https://doi.org/10.7427/DDI.24.05

5. Heavy Metals in Aquatic Macroinvertebrates and Danube River Sediments

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Abstract: Actual study is focused on assessment of heavy metals (cadmium, lead, chromium, nickel, and zinc) concentration from Danube river sediment and aquatic macroinvertebrates. The sampling point is located along the Danube river. Ecosystem characteristics and anthropogenic influences located in the area were taken into consideration when selecting the sites. We sampled both sediment and macroinvertebrates in a single occasion, in May 2018. In the sediments of the studied Danube sectors, maximum allowed values were exceeded for cadmium and nickel. It was also found that the highest concentrations of nickel in the sediment are located downstream of the urban agglomerations and industrial ports on the two banks of the Danube. The bioconcentration factor (BCF) was used in order to evaluate the transfer and accumulation of the analysed heavy metals in aquatic macroinvertebrates. The overall analysis of the BCF's revealed no common pattern regarding the transfer of heavy metals between different macroinvertebrate groups and riverine sediments.
Keywords: heavy metals, Danube river, aquatic macroinvertebrates, sediments

INTRODUCTION

Under natural conditions, river sediments can accumulate and retain large amounts of water pollutants, acting as a trap. But when resuspension processes occur a part of this pollutants ends up in the water column. In particular, fine sediments (<63 µm) can accumulate large amounts of heavy metals on the surface of mineral and organic particles (Laane, 1992). At the same time, the sediments accumulate insoluble metal compounds, which can under certain conditions be released into the interstitial water, thus adding to the soluble or suspended metals in the water column.
Starting from the accumulation of heavy metals in sediments, the risk of contamination occurs in animal organisms, especially aquatic macroinvertebrates. Living at the sediment-water interface benthic macroinvertebrates are exposed sediment bound pollutants acting also as a transfer channel to other aquatic organisms via food-chain.
The importance of carrying out this study started from the need to know the present level of accumulation of heavy metals in the Danube sediments and their transfer to the main aquatic macroinvertebrate groups.

MATERIALS AND METHODS

In order to quantify the transfer rate of some heavy metals from the Danube sediments to the main aquatic macro-invertebrates groups, 27 sampling points have been selected. Ecosystem characteristics and anthropogenic influences located in the area were taken into consideration when selecting the sites. Thus, depending on the geographic location the sampling points were located in 3 different Danube sectors with distinct characteristics and influences (figure 1).
Sediment and macroinvertebrate samples have been collected using an Eckmann dredge with a surface of 0.132 m^2. A 500 µm sieve was used to separate invertebrates from the sediment.

To determine the metal concentration, the samples were mineralized to the Anton Paar oven. The mineralization step was performed differently, depending on the type of sample, respecting the standard procedures and the recommendations suggested by microwave own manufacturer. A detailed description of the methods is given in Burada et al. (2017)

Figure 1. Three Danube sectors where the 27 sampling point are located

RESULTS AND DISCUSSION

Cadmium (Cd)

Cadmium is naturally found in rocks and soils, usually in concentrations less than 0.1 mg/kg. In aquatic ecosystems, it is present in soluble form of cadmium ions and most of the times have anthropogenic origin (Drzewiecka et al., 2010).

Analysing the cadmium accumulations in the three groups of benthic macroinvertebrates (Bivalvia, Odonata, and Gasteropoda) (Figure 2), it is noted that they have an accumulation capacity that differs from a sampling point to another and seems to be correlated with the average concentration of cadmium in the sediment of the Danube sector from which they were sampled.

The analysis of the results obtained for cadmium concentrations (Figure 2), according to the legislation in force, shows that this element has exceeded the quality standard, of 0.8 mg/kg, in each studied Danube sector. The highest value of 1.303 mg/kg, identified at point P6 - Upstream Isaccea town. The differences between sampling points can be attributed to the sedimentation process that is performed differently from one area to another.

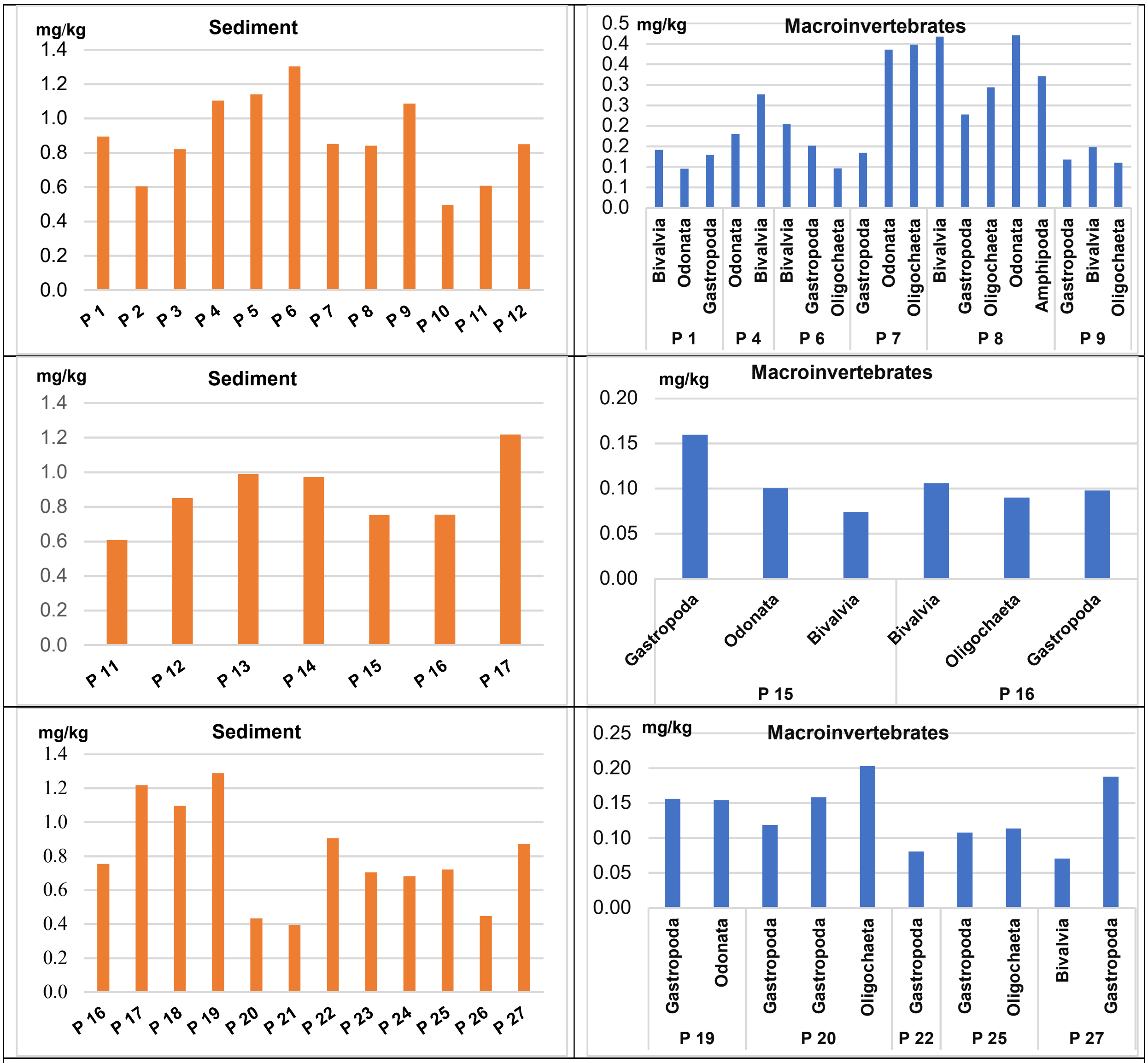


Figure 2. Cadmium concentration in sediments and aquatic macroinvertebrates

Lead (Pb)

In the aquatic environment, lead suffer little transformation and will be found as Pb^{2+} ions or as an insoluble lead compound with a high tendency to associate with suspended matter. Changes in the aquatic environment depend on some processes of precipitation and dissolving depending on the particular conditions of each ecosystem. The highest lead concentrations have been determined in the points P5 - Downstream Reni, P6 - Upstream Isaccea, P12 - Ceatal Chilia, P20 - Downstream Mahmudia river meander) and P21 on river meander downstream Mahmudia village. It is worth noting that all of these points are characterized by low flow velocity and implicitly high sedimentation rates.

Taking into account the common characteristics of the above-mentioned points, as well as the strong association of lead with small suspended particulate matter (Iordache et al., 1998) we consider that higher lead values are due to the increased proportion of the finer fractions in the sediment associated with these points. According to the quality standard, the maximum allowed value of 85 mg/kg was not exceeded in any of the sectors studied.

Lead concentrations (Figure 3), from the macroinvertebrates tissues taken from the most downstream section of the Danube, showed a higher value compared with the two upstream sectors. This upstream-downstream increase in lead concentrations could most likely be caused by the strong association of lead with small sized particulate matter (Iordache, 1997), which is much more intense in the area of Danube river mouth. In the case of Bivalvia group, concentrations range was between 5.788 mg/kg (P6 – Downstream Reni) and 0.148 mg/kg (P9 - Downstream Somova-Parcheş inlet). The Odonata group showed a similar lead retention capability, reaching peak values of 3.867 mg/kg (P15 - Downstream Tulcea Industrial Harbour) The lead concentrations were found in the Oligochaeta (6.059 mg/kg) and Gastropoda (9.639 mg/kg), sampled closed to the river mouth.

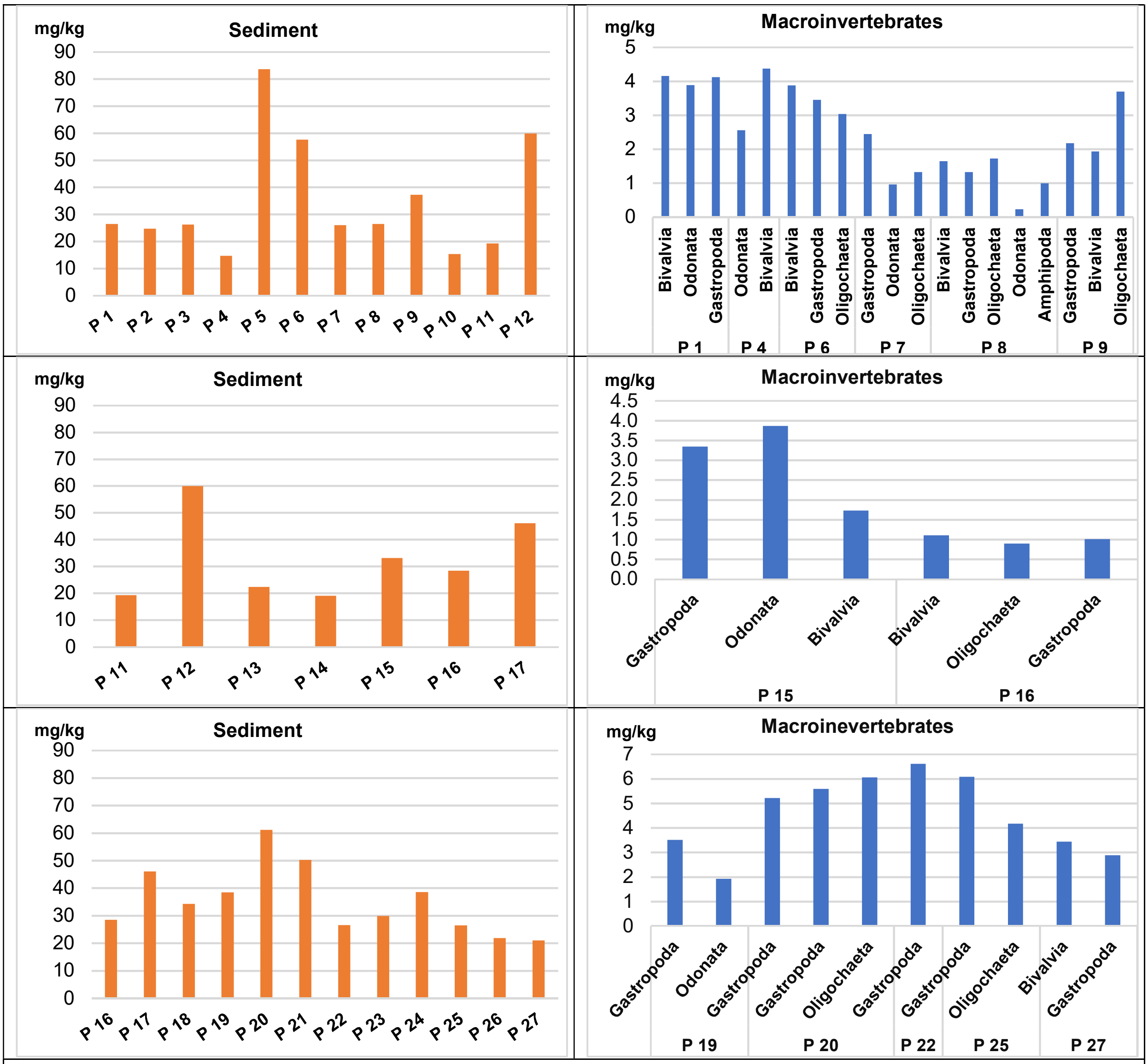


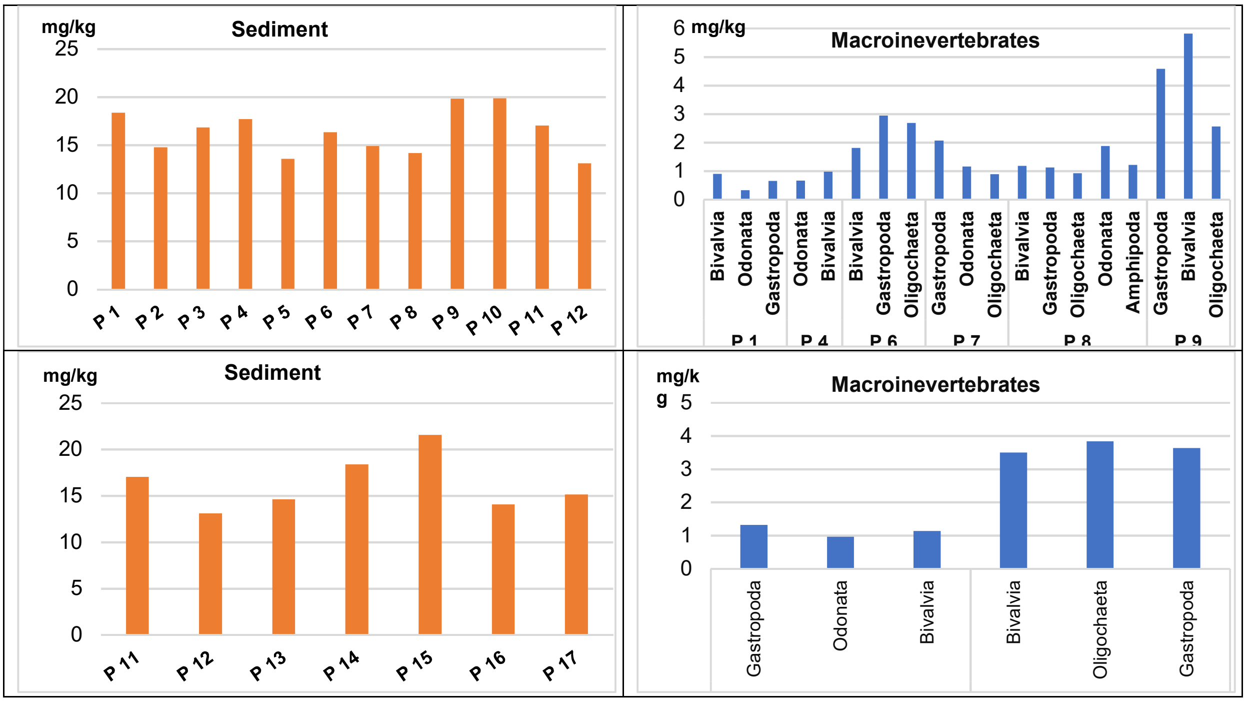
Figure 3. Lead concentration in sediments and aquatic macroinvertebrates

Chromium (Cr)
Environmental Chromium can exist in oxidized states with the most common forms being trivalent (Cr^{+3}) or hexavalent (Cr^{+6}) oxidized forms (Wright & Welbourn, 1994).

Our results revealed a relative uniformity of concentrations in all three studied Danube sectors The values ranged between 13,579 mg/kg and 19,820 mg/kg, well below the maximum admissible limit of 100 mg/kg, provided by the current legislation (figure 4) The small differences of up to 6.20 mg/kg between the three studied sectors can be attributed to the natural background and uneven distribution of pollutants in the sediment.

Although the results obtained in our study showed values well below the quality standard, it should be taken into account that there are still large "stocks" in the Danube sediments that can be remobilized during the floods. In a study (Ilie et al., 2017) on the sediments of the Danube between kilometers 184 and 348, values up to 126.52 mg/kg have been found. Also, in samples taken by Joint Danube Survey 3- (Liška et al., 2013) chromium is a common feature, with high values upstream of the Danube's entry into Danube Delta Biosphere Reserve.

In the first sector of the Danube, the Bivalvia group presented values ranging from 0.897 mg/kg (P1 – Downstream Cotul Pisicii) and 5.821 (P9 – Downstream Somova-Parches inlet). In Odonata, the smallest concentrations were recorded at (P1 – Downstream Cotul Pisicii) 0.328 mg/kg and the highest was1.874 mg/kg (P8 – Downstream Isaccea town). For Gasteropoda and Oligochaeta, maximum values were up to 4.579 mg/kg and 2.564 mg/kg respectively. In the second sector, the Gasteropoda and Bivalvia groups showed accumulation differences of approximately 2.3 mg/kg between the two sampling points and Odonata and Oligochaeta, presenting values of 0.961 mg/kg and 3.836 mg/kg respectively. The third sector showed the smallest differences in chromium accumulation between the three groups of macroinvertebrates studied (Gasteropoda, Odonata, and Oligochaeta).



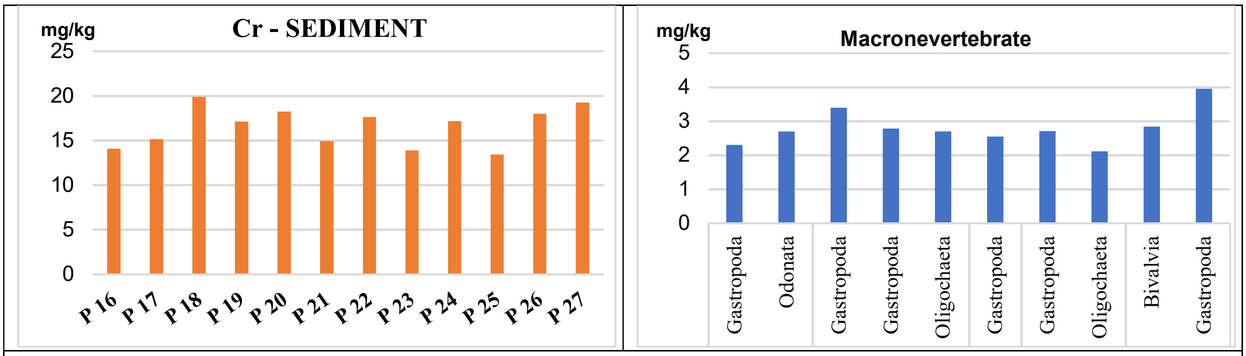


Figure 4. Chromium concentration in sediments and aquatic macroinvertebrates

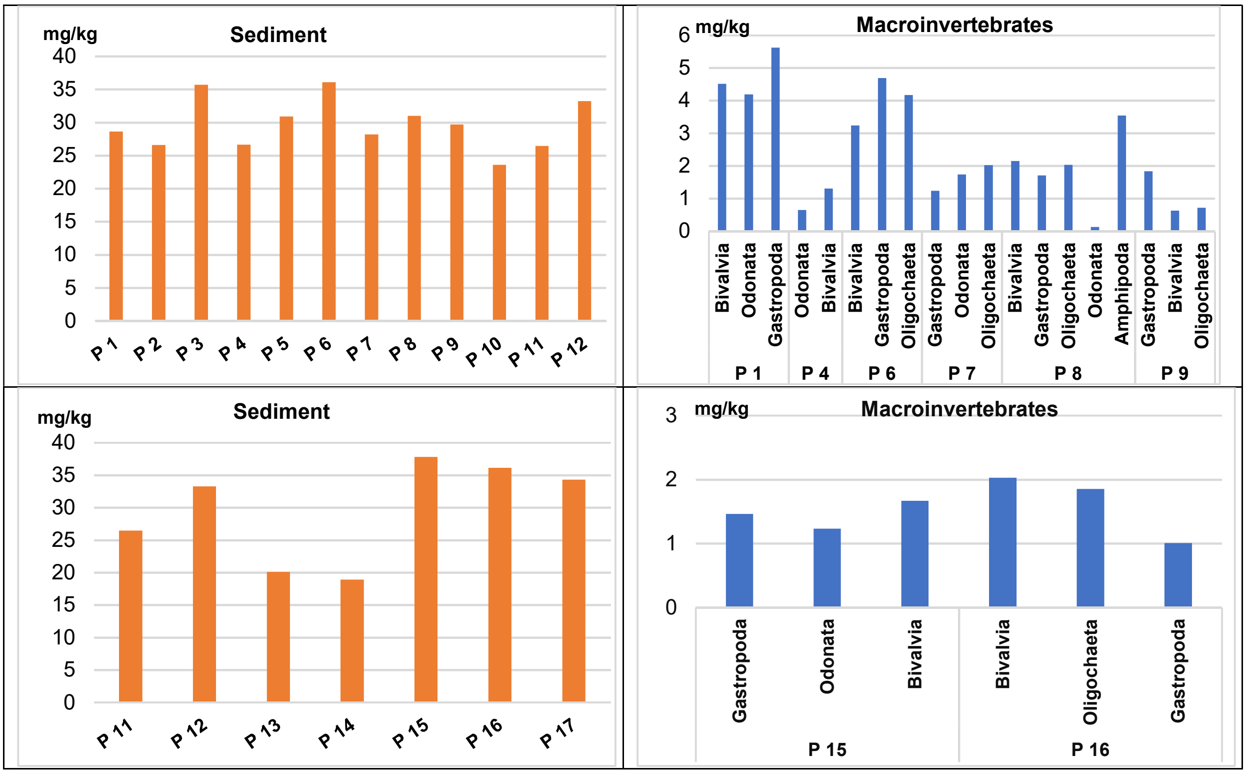
Nickel (Ni)

Soil erosion, atmospheric deposition of fossil fuel combustion, galvanic coating processes are the main sources for nickel in aquatic ecosystems. Nickel is also a component of sludge generated by sewage treatment plants that can be combined with chlorine, sulphur, and oxygen (Irwin et al., 1997).

Analysing the spatial distribution of nickel concentrations, it can be noted that the highest concentration in the sediment was identified downstream of the urban agglomerations and industrial ports on the two banks of the Danube.

According to the normative limit values of 35 mg/kg, was exceeded at points P3 – Downstream Prut river mouth (35.690 mg/kg), P6 – Upstream Isaccea town (36.078 mg/kg), P15 – Downstream of Tulcea industrial harbour (37.815 mg/kg) and P16-Ceatal Sf. Gheorghe (36.111 mg/kg).

Macroinvertebrates from the first studied Danube sector (P1 – P12) (Figure 5), had a different "response" regarding the concentrations of this element in the tissues, which varied from one sampling point to another. Thus, for the sampling points, P1 – Downstream Cotul Pisicii and P6 – Downstream Reni the accumulation level of this metal was proportional to the concentration identified in the sediment. The rest of the points showed significant variations that cannot be correlated with the contamination level of the substrate. It is quite likely that this phenomenon is influenced by the mobility of this metal, which is influenced by several parameters including the substrate type.



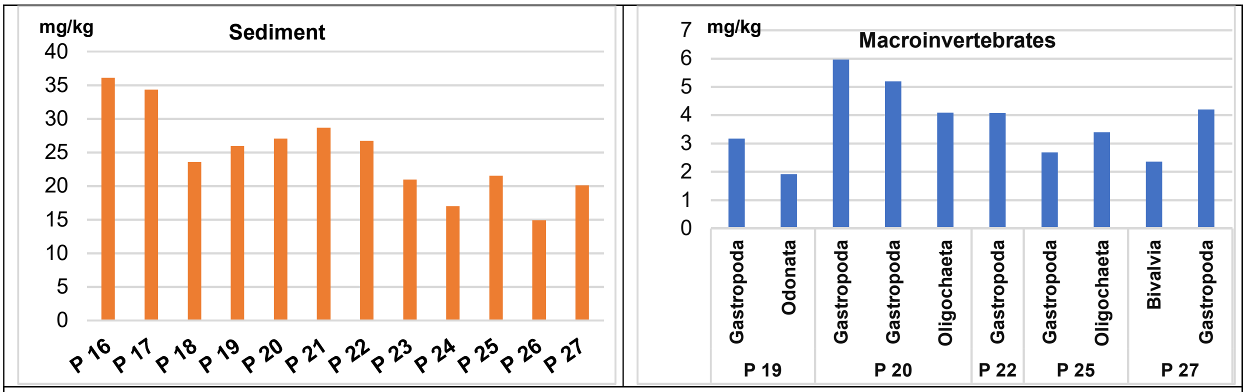


Figure 5. Nickel concentration in sediments and aquatic macroinvertebrates

Zinc (Zn)

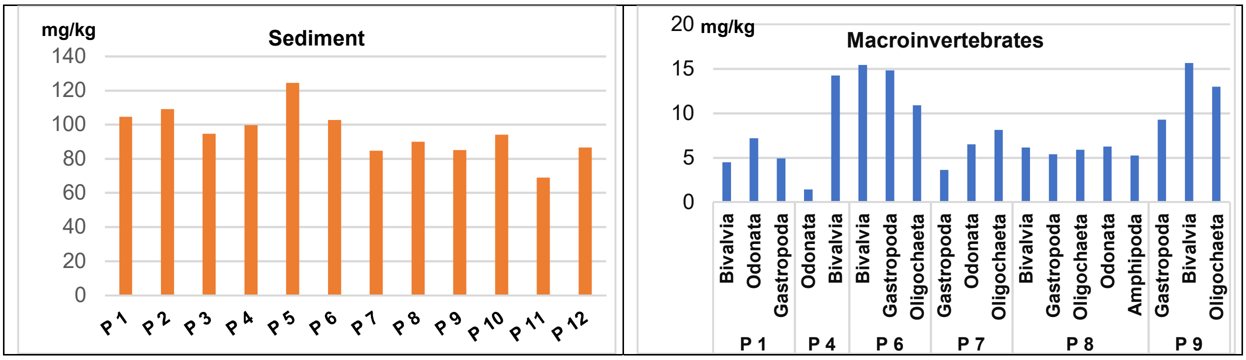
Zinc is an essential element for organisms, being a part of various metalloenzymes. Main anthropic inputs of zinc into aquatic ecosystems are represented either by the metallurgical industry or from a number of manufacturing processes. At the same time, zinc compounds may contain cadmium impurities (Iordache et al., 1998).

Zinc concentrations showed a similar pattern to nickel, showing a decrease of concentrations on an upstream-downstream direction (Figure 6).

The maximum value was recorded in the sediments of P5 – Downstream Reni (124.356 mg/kg). According to the quality standard, the maximum admissible value of 150 mg/kg was not exceeded in any of the investigated points. Although at these concentrations, zinc does not pose a hazard to the health of aquatic organisms, it should be considered that a reduction in the dissolved oxygen concentration from 7 mg/L to 2 mg/L leads to an increase in zinc toxicity by approximately 50% (Lloyd, 1960).

Zinc concentration in the analysed Danube sectors can be attributed to the natural background and we can safely conclude that urban agglomerations and port activities on both sides of the Danube have no influence on zinc concentrations in the studied area.

The highest values of zinc concentrations in aquatic macroinvertebrates were recorded at points P4 (Downstream Prut river mouth) and P6 (Downstream Reni). The gastropod *Viviparus sp*. and the bivalve *Dreissena polymorpha* had concentrations of 14 mg/kg and 16 mg/kg respectively. The values determined in the studied areas are much lower than the values obtained from similar studies from other regions. Thus, concentrations in the Ontario Lake were between 100 mg/kg and 171 mg/kg in *Dreissena polymorpha* tissue without any adverse effects (Johns & Timmerman, 1998).



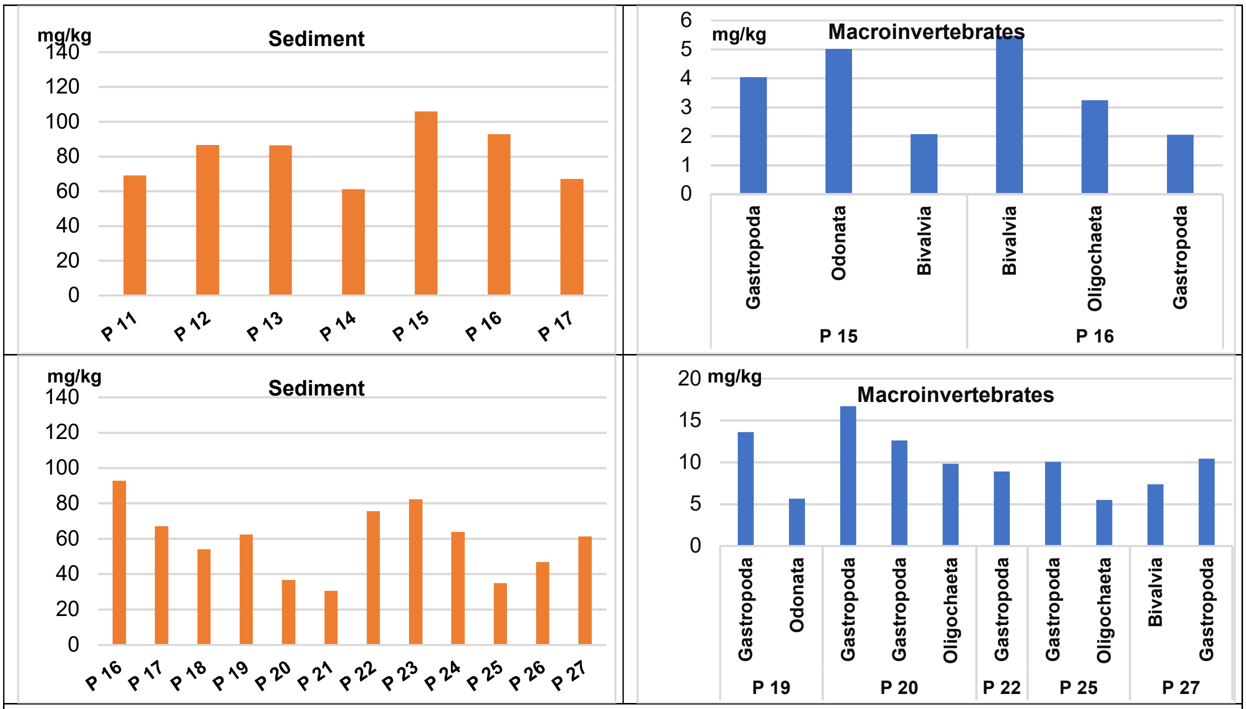


Figure 6. Zinc concentration in sediments and aquatic macroinvertebrates

Bioconcentration factor

The bioconcentration factor (BCF), defined as the ratio between the concentration of a metal in benthic macroinvertebrates and its concentration in the source substrate (Majid et al., 2014), is a particularly important indicator used to determine the take-up rate and storage of metal in the biotic compartment (Klancko, 2006). If the bioconcentration factor (BCF) values are greater than 1, the macroinvertebrates group may be included in the heavy metal hyperaccumulators category(Majid et al., 2014).

Depending on the value of the transfer factor, the pollutants can be transferred to the trophic network by accumulation (factor <1) or by concentration (factor> 1). The increasing the concentration of the pollutant in the succession of the trophic chain levels is not a rule (Iordache, 2009).

Understanding bioaccumulation/accumulation is an important process. The persistence of the pollutant can increase thus representing a potential risk for the health of aquatic ecosystems because of long-term effects. Even if laboratory toxicity tests offers a solid method for investigating heavy metals effects on macroinvertebrates long-term effects are difficult to asses in the laboratory. On the other hand, a high bioaccumulation potential does not necessarily imply a high potential for toxicity (Streit, 1992), and therefore toxic effects should be estimated separately. In addition, a distinction has to be made between accumulation on a narrow range that occurs due to physiological needs (zinc case), and apparently uncontrolled accumulation (cadmium case).

The BCF values of the main groups of aquatic macro-invertebrates from the investigated Danube sites are shown in Table 2.

Therefore, the analysis of the results obtained by calculating the bioconcentration factor (BCF) in the Danube sector between Cotul Pisicii and Ceatal Chilia (Table 2), revealed that the different macroinvertebrate groups have a bioconcentration capacity that differs from a metal to another.

For cadmium, the Bivalvia group presented values ranging from 0.136-0.496 for the Danube sector between Cotul Pisicii - Ceatal Chilia, 0.098 and 0.141 for the Danube sector between Ceatal Chilia-Ceatal Sf. Gheorghe and 0.081 for the last studied sector.

Lead BCF showed lower values compared to cadmium in all three analysed segments. For copper and nickel, similar values were obtained at all monitored sites. Of the investigated macroinvertebrate

groups, the Bivalvia has, with two exceptions, all of the BCF values less than 1, indicating that the transfer of the metals is done by accumulation.
The Odonata group generally presented subunit values (<1) with the exception of the P1-Aval Cotul Pisicii, where BCF values were greater than 1 for lead, copper, and zinc. We estimate that these values may be attributed to external factors that favour the bioavailability of these heavy metals. Likewise, a similar situation was noted for Gastropoda and Oligochaeta, for P6 - Aval Reni.
The overall analysis of the BCF's revealed no common pattern regarding the transfer of heavy metals between different macroinvertebrate groups and riverine sediments. The results show that the transfer of heavy metals differs from one Danube's sector to another (Table 1).
Comparing the average results obtained from the calculation of the bioconcentration factor for the 3 sectors of the Danube, a downward trend was determined: **BCF_{Cd}, BCF_{Pb} BCF_{Cr} BCF_{Cu} BCF_{Ni} BCF_{Zn} Cotul Pisicii – Ceatal Chilia < BCF_{Cd}, BCF_{Pb} BCF_{Cr} BCF_{Cu} BCF_{Ni} BCF_{Zn} Ceatal Sf. Gheorghe – river mouth < BCF_{Cd}, BCF_{Pb} BCF_{Cr} BCF_{Cu} BCF_{Ni} BCF_{Zn} Ceatal Chilia – Ceatal Sf. Gheorghe.**

Table 1. Mean BCF values in different macroinvertebrate groups from Danube River

	BCF_{Cd}	BCF_{Pb}	BCF_{Cr}	BCF_{Ni}	BCF_{Zn}
Cotul Pisicii – Ceatal Chilia	0.339	0.248	0.256	0.745	0.486
Ceatal Chilia – Ceatal Sf. Gheorghe	0.139	0.063	0.156	0.042	0.037
Ceatal Sf. Gheorghe – river mouth	0.451	0.135	0.164	0.152	0.223

Table 2 Heavy metals BCF's in different macroinvertebrate groups from Danube River

	TAXONOMIC GROUP	BCF_{Cd}	BCF_{Pb}	BCF_{Cr}	BCF_{Ni}	BCF_{Zn}
P 1	Bivalvia	0.158	0.128	0.049	0.158	0.043
	Odonata	0.528	1.519	0.491	6.509	1.029
	Gastropoda	0.468	0.942	0.664	0.196	0.345
P 4	Odonata	0.163	0.174	0.038	0.024	0.014
	Bivalvia	0.351	1.130	0.543	0.404	0.922
P 6	Bivalvia	0.157	0.067	0.111	0.490	0.150
	Gastropoda	0.116	0.060	1.424	0.787	4.077
	Oligochaeta	0.074	0.053	2.396	0.164	1.669
P 7	Gastropoda	0.157	0.094	0.139	0.144	0.043
	Odonata	0.453	0.037	0.078	0.062	0.077
	Oligochaeta	0.468	0.051	0.060	0.072	0.096
P 8	Bivalvia	0.496	0.062	0.083	0.069	0.068
	Gastropoda	0.270	0.050	0.080	0.055	0.060
	Oligochaeta	0.349	0.065	0.065	0.065	0.065
	Odonata	0.500	0.009	0.132	0.004	0.069
	Amphipoda	0.382	0.037	0.086	0.114	0.058
P 9	Gastropoda	0.108	0.058	0.231	0.162	0.109
	Bivalvia	0.136	0.052	0.294	0.021	0.184
	Oligochaeta	0.101	0.099	0.129	0.024	0.153
P 15	Gastropoda	0.212	0.101	0.061	0.039	0.038
	Odonata	0.134	0.117	0.045	0.033	0.047
	Bivalvia	0.098	0.052	0.053	0.044	0.020
P 16	Bivalvia	0.141	0.039	0.249	0.056	0.059
	Oligochaeta	0.119	0.032	0.273	0.051	0.035
	Gastropoda	0.130	0.035	0.258	0.028	0.022
P 19	Gastropoda	0.121	0.091	0.134	0.122	0.219
	Odonata	0.119	0.050	0.157	0.074	0.091
P 20	Gastropoda	0.884	0.085	0.186	0.220	0.455
	Gastropoda	0.182	0.091	0.153	0.192	0.343
	Oligochaeta	0.516	0.099	0.148	0.151	0.267
P 22	Gastropoda	0.089	0.249	0.144	0.152	0.117
P 25	Gastropoda	0.149	0.230	0.202	0.125	0.288
	Oligochaeta	0.158	0.158	0.158	0.158	0.158
P 27	Bivalvia	0.081	0.164	0.147	0.117	0.120
	Gastropoda	0.215	0.137	0.206	0.209	0.170

CONCLUSIONS

From the investigations carried out on sediments of the three studied Danube sectors, maximum allowed threshold exceedances for cadmium and nickel have been identified. It was also found that the highest concentrations of nickel in the sediment are located downstream of the urban agglomerations and industrial ports on the two banks of the Danube.

Regarding the level of accumulation of heavy metals in the main macroinvertebrates groups, cadmium exhibited variations ranging from 0.14 mg/kg in snails (Gastropoda) and 0.22 mg/kg in dragonflies (Odonata). The latter group, represented by one species, namely *Gomphus flavipes*, recorded the lowest values also for the other metals analyzed. The presence of this species, which is sensitive to pollution, in many of the analyzed points demonstrates that recorded values of heavy metal concentrations do not pose a risk to aquatic invertebrate populations. The highest cadmium values were identified in the P8 - Isaccea downstream, in all groups of organisms.

In the case of lead, chromium, copper and nickel, in aquatic macroinvertebrates, the magnitude of variation between the minimum and maximum mean values is small, ranging between 1.21 mg/kg and 2.28 mg/kg, with no significant differences between the groups of organisms.

ACKNOWLEDGMENTS
This work was supported by Romanian National Authority for Scientific Research and Innovation, ”Program Nucleu contract no, 14N/2018”

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Received 5.04.2019
Revised 9.04.209