METAL POLLUTION INDEX: PROPOSAL FOR FRESHWATER MONITORING BASED ON TRACE METAL ACCUMULATION IN FISH

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Abstract. Different fish species (benthivore and piscivore) from the Channel System Danube - Tisza - Danube in Yugoslavia have been analysed for body trace metal burden with an aim to establish freshwater metal pollution biomonitoring. New Metal Pollution Index (MPI) which distinguishes polluted from unpolluted ecosystems is proposed, based on acquired knowledge on metal bioavailability, bioconcentration and bioaccumulation patterns. This simple mathematical model is calculated upon logarithmically transformed values of metal concentrations in fish tissue, in order to achieve normal distribution of the element values and to diminish the more than thousand-fold differences between the least and the most abundant elements. MPI is the simplest way which enables presentation of all results from the metal concentrations as one value, by using a normalizer (reference values for lower part of the Danube Basin) to account for biological variation in non-polluted areas. Since no biomagnification within fish trophic chain has been observed, benthic fish seem to be, due to the highest metal burden, more suitable for evaluation. Furthermore, *Carassius auratus gibelio*, being presently one of the most abundant species inhabiting the Yugoslav part of the Danube watershed is being proposed as sentinel organism.

Key words: trace metals, Metal Pollution Index, freshwater monitoring, fish, Danube Basin

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Introduction

International waters, particularly large watersheds as the Danube river basin is, are subjected to both huge amounts of waste water input and different legislative and enforcement measures. Therefore, there is a need to establish internationally standardised methods for freshwater monitoring, which seems not to be a problem when considering chemical analysis of sediments, waters and wastewaters. However, biological monitoring (using bioindicators) is highly dependent on biodiversity and species richness within the region. Therefore, the objective of this paper is to contribute to finding the most appropriate model and applicable sentinel organisms for trace metal pollution monitoring within the lower part of the Danube river basin.

Trace metal accumulation patterns in aquatic biota, along with bioconcentration and biomagnification processes have been excessively studied in the Danube Basin (Salanki *et al.* 1982, Pujin *et al.* 1990, Wachs 1991, Maletin *et al.* 1992). Some valuable attempts have been made (Wachs 1992/93) in introducing the classifications of freshwater ecosystems according to fish muscle metal burden. Such evaluations have even been applied for the Yugoslav part of the Danube watershed (Maletin *et al.* 1996, Djukic *et al.* 1998a), with an idea that fish muscle metal burden could be more reliable water quality indicator than chemical analysis of water-column and sediment. Fish tissue metal content has also been successfully used in estimations of trace metal input into large European and American rivers. (Allen-Gil and Martynov 1995, Chevreuil *et al.*

1995, Saiki *et al.* 1995, Carru *et al.* 1996). However, the cited literature reviled extremely diverse approaches to choosing the most appropriate fish group, species and tissue for these kind of monitoring studies. Considering that fish tissue preparation methods and analytical methods for metal determinations as well have been standardised so far (APHA 1989, U.S. EPA 1991), the attempt herein has been made in finding the most suitable species and tissues for bioaccumulation studies and particularly the simplest mathematical model for evaluation and comparison of results, at least within the region.

Material and methods

The fish for this study was caught in spring 1997, in irrigation channel Danube - Tisza - Danube at the dam in Becej (separating Tisza from the channel) and in microreservoir "Moharac" which, due to its position (remote from industry, urban zones and major roads) served as reference site. The sample consisted of piscivore - *Silurus glanis* (from both locations), *Tinca tinca* (from the channel) and *Carassius auratus gibelio* and *Abramis brama* (from the microreservoir). Investigated tissues included muscle, gills, liver and kidney. Tissue digestion and sample preparation was done according to standard procedure (U.S. EPA 1991) and Perkin Elmer AAS (flame and graphite furnace with background correction) was used for Cd, Zn, Cu, NI, Cr, Pb and Al determinations (APHA 1989). All results are presented on a wet weight basis, as mg/kg, but recalculation factors from wet to dry weight basis are presented for comparative purposes: muscle 5; liver 2.6 and kidney 5.5. Means ± SD are chosen in the presentation of data, statistical significance was assigned at $p \leq 0.05$ after one way ANOVA analysis. In all statistical analyses, values bellow the detection limits were replaced by half of the detection limit. Fish muscle metal burden has been used for environmental evaluation (Teodorovic *et al.* 1998). Metal Pollution Index (MPI) has been introduced and calculated for the chosen site, yet the calculation will be explained further in the text.

Results and Discussion

Three species from reference site (microreservoir "Moharac") with total number of 25 specimens, and two species from the chosen site of the Danube - Tisza - Danube (Becej dam) Channel with 20 specimens in total, were analysed on metal body burden. As the literature revealed very few differences in heavy metal concentrations between sexes (Scharenberg *et al.* 1994), this parameter was not included in the data handling. The available data (number of samples) are too few to allow the analysis of age/size/weight dependence. However, grand scale studies (Strip *et al.* 1990, Spry et Wiener 1991, Dietz *et al.* 1996) showed that concentrations of metals within a fish population (with exemption of Hg) do not typically increase with increasing age or body size. Consequently, these parameters were also omitted in the data handling.

The results presented in Figures 1-5 show tissue/organ distribution pattern of metal accumulation in wels. Generally, the patterns in other species are quite similar, although the sites for accumulation vary with route of uptake and are, to a certain extent, species-specific. The highest concentrations of Cd (0.82 mg/kg wet wt.) were detected in kidneys, then in liver (0.19 mg/kg. wet wt.) which is in accordance with previous findings (Kraal *et al.* 1995, Allen 1995, Djukic *et al.* 1998b). Cu, Pb, and Zn accumu-

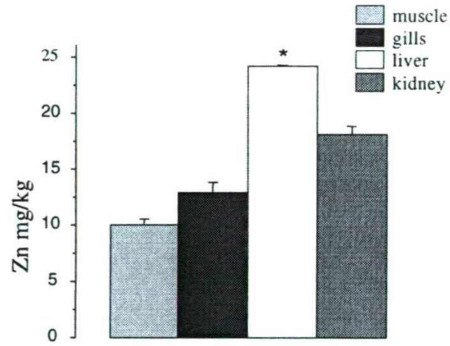

Fig. 1 Distribution of Zn in the tissues of wels from DTD Hydrosystem (sampling site Becej dam). Values represent mean±SD (n=10); * -significant difference p≤0.05

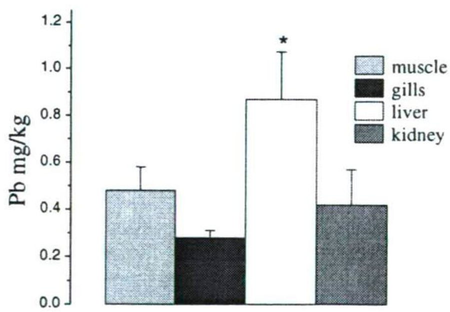

Fig. 2 Distribution of Pb in the tissues of wels from DTD Hydrosystem (for legends see Fig. 1)

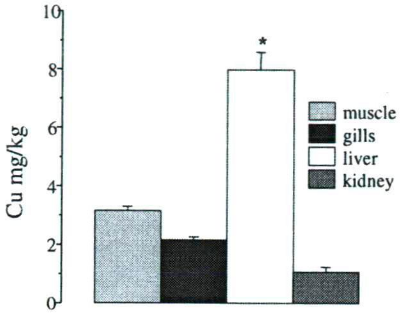

Fig. 3 Distribution of Cu in the tissues of wels from DTD Hydrosystem (for legends see Fig. 1)

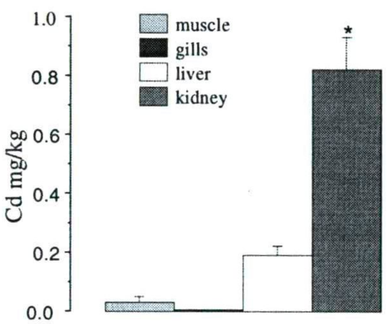

Fig. 4 Distribution of Cd in the tissues of wels from DTD Hydrosystem (for legends see Fig. 1)

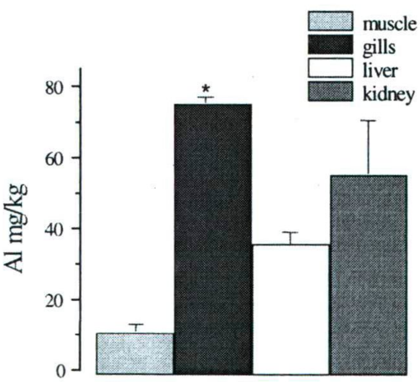

Fig. 5 Distribution of Al in the tissues of wels from DTD Hydrosystem (for legends see Fig. 1)

lated chiefly in liver, (8.01, 0.87, and 24.2 mg/kg wet wt. respectively) which is also reported to be the pattern. (Salanki *et al.* 1982, Pujin *et al.* 1990, Jorgensen and Pedersen 1994).

The highest Al concentrations were found in gills (76 mg/kg wet wt.) and these findings support the existing theory (Poleo *et al.* 1997) that fish exposed to aqueous Al (both in laboratory and field) readily accumulate the metal in and on the gill, and that concentrations of the analyte are much less in blood and internal organs. According to our results, muscle proved to be the tissue with low metal burden of all analytes, which supports the thesis that metal contamination of fish, particularly with cadmium and lead, generally need not to be considered as a significant health risk to human consumers (Spry and Wiener 1991).

Comparison of muscle metal burden in piscivore (wels) and benthivore (tench) is shown in Fig. 6 Zn, Cd, Al and Ni content was slightly while Pb concentration was significantly higher in tench.

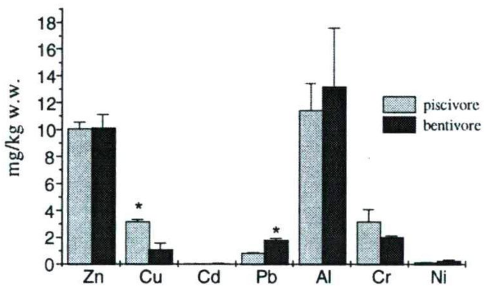

Fig. 6 Muscle metal burden in piscivore (wels) and benthivore (tench) from the DTD Channel. Values represent mean±SD (n=10 for piscivore and 15 for benthivore); * -significant difference p≤0.05

Essential micronutrients Cu and Cr were detected in higher concentrations in wels, which could be explained by species-specific requests and regulation of these elements. These results lead to a conclusion, which stands in accordance with previously published data (Scharenberg *et al.* 1994, Carru *et al.* 1996, Djukic *et al.* 1998b), that no biomagnification within the fish trophic chain could be observed Consequently, benthivore fish muscle metal content has been used for environmental evaluation.

According to Wachs (1991, 1992/1993), metal concentrations in freshwater fish muscle could be used in hydroecosystem classification. Consequent-ly, the cited author introduces an environmental classification of freshwater ecosystems into 7 classes, based on muscle metal burden of the Danube fish, with no specification of species. In previously published papers (Djukic *et al.* 1998c, Teodorovic *et al.* 1998) we slightly revised the mentioned classification and suggested a new one, more suitable for the lower part of the Danube. Moreover, our opinion is that this newly proposed classification of freshwater ecosystems (also based on fish muscle metal content) more precisely takes into account standard detection limits for chosen metals, influence of local/regional geochemistry and food standard limits within the region. Basically, detection limits (APHA, 1989, US EPA, 1990) are set as an ambient standard for I class waters. Ranges for I-II class waters are set to cover minimal detected Cu, Cr and Zn concentrations (0.33. 0.1 and 5 mg/kg wet wt., respectively) in fish muscle from unpolluted reference site (Moharac microreservoirs). (Teodorovic *et al.* 1998). These values represent the lowest concentrations measured after analysing more than 20 locations within the Yugoslav part of the Danube basin. (Pujin *et al.* 1990, Maletin *et al.* 1992, Maletin *et al.* 1996, Djukic *et al.* 1998a, b, c, Teodorovic 1997, Teodorovic *et al.* 1998). Therefore, microreservoir Moharac has been chosen as a reference site to provide information on natural metal levels in non-contaminated fish, from non-polluted areas located within Yugoslav (e.g. lower part of the Danube River Basin). National food standard limits for edible fish tissues (Cd - 0.1 mg/kg wet wt. and Pb - 1 mg/kg wet wt. - Anon. 1992) are set as upper ambient standard values for III class waters.

Table 1, therefore, represents the environmental evaluation of Danube - Tisza - Danube Channel (sampling site Becej) based on tench muscle metal burden. Such classification could be useful in human health risk evaluation, providing it deals only with edible fish tissue metal load. It should be pointed out that Pb concentration in tench from the mentioned location exceeds Yugoslav food limits for edible fish tissue (1 mg/kg wet wt).

However, there are many problems with implementation of this classification. First of all, in total score, it treats highly toxic (Cd, Pb) and essential metals (Zn, Cu) equally. Further on, it doesn't represent the actual state of the chosen ecosystem as it is made on muscle burden basis, although it has been showed that other tissues (liver, kidneys and gills) could be basically refereed to as "target zones". (Figs 1-5). Moreover, it doesn't calculate for biological variability, since the exact sentinel species hasn't been chosen.

Therefore, we made an attempt in introducing Metal Pollution Index (MPI) as a mathematical model which could solve some of the highlighted problems. MPI has been calculated to enable presentation of all results from the metal concentrations (Cd, Cu, Zn, Pb and Al) as one value if possible, yet overcoming the difficulties with both application and understanding of demanding statistical analysis. According to Jorgensen and Pedersen (1994), this implies that the five metal concentrations must be normalised to make it possible to sum up and average the different metal concentrations into one value. We have chosen the average values of fish tissue burden (liver for Cu, Zn and Pb, gills for Al and kidney for Cd) from the reference site. (Moharac microreservoir) Such normaliser is used to account for the biological variation in a non-polluted area. Since no significant difference has been found between metal concentration in wels, prussian carp and bream from the reference site, (Teodorovic 1997, Teodorovic 1998 - unpublished) the sample has been pooled so the reference values represent the mean of 25 specimens. (Table 2). Furthermore, the data were logarithmically transformed to achieve normal

Table 1. Ecosystem classification based on fish muscle metal burden and environmental evaluation of DTD channel (at Becej dam)

class	Cd	Cu	Ni	Cr	Pb	Zn	
I	<0.015	<0.3	<0.03	<0.1	<0.01	<5	
I-II	0.015-0.02	0.3-0.5	0.03-0.1	0.1-0.5	0.01-0.05	5-10	
II	0.02-0.05	0.5-1	0.1-0.4	0.5-1	0.05-0.1	10-15	
II-III	0.05-0.08	1-2	0.4-1	1-1.5	0.1-0.5	15-20	
III	0.08-0.1	2-3	1-2.5	1.5-3	0.5-1	20-25	
III-IV	0.1-0.5	3-5	2.5-4	3-5	1-2	25-35	
IV	>0.5	>5	>4	>5	>2	>35	
muscle content tench mean±SD n=10	0.02±0.005	1.07±0.5	0.23±0.1	1.99±0.1	1.78±0.12	10.14±1	TOTAL
class	I-II	II-III	II	III	III-IV	II	II-III

Table 2. Metal Concentrations in wels and tench tissues; Reference values and MPI

	tissue	wels mg/kg wet wt. Mean (n=10) ±SD	(x) - tench mg/kg wet wt mean (n=10) ±SD	reference value (ref.)	x/ref.
Zn	liver	24.2± 1	34 ± 9*	20	1.7
Cu	liver	8 ± 0.6	13.7 ± 2.5*	2	6.85
Pb	liver	0.87 ± 0.3	3 ± 0.6*	0.2	15
Cd	kidney	0.82 ± 0.3	0.9 ± 0.2	0.1	9
Al	gills	76 ± 1	85 ± 3*	20	4.25
				Σ	36.8
			MPI=	log Σ	1.57

*$p \leq 0.05$ - significantly different from wels; reference values mean (n=25) concentrations in pooled sample from Moharac reservoir

distribution of the element values and, what is more important, to diminish the more than thousand-fold difference between the least and the most abundant elements. Without such transformation, the least abundant elements would be without influence on the results. (Julshamn and Grahl-Nielsen 1996).

MPI has been calculated as:

$$MPI = \log \sum_{i=1}^{n=5} \frac{[\bar{x}]}{ref_i}$$

where ref_i represents a normalizer, or a reference value for each of five chosen metals (Cd, Cu, Pb, Zn and Al) in selected tissues, while x represents mean value (n≥10, SD up to 30%) of metal concentration in the same tissues from the chosen sampling site. If calculated as proposed, MPI distinguishes „polluted" from „non-polluted" ecosystem: if this combined index is above 1 the concentrations of trace metals would be considered elevated and ecosystem could be regarded as „polluted".

Table 2 presents the liver, kidney and gill concentrations of Zn, Cu, Pb, Cd and Al in wels and tench from Channel Danube — Tisza — Danube (location Becej) and the reference values from unpolluted site. As all metal concentration in selected tissues were higher in tench than in wels (all but for Cd significantly), tench metal load has been used in MPI calculation. Also, the values of x/ref. ratio are presented and MPI is calculated. According to its value (1.57) location Becej could be regarded as „polluted" when trace metals are concerned. As it is obvious from the Table 2, the main component of the index is the Pb concentration, followed by Cd. The rest of the concentrations (essential and in the same time the most abundant metals) tend to contribute to a smaller extent. Our opinion is that this is the point where the muscle metal burden - based classification failed, while MPI succeeded: the most toxic and hazardous elements (Pb and Cd) contribute the most to this combined index.

Although fish do not fulfil all requirements for indicator organism (e.g., they are not sedentary) OECD and ICES agreed upon using trace metal concentrations in stationary fish as possible indicators in areas affected by human activities. (Jorgensen and Pedersen 1994). As benthivore fish proved to accumulate higher amounts of trace metals than piscivores, our opinion is that adequate sentinel species could be chosen from this group. To avoid possible species-specific differences, our suggestion is that *Carassius auratus gibelio*, which, according to recently published data (Jankovic 1994, Maletin *et al.* 1997) makes up to 50% out of total catch in Yugoslav part of the Danube Basin, could successfully serve as sentinel species. Besides, to diminish possible age/size influence on trace metal content, our suggestion is that only specimens belonging to same age group must be used in MPI calculations. Moreover, as seasonal variations in metal content has been observed (Balogh *et al.* 1985, Kock *et al.* 1996), the sampling has to be undertaken always within the same season, at precisely the same location. Our opinion is that MPI, calculated as proposed, with all applied constrains, could serve, in future, for time trend analysis of metal pollution within the region. In spite of indisputable importance of established chemical, biochemical and biological methods, our stingiest belief is that MPI might be included in complex freshwater monitoring programmes since it could produce some additional information on metal bioavailability, bioconcentration and metal input into the environment.

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