ± 1.79
	Liver	1.41 ± 0.65	1.72 ± 0.03	1.65 ± 0.007
	Gill	3.56 ± 0.93^{a}	3.71 ± 0.04^{a}	5.99 ± 0.63^{b}
	Gonad	1.39 ± 0.36	1.08 ± 0.04	0.85 ± 0.16
Sparus aurata	Muscle	0.64 ± 0.01	2.44 ± 2.27	1.11 ± 0.36
	Liver	3.92 ± 0.007^{a}	1.87 ± 0.02^{b}	2.33 ± 0.007^{c}
	Gill	4.51 ± 0.01	3.86 ± 1.96	4.62 ± 0.007
	Gonad	1.10 ± 0.007	1.07 ± 0.16	0.81 ± 0.007
Mugil cephalus	Muscle	1.07 ± 0.90	0.49 ± 0.15	1.19 ± 0.44
	Liver	3.12 ± 4.68	2.12 ± 0.007	1.85 ± 0.11
	Gill	2.67 ± 1.14	4.54 ± 0.02	6.75 ± 5.23
	Gonad	0.41 ± 0.62^{a}	1.88 ± 0.007^{b}	1.22 ± 0.005^{ab}

[a,b,c] Show differences among seasons ($P < 0.05$).

1976). The gills are the uptake site of waterborne ions, where metal concentrations increase especially at the beginning of exposure, before the metal enters other parts of organism (Jezierska & Witeska, 2001).

Levels of the essential metals in the fish samples were higher than those of the non-essential metals except Cu. The Tuzla lagoon was comparable to those of other studies carried out in polluted areas. For example, the Zn concentrations found in this study were higher than those reported by Tayel (1995) in fish from the Bay of Egypt and by Usero et al. (2003) in fish from salt marshes on the southern Atlantic coast of Spain. Quazi, Banu, Rahman, and Sayeed (1995) also found that the iron and zinc concentrations in the muscles of various marine fishes from Bangladesh ranged between 8.50 and 22.2 ppm w.w. for iron and 7.40 and 22.5 ppm w.w. for zinc. The present study also showed that iron and zinc concentrations also varied among the fishes and in fish caught off the coast of the United Arab Emirates (UAE) in the Arabian Gulf, an area polluted by hydrocarbons and heavy metals, the levels of Zn (34–70 mg/kg) and Cd (0.51–0.63 mg/kg) were generally found to be lower than those reported by our study (Al-Yousuf, El-Shahawi, & Al-Ghais, 2000). Kalay, Ay, and Canlı (1999) studied the heavy metal concentrations in different fish species from İskenderun Bay in 1996. Compared with the results of this study in general, our results were lower. Similarly, Türkmen, Türkmen, Tepe, and Akyurt (2005) studied the heavy metal concentrations in different fish species from İskenderun Bay in 2003. The present results were generally lower for Fe, Cd, Cu, and Pb than theirs. The results reported by Tamira, Shane, and Ambrose (2001) from California Lagoons were generally higher than the present results.

The Turkish legislation establishes maximum levels for four of the metals studied, above which human consumption is not permitted; 0.1 mg/kg for Cd, 1.0 mg/kg for Pb, 20.0 mg/kg for Cu, and 50 mg/kg for Zn (Anonymous, 1996). Food and Agricultural Organization limits for Cd and Pb 0.5 mg/kg, for Cu and Zn 30 mg/kg ((Food and Agriculture Organization) FAO, 1983). The concentrations of these metals measured in the muscles of the three species studied were generally lower than the levels issued by FAO and Turkish legislation. Yet, in some seasons Zn, Cd and Pb concentrations in the muscle tissues were higher than the maximum levels set by law. Especially, for each species in spring, high level of Zn were measured; for *D. labrax* and *M. cephalus* in spring and for *S. aurata* in winter, high level of Pb were measured; for *S. aurata* in spring and for *M. cephalus* in winter high level of Cd for human consumption were measured.

Human beings have been exposed to heavy metal toxins for an immeasurable amount of time. Industrialization of the world has dramatically increased the overall environmental 'load' of heavy metal toxins, to the point that our societies are dependent upon them for proper functioning. Today, heavy metals are abundant in our drinking water, air and soil due to our increased use of these compounds. It is very difficult for anyone to avoid exposure to any of the many harmful heavy metals that are so prevalent in our environment. Heavy metal toxins contribute to a variety of adverse health effects. There exist over 20 different heavy metal toxins that can impact human health and each toxin will produce different behavioral, physiological, and cognitive changes in an exposed individual.

Knowledge of heavy metal concentrations in fish is important both with respect to nature management and human health. The toxicity of metals most commonly involves the brain and the kidney, but other manifestations occur, and some metals, such as arsenic, are clearly capable of causing cancer. An individual with metals toxicity, even if high dose and acute, typically has very general symptoms, such as weakness or headache. In this research, high levels of the concentrations of Zn, Cd and Pb were measured for human consumption in some seasons. The general body of the literature on lead toxicity indicates that, depending on the dose, lead exposure in children and adults can cause a wide spectrum of health problems, ranging from convulsions, coma, renal failure and death at the high end to subtle effects on metabolism and intelligence at the low end exposures (US Agency for Toxic Substances & Disease Registry, 1999). In contrast to children, adults are generally allowed by regulations to be exposed to higher amounts of lead. The health implications of cadmium exposure are exacerbated by the relative inability of human beings to excrete cadmium. Acute high dose exposures can cause severe respiratory irritation. The occupational levels of cadmium exposure prove to be a risk factor for chronic lung disease and testicular degeneration (Benoff, Jacop, & Hurley, 2000). Lower levels of exposure are mainly of concern with respect to toxicity of the kidney (Satarug, Haswell-Elkins, & Moore, 2000). Zinc in low to moderate amounts is of very low toxicity in its ordinary compounds and in low concentrations is an essential element in plant and animal life. In humans, prolonged excessive dietary intake of zinc can lead to deficiencies in iron and copper, nausea, vomiting, fever, headache, tiredness, and abdominal pain. Zinc is a human skin irritant. There are no reports on the possible carcinogenicity of zinc and compounds on humans (Fosmire, 1990).

5. Conclusion

Health-conscious people have repeatedly been told that fish is good for the heart. However, there is growing concern that some seafood lovers are consuming high doses of heavy metals along with their fish dishes and could be suffering from health problems as a result. This study emphasizes that some metal levels are higher than the acceptable values for human consumption set by various health organizations. The three fish species with different ecological needs from the Eastern Mediterranean showed high metal concentrations in their tissues. Results generally showed that metal concentrations were lowest in the muscle and highest in the gill and liver. This is probably due to their physiological roles in fish metabolism. The results show that metal accumulation in spring and winter seasons was higher than in the other season. This high level accumulation could be due to heavy rainfall during these seasons, which increases the metal content of water by washing down the agricultural wastes.

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