Coll. Antropol. **27** Suppl. 1 (2003) 31–39
UDC 614.31:614.777:597
Original scientific paper

Toxic Metals in Freshwater Fish from the Zagreb Area as Indicators of Environmental Pollution

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ABSTRACT

The aim of this investigation was to determine the levels of heavy metals and metalloids in freshwater fish from the Zagreb area. A total of 216 freshwater fish samples from 5 sites were examined: Sava river upstream from Zagreb, Sava river at Zagreb, Sava river downstream from Zagreb, Jarun Lake, and 5 »ecologic« fishponds from the Zagreb surroundings. The metals lead, cadmium, mercury and the metalloid arsenic were determined by the method of atomic absorption spectrometry. The mean pooled levels of lead, cadmium and arsenic in all fish samples were 112.3 ± 95 μg/kg, 8.5 ± 11 μg/kg and 23.5 ± 36 μg/kg, and did not exceed the allowed levels of 1,000 mg/kg, 100 μg/kg and 200 μg/kg, respectively. In 4 fish samples, the levels of mercury exceeded the allowed limit of 500 μg/kg (509, 596, 605 and 788 μg/kg), however, the pooled mean level of mercury was 127.8 ± 90 μg/kg. There was no major difference in the levels of heavy metals between the two fish families observed, although the levels of lead, cadmium and mercury were higher in the family Ictaluridae (144 vs. 107 μg/kg, 10.4 vs. 8.2 μg/kg, and 153 vs. 124 μg/kg, respectively), whereas the level of arsenic was higher in the family Cyprinidae (23.8 vs. 21.8 μg/kg). Although the Sava river at Zagreb is the main recipient of sewage and wastewater in the Republic of Croatia, the levels of heavy metals were within the allowed limits in all groups of freshwater fish samples, with the exception of 4 samples that contained moderately elevated levels of mercury. Study results suggest that only mercury could be considered a good indicator of environmental pollution, because higher levels of mercury were measured in the fish from the Sava

Received for publication January 7, 2003

river than in the fish from the Jarun Lake and fishponds from the Zagreb surroundings, considering both pooleded and fish family specified data.

Key words: *heavy metals, freshwater fish, environment, pollution indicators, Zagreb, Croatia.*

Introduction

Toxic metals have been increasingly investigated by a number of scientific disciplines, especially toxicology and epidemiology, as they occur in all settings and have a great although yet inadequately recognized impact on every living thing. Toxic metals and metalloids are chemical elements of a relative weight of 5.0. In the periodic table of the elements, 65 of them have the properties of metals, i.e. high thermal conductivity, high density, and forge ability. The increased interest in the impact of metals on human health as well as on the ecosystem has been aroused by numerous cases of poisoning with some of toxic metals recorded over the last few decades. Every naturally found or occurring element has a specific biochemical cycle, which determines its circulation in nature, from the atmosphere and hydrosphere through geosphere. Metals differ in their environmental motility and toxicological action on the plants, animals and man, depending on the metal type and chemical characteristics. Although particular inorganic species have different chemical characteristics, the highest differences result from the metal – carbon bonds, i.e. from the formation of organometallic compounds[1]. A considerable proportion of toxic metals and metalloids are taken to the human body with food of vegetable and animal origin. The level of metals in plants depends on the type, dominant tissue, level and availability of elements in the soil, distance from the source of emission, season, and weather conditions. A plant can receive metals directly through the leaves and sprouts, or *via* its roots from the soil, which is a more common route upon their conversion to more soluble forms[2].

On an average, sedimentation of metals in the atmosphere occurs within 10 km from the source of emission, however, mercury can be traced to a distance of 2,000 km because of its high vapor pressure. Generally, the metal sedimentation rate and distance from the source of emission depend on weather conditions, size of particles, topography, and vegetation. Emission can be natural as a consequence of volcanic eruption and forest fire, or may result from human activities. Lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) are released on oil and coal burnout, whereas lead is also released on metallurgic processes and ethylated oil burnout[3].

In comparison with ocean sediments, freshwater sediment contains less arsenic, more lead and mercury, and approximately the same level of cadmium. The plankton accumulates toxic metals, transport them and precipitate them in the sediment. Due to the effects of microbiologic activity and depending on the pH and physicochemical processes, these metals are then released from the sediment to the environment, or organic metal compounds of even higher toxicity may form[4-6]. Metals are constituents of the rocks and soil but can also occur as external contaminants. The primary sources of metal emission to the soil include mineral fertilizers and organic manure for Cd, As and Hg, lime and pesticides for Pb, As and Hg, whereas sewage sludge contains highest levels of Pb and Cd but less As and Hg. Contaminated irrigation water is another primary source of soil contamination with metals. Secondary sources of emission are smelting plants, exhaust gases, coal burnout, mining industry, garbage disposal, etc. The ability of man to utilize nature and its resources has resulted in the modern life amenities. However, the natural substances utilized by man are found in the soil, air and water, in both processed and natural form. When these substances have a toxic potential, they do not only interfere with the natural cycle integrity but also enter the nutritional cycle and may pose serious threat to all living beings[7-13].

Fish has been ever more used in diet for its energy and biologic nutritive value, first of all for its content of unsaturated fatty acids, ready and complete digestability, and presence of oligoelements[14,15]. Although dietary use of fish is related to traditional habits and geographic area, total fish consumption including freshwater fish is on a steady increase. In Croatia, *per capita* fish consumption is still quite low, however, a continuous rise has recently been recorded[16]. This trend is in part stimulated by the possibility of fishing as a recreation and sports activity, whereby the economic aspect of this type of fishing should by no means be neglected as a means of ensuring free 'meat meal' for the family, especially in view of the living standard decline during and after the war.

In line with this, the aim of the study was to determine the levels of heavy metals in the fish from the Sava river at and downstream from Zagreb because, after several decades, the number of anglers at the banks of the Sava river, its branches and adjacent lakes has recently been observed to increase. The City of Zagreb is not only the biggest urban agglomerate in the Republic of Croatia but also the largest industrial zone and communication junction, with the Sava river as the main wastewater recipient. Another aim was to investigate whether dietary habits and metabolic differences between the two large fish families living in these waters had an impact on their content of metals. The ultimate goal, however, was to determine the possible differences between the levels of heavy metals in the fish from the Sava river at Zagreb and those from the predictably less contaminated sites such as Sava river upstream from Zagreb, Jarun Lake connected with Sava, and several ecologic fishponds with no water connection with Sava.

Material and Methods

Study material included 216 freshwater fish from 5 sites in the broad Zagreb area. 38 fish represented group 1 from the Sava river upstream from Zagreb. Groups 2 and 3 included 34 and 31 fish from the Sava river at Zagreb and downstream from Zagreb, respectively. Group 4 had 82 fish from the Jarun Lake, which is connected with the Sava river by underground waters. Group 5 included 31 fish from the five ecologic fishponds in the Zagreb surroundings. These fishponds are free from contaminants and are primarily used for game fishing.

The fish from these locations belonged to two large families, *Cyprinidae* with 185 fish samples and *Ictaluridae* with 31 fish samples in total. The family *Cyprinidae* comprises the carp genera, characterized by the absence of teeth, instead of which they have a bone deep in the pharynx bilaterally, supplied with several teeth for food mastication, pushing, crumbling and chopping. The body is mostly covered with scales. These fish live in still water, at the bottom and in sand in search for food, and include the following species: chub (*Leucius leucius L.*), roach (*Rutilus rutilus L.*), bream (*Abramis brama L.*), barbel (*Barbus barbus L.*), goldfish (*Carassius auratus gibelio Bloch*), etc. The family *Ictaluridae* includes fish with no scales but naked body, spiked spines on the front and back fins, and eight barbs around the mouth. In the study material, only the pygmy catfish (*Ictalurus nebulosis Lesneur*) belonged to this family[17,18].

The levels of toxic metals and metalloids were determined by the method of atomic absorption spectrometry (AAS) on a Perkin-Elmer 4100 Zeeman ZL. FIMS-400 atomic absorption spectrometer (Überlingen, Germany)[19]. The level of detection was 25 μg/kg for Pb, 2.5 μg/kg for Cd, 7 μg/kg for As, and 1.5 μg/kg for Hg[20].

Results

Results are presented in Tables 1 and 2. The levels of lead, cadmium and arsenic did not exceed the maximal allowed levels in any of the 216 fish samples, whereas the level of mercury exceeded the maximal allowed level in only 4 fish samples.

The mean pooled level of lead in all fish samples was 12.3 ± 95 μg/kg and did not exceed the allowed level of 1000 μg/kg in any of the study samples. A higher level of lead was measured in the fish of the family *Ictaluridae* than in those of the family *Cyprinidae* (144 *vs.* 107 μg/kg). Among fish samples of the family *Ictaluridae*, the highest level of lead was found in the fish from the Sava river at Zagreb (193 μg/kg), and lowest in the fist from the Sava river upstream from Zagreb (36 μg/kg). Among fish samples of the family *Cyprinidae*, the highest and lowest levels of lead (156 *vs.* 82 μg/kg) were recorded in the fish from the Sava river downstream from Zagreb and from ecologic fishponds near Zagreb, respectively (Table 2).

TABLE 1
LEVELS OF TOXIC METALS (PB, CD AND HG) AND A METALLOID (AS) IN FISH FROM THE SAVA RIVER UPSTREAM FROM ZAGREB, AT ZAGREB AND DOWNSTREAM FROM ZAGREB, JARUN LAKE AND FISHPONDS WITH NO UNDERGROUND WATER CONNECTION WITH THE SAVA RIVER, ACCORDING TO FISHING SITES

Fishing site		Pb (μg/kg)	Cd (μg/kg)	As (μg/kg)	Hg (μg/kg)
Sava upstream from Zagreb (N = 38)	Min.	3	1	1	98
	Max.	228	53	282	605
	X (± SD)	84±57	11±13	36±58	168±102
Sava at Zagreb (N = 34)	Min.	1	1	0.3	51
	Max.	244	28	57	318
	X (± SD)	98±147	7±6	12±18	171±85
Sava downstream from Zagreb (N = 31)	Min.	59	2	1	56
	Max.	374	20	245	788
	X (± SD)	157±81	6±5	21±44	163±179
Jarun lake (N = 82)	Min.	3	1	0.1	19
	Max.	495	79	155	596
	X (± SD)	134±105	11±13	15±30	76±81
Fishponds without water connection with Sava river (N = 31)	Min.	3	0.1	0.3	11
	Max.	298	28	157	113
	X (± SD)	89±71	8±15	35±43	56±25
Maximal allowed level		1,000	100	2,000	500

TABLE 2
LEVELS OF TOXIC METALS (PB, CD AND HG) AND A METALLOID (AS) IN FISH FROM THE SAVA RIVER UPSTREAM FROM ZAGREB, AT ZAGREB AND DOWNSTREAM FROM ZAGREB, JARUN LAKE AND FISHPONDS WITH NO UNDERGROUND WATER CONNECTION WITH THE SAVA RIVER, ACCORDING TO FISHING SITES AND FISH FAMILIES

Fishing site	Fish family		Pb (μg/kg)	Cd (μg/kg)	As (μg/kg)	Hg (μg/kg)
Sava upstream from Zagreb	*Ictaluridae* (N = 2)	Min.	1	9	3	136
		Max.	71	17	19	239
		X (± SD)	36±79	13±6	11±34	187±176
	Cyprinidae (N = 36)	Min.	3	1	1	98
		Max.	228	53	282	605
		X (± SD)	87±56	11±13	37±58	167±98
Sava at Zagreb	*Ictaluridae* (N = 4)	Min.	131	4	1	89
		Max.	300	21	31	393
		X (± SD)	193±86	14±8	15±15	214±136
	Cyprinidae (N = 30)	Min.	1	1	0.3	51
		Max.	244	28	57	318
		X (± SD)	85±155	6±6	12±18	165±78
Sava downstream from Zagreb	*Ictaluridae* (N = 4)	Min.	100	3	19	74
		Max.	308	6	245	509
		X (± SD)	167±96	5±1	77±112	191±212
	Cyprinidae (N = 27)	Min.	59	2	1	56
		Max.	374	20	245	788
		X (± SD)	156±79	6±6	13±34	159±174
Jarun lake	*Ictaluridae* (N = 18)	Min.	71	3	1	35
		Max.	495	65	39	214
		X (± SD)	165±107	15±17	4±9	81±75
	Cyprinidae (N = 64)	Min.	3	1	0.1	19
		Max.	337	79	155	596
		X (± SD)	125±104	10±12	18±38	75±83
Fishponds without water connection with Sava	*Ictaluridae* (N = 3)	Min.	98	2	1	87
		Max.	190	7	2	97
		X (± SD)	157±51	5±20	2±0.4	91±5
	Cyprinidae (N = 28)	Min.	3	0.1	0.3	11
		Max.	298	28	157	113
		X (± SD)	82±73	8±14	39±47	52±27
Maximal allowed level			1,000	100	2,000	500

The mean pooled level of cadmium was 8.5 ± 11 μg/kg and did not exceed the allowed limit of 100 μg/kg in any of the fish samples. The fish of the family *Ictaluridae* contained a mean of 10.4 μg/kg cadmium, the highest level being measured in the fish from the Jarun Lake (15.0 μg/kg) and lowest in the fish from the Sava river downstream from Zagreb and from the ecologic fishponds (5.0 μg/kg). In the fish of the family *Cyprinidae*, the mean level of cadmium was 8.2 μg/kg.

The highest levels were recorded in the fish from the Sava river upstream from Zagreb (11 μg/kg) and lowest in the fish from the Sava river at Zagreb and downstream from Zagreb (6 μg/kg) (Table 2).

The level of arsenic did not exceed the allowed limit of 200 μg/kg in any of the fish samples, whereas the mean pooled level of arsenic was 23.5 ± 36 μg/kg. The mean level of arsenic in the fish of the family *Cyprinidae* was 23.8 μg/kg, the highest level being measured in the fish from ecologic fishponds (39 μg/kg), and lowest in the fish from the Sava river at Zagreb (12 μg/kg). The mean level of arsenic in the fish of the family *Ictaluridae* was 21.8 μg/kg, the highest (77 μg/kg) and lowest (2 μg/kg) levels being recorded in the fish from the Sava river downstream from Zagreb and from ecologic fishponds, respectively (Table 2).

Mercury levels higher than the allowed limit of 500 μg/kg were measured in 4 fish samples, however, the mean level of mercury was 127.8 ± 90 μg/kg. The mean level of mercury was higher in the fish of the family *Ictaluridae* (153 μg/kg), ranging from 81 μg/kg in the fish from the Jarun Lake through 214 μg/kg in the fish from the Sava river at Zagreb. The mean level of mercury in the fish of the family *Cyprinidae* was 124 μg/kg, ranging from 52 μg/kg in the fish from the ecologic fishponds through 167 μg/kg in the fish from the Sava river upstream from Zagreb.

Statistical testing of differences could not be performed because of the small number of fish of the family *Ictaluridae*.

Discussion

Zagreb, the capital of Croatia, with the surrounding districts (Zagreb County) has a population of >1.2 million. Zagreb is also the largest industrial zone with around 90,000 industry workers. Food industry, chemical industry and engineering industry prevail. In addition, Zagreb is an intersection of both domestic and international highway and railroad communication, and a city with the highest number of cars and other vehicles[21]. The City of Zagreb is located over water springs that are being supplied with water from the Sava river[22].

The long lasting uncontrolled draining of sewage and wastewater to the Zagreb underground and Sava river had led to contamination of some of the water springs on the one hand, and to considerable loading of the Sava waters, which resulted in condemning any bathing or fishing activities in the Sava river due to the lack of safety[22]. The polluters from Slovenia, using the Sava river upstream from Zagreb as a wastewater recipient, also contributed to this disturbing situation, which persisted for several decades with complete destruction of any form of life pending over the river.

With the establishment of the independent states of Croatia and Slovenia about a decade ago, both countries have tended to meet the standards of the industrialized European countries. In line with this, many plants in the two countries were closed or started working at a lower capacity because they were not competent on the international market anymore. Consequently, the waters of the Sava river have gradually turned ever more clear and acceptable in color, which has been confirmed by numerous freshwater analyses, at the same time pointing to lower water loading[21]. This sequence of events has resulted in the resumption of game fishing on the river banks, however, accompanied by a dilemma whether or not the fish from the Sava river is safe for human use, taking into consideration the fish lifespan and feeding habits. On these analyses, however, it should have been taken into account that the Zagreb population has not decreased, on the contrary, and that neither wastewater nor sewage have been submitted to cleansing prior to draining into the Sava river.

The results of our study showed the level of lead to be highest in the fish samples from the Sava river downstream from Zagreb. This is consistent with the reports relating the levels of lead in fish with the wastewater draining into, e.g. the Seine river, some English rivers, and some lakes in Hungary[23,24]. In the Zagreb area, there are two lead processing plants, one in the eastern and western part of the city each[21]. Thus, the load due to the wastewater draining from the plant in the western part of the city is additionally augmented by the load from the eastern part of the city, which then results in the expectedly highest load upon the waters of the Sava river downstream from Zagreb.

A quite opposite pattern was observed in case of cadmium. The fish from all sites showed very low levels, i.e. 10 times lower than the allowed levels of cadmium. The lowest level of cadmium was recorded in the fish from the Sava river at and downstream from Zagreb, indicating the entire Zagreb area to have been exposed to a very low contamination with cadmium. Nevertheless, it should be taken into account that the liver and kidneys are target organs for this metal, where its levels exceeding the allowed one can be expected, as demonstrated elsewhere[25-29]. The present study was focused on the analysis of the edible parts of fish as a foodstuff.

The metalloid arsenic showed an even more unexpected pattern in the Zagreb eco-system. The very low levels of arsenic in the Sava river at and downstream from Zagreb, and especially upstream from Zagreb as well as in the »clean ecologic« fishponds, support the role of the natural soil composition[25,30] instead of industry, the more so as the mean pooled levels were also by 10 times below the allowed limit. Thus, it appears that there is no major risk of the eco-system pollution with arsenic in the long run.

Mercury as the most common and most important fish contaminant was detected in the fish from all sites. The more so, in some fish samples, the levels of mercury exceeded the maximal allowed limit. In addition, the fish from all three Sava river sites contained more than twofold levels of mercury recorded in the fish from the Jarun Lake and ecologic fishponds. The results clearly call for caution and need of technologic improvement in the existing industry plants, along with the need of wastewater cleansing, in order to halt the growing trend of this contaminant in the Zagreb area. Other reports also describe mercury as a common and still actual contaminant of freshwater fish *via* draining of uncleanness wastewater into the stream, e.g., in Artic lakes[31], in the neighboring Hungary[32], and in India, where the situation is most serious, with the levels of mercury in freshwater fish reaching as much as 1,380 mg/kg[33].

In conclusion, the decline in industrial output as a consequence of transition and privatization has entailed a favorable impact on the freshwater fish contamination with heavy metals, lead and mercury in particular. However, the industrial production will certainly increase with time, therefore continuous monitoring is obviously needed, along with insisting on efficient cleansing of the wastewater from all potential industrial polluters. These tasks and the need of construction of the Zagreb sewage collector are the minimal and basic measures for the Zagreb area water eco-system to preserve at least in the present condition.

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TOKSIČNI METALI U SLATKOVODNIM RIBAMA ZAGREBAČKOG PODRUČJA KAO POKAZATELJI ONEČIŠĆENJA OKOLIŠA

S A Ž E T A K

Cilj ovog istraživanja bio je utvrditi količine teških metala i metaloida u slatkovodnim ribama zagrebačkog područja. Ukupno je ulovljeno i obrađeno 216 uzoraka slatkovodne ribe s 5 lokacija: rijeke Save uzvodno od Zagreba, rijeke Save kod Zagreba, rijeke Save nizvodno od Zagreba, jezera Jarun i 5 »ekoloških« jezera u okolici Zagreba. Metali: olovo, kadmij, živa i metaloid arsen određivani su metodom atomske apsorpcijske spektrometrije. Srednja vrijednost olova, kadmija i asrena u svim ribama bila je 112.3 ± 95

µg/kg, 8.5 ± 11 µg/kg i 23.5 ± 36 µg/kg i niti u jednom uzorku nije prelazila dozvoljenih 1,000 µg/kg, 100 µg/kg i 200 µg/kg. Kod 4 uzorka nađene su vrijednosti žive koje su prelazile dozvoljenih 500 µg/kg (509, 596, 605 i 788 µg/kg), ipak ukupna prosječna vrijednost žive bila je 127.8 ± 90 µg/kg. Ne postoji bitna razlika u količini utvrđenih teških metala između dvije promatrane porodice riba, premda su količine olova, kadmija i žive više u porodici *Ictaluridae* (144 prema 107 µg/kg, 10.4 prema 8.2 µg/kg i 153 prema 124 µg/kg), a arsena u porodici *Cyprinidae* (23.9 prema 21.8 µg/kg). Unatoč činjenici da je rijeka Sava kod Zagreba najveći recipijent komunalnih i industrijskih otpadnih voda u Republici Hrvatskoj, količine teških metala u svim skupinama uzoraka unutar su dozvoljenih vrijednosti (osim kod 4 uzorka koja su sadržavala umjereno povišene vrijednosti žive). Jednako tako tek se, prema našim istraživanjima, samo za živu može reći da je dobar indikator onečišćenja okoliša, jer su ukupno i kod obje porodice riba vrijednosti žive više u rijeci Savi u odnosu na jezero Jarun i ekološka jezera.