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Assessment of the Heavy Metal Contamination in the Danube Delta from the Bioaccumulation Perspective

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Strictly as per the compliance and regulations of:

Assessment of the Heavy Metal Contamination in the Danube Delta from the Bioaccumulation Perspective

Gati Gabriel [α], Cristian Pop [σ], Florin Brudașcă [ρ], Anca Elena Gurzău [Ѡ] & Marina Spînu [¥]

Abstract- The objective of this work is to assess the heavy metal contamination of water and sediment in the Danube Delta, approaching also the bioaccumulation and trophic transfer issue. It was conducted in15 sampling sites in Sf. Gheorghe Branch, were collected water, sediment and biological samples from two species of fish with different habitat and trophic level (Crucian carp - Carassius auratus gibelio and Zander - Sander lucioperca). All samples, were analyzed for Pb, Cd, As and Hg by atomic absorption spectrophotometry. The bioaccumulation and trophic transfer assessment was done calculating the bioaccumulation (BAF) and biomagnification factor (BMF). Our pilot study showed that the heavy metal concentration varies from sediment to water where a significant correlation between water-sediment metals concentration was only in case of As. The distribution of the metals in fish organs shows differences between the two investigated species: in Crucian carp the concentrations of metals were Pb>As>Cd>Hg, Pb and As being predominant in external organs and in Zander the concentrations of metals were Pb> Cd>As> Hg, Cd,As and Hg being predominant in internal organs. BAFs showed a fairly high rate for mercury and lead and low arsenic rate in both species.

Keywords: danube delta, heavy metals, contamination, bioaccumulation.

I. Introduction

Heavy metal pollution constitutes an actual problem in the entire world.

Situated in the eastern extremity of Romania the Danube Delta, is part of the Danube Delta Biosphere Reserve. The Danube Delta's plain covers an area of over 5.800 km2, which includes the 1.800 km2 marine delta plain (Panin, 2003) and it starts from the first bifurcation of the Danube, at Ceatal Izmail, forming the Chilia (Kilia), and the Tulcea distributaries, which 17 km downstream divides again to form the Sulina and Sf. Gheorghe (St. George) distributaries (Panin, 2003).

The objective of this work is to assess the heavy metal contamination of water and sediment and the correlation between them in the Danube Delta. Also, this paper approaches the bioaccumulation and trophic transfer issue, the tendencies that are emerging in the Danube Delta after the pilot study and it has developed an experimental model (pilot study), the establishment of the best fitted sampling and analysis methods for heavy metals, and the choice of the right exposure biomarkers.

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II. Materials and Methods

a) Study area

The Danube Delta sampling location was settled in the Sf. Gheorghe Branch area, between the Turcesc and Central Channels, nearby Sf. Gheorghe locality (44°53'N & 29°36'E). it is the area with the fewest point sources of contamination being the place of choice for studying the general heavy metal contamination of the Danube Delta.

Water, sediment and fish samples were collected in October 2012.

b) Sampling

There were 15 sampling sites, and there were collected water and sediment samples for each one, resulting in 15 water samples and 15 sediment samples. Water samples were collected at 20-30 cm under the water surface in 50 ml polyethylene demineralised containers, and conserved through acidification at pH<2 with 0.25 ml of concentrated HNO_3 solution, kept at 4°C until the transportation to the laboratory.

Sediment samples were collected from the margin and bottom of the main branch and channels, in 100g amounts, kept at 4°C in metal free plastic bags.

Biological samples were collected from two fish species with different habitat and trophic level. The first one is the Crucian carp (Carassius auratus gibelio), representing a benthic, omnivorous fish with a low-medium trophic level, and the other is the Zander (Sander lucioperca), representing a pelagic, top-carnivorous fish. The sampling consists of liver, spleen, gonads, skin, scales and fins. The samples were frozen immediately.

c) Analytical methods

All samples, of each type, were analyzed for Pb, Cd, As and Hg.

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The water samples were analysed using a Zeenit 700P Spectrophotometer (AAS). HG-AAS (Hydride Generation) was used for the analysis of As, supplemented with amalgamation and CVAA (Cold Vapor Atomic Absorption) method for Hg, and respectively the ET-AAS (Electrothermal) method for Pb and Cd.

The sediment samples were subjected to a microwave digestion in suprapure nitric acid, using a Mars 6 Microwave digester. After that, the metals were analysed using a Zeenit 700P (AAS), As, Pb and Cd with the ET-AAS and Hg with the HG-AAS with Amalgamation/CVAA method.

The biological samples were also mineralized in suprapure nitric acid in a microwave digester. These were analysed using a Zeenit 700P Spectrophotometer (AAS), through ET-AAS method for Pb and Cd, HG-AAS Method for As and HG-AAS with Amalgamation/CVAA method for Hg.

An external standard curve method was used for the calculation of the concentrations.

Quality control was made through specific methods, to evaluate the accuracy of our analyses, meeting the RENAR (the Romanian Accreditation Association) standards and certifications.

d) Data analysis

The bioaccumulation and trophic transfer assessment was done by the calculation of the bioaccumulation factor (BAF) (1) and respectively the biomagnification factor (BMF) (2). These were calculated following Gobas & Morrison`s (2000) formulas:

1. BAF= CB/CW
2. BMF= CB/CD

The BAF is expressed in the form of the ratio between the chemical concentrations in the organism (CB) and the chemical concentrations in the water (CW), respectively the BMF is the ratio of the chemical concentrations in the organisms to the concentration in the organism`s diet (CD) (Gobas & Morrison, 2000). The chemical concentration in the organism is usually expressed in units of mass of chemical per kg of organism, whereas the concentration in water is expressed in mass units per litre. Therefore, the unit for BAF is L/kg, while BMF is unitless. The weight of the organism can be expressed on a dry weight (DW), wet weight (WW) or lipid weight (LW) basis (Idem). For the use of other studies as reference in the comparison of the BAF and BMF, the weight of the organism is expressed in a wet weight basis. Geographical representation through GIS allows a more efficient approach for the integrated analysis of the spatial-temporality aspect. The maps were created using Open Source GIS Software – Q-GIS 1.8 (Quantum GIS, 2012) and the Geographic Resource Analysis Support System (GRASS) 6.4.2 (GRASS, 2012).

To highlight the spatial distribution of the heavy metal concentrations from the water and sediment samples, these were rendered in a geographic information system (GIS). Metal concentrations are represented by bar charts.

The analyses results (heavy metals) of water samples were correlated to the sediment samples from the same sampling sites. The correlations were verified through the Pearson`s correlation coefficient ("r"), which is a measure of the strength and direction of the linear dependence between two variables. Pearson`s correlation coefficient can take values between -1 (perfect anti-correlation) and 1 (perfect correlation).

III. Results & Discussions

a) Water and sediment results

The heavy metal concentration and distribution of the samples varies from sediment to water. Higher values were found in sediments mainly close to the Sf. Gheorghe Branch, as can be seen in Figure 2. The sediment analysis revealed lead (18,18 mg/kg DW +/- s.d. 9,8), arsenic (7.97 mg/kg DW +/- s.d. 5,6) and mercury (0,04 mg/kg DW +/- s.d. 0,04), while the water analysis revealed only lead (1,01 µg/L +/- s.d. 0,3) and arsenic (1,96 µg/L +/- s.d. 0,6). The detection limits of our methods were 0,1 mg/kg DW and 0,5 µg/L for cadmium in sediment and water, respectively 0,1 µg/L for mercury in water. There was no concentration exceeding recorded, according to the 161/2006 Romanian Normative framework.

Even if there aren`t any major contamination point sources identified in the sampling area, heavy metal concentrations were found in the water and sediment. Due to the emission of heavy metals along the international course of the Danube River system and to the sedimentation and re-suspension hydrodynamic conditions that occur, the deposition is taking place far downstream of the discharge. Even so, compared to the results of Woitke et al. (2003), regarding the range of metal concentrations through the entire Danube River and its Delta, Pb, Cd, As and Hg concentrations of our analysed sediment samples fall in the lower limits or even slightly below the Danube River`s concentration range (As [9-68,9], Cd [<1,1-25,9], Pb [14,7-107,6] and Hg [<0,1-2,37], expressed in mg/kg DW).Our results of metal concentrations in sediment, are also comparable to those in the Chilia Branch and it`s secondary delta from the territory of Ukraine (Vignati & Berlinsky 2010) being slightly lower in a few places.

Table 1: Pearson Correlation for water-sediment samples

	Pb in sediments	As in sediments
Pb in water "r"	-0.344	
As in water "r"		0.537

From our Pearson calculated statistics data (Tab.1) for heavy metal concentrations, we can say that there is a low and negative ("Pearson") correlation between the sediment-water sample concentrations for Pb (-0,344), meaning that there is an anti-correlation. In the case of As, the correlation is higher and positive

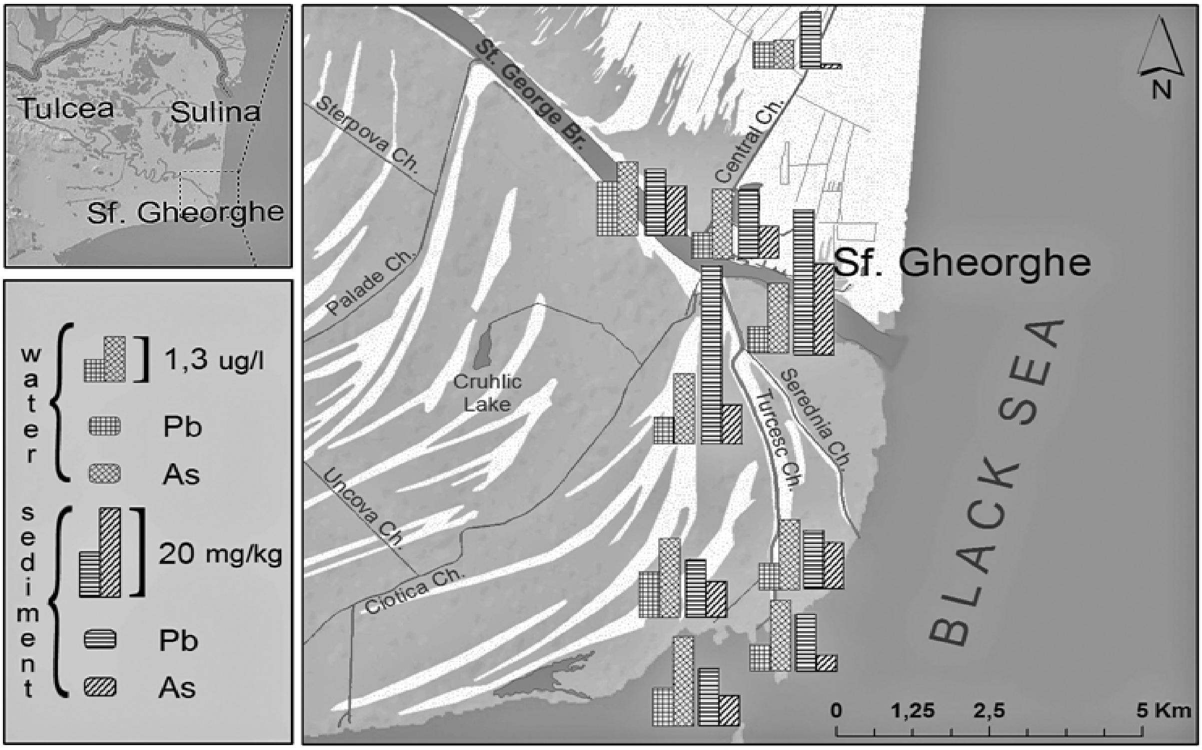

Fig. 2 : Above is the example of single column image. Images must be of very high quality

(0.537). These results are suggesting that there is mobilisation and balance between these two matrices. While the sediment analysis revealed Pb>As>Hg, the water analysis revealed only As>Pb.

This can happen because of the different physico-chemical conditions that occur in the Danube Delta, due to the low water speed(0,3 m/s - shallow channels to no more than 2 m/s - deep parts of the main branches), the floodplain width and specific flow hydraulics. In wider floodplain sections, the heavy metals tend to accumulate near the channel margins, from where these can be remobilised due to bank erosion processes (Wyzga & Ciszewski, 2010).

b) Fish sample results

Heavy metals tend to be absorbed by some fish species through the food, water and sediments, regardless of their biological needs or nutritional category (Yilmaz, 2003, Mendil et al., 2010).

In the figure 3 we present the mean heavy metal concentrations in internal (liver, spleen, gonads) and external (skin, scales, fins) organs of the sampled fishes. Overall, for each studied heavy metal, the concentrations in all the analysed organs were higher in Crucian carp than in Zander.

Our sampled Crucian carps revealed (Fig. 3) higher levels of Pb (0.85 mg/kg WW), compared to As (0.38 mg/kg WW) and Cd (0.13 mg/kg WW) levels; the Hg concentration was the lowest between the four measured elements (0.08 mg/kg WW). The predominant bioaccumulation of Pb and As are in external organs, while the Hg has been found in higher concentrations in internal organs. Related to Cd, this metal was similarly bioaccumulated in external and internal organs.

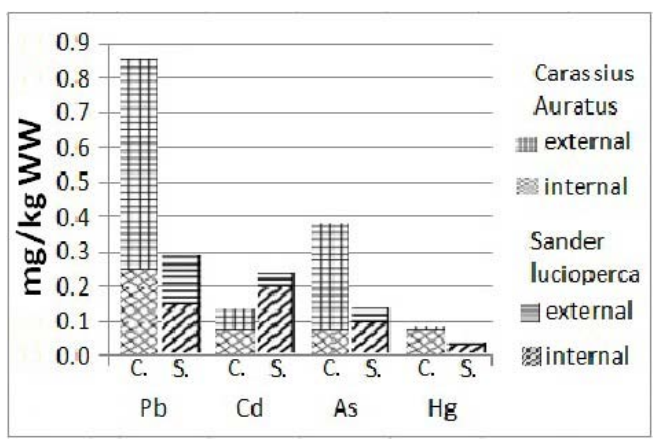

Fig. 3 : Mean metal concentrations in sampled fish in internal and external analysed tissue

Detailing the bioaccumulation of studied metals in organs, it has to be mentioned that the concentration of cadmium from the gonads (0,18 mg/kg WW) and scales (0,12 mg/kg WW), and of lead from the liver (0,57 mg/kg WW) were over the maximum levels accepted for human consumption according to the European references (Commission Regulation (EC) 1881/2006). The order of the affected tissue by the highest quantity of heavy metals is liver>gonads>scales>skin>fins> spleen.

It is known that benthic fish species are dietary exposed to heavy metals through consumption of zoobenthic biota (e.g. shellfish, worms) from contaminated sediment (Sakurai et al., 2009; Clearwater et al., 2002)

Our sampled Zander individuals (Fig. 3) had higher concentrations of Pb (0,30 mg/kg WW) and Cd (0.24 mg/kg WW), compared to lower concentrations of As (0,13 mg/kg WW) and Hg (0,03 mg/kg WW). The distribution of the metals in Zander organs shows differences compared to the Crucian carp. Cd, As and Hg are predominant in internal organs, while the Pb similarly bioaccumulated in external and internal organs.

Detailing the bioaccumulation of studied metals in organs, it has to be mentioned that cadmium measured in gonads (0,31 mg/kg WW), liver (0,22 mg/kg WW), scales (0,12 mg/kg WW) and spleen (0,055 mg/kg WW) and lead in skin (0,35 mg/kg WW) exceeded the maximum levels accepted for human consumption according to the European references. The order of the affected tissue by the highest quantity of heavy metals is liver>gonads>skin>spleen>scales> fins.

In the Danube River and Delta, there are few studies on accumulation of heavy metals in fish species to make reference to. Zelika et al. (2010) showed high concentrations of metals in Pontic shad (Alosa immaculata Bennet 1835) gills, liver and lower in the muscles, mainly Cd and As levels were higher than the maximum acceptable concentrations for human consumption according to the European references. Jaric et al., 2011 found high levels of heavy metals in sterlet (Acipenser ruthenus) liver and lower levels in the muscles, acceptable for human consumption according to the European references, excepting cadmium. The sheatfish (Silurus glanis) was measured with the lowest levels of all metals (Pantelica et al., 2012). Filazi et al., 2003 revealed that many fish species have high concentrations of Cu, Pb and Cd, especially in the liver. The highest concentrations were found in August and the lowest in May.

The metal bioavailability has a great effect over the bioconcentration and bioaccumulation in the aquatic biota.

The bioaccumulation factors (BAFs) calculated for our sampled fish (Fig. 4) showed a fairly high rate for mercury, followed by lead and cadmium in the case of the Crucian carp, and a high rate for cadmium, followed quite closely by mercury and lead in the case of the Zander. The arsenic showed a low BAF in both species. Different studies show that the heavy metals bioaccumulation factors for all organisms are highest in the plankton, followed by the zoobenthos, predator fish species and herbivorous fish species depending on the organisms place in the food chain, their feeding behaviour specific habits, physico-biochemical characteristics and age (Culioli et al., 2009, Tao et al., 2012, Pantelica et al., 2012). In several studies, the highest bioaccumulation factor in the fish organs and

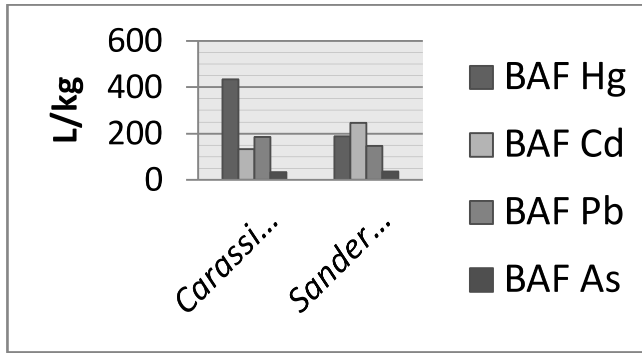

Fig. 4 : Bioaccumulation factors for sampled fish (mean)

tissues is shown by Fe and Zn, followed by Cu, Pb, Cd and As (Uluturhan & Kucuksezgin, 2007; Ayotunde et al., 2012; Nwani et al., 2010; Farombi et al., 2007).

Classing our resulted BAFs by specific metal and fish tissue, arises the following remarks: Hg and As revealed the highest values in the liver of both species (1092 L/kg Hg for Crucian carp and 361,4 L/kg Hg for Zander, respectively 89,6 L/kg As and 86,1 L/kg As); Cd showed the highest values in the gonads of both species (356,4 L/kg for Crucian carp and 629,1 L/kg for Zander); Pb showed the highest values in the liver of the Crucian carp (564,3 L/kg) and in the skin of the Zander (343,7 L/kg).

Several authors observed that fish from various locations show similarities regarding the assimilation and bioaccumulation of metals in different parts of their organisms. The concentration of heavy metals was observed to decrease in the following order: liver> gills> skin and gonads> muscles and other measured tissues (Bashir et al., 2012; Cogun et al., 2006; Dural et al., 2006; Yilmaz, 2003).

Assuming that the Crucian carp signs up as a potential prey of the Zander, the calculation of the trophic transfer, based on our sampled fish, can be made through the biomagnification factors. The results revealed that there is a high biomagnification potential for cadmium (1,857), an even rate for arsenic (1,039) and a low rate for lead (0,795) and for mercury (0,431). Based on a study of Clearwater et al. (2002), this can also mean that the Zander is more exposed to cadmium, the two species are relatively equally exposed to arsenic, while the Crucian carp is more exposed to mercury and lead, since the exposure is not strictly dietborne. Furthermore, the dietary exposure is not strictly given by the consumed pray, but also from the ingestion of sediments that can occur in many forms (e.g. in the digestive system of the pray, attached to the pray`s body, etc.), (Clearwater et al., 2002).

Adams et al. (2000) mention that for aquatic organisms, the bioaccumulation process is the most significant route of uptake for most metals. Only a few metals, like mercury, are believed to have a higher uptake through food than through the water component. Fish closely regulate their internal levels of essential metals mainly through bioaccumulation. Non-essential metals are often regulated to varying degrees because these regulating mechanisms are not metal-specific (Adams et al., 2000). As a result of these processes an inverse relationship exists between the metal concentration from the water and the specific bioaccumulation factor. Therefore, at low metal concentrations in water, aquatic organisms are accumulating essential metals, and non-essential metals along with these, to meet their metabolic needs. At high concentrations, only fish with active regulation mechanisms are able to excrete excess metals and try to limit the uptake (Brix et al., 2001). As a result, the bioaccumulation factor is not always an intrinsic property of the exposure level. Usually, higher values tend to appear at low water concentrations and conversely.

In order to make a more efficient assessment and control of the level of contaminants in fish products designated for human consumption, the European legislation must be thoroughly reviewed and complemented (Jaric et al., 2011).

IV. Conclusion

- Our pilot study showed that the heavy metal concentrations and distributions of the samples varies from sediment to water.
- A significant correlation between water-sediment concentrations of studied metals was significant only in the case of As, suggesting mobilisation and balance between these two matrices.
- The distribution of the metals in external and internal fish organs shows differences between the two investigated species. In Crucian carp the concentrations of metals were Pb>As>Cd>Hg, Pb and As being predominant in external organs. In Zander the concentrations of metals were Pb> Cd>As> Hg, Cd, As and Hg being predominant in internal organs. There was recorded an exceeding of the European references, for cadmium and lead.
- The order of the affected tissue by the highest quantity of heavy metals, even different for the two fish species investigated highlighted that liver and gonads are the main internal organs bioaccumulating metals.
- The bioaccumulation factors (BAFs) showed a fairly high rate for Hg>Pb>Cd in the case of the Crucian carp, and a high rate for Cd>Hg>Pb in the case of the Zander.
- The bioaccumulation of heavy metals and metalloids in fish species is proved to be species, tissue and element dependent, our study revealing higher concentrations of metals in Crucian carp compared to Zander.

V. Acknowledgement

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