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Chapter 4

Metals Toxic Effects in Aquatic Ecosystems: Modulators of Water Quality

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Additional information is available at the end of the chapter

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Abstract

The topic of this work was based on the assessment of aquatic systems quality related to the persistent metal pollution. The use of aquatic organisms as bioindicators of metal pollution allowed the obtaining of valuable information about the acute and chronic toxicity on common Romanian aquatic species and the estimation of the environment quality. Laboratory toxicity results showed that Cd, As, Cu, Zn, Pb, Ni, Zr, and Ti have toxic to very toxic effects on *Cyprinus carpio*, and this observation could raise concerns because of its importance as a fishery resource. The benthic invertebrates' analysis showed that bioaccumulation level depends on species, type of metals, and sampling sites. The metal analysis from the shells of three mollusk species showed that the metals involved in the metabolic processes (Fe, Mn, Zn, Cu, and Mg) were more accumulated than the toxic ones (Pb, Cd). The bioaccumulation factors of metals in benthic invertebrates were subunitary, which indicated a slow bioaccumulation process in the studied aquatic ecosystems. The preliminary aquatic risk assessment of Ni, Cd, Cr, Cu, Pb, As, and Zn on *C. carpio* revealed insignificant to moderate risk considering the measured environmental concentrations, acute and long-term effects and environmental compartment.

Keywords: metals, fish, crustaceans, benthic invertebrates, toxicity, LC50, MATC, bioaccumulation, risk

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1. Introduction

Metals are constantly released in aquatic systems from natural and anthropic sources such as industrial and domestic sewage discharges, mining, farming, electronic waste, anthropic accidents, navigation traffic as well as climate change events like floods (**Figure 1**) [1, 2]. Moreover, metals are easily dissolved in water and are subsequently absorbed by aquatic organisms such as fish and invertebrates inducing a wide range of biological effects, from being essential for living organisms to being lethal, respectively. In spite of the fact that some metals are essential at low concentrations for living organisms, such as (i) micronutrients (Cu, Zn, Fe, Mn, Co, Mo, Cr, and Se) and (ii) macronutrients (Ca, Mg, Na, P, and S); at higher concentrations, they could induce toxic effects disturbing organisms' growth, metabolism, or reproduction with consequences to the entire trophic chain, including on humans [3]. In addition, the non-essential metals such as Pb, Cd, Ni, As, and Hg enhance the overall toxic effect on organisms even at very low concentrations. High levels of metals in the environment could be a hazard for functions of natural ecosystems and human health, due to their toxic effects, long persistence, bioaccumulative proprieties, and biomagnification in the food chain [4, 5]. In this context, metal pollution is a global problem; therefore, the international regulations demanded for water quality compliance with the quality standards both in surface water or groundwater and in biota [6–9]. Currently, in accordance with the European Water Framework Directive (EU-WFD, 2000/60/EC), the ecological status of water bodies is assessed based on five biological indicators such as phytoplankton, macrophytes, phytobenthos, benthic invertebrates, and fish alongside with chemical and hydromorphological quality elements. Due to the fact that biota has the ability to accumulate various chemicals, it has been extensively used to measure the effects of metals on aquatic organisms as an essential indicator of water quality [10]. The mollusks [11–14] and fish [3, 15] are the most used organisms as bioindicators of metal pollution in water or sediment.

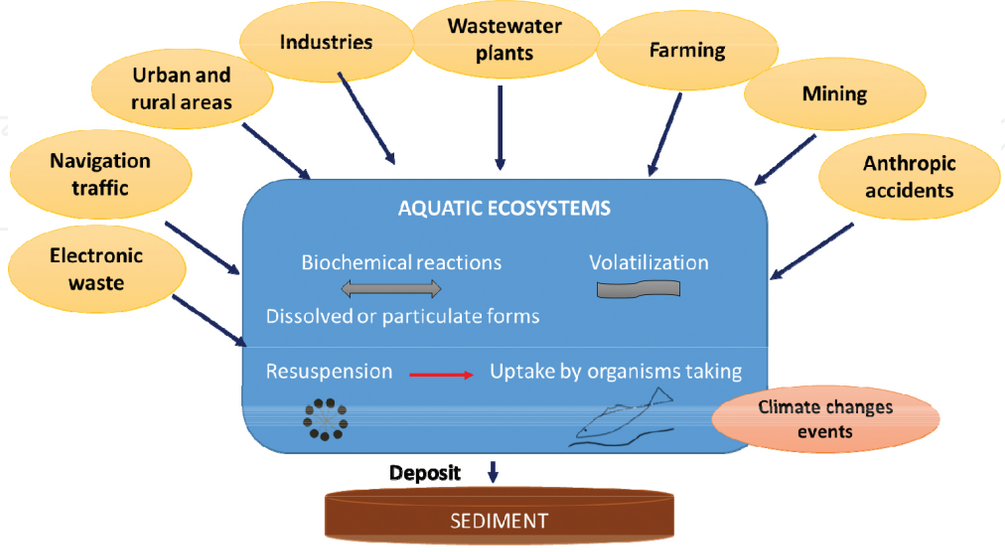


Figure 1. Sources of metal contamination affecting aquatic ecosystems.

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The proposed topic of this chapter is based on the assessment of aquatic systems quality linked to persistent metal pollution. The chapter includes an extensive literature review concerning the impact of heavy metals on aquatic systems followed by an experimental part based on metal distribution and toxicity effect on the Romanian surface waters. Due to the European economic and strategic importance of Danube Delta, the final receptor of Danube's flow, the toxic effect of various metal concentrations (Ni, Zn, Cu, Cd, As, Cr, Pb, Co, Ti, Zr, Fe, Mn, etc.) was analyzed.

2. Metal ecotoxicology

2.1. Heavy metal bioavailability to aquatic organisms

Unlike organic chemicals, the majority of metals cannot be easily metabolized into less toxic compounds, a characteristic of them being the lack of biodegradability. Once introduced into the aquatic environment, metals are redistributed throughout the water column, accumulated in sediments or consumed by biota [16]. Due to desorption and remobilization processes of metals, the sediments constitute a long-term source of contamination to the food chain. Metal residues in contaminated habitats have the ability to bioaccumulate in aquatic ecosystems—aquatic flora and fauna [17], which, in turn, may enter into human food chain and result in health problems [18]. Metal accumulation in sediments occurs through processes of precipitation of certain compounds, binding fine solid particles, association with organic molecules, co-precipitation with Fe or Mn oxides or species bounded as carbonates—according to the physical and chemical conditions existing between the sediment and the associated water column [19, 20].

Metal bioavailability is defined as the fraction of the total concentration of metal which has the potential to accumulate in the body. The factors that control the bioavailability of metals (**Figure 2**) are the following: the organism biology (metals assimilation efficiency, feeding strategies, size or age, reproductive stage); metal geochemistry (distribution in water—sediment, suspended matters, and metal speciation) [21, 22]; physical and chemical factors (temperature, salinity, pH, ionic strength, concentration of dissolved organic carbon, total suspended solids) [23, 24].

Metal bioavailability controls their accumulation in aquatic organisms. The metals uptaken paths are through the permeable epidermis if metals are in dissolved forms or through the food ingestion if metals are in particulate forms. Metal speciation, the presence of organic or inorganic complexes, pH, temperature, salinity, and redox conditions [24] are the main factors that could modulate metal toxicity. The ingestion uptake depends on similar factors, plus the rate of feeding, intestinal transit time, and the digestion efficiency [25].

Many studies have shown that the free hydrated metallic ion is the most bioavailable form for Cu, Cd, Zn [26], and Pb [27], but some exceptions have been reported [28]. Thus, the importance of other chemical forms of dissolved metals and complexes formed with suitable organic ligands with low molecular weight should not be neglected. It has been found that the presence

of organic binders increases the bioavailability of Cd in mussels and fish, by facilitating the diffusion of the hydrophobic compound in the lipid membrane. The organic compounds of metals could be more bioavailable than the ionic forms [29]. For instance, the organic mercurial compounds are lipid-soluble and penetrate quickly the lipid membranes, increasing the toxicity compared to mercuric chloride which is not lipid-soluble [30].

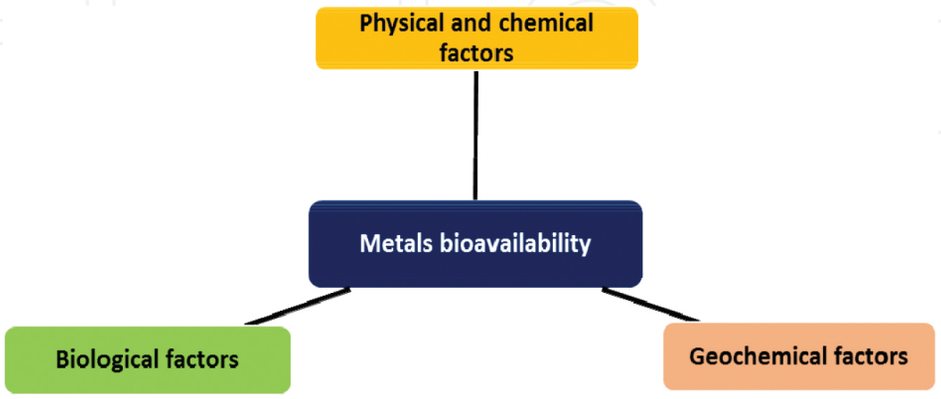


Figure 2. The main control factors that influence metal bioavailability.

The adsorption on suspended solids affects the total concentration of metals present in water. The association between solid particles and metals is also critical for the metal uptake into organisms through food ingestion [31]. The suspended solids accumulate the insoluble metal compounds, but under certain conditions, the metal reached the interstitial water being dissolved. Heavy metal concentrations from sediments or suspended solids are much higher than in water, so a small fraction of them could be an important source for bioaccumulation in planktonic and benthic organisms [32]. The dynamics of different forms of metals in the aquatic environment is not fully understood, so new studies are required to analyze the different accumulation/bioaccumulation pathways based on dissolved or suspended metal forms.

Other studies highlighted that bioavailability of metals in bivalve mollusks depends on sediment particle size due to their filter feeding behavior. If the particles were coated with bacterial extracellular polymers or fulvic acids, the Cd, Zn, and Ag bioavailability was significantly increased. Overall, the binding of metal decreased the bioavailability of metals from the sediment [28, 33].

2.2. Metal toxicity effects

After metal ingestion, this is specifically transported by lipoproteins into different body compartments (organs, blood, or other physiological structures) where they can be specifically oriented to different centers: (i) *action centers* where the toxic metal interacts with an endogen macromolecule (protein or ADN) or a certain cellular structure inducing toxic effects for all body; (ii) *metabolism centers* where the metal is processed by detoxified enzymes; (iii) *storage centers* where the metals are collected in a toxic inactive state; and (iv) *excretion centers* where the metals are disposed.

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The heavy metal overload has inhibitory effects on the development of aquatic organisms (phytoplankton, zooplankton, and fish) [34, 35]. The metallic compounds could disturb the oxygen level and mollusks development, byssus formation, as well as reproductive processes. Several histological changes such as gill necrosis or fatty degeneration of the liver occur in the fish and crustaceans [36, 37]. Assessments at the cellular level enable to understand the action of toxic metals on the enzymatic metabolism and physiology of the aquatic organisms.

The lethal effects of metals in crustaceans were induced by the inhibition of enzymes involved in cellular respiration. The histological changes observed in fish and crustaceans after chronic exposure to metals are the result of antioxidant enzymes inhibition [38–41]. The effects on organisms' growth and development were triggered by the inhibition of enzymatic systems involved in protein synthesis and cell division. The metal type modulates the bioaccumulation level and enzymatic systems vulnerability generating a multitude of effects, toxic or not [42, 43].

In order to understand the interaction mechanism between the toxic metals and the aquatic organisms and how organisms answer to metal contamination, more information on bioavailability is needed [44].

At the present, many studies on the assessment of acute and chronic toxicity of metals mentioned the following parameters: survival, growth, development, reproduction, behavior, accumulation, effects on enzyme systems, etc. In **Table 1**, the values of acute (LC50) and chronic (MATC/NOEC/LOEC) toxic concentration for fish and planktonic crustaceans according to PAN Pesticide Database—Chemical Toxicity Studies on Aquatic Organisms [45] are exemplified. The studies highlighted that the toxic concentration intervals depend on the species, exposure time, age of specimens, type of toxicity test type, and laboratory conditions.

Metal	Fish (*Cyprinus carpio*)		Crustaceans (*Daphnia magna*) EC50 (48 h)
	LC50 (96 h)	MATC/NOEC/LOEC	
Ni	1.3–10.4 mg/L	NOEC 50 μg/L LOEC 3.50; 13 μg/L; 1.97 mg/L	1 g/L
Zn	0.45–30 mg/L	NOEC 2.60 μg/L; 0.43; 4.20 mg/L	0.35–3.29 mg/L
Cd	2–240 μg/L 3–17.05 mg/L	NOEC 0.02–37 μg/L LOEC 6.7–440 μg/L	24–355.4 μg/L
As	0.49 mg/L (*Carassius sp.*) 0.9 mg/L (*Pimephales sp.)*	LOEC 25 μg/L	3.8 mg/L
Cr	14.3–93 mg/L	NOEC 0.19–17 μg/L LOEC 25 μg/L	22–160 μg/L
Pb	0.44–2 mg/L	NOEC 0. 07; 128 μg/L LOEC 0.03–128 μg/L	4.4; 5.7 mg/L (*Daphnia sp.*)
Sb	6.2–8.3 mg/L (*Cypridon sp.*)	NOEC 6.2 mg/L	>1 g/L
Mn	0.1–15.61 mg/L (*Oncorhynchus sp.)*	–	40 mg/L

LC (EC) 50—lethal concentrations for 50% of tested organisms after 96 or 48 h; MATC—maximum acceptable toxicant concentration in aquatic systems; NOEC—no observed effect concentration; LOEC—low observed effect concentration.

Table 1. Literature acute and chronic toxicity values [45–47].

2.3. Metal bioaccumulation and bioamplification

Monitoring the toxicity and accumulation of metals into the aquatic biota or sediment is mainly performed for assessing both the surface water quality and ensure food safety, respectively, as well as for the compliance with the directives. The toxicity and accumulation parameters are used for various environment monitoring programs such as wastewater discharges or various risk assessments of natural and anthropogenic events (floods or dredging activities) and also for identifying the source of metal contamination [48, 49].

According to the European document COM (2011)—876 final—2011/0429 (COD) (2012/C 229/22) amending the Directive 2000/60/EC and 2008/105/EC on priority substances in the field of water policy, new concentration limits of a number of harmful chemical compounds were allowed for the aquatic biota (fish, mollusks, or crustaceans). For instance, diphenyl brominated, fluoranthene, hexachlorobenzene, hexachlorobutadiene, benzene compounds, dicofol, perfluorooctane sulfonic acid and its derivatives, dioxins and dioxin-type compounds, cyclohexa-bromo-dodecane, heptachlor epoxide, and heptachlor have a concentration values in the range 6.7×10^{-3} to 167 mg/kg wet weight. The rest of the chemical compounds were not yet amended, which represents a considerable research opportunity to assess their chronic toxicity, bioaccumulation, and subsequently to set their maximum permissible concentration limits in aquatic organisms. Furthermore, the Directive 2008/105/EC of Environmental Quality Standards (EQS) entail values for various chemicals in biota.

More and more studies of various organic and inorganic chemical bioaccumulation/bioconcentration in freshwater organisms revealed induced harmful effects, especially of heavy metals (Hg, As, Cd, Zn, Fe, Pb, Fe, Mn, etc.) [10, 50, 51] and metal nanoparticles [52]. Bioaccumulation remains to be an ongoing highly debated subject. According to United States Geological Survey (USGS) Toxic Substances Hydrology Program, the bioaccumulation represents "*the biological sequestering of a substance at a higher concentration than that at which it occurs in the surrounding environment or medium*." Pollutants can be uptaken by organism directly from the environment or through ingestion of particles [53], and the accumulation occurs when an organism absorbs toxic chemical with a rate faster than the chemical is metabolized. On the contrary, the bioconcentration refers to the chemical uptake from the water only, which could be assessed in the laboratory conditions. The value of the concentration factor index gives information if there is a bioaccumulation (the concentration factor of <1) or a bioconcentration (concentration factor >1) [54]. The understanding of the bioaccumulation process is important because persistent pollutants (such as metals) could increase the toxic potential risk by bioaccumulation in the ecosystem, triggering a long-term effect on the ecosystem which cannot be assessed by laboratory toxicity tests [54]. It is considered that a high bioaccumulation potential does not necessarily imply a high potential for toxicity, and as a result, the toxic effects should be estimated separately. In addition, it was made a distinction between accumulation in a small concentrations range, which occurs due to physiological needs (e.g., Zn) and apparently uncontrolled accumulation (e.g., Cd) [55].

It was observed that the mollusks from the Black Sea have shown a great tendency to accumulate in high concentration Cd and Cu from sediments as well as Cd, Ni, and Cu from water. Data showed that the highest concentrations of heavy metals were found in the digestive tract

of fish [56]. Also, the fish *Cyprinus carpio* could differentially bioaccumulate metals inform one organ to another: Zn > Cr > Pb > Cu in muscle; Pb > Cr > Zn > Cu in gills; Pb > Cr > Zn in liver [10]. Moreover, for the same species, it has been shown that gills and liver or kidney were accumulating the following metals: Pb > Cd > Cr > Ni and Pb > Cd > Ni > Cr. On the other hand, bioaccumulation of Pb and Cd was significant in all *C. carpio* tissues [57].

Metal transfer in the aquatic food chain is another interesting environmental topic for many reasons such as the accumulation of metals in aquatic organisms that could transfer up to humans, leading to a potential risk of public health through consumption of contaminated fish [58, 59]. It is known that aquatic organisms can be exposed to high or low concentrations of metals as a result of continuous or accidental release, causing long-term effects. The main uptake pathways of metals in aquatic organisms are direct through the food or sediment particles ingestion and water via epidermis and gills then they are transported inside the cells through biological membranes and ionic channels [60]. Bioconcentration and bioaccumulation of metals into the trophic chain occur if metals are excreted into the water or the contaminated organisms are food for some predator's organisms [61, 62].

The study named "*Ecotoxicology of heavy metals in the Danube meadow*" [55] revealed that the amplification of metal concentrations in the food chains of ecosystems depends on the type of metal and the food chain. The metal accumulation in plants depends on the species, metal type, and ecosystem, especially for species which predominantly take metals from soil/sediment. Benthic gastropods tend to differentially accumulate the metals. The populations which are using the seston as energy source concentrate in many cases metals from different sources: Bivalves shell accumulate Pb, the tissue—Mn, Zn, Cd, and sometimes the amphibians in young stages accumulate Cd, Mn, and Zn.

Metal concentrations such as Fe, Mn, Cu, Cr, and Pb were not amplified in the food chain (benthic fauna-fish-birds), but they were amplified for Zn and Cd. Concentrations of metals were greater at the end of the trophic chains, as follows: vegetation/detritus—terrestrial invertebrates phytophase/detritophage—terrestrial invertebrates' predators—amphibians (Cd, Cr, Pb, and Cu in case of detritus chain and Zn in case of vegetation). The fish always accumulate metals, with some exceptions in the case of Cd, for which the transfer coefficient indicates accumulation in muscle and liver. The transfer of metals from benthic invertebrates to omnivorous fish revealed concentration of Zn and Cu in the liver and Zn in muscle. The Mn, Cr, and Cd metals transfer from omnivorous fish (muscle) to predatory fish, more specifically in their muscles and liver. At the end, the birds that are using contaminated fish as food source will accumulate Fe, Mn, Zn, Cu, and Cd in muscle and all metals (except Cr) in the liver [55].

3. Experimental part

3.1. Occurrence of metals in surface water and sediments

Several monitoring studies performed by INCD ECOIND Bucharest researchers during 2003–2013 in the Danube Delta—Sfantu Gheorghe Branch (sampling points: Mahmudia,

Murighiol, and Uzlina) emphasized some heavy metal concentration patterns in the study area (**Table 2**). The metal concentrations in water were within the limits of Romanian legislations, for class I and class II quality (according to the EU-WFD and the requirements set by the Romanian Law 310/2004 which amends the Law 17/1996). Cu and Ni showed the highest total concentration (**Table 2**, marked lines) among the determined metals.

Metal	Mahmudia (2009–2013)				Murighiol (2003–2013)				Uzlina (2003–2013)			
	Min	Max	Average	SD	Min	Max	Average	SD	Min	Max	Average	SD
Ni	<1.0	24.0	**4.02**	5.79	<1.00	68.1	**12.4**	18.6	<1.00	10.3	**2.60**	2.85
Fe	<20	880	**300**	270	112	3400	**710**	750	80.0	1040	**350**	280
Mn	<2.0	30.0	**10.0**	10.0	3.00	290	**70.0**	80.0	5.00	50.0	**20.0**	10.0
Cd	0.40	0.40	**0.40**	0.00	<0.10	0.50	**0.36**	0.14	<0.10	0.50	**0.37**	0.13
Cr	<0.5	6.00	**2.09**	1.74	<0.50	21.0	**5.38**	6.00	<0.50	21.0	**3.52**	5.33
Cu	2.50	10.5	**5.74**	2.67	0.012	55.3	**12.9**	17.8	0.03	123	**14.5**	26.7
Pb	<2.0	3.20	**2.08**	0.31	<2.00	5.00	**2.15**	1.29	<2.00	5.00	**2.17**	1.27
As	<2.0	2.20	**2.01**	0.05	<2.00	3.90	**1.82**	0.88	<2.00	2.64	**1.73**	0.59
Hg	<0.1	0.24	**0.32**	0.06	<0.10	0. 77	**0.15**	0.20	<0.10	0.14	**0.22**	0.10
Zn	<2.0	24.7	**10.1**	7.07	<2.00	56.0	**9.58**	11.4	<2.00	57.0	**8.15**	11.7
Co	<0.5	1.30	**0.66**	0.33	<0.50	5.00	**1.17**	1.65	<0.50	5.00	**1.18**	1.65

Min—the minimum detected concentration; Max—the maximum detected concentration; SD—standard deviation.

Table 2. Occurrence of metals in water of Danube Delta—Sf. Gheorghe (2003–2013) in μg/L [63, 64].

The studies revealed that metals Cu, Pb, Zn, Cr, Ni, Cd, Mn, and Fe were the most abundant in the sediments of the Danube Delta—Sf. Gheorghe Branch sampling sites. The concentrations of these metals ranged with the sampling location and seasonal or natural events, as follows: Cu 4.65–194 mg/kg d.m (dry matter), Pb 4.76–51.3 mg/kg d.m., Zn 17.7–218 mg/kg d.m., Cr 7.5–61.9 mg/kg d.m., Ni 10.8–111 mg/kg d.m., Cd <0.01–1.5 mg/kg d.m. (**Figure 3**). The average value in the period 2009–2013 for Mn was 614.03 mg/kg d.m. and for Fe, it was 20 987 mg/kg d.m. [64, 65].

Alongside metal concentration, several chemical (nutrients, oxygen and pH regime, pesticides, petroleum products, polychlorinated biphenyls) and biological (phytoplankton, zooplankton, and benthic macroinvertebrates) elements were investigated, showing that the organochlorine pesticides and petroleum products exceeded the maximum allowed limits [63, 66].

In addition, other studies performed along Romanian rivers showed that the mining activities had a great impact on sediment ecosystems due to metal pollution. For instance, a study performed during 2003 in Baia Mare (in North Vest of Romania) mining area after a pollution accident showed a high content of heavy metals in Somes River sediment (Cu 104–339 mg/kg, Pb 59–465 mg/kg, Zn 56–2060 mg/kg, Cd 0.05–14.14 mg/kg, CN 0.33–15.86 mg/kg). The detected concentration affected the aquatic ecosystem where the microalgae species disap-

peared and the number of fish species decreased dramatically compared to the period before the incident. Also many species of mollusks disappeared because their capacity to accumulate large amount of heavy metals was exceeded [67]. In addition, in Rosia Montana area (in the West part of Romania), significant water contamination with heavy metals occurred due to the mining acidic waters from area on two water courses: Rosia and Corna stream. The results showed exceedances of Cu, Cd, Fe, Ni, and Cr, in particular in the Rosia Montana water stream [68]. Along Jiu River (in south of Romania) sediments, heavy metal pollution in most sampling points was recorded according to the pollution load index (PLI) [69].

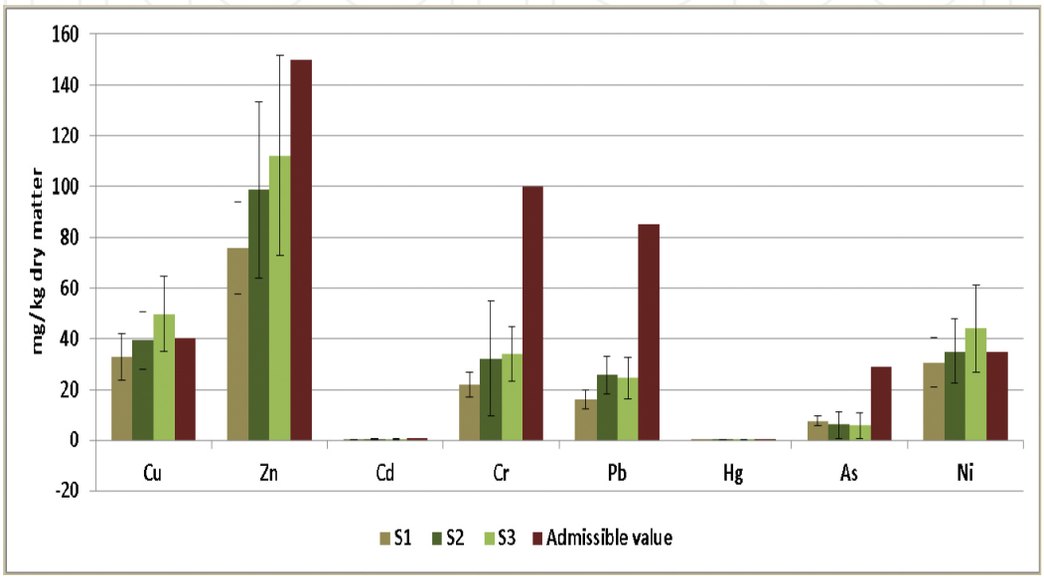


Figure 3. Occurrence of metals in sediments of Danube Delta—Sf. Gheorghe (2003–2013) (average values). S1—Mahmudia, S2—Murighiol, and S3—Uzlina [65].

In the above context, in the following sections will be presented some data concerning the metals effects on freshwater organisms (fish, planktonic crustacean, and mollusks), obtained through laboratory testing or by biological samples collected from contaminated fields.

3.2. Laboratory tests: acute and chronic effects of metals

3.2.1. Materials and methods

The assessment of metals acute and chronic effects was based on fish (*C. carpio*) and planktonic crustacean (*Daphnia magna*) laboratory data. The tested organisms were those recommended by the international ecotoxicology protocols (OECD or ISO), and they are frequently found in Romanian surface waters, easily to acclimatize in laboratory and sensitive to various contaminants. *C. carpio* are in particular the most affected organisms due to the fact they ingest both planktonic and benthic organisms, respectively, and thus, they especially accumulate the contamination from water and sediment. The tests were performed on the following metals: Ni, Zn, Cu, Cd, As, Cr, Pb, Sb, Mn, Ti, and Zr, which were usually detected in the aquatic systems.

3.2.1.1. Sample preparation

For stock solution preparation, a known quantity of metals test as $NiSO_4$, $ZnSO_4$, $CuSO_4$, $CdCl_2/CdSO_4$, As_2O_3, $K_2Cr_2O_7$, $Pb(NO_3)_2$, $SbCl_5$, $MnCl_2x4H_2O$, TiO_2, $ZrCl_4$ was dissolved into the specified volume of dilution water or growth medium. No added solvents have been used, and all substances have been tested under their maximum solubility. The solutions were stirred for 24 h, in the dark at 25°C. The testing solutions were prepared by mixing the appropriate volumes of stock solution with dilution water or growth medium in order to obtain the final concentrations used for testing. Finally, the pH values of tested solutions were situated between 6.5 and 8.5 units.

3.2.1.2. Fish toxicity test procedure

Using OECD methodologies for acute toxicity, the lethal concentrations for 50% of tested organisms were estimated. Metals' long-term toxicities on fish were conducted using an in-house methodology based on the changes in some physiological indicators such as growth rate, mortality, biomass, production, food use and biochemical indicators, hepatic enzyme activity, respectively. **Table 3** presents the technical parameters of fish toxicity tests.

3.2.1.3. Crustacean toxicity test procedure

The toxicity test determined the metal concentration that immobilizes or kills 50% (LC50) of *D. magna* crustacean, after chemical exposure at 20°C ± 2°C in the dark for 24 or 48 h. The test procedure was performed according to OECD 202 using the microbiotest Daphoxkit F Magna provided by MicroBioTests Inc., Belgium. Briefly, the test was performed in three replicates, in multiwall test plates (six rinsing wells and 24 wells for toxicant dilutions) using 20 organisms per each concentration (at least five different concentrations for each metal) and control (untreated standard freshwater). The mortality/immobility percentage of organisms was registered after 24 and 48 h.

3.2.1.4. Data processing and statistics

The acute effect concentration values in the fish and crustacean tests were calculated using probity analysis method, based on exponential regression relationship between cumulative percentages of mortality (expressed as probity units) for each exposure period against logarithmic concentrations of test substance. For each result, standard deviations were calculated.

3.2.2. Results and discussion

3.2.2.1. Fish toxicity

Acute toxicity tests provide a measure of toxicity for a target species under specific environmental situations and could suggest a rapid and severe effect of contaminants. Acute and chronic toxicity test mimicked the metals accidental release or long-term accumulation in sediment [67–70]. The carp fish LC50-96h values showed different responses in direct corre-

lation with the metals type and concentration. The LC50-96h values were 0.16, 0.28, 0.31, and 0.40 mg/L for Cd, Ti, Zr, and As (**Figure 4**), 2.17, 12.2, 30.10, and 65.8 mg/L for Cu, Zn, Pb, and Ni (**Figure 5**), 120, and 758 mg/L for Cr and Sb, obtained from two replicates for each metal (**Table 4**).

Test conditions	OECD 203 (acute tests)	In-house procedure (chronic tests)
Holding of fish	Acclimatization of fish in laboratory tanks for 3 weeks	
Limit test	One concentration selected according to scientific literature	MATC estimated = LC50-96 h × 0.1
Definitive test		
Test concentrations in definitive test	Five concentrations in a geometric series	Two concentrations (under or over the estimated MATC)
Type of test	Static	Discontinuous (renewal solutions at 24 h)
Time of exposure	96 h	60 days
Fish species and characteristics	*Cyprinus carpio* (1 year) 10 exemplars/test solution, 5–7 cm, 10–15 g/exemplary	*Cyprinus carpio* (2 years) 20–30 exemplars/test solution, 12–14 cm, 25–30 g/exemplary
Fish source	Romanian specialized fish farm	
Testing vessels	10 L	100 L
Temperature, oxygen concentration, pH, light	18–25°C, ≥4 mgO_2/L, pH 6.5–8.5 (daily measuring), 12- to 16-h photoperiod daily. Mean of water total hardness 13 mg/L $CaCO_3$	
Feeding	Not food	2% from the surviving lot weight/day
Control test	All toxicity tests were carried out in the same time with a control test	
Replicates	Two replicates/test/metal	
Analytical control in test solutions	Inductively coupled plasma atomic emission spectrometry (ICP-OES)	
Toxicity criteria	Organisms mortalities and visible abnormalities (at 24, 48, 72, and 96 h)	Growth instant rate, mortality rate, biomass mean, production, used food rate, and biochemical indicators—hepatic enzymes activity—GOT and GPT
Results treatment	Probity analysis method based on the exponential regression model between the mortality (probity units) and the log of concentrations of the metal	Comparative analyses with the controls
End points	Lethal concentrations for 50% of tested fish after 96 h of exposure (LC50-96 h)	Maximum acceptable toxicant concentration in aquatic systems (MATC)

Table 3. Test conditions of acute and chronic toxicity tests.

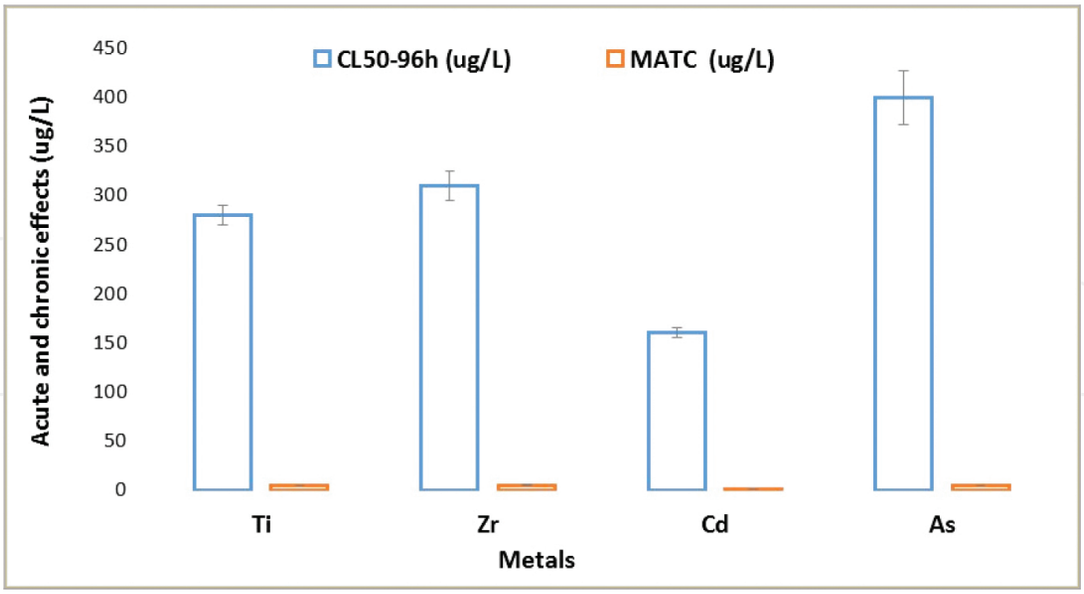


Figure 4. Acute and chronic toxicity of Ti, Zr, Cd, and As classified in very toxic class for *Cyprinus carpio*.

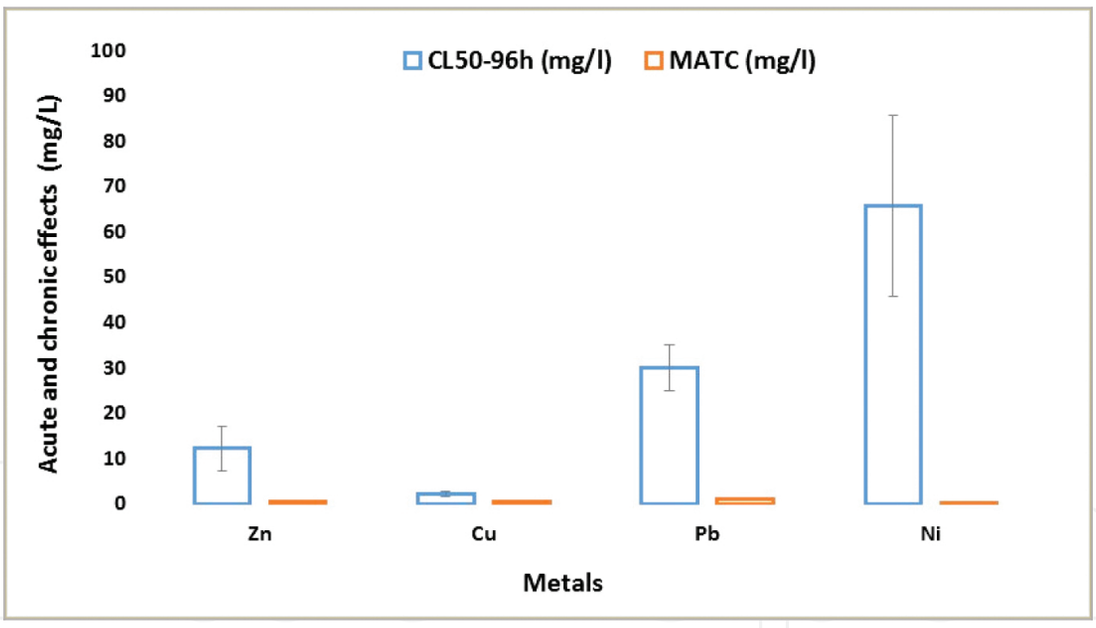


Figure 5. Acute and chronic toxicity of Zn, Cu, Pb, and Ni classified in toxic class for *Cyprinus carpio*.

According to Global Harmonization System for chemical classification and labeling, Cd, Ti, Zr, and As were the most toxic metals for fish. Cd, Ti, Zr, and As showed to be very toxic compared with the other analyzed metals. Research studies revealed similar acute toxicity intervals: 6.16–47.58 mg/L for Ni, 0.15–21.4 mg/L for Zn, 0.28–34.5 mg/L for Cu, 0.005–7.92 mg/L for Cd and 90 to >139 mg/L for Cr [71]. The maximum acceptable toxicant concentration (MATC) is a value calculated from chronic toxicity tests [72] in order to set water quality norms for aquatic life protection.

Metals	*Cyprinus carpio*		*Daphnia magna*	G.D. 351/2005[a] (μg/L)	Directive 105[b] (μg/L)	National plan[c] (μg/L)	Toxicity class[d]
	LC50-96h (mg/L)	MATC (mg/L)	LC50-48h (mg/L)				
Ti (TiO_2)	0.28 ± 0.01	0.005 ± 0.001	5.56 ± 0.8	–	–	–	Very toxic—fish
Zr ($ZrCl_4$)	0.31 ± 0.01	0.005 ± 0.002	91.20 ± 10	–	–	–	Very toxic—fish
Ni ($NiSO_4$)	65.8 ± 20.0	0.10 ± 0.02	–	20	20	4–34	Toxic—fish
Zn ($ZnSO_4$)	12.2 ± 5.0	0.60 ± 0.01	–	5	–	11.80–73	Toxic—fish
Cu ($CuSO_4$)	2.17 ± 0.50	0.05 ± 0.01	–	100	–	1.22–10	Toxic—fish
Cd ($CdCl_2$/ $CdSO_4$)	0.16 ± 0.001	0.001 ± 0.0005	0.14 ± 0.01	5	0.2	–	Very toxic—fish and daphnia
As (As_2O_3)	0.40 ± 0.02	0.005 ± 0.001	–	10	–	49	Very toxic—fish
Cr ($K_2Cr_2O_7$)	120 ± 22	1.00 ± 0.01	0.81 ± 0.02	50	–	8.8	Very toxic—daphnia
Pb ($Pb(NO_3)_2$)	30.1 ± 5.0	1.00 ± 0.02	–	10	7.2	–	Toxic—fish
Sb ($SbCl_5$)	758 ± 24	0.060 ± 0.001	148 ± 21	5	–	–	Non-toxic—fish and daphnia
Mn ($MnCl_2 \times 4H_2O$)	>53 ± 8	–	–	–	–	–	Non-toxic—fish

[a] Governmental Decision no. 351/2005 concerning the hazard chemical discharge.
[b] Directive 2008/105/EC on environmental quality standards in the field of water policy.
[c] National Plan of River Basin Management (2016-2021)—Annex 6.1.3B.
[d] According to REACH 1907/2006; Regulation (EC) 1272/2008; Regulation (EU) 286/2011; Global Harmonization System for chemical classification and labeling (GHS) Revision 2011. The toxicity class was decided on the highest toxicity of target organisms.

Table 4. In-house toxicity data of metals for fish and crustacean in relation with the national and international norms for metals limits in surface water.

Experimental exposure of fish for 60 days to different concentrations of metals revealed different long-term effects. The final results showed no effects concentrations on target organisms, assessment of environmentally safe concentrations, respectively. The calculation of MATC values started by multiplication of the LC50-96h of each metal with an application factor of 0.1 (**Table 2**). The monitored physiological parameters from chronic test revealed that Cd is non-toxic at 0.001 mg/L, Ti, Zr, and As were safety to 0.005 mg/L, Cu at 0.05 mg/L, Sb at 0.06 mg/L, Ni at 0.10 mg/L, Zn at 0.60 mg/L, Cr and Pb at 1.00 mg/L, comparative with the controls (**Figures 4** and **5**, **Table 4**). Similar values for Cu (0.012 mg/L) and Zn (0.5 mg/L) were also obtained in other studies [73].

3.2.2.2. Crustaceans toxicity

Toxicity tests on *D. magna* crustaceans showed various toxicities of metals; the LC50-48h showed 0.14 mg/L for Cd, 0.81 mg/L for Cr, 5.56 mg/L for Ti, 91.2 mg/L for Zr, and 148 mg/L for Sb. Cd and Cr showed the highest toxicities and were classified in very toxic chemicals class for *Daphnia* sp. (**Figure 6**). Similar literature values were reported for Cr between 0.02 and 0.05 mg/L [71] and for Cd between 0.024 and 0.355 mg/L [45].

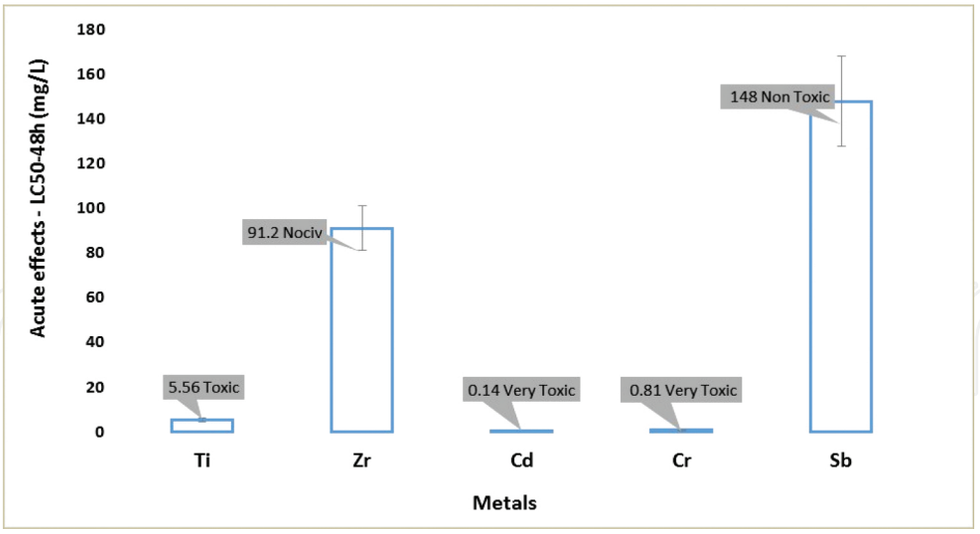


Figure 6. Acute toxicity of Ti, Zr, Cd, Cr, and Sb for *Daphnia magna*.

The surface water quality norms require specific limits only for few very toxic and toxic metals. For example, Ti, Zr, Cd, and Pb norms are not established by the National Plan of River Basin Management—Annex 6.1.3B, despite of their acute toxic effects at very low concentrations (**Table 4**). Also the Directive 2008/105/EC on environmental quality standards in the field of water policy sets limits only for Ni, Cd, and Pb. The present limits assure the protection of aquatic organisms, especially for fish and planktonic crustaceans.

3.3. Field test: bioaccumulation

In order to assess the impact of metals in the field, the following sections present some preliminary data concerning the metal bioaccumulation into benthic invertebrates (mollusks).

3.3.1. Materials and methods

3.3.1.1. Studied area characterization

The studied area was focused on a highly sinuous channel, located on the southeast area of the Danube Delta (Sf. Gheorghe Branch) receiving 22% of Danube's water flow. The Sf. Gheorghe Branch has a width varying between 150 and 550 m, and the water depth varies between 3 and 27 m. The sampling sites location was selected taking into consideration the changes in the Sf. Gheorghe Branch morphology as a result of the pressure from anthropic and environmental factors. Iron Gates I dam construction on Danube River led to a 10% decrease in the suspended sediment amount at Isaccea station. Moreover, the Iron Gates II dam building induced a 50% decrease in suspended sediment at Isaccea. These constructions alongside meander modification (during the years 1984–1988) have produced major changes in sediment distribution. The establishing of space location was performed using GPS type system map 60CSx—Garmin [74].

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In addition, the anthropic activities undertaken to strength the banks against coastal erosion led to meanders cutoff, which in turn caused continuous biotope degradation. These changes negatively impacted the ecosystem functions by reducing the structure of the main and constant ecological communities, the benthic invertebrates. So that, to characterize metal bioaccumulation (in benthic invertebrates), two representative sampling sites were selected considering the pressure resulted from anthropic and environmental factors (Murighiol and Uzlina)—**Figure 7**. At temporal scale, this study was conducted during summer and autumn of 2013.



Figure 7. Location of sampling sites in Danube Delta (Sfantu Gheorghe Branch) (St 1—Murighiol; St 2—Uzlina).

3.3.1.2. Sampling collection

The sediment samples for both benthic invertebrates and metal analysis were collected in two replicates using a Van Veen grab, according to the following methodologies: EN ISO 5667-1:2008, ISO SR 5667-6:2009, SR ISO 5667-12:2001 and EN ISO 9391:2000. Surface sample unit was of 255 cm^2, and the sampling depth was of 10 cm. The analysis of benthic invertebrates was performed according to SR EN ISO 8689-1:2003. The species identification was performed using a Motic stereomicroscope. The results were calculated taking into consideration the wet biomass.

3.3.1.3. Sample preparation

The biota samples were dried at 40°C (24 h) and crushed then about three grams of biological sample were dissolved in aqua regia (a mixture of suprapure acids HCl 30 and 65% HNO_3 in the report 21–7 mL). The mixture was mineralized using a sand bath until complete dissolution. After cooling, the samples were filtered on paper filter (porosity <45 μm) in a 50-mL volumetric flask and filled with ultrapure water. The metal content in the samples was determined by inductively coupled plasma optical emission spectrometry. A calibration curve in the range of 0.1–0.5 mg/L (As, Se, Sb, Cd, Cr, Cu, Co, Fe, Mn, Mg, Ni, Pb, Zn) was performed using a

Certified Reference Material solution (100 mg/L Multi Element Standard Solution, Certipur, Merck). The quality control of the data was carried out according to Quality Control Standards 21A, 100 mg/L, produced by PerkinElmer. A reagent blank in order to estimate the metal contents from acids was prepared.

The mollusks (two bivalves' species: *Unio pictorum* and *Anodonta cygnea*) and one gastropod species (*Viviparus viviparus*) were selected in this study (**Figure 8**) as they prevail in the total biomass of benthic invertebrate community structure, and they are widely used as bioindicators for water quality. Their shells were subjected to metals detection, because they are formed throughout mollusks life and their chemical composition is an integral index to describe the composition of the aquatic environment over time [75]. Bioaccumulation factors of metals were calculated for each tested species.

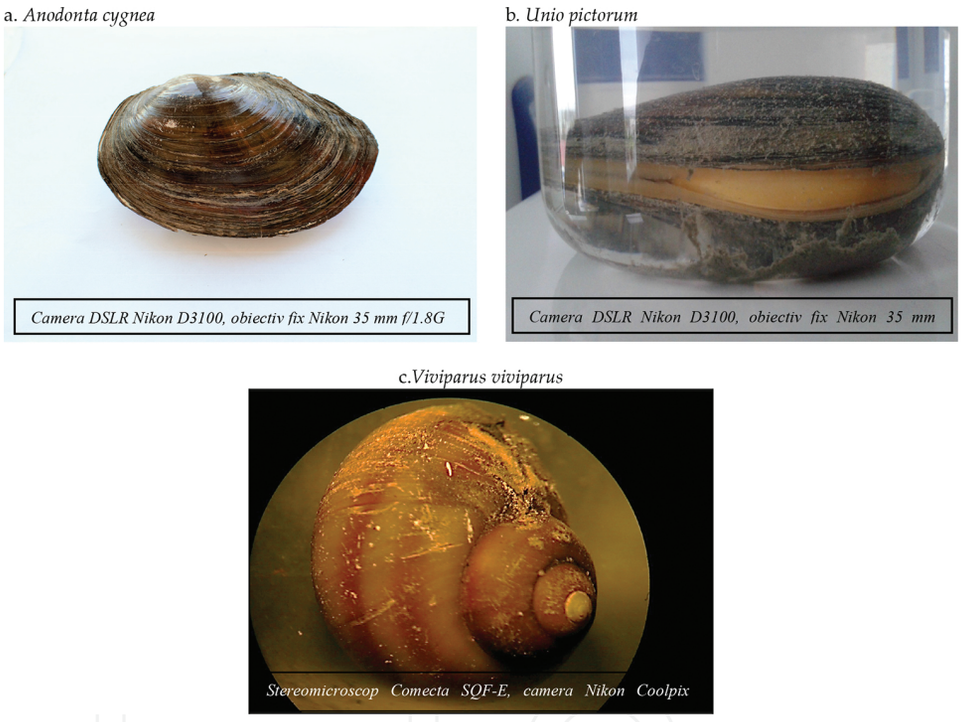


Figure 8. The analyzed benthic macroinvertebrates species.

3.3.2. Results and discussion

3.3.2.1. Metal accumulation in benthic organisms

This study included metal analysis results in the bivalves and gastropod shells from Murighiol and Uzlina sampling site. Other researchers [76–79] performed their studies as well using the same biological model, mollusk shells, for the metal accumulation analysis.

The mollusks have the largest representation and are the most valuable groups among the benthic invertebrates' communities due to the fact they are dominant in the total benthic community biomass and represent a basic food for the next trophic level (e.g., fish).

Two types of bivalve species identified at Uzlina and Murighiol were selected for metal analysis, respectively: *U. pictorum, A. cygnea*. Also from Gasteropoda, the species *V. viviparus* were selected (**Figure 8**). For each species, the dry and wet biomasses were determined (**Table 5**).

Sampling point/month	Species	Wet biomass (g)	Dry biomass (g)
Murighiol/July	*Unio pictorum*	25.38	24.49
Uzlina/July	*Viviparus viviparous*	10.96	0.88
	Unio pictorum	39.64	35.44
Uzlina/September	*Anodonta cygnea*	32.73	30.16
	Unio pictorum	19.68	18.58

Table 5. Dry and wet biomass values of the selected species.

The Biota Sediment Accumulation Factor (BSAFsed) was calculated using the equation: $BSAFsed = Cb/Csed$, where *Cb* is the metal concentration in biota/organism and *Csed* is the metal concentration in the sediment sample [80].

At Murighiol sampling site, in the *U. pictorum*, shells (collected in July) were recorded the highest values for Cu, Ni, and Zn. Moreover, the Cu concentration in sediment was 47 mg/kg d.m. over the set limit. It was estimated that 4% of the Cu concentration, 2% of Zn, and 1% of Ni were found in *U. pictorum* shell species. The BSAFsed values were <0.05 (**Table 6**). Also, various metals were detected in the *U. pictorum* and *V. viviparous* shells from Uzlina, in July (**Table 7**).

Metal	Cb* *Unio pictorum*	Csed*	Csed*	BSAFsed 2009–2013**
As	<0.05	12.2	9.61	0.004
Cd	<0.01	–	0.50	–
Cu	1.97	47.0	35.1	0.04
Cr	0.12	27.6	31.6	0.004
Co	0.05	9.41	8.37	0.005
Fe	73.2	–	14,895	–
Mn	230	–	464	–
Ni	0.60	35.0	30.8	0.02
Pb	<0.05	25.6	22.3	0.002
Se	0.44	–	–	–
Sb	<0.05	–	–	–
Zn	1.17	91.7	88.5	0.01
Mg	62.5	–	–	–

* Average of metal concentrations (for two replicates) expressed in mg/kg d.m.
** Csed 2009–2013—average of the metal concentration detected in sediment from 2009 to 2013 [63, 65].

Table 6. Metal concentration (mg/kg d.m) in the shell of *Unio pictorum* at Murighiol in July 2013.

Metal	Cb* *Unio pictorum*	BSAFsed	Cb* *Viviparus viviparus*	BSAFsed	Csed*	Csed* 2009–2013**
As	<0.05	0.006	<0.05	0.006	7.75	9.30
Cd	<0.01	–	<0.01	–	–	0.51
Cu	2.61	0.05	2.60	0.05	54.7	47.0
Cr	<0.01	0.0003	0.42	0.01	29.6	29.2
Co	0.11	0.01	0.19	0.02	10.8	9.84
Fe	140	–	279	–	–	20987
Mn	58.7	–	30.0	–	–	614
Ni	0.34	0.0085	0.58	0.015	40.0	39.3
Pb	<0.05	0.002	0.15	0.006	26.7	21.3
Se	<0.09	–	<0.09	–	–	–
Sb	<0.05	–	<0.05	–	–	–
Zn	0.90	0.006	3.87	0.02	158	96.9
Mg	154	–	211	–	–	–

* Average of metal concentrations (for two replicates) expressed in mg/kg d.m.
** Csed 2009–2013—average of the metal concentration detected in sediment from 2009 to 2013 [63, 65].

Table 7. Metal concentration (mg/kg d.m) in the shell of *Unio pictorum* and *Viviparus viviparus* at Uzlina in July 2013.

Concentrations of As, Cd, Cr, Fe, Pb, Se, Sb, Mg did not showed significant values in shells of analyzed benthic organisms. The metals Cu, Ni, and Zn were present in sediment over the set limits of national norms inducing their accumulation in shells. The highest values of Cu and Zn were both in *U. pictorum* and *V. viviparus* (**Table 7**). Similar concentrations of Zn, Cu, Pb, Cd, and Co in *V. viviparus* were found in the River Dnieper in the same gastropod shells [81].

Metal concentrations showed a lower magnitude in mollusk shells than in their bodies, and this result could be explained by the fact that metals were accumulated in shell only after they were absorbed by the body. The bioaccumulation selectivity of metals in gastropod shells follows the next order: Fe > Mn > Zn > Cu > Pb > Co > Cd. Thus, the quantitative distribution of metals in mollusk shells is considered by the level of biochemical involvement, metabolic processes, their toxicity degree as well as the bioavailability to aquatic organisms [81].

Some studies [82] revealed that Fe belongs to metals which play an important role in body metabolism and is not toxic. The Mn, Mg, Co, Cu, Zn, and Ni are involved in growth, development, and reproduction process, but in high concentrations can show toxic effects (see the above section "*Laboratory tests—acute and chronic effects*"). Pb and Cd are not involved in metabolic processes; thus, they are highly toxic at low concentrations and have a great storage capacity in the organisms at long-term exposure. The results on metal concentrations in *U. pictorum* and *A. cygnea* shells, metal detection in sediment samples (2013), average of metals detection in sediment in period of 2009–2013 at Uzlina and BSAFsed values are presented in

Table 8. The metals were determined in both bivalve species *U. pictorum* and *A. cygnea* shells, in September 2013 (**Table 8**).

Metal	Cb* *Unio pictorum*	BSAFsed	Cb* *Anodonta cygnea*	BSAFsed	Csed*	Csed* 2009–2013**
As	<0.05	0.007		0.008	6.60	9.30
Cd	<0.01	–	<0.01	–	–	0.51
Cu	2.57	0.05	4.63	0.09	48.9	47.0
Cr	<0.01	0.0004	0.14	0.005	28.2	29.2
Co	0.13	0.01	<0.01	0.001	9.47	9.84
Fe	153	–	97.8	–	–	20,987
Mn	248	–	157	–	–	614
Ni	0.29	0.008	0.24	0.007	35.6	39.3
Pb	<0.05	0.002	<0.05	0.001	30.2	21.3
Se	<0.09	–	<0.09	–	–	–
Sb	<0.05	–	<0.05	–	–	–
Zn	0.59	0.006	1.27	0.01	91.2	96.9
Mg	42.8	–	79.6	–	–	–

* Average of metal concentrations (for two replicates) expressed in mg/kg d.m.
** Csed 2009–2013—average of the metal concentration detected in sediment from 2009 to 2013 [63, 65].

Table 8. Metal concentration (mg/kg d.m) in the shell of *Unio pictorum* and *Anodonta cygnea* at Uzlina in September 2013.

In this case, the highest metal concentration was recorded for Cu, Ni, and Zn. The Cu and Ni concentrations from sediment exceed the allowed limit values both in September 2013 and as well as during 2009–2013 monitoring period. As shown in **Table 8**, the *A. cygnea* were found to have a greater capacity for metal accumulation (especially for Cu, As, Cr, Zn) than *U. pictorum* shells.

The bioaccumulation level varied depending on species, metals type, and sampling sites. No significant differences were observed between bioaccumulation factors of Cu, Zn, and Ni calculated for *U. pictorum* collected in July and September. The BSAFsed values were subunitary maintained. It was observed a difference considering the sampling points, respectively, at Murighiol the bioaccumulative metals impact (Ni and Zn) was greater compared to Uzlina. This aspect may be explained by the dredging works for the canal enlarging/widening to facilitate navigation, allowing a better water circulation from the branch inside the canal.

This preliminary study for the metal bioaccumulation capacity in the shell mollusks from Danube Delta aquatic system showed that essential metals involved in metabolic processes (such as Fe, Mn, Zn, Cu, and Mg) have a greater storage capacity than those toxic (such as Pb and Cd). The statement was also confirmed in other studies [83–85].

All the biota sediment bioaccumulation factors were subunitary, which indicated a slowly bioaccumulation process occurred in the studied aquatic ecosystems.

3.4. Preliminary risk assessment

Risk characterization is required for all chemicals as an estimation of their exposure and adverse effects on the environmental compartment. Generally, this is based on Predicted Environmental Concentration (PEC) and Predicted No Effect Concentration (PNEC) calculation, in terms of exposure and assessment of effects [86].

In order to estimate the current contamination of Danube surface water and sediment with metals, we use the average of the measured environmental concentrations (MEC) as PEC values, for the period 2009–2013 at Murighiol and Uzlina. The PNEC value calculation was made using an assessment factor (AF) of 1000 applied for acute toxicity values—LC50 (96 h) or 10 applied for chronic toxicity values—MATC for *C. carpio* (our laboratory tests), which expresses the degree of uncertainty in the actual environmental extrapolation [87]. The risk quotients (RQs) between MEC values and acute or chronic PNECs were calculated, and the level of risk was expressed as: insignificant risk (RQs <0.1); low risk (RQs <1); moderate risk (RQs <10), and high risk (RQs >10). The estimated RQs for the most detected metals in Danube water and sediment (Ni, Cd, Cr, Cu, Pb, and Zn) were summarized in **Table 9**.

Metal	MEC (μg/L)*		PNEC (μg/L)		RQs acute		Risk level	RQs chronic		Risk level
	S7	S8	Acute (AF = 1000)	Chronic (AF = 10)	S7	S8		S7	S8	
Surface water										
Ni	12.4	2.60	65.8	10.0	0.18	0.03	L/I	1.24	0.26	L
Cd	0.36	0.37	0.16	0.10	2.25	2.31	M	3.60	3.70	M
Cr	5.38	3.52	120	100	0.04	0.02	I	0.05	0.04	I
Cu	12.9	14.5	2.17	5.00	5.94	6.68	M	2.58	2.90	M
Pb	2.15	2.17	30.1	100	0.07	0.07	I	0.02	0.02	I
As	1.82	1.73	0.40	0.50	4.55	4.32	M	3.64	3.46	M
Zn	9.58	8.15	12.2	60.0	0.78	0.66	L	0.16	0.14	L
Sediment										
Ni	30.8	39.3	65.8	10.0	0.46	0.59	L	3.08	3.93	M
Cd	0.50	0.51	0.16	0.10	3.12	3.18	M	5.00	5.10	M
Cr	31.6	29.2	120	100	0.26	0.24	L	0.32	0.29	L
Cu	35.1	47.0	2.17	5.00	16.2	21.7	H	7.01	9.41	M
Pb	22.3	21.3	30.1	100	0.74	0.70	L	0.22	0.21	L
As	9.61	9.30	0.40	0.50	24.0	23.3	H	19.2	18.6	H
Zn	88.5	96.9	12.23	60.0	7.23	7.92	M	1.48	1.62	M

* Average of concentrations in period of 2009–2013; I—insignificant risk; L—low risk; M—moderate risk; H—high risk.

Table 9. Estimated acute and chronic RQs at Murighiol (S7) and Uzlina (S8) for *Cyprinus carpio*.

The results showed different levels of risk in accordance with detected environmental concentration of metals, the acute and chronic toxicity and the environmental compartment (water or sediment). In water, Cr and Pb showed insignificant risk; Ni and Zn showed a low risk; and Cd, Cu, and As highlight a moderate risk considering both acute and chronic effects on *C. carpio*. Variations of the RQs depending on sampling location are not observed.

As we expected, the risk level increases within the sediment compartment. The sediment contamination revealed low-to-moderate risk, exception for As and Cu. Therefore, Cr and Pb showed low risk; Ni, Cd, Zn and Cu highlighted moderate risk; and As and Cu could express a high risk on fish *C. carpio*. Cu, Zn, and Ni were constantly present in sediment over the set limits of national norms inducing also their accumulation (see the section "*Field tests—bioaccumulation*"). No variation is observed of the RQs depending on sampling location. Using long-term toxicities in PNECs estimation, the RQs increased for Ni, Cd, and Cr and decreased in case of Cu, Pb, As, and Zn, due to the use of a small applied factor (AF = 10) to chronic toxicities.

The results highlighted a pessimistic view concerning the quality of aquatic ecosystem needed to support the carp fish survival. The concern is related to the constantly presence of metal concentrations especially in sediments (the food provider compartment) which could determinate the bioaccumulation. The same statement was made in a Romanian study named "*Ecotoxicology of heavy metals in Danube meadow*" [55].

4. Conclusions

The topic of this chapter was based on the assessment of aquatic systems quality related to persistent metal pollution. The toxic metals are the most frequently detected pollutants in the aquatic environmental, and their effects identification are essential to protect the ecosystems integrity as well as human health. Metal pollution is a global problem; thus, the international regulations with regard to the water quality demand compliance with the quality standards in surface water, groundwater, and biota. The use of organisms (such as fish, crustacean, and mollusks) as bioindicators of metal pollution allowed us to obtain valuable information about the effects on the Romanian common species and to estimate the quality of their environment. The results from laboratory toxicity tests showed the highest concentration values that are not relevant for the detected metal concentrations into surface water, but the metals accidentally released and long-term accumulation could create similar conditions to the results of applied tests. Cd, As, Cu, Zn, Pb, Ni, Zr, and Ti have a very toxic and toxic effects for *C. carpio* and could raise concerns because of its importance for human as a fishery resource. Benthic invertebrates' analysis of the bioaccumulation level varied between species, metals type, and sampling sites. The metal analysis in mollusks shell showed that the metals involved in the metabolic processes (Fe, Mn, Zn, Cu, and Mg) had greater storage capacity than the toxic one (Pb, Cd). In case of *V. viviparus* shell, the selectivity of the metal concentration was represented as follows: Fe > Mn > Zn > Cu > Pb > Co > Cd, while the shell of *A. cygnea* had a greater accumulation capacity for Cu, As, Cr, Zn compared to *Unio* sp. The bioaccumulation factors

of metals in benthic organisms were subunitary, which indicated a slowly bioaccumulation process occurred in the studied aquatic ecosystems. This conclusion highlighted a bioaccumulation process that can increase the persistence of metals in the ecosystem, with a long-term potential risk in trophic chain. The preliminary aquatic risk assessment calculated for *C. carpio* for the most detected metals both in water and in sediment (Ni, Cd, Cr, Cu, Pb, As, and Zn) revealed insignificant to moderate risk considering the metals measured environmental concentrations, acute and long-term effects. The results highlighted a pessimistic view concerning the quality of aquatic ecosystem needed to support the carp survival. The concern is related to the constant presence of metal concentrations especially in sediments which is the principal food provider, leading to bioaccumulation processes and trophic chain transfer. Future studies have been initiated to understand the long-term effects of metals in aquatic biota and to complete the aquatic risk assessment considering the abiotic factors.

Abbreviations:

Cd	cadmium
As	arsenium
Cu	copper
Pb	lead
Ni	nickel
Zr	zirconium
Ti	titanium
Fe	iron
Zn	zinc
Mn	manganese
Mg	magnesium
Cd	cadmium
Co	cobalt
Cr	chromium
Mo	molybdenum
Se	selenium
Na	sodium
P	phosphorus

S	sulfur
Hg	mercury
CN	cyanide
LC (EC) 50	lethal concentrations for 50% of tested organisms after 96 or 48 h
MATC	maximum acceptable toxicant concentration in aquatic systems
NOEC	no observed effect concentration
LOEC	low observed effect concentration
GOT	glutamic oxaloacetic transaminase
GPT	glutamic pyruvic transaminase
OECD	Organization for Economic Co-operation and Development
PNEC	predicted no-effect concentration
PEC	predicted exposure concentration
MEC	measured environmental concentration
RQ	risk quotient

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