The assessment of metal contamination in water and sediments of the lowland Ilova River (Croatia) impacted by anthropogenic activities

Tatjana Mijošek[1], Vlatka Filipović Marijić[1], Zrinka Dragun[1], Dušica Ivanković[1], Nesrete Krasnići[1], Zuzana Redžović[1], Mirela Sertić Perić[2], Neda Vdović[1], Niko Bačić[1], Jelena Dautović[1], Marijana Erk[1]

[1]Ruđer Bošković Institute, Division for Marine and Environmental Research, Bijenička cesta 54, 10000 Zagreb, Croatia

[2] University of Zagreb, Faculty of Science, Department of Biology, Division of Zoology, Rooseveltov trg 6, 10000 Zagreb, Croatia

*Corresponding author:
Tatjana Mijošek
Laboratory for Biological Effects of Metals
Division for Marine and Environmental Research
Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia
Phone: 00385-1-4680216; E-mail: tmijosek@irb.hr

Acknowledgements

The study was funded by the Croatian Science Foundation, within the project "Accumulation, subcellular mapping and effects of trace metals in aquatic organisms" AQUAMAPMET (IP-2014-09-4255).

Abstract

The aim of the present study was to assess physico-chemical water parameters, granulometric sediment characteristics and concentrations of trace and macro elements in the water and sediments of the Ilova River. Samplings were conducted at three sampling sites (near villages Maslenjača, Ilova and Trebež) along the Ilova River, differing in the source and intensity of the anthropogenic influence.This study indicated disturbed environmental conditions, most pronounced in the downstream part of the river (Trebež village) impacted by the activity of fertilizer factory. Water from the Ilova and Maslenjača villages was of good quality, whereas COD, nitrates and phosphates exceeded the good quality levels in Trebež village. Trace and macro element concentrations in water were mostly below thresholds set by environmental quality standards at all locations, but levels of Al, As, Cd and Ni were few times higher in Trebež village than at other locations. Metal contamination assessment of sediments (trace and macro elements concentrations, contamination and enrichment factor, pollution load index) confirmed deteriorated environmental quality in Trebež village. However, the overall assessment performed in this study revealed that anthropogenic impact was still not particularly strong in the Ilova River ecosystem. Nevertheless, the observed water and sediment characteristics serve as a warning and suggest that stricter protection measures should be initiated, including continuous monitoring and comprehensive quality assessment of the downstream part of the Ilova River, especially because it is a part of the protected area of the Lonjsko Polje Nature Park.

Keywords: environmental quality of water and sediment, trace elements, macroelements, metal exposure, monitoring, wastewaters, Ilova River

Introduction

Intensive anthropogenic activities including industry, urbanization, aquaculture, agriculture and consequential wastewater disposal have nowadays significantly increased metal contamination in aquatic ecosystems, and represent a critical environmental issue due to the metal persistence, toxicity and possible bioaccumulation in biota (Campbell et al. 2006). Therefore, it is suggested that environmental quality assessments involve analysis of physico-chemical descriptors of water and sediment, but also the measurements of metal concentrations in water, sediments and biota.

Sediments are considered as the main sink of organic substances and different chemical contaminants, including metals, in aquatic ecosystems (Alvarez-Guerra et al. 2007). However, apart from being only the sink, they are also a well known source of contaminants in freshwater environments (Burton 2002). Metals, as one of the major concerns, are not permanently bound to sediments but can be released to the water column depending on both natural and anthropogenic variations of physico-chemical environmental factors, such as pH value, temperature, river flow or bioturbation, which could affect the metal equilibrium and remobilization between the sediments and water (Crane 2007; Zhang et al. 2009). The size of sediment particles can also significantly contribute to the metal remobilization and sediment resuspension (Chon et al. 2012; Fiket et al. 2017).

Established evaluation criteria for the ecological status of natural waters comprise the concentrations of trace metal cations (Cd, Hg, Ni, Pb as priority metal pollutants), physico-chemical water parameters, as well as hydromorphological characteristics (WFD 2016). The previous assessment of the stream morphological features within the Ilova catchment, conducted according to the relevant EU Water Framework Directive (WFD) and Croatian Waters' Methodology for stream monitoring and assessment, revealed that 38.5% of the total Ilova watercourse did not meet the WFD objectives. The streams in the Ilova River catchment were classified as moderate, poor or bad with respect to morphological condition (Plantak et al. 2016), suggesting a significant human impact emerging from the extensive river training works, construction of canals and fish farms. Apart from that, middle and lower parts of the Ilova watercourse are influenced by municipal wastewaters of the Town of Kutina, as well by industrial wastewaters from the fertilizer factory.

Previous studies within this area (Durgo et al.2008; Radić et al. 2013) mostly covered the investigations of the (geno)toxic potential of the Ilova River surface water contaminated by the effluents of the fertilizer factory, reporting a considerable oxidative stress in model organisms as well as enhanced bioaccumulation of some metal cations (Cd, Cr, Cu, Fe, Ni, Pb and Zn). However, the overall assessment of the metal exposure from different anthropogenic sources has not yet been conducted in this region, which is especially relevant because of the nearby Lonjsko Polje Nature Park protected area.

Thus, to evaluate the environmental conditions of the lowland Ilova River, which is affected by various sources of anthropogenic influence - mainly aquaculture (fish ponds), agriculture (farms) and industry (fertilizer production), several parameters were measured in samples of water and surface sediments at three sampling sites stretched along the Ilova River and differing in the degree of anthropogenic influence: i) the concentrations of trace and macroelements cations dissolved in the water and present in the sediments, ii) physico-chemical water parameters, iii) granulometric characteristics of sediments, and iv) the concentrations of total organic carbon in the sediments. Data on trace and macro elements levels in water and sediments, as well as on their spatiotemporal variations, and the calculation of contamination and enrichment factors (CFs and EFs) were used to enable the distinction of natural and anthropogenically elevated metal levels in this ecosystem. The obtained results can contribute to the future developments of water management plans and monitoring programmes in the protected area of Lonjsko Polje Nature Park and similar freshwater ecosystems in general.

Materials and methods

Study area

The study was conducted in the lowland Ilova River watercourse in the continental part of Croatia (Fig. 1). The Ilova River rises at the altitude of about 200 m a.s.l., it is about 85 km long and drains an area of about 1,049 km^2, including its largest tributaries Rijeka and Toplica. Many wetland areas of the Ilova River are converted into fish ponds and are thus active regions of both fish farming (aquaculture production) and recreational and sport tourism. Freshwater fish farms Končanica (9.43 km^2) and Poljana (13 km^2), located at the Ilova River close to our study sites (Fig. 1), are the biggest freshwater aquaculture fisheries in Croatia. Considering our research, three sampling sites were selected. The first sampling site at the Ilova River near the Maslenjača village (45°39'35.70''N 17°16' 20.73''E; Fig.1) was located in the uppermost watercourse area with a low anthropogenic influence (i.e., upstream from the fish ponds, industrialised and agricultural area). At the second sampling site located near the Ilova village (45° 26' 45.08'' N 16° 49' 43.34'' E; Fig. 1), the Ilova River receives effluents from the upstream fish farms. The effluent constituents of greatest concern are suspended solids, organic matter, phosphorus and nitrogen. The effluents from aquaculture can have an adverse effect on the surrounding environment. At the location about 1 km downstream from the Town of Kutina, the Ilova River receives contaminating inputs from a precipitation and wastewater through the Kutinica River that discharges approximately up to 12,000 m^3 of untreated industrial wastewater per day (Radić et al. 2013). The discharged wastewater originates mostly from a fertilizer factory producing nitrogenous fertilizers, mineral NPK fertilizers, carbonblack, bentonites, additives for foundries and cattle feed additives; from municipal wastewater (average daily input – 8,000 m^3 of treated sewage and industrial water); and from runoff from soil contaminated by agricultural practices (Radić et al. 2013). Downstream of the confluence with Kutinica, the Ilova River flows into the Lonja River, which forms wetland area protected as a Nature Park(http://www.pp-lonjsko-polje.hr/new/english/index.html). Therefore, the third sampling site, presumed as the most contaminated one, was located near the Trebež village (45° 21' 21.21'' N 16° 46' 26.16'' E; Fig. 1) about 8 km downstream from the confluence of the Kutinica River. A previous study in this region (Radić et al. 2013) revealed elevated concentrations of Fe, Cd, Pb, Cr, Hg, Zn, Cu, and Ni at the location downstream of the Town of Kutina (near the fertilizer factory) in comparison to their reference location.

Water and sediment sampling

In the present study, the river water samples were collected in triplicates in acid pre-cleaned polyethylene bottles at each sampling site in two seasons – autumn 2017 and spring 2018. Sampled water was filtered at site through a 0.45 µm pore diameter cellulose acetate filter (Sartorius, Germany) mounted on syringes. Aliquots of these samples that contained dissolved metal cations, small inorganic and organic metal complexes and colloids were transferred into acid pre-cleaned 20 mL polyethylene bottles and acidified with concentrated nitric acid (HNO_3) (Rotipuran Supra 69 %, Carl Roth, Germany) and stored at 4 ºC for metal analysis. An additional 1 L water sample was collected and stored at 4 °C for the subsequent analysis of several physico-chemical parameters in the laboratory. The sampling of the surface sediments was performed manually in the shallow parts of the river at the distance of about 2 m from the river bank. To ensure that the substrate sample is fully representative, according to the recommendations by Loring and Rantala (1992), five sampling spots were chosen in the diameter of 10 m. On each spot, about 0.5 kg of surface and near surface sediment was taken using plastic spatula, and stored into a plastic bag avoiding the leakage of fine-grained sediment from the spatula and bag. In this way, the composite sample of approximately 2.5 kg of the surface sediment was sampled at each of three selected sites in autumn 2017. Samples were transported to the laboratory at 4°C and then stored at -20°C until analysis.

Determination of physico-chemical parameters in water

Water temperature (T), concentration and saturation of dissolved oxygen (DO), pH, oxidation/reduction potential (ORP), conductivity (Cond) and total dissolved solids (TDS) were measured *in situ* using the respective portable field meters which were calibrated before samplings (oximeter OXI 96, WTW GmbH, Weilheim, Germany for T and DO; pH-meter 330i, WTW GmbH, Weilheim, Germany for pH and conductometer Sension 5, Hach, Loveland, Colorado, USA for Cond and TDS). Other parameters were measured in the laboratory. Concentrations of nitrite ($N\text{-}NO_2^-$), nitrate ($N\text{-}NO_3^-$) and orthophosphate ($P\text{-}PO_4^{3-}$), chemical oxygen demand (COD_{KMnO4}), alkalinity (Alk) and total water hardness (TWH) were determined 24 h after the sampling following the standard methods of APHA (1985). Nitrite, nitrate and orthophosphate were all determined spectrophotometrically, applying diazotization method (using sulfanilic acid and 1-naphthylamine) for determination of nitrites, sodium salicylate method for nitrates, and molybdenum blue phosphorus method formeasuring orthophosphates (APHA 1985). Calibration curves were made for each ion from a series of 8 standard solutions within the following ranges: 0.001 – 0.1 $N\text{-}NO_2^-$/mg L^{-1}; 0.05 – 1 $N\text{-}NO_3^-$/mg L^{-1}, 0.01 – 0.15 $P\text{-}PO_4^{3-}$ /mg L^{-1}. The nitrite ion concentration was determined by measuring the absorbance of the azo complex at λ = 520 nm; nitrate ion concentration by measuring the absorbance of the yellow nitrosalicylic acid complex at λ = 420 nm; and orthophosphate ion concentration by absorbance measurement of the molybdenum blue complex at λ = 690 nm. Acid titration (0.1N HCl) with methyl orange was used to determine the alkalinity, while TWH was determined using standard ethylenediaminetetraacetic acid (EDTA) solution (Kompleksal® III, Kemika, Croatia) and the respective indicator buffer tablets (Kompleksin®, Kemika, Croatia). Finally, total chemical oxygen demand (COD_{KMnO4}) was measured by standardized acidic potassium permanganate titrimetric method (Deutsches Institut für Normung 1986). To assess the river water quality of the Ilova River, we have made comparisons of our data with the limiting values of ecological status categories for basic physico-chemical indicators for specific type of river (Directive on Water Quality Standard of the Government of the Republic of Croatia; GRC 2019). According to the classification of the Croatian rivers (GRC 2013), the Ilova River belongs to lowland medium and large river type (type: HR-R_4) in the Pannonian ecoregion (Hungarian lowlands) (EEA 2009). The classification is based on zoogeographical features according to Illies (1978) (Pannonian and Dinaric ecoregions) and on the obligatory abiotic factors of the respective ecosystem (EEA 2009).

Sediment preparation and analyses

Sediment samples were defrosted and homogenized by mixing, and then one portion was taken for the analysis of particle size distribution (granulometry). Granulometric analysis of the sediments was performed by a laser diffraction particle size analyzer (LS 13320, Beckman Coulter, USA). Sediments were categorized according to the Wentworth's grade scale (1922).

Remaining sediment samples were dried by the process of lyophilisation (FreeZone® 2.5L, Labconco, USA), put through 1 mm sieve and the resulting <1 mm fraction was ground to dust in agate mortar and used for the metal and total organic carbon (TOC) determination. Prior to the trace and macroelements determination, portions of around 100 mg of ground subsamples were digested using a microwave oven (Multiwave 3000, Anton Paar, Austria) in a two-step digestion procedure: the first step consisting of addition of a mixture of 4 mL nitric acid (HNO_3, 65%; pro analysi, Kemika, Zagreb, Croatia), 1 mL hydrochloric acid (HCl, 36.5%; pro analysi, Kemika, Zagreb, Croatia) and 1 mL hydrofluoric acid (HF, 48%; pro analysi, Kemika, Zagreb, Croatia) followed by the second step consisting of addition of 6 mL (40 g L^{-1}) of boric acid (H_3BO_3; Fluka, Steinheim, Switzerland). Afterwards, samples were appropriately diluted with Milli-Q water to obtain optimal acid concentrations for HR ICP-MS measurements. Details of the procedure are given by Fiket et al. (2017).

TOC content was determined from lyophilised, well homogenised sediment samples using a Shimadzu SSM-5000A connected to a TOC-V_{CPH} analyzer, by high-temperature (900 °C) catalytic (mixture of Pt/silica and CoO) oxidation method with IR detection of CO_2 and calibrated with D (+) glucose (Merck, Germany) (Lučić et al. 2019).

Determination of dissolved trace and macro elements concentrations in water and sediments

All together, the concentrations of total dissolved chemical forms of 26 trace and macro elements were measured in filtered and acidified samples of river water. Macroelements were determined in 10 times diluted samples due to their higher concentrations, whereas trace elements were measured directly in the prepared water samples. High resolution inductively coupled plasma mass spectrometer (HR ICP-MS, Element 2; Thermo Finnigan, Germany) equipped with an autosampler SC-2 DX FAST (Elemental Scientific, USA) was used for element analysis. Low resolution mode was used for the measurements of ^{82}Se, ^{85}Rb, ^{98}Mo, ^{11}Cd, ^{121}Sb, ^{133}Cs, ^{205}Tl, ^{208}Pb, ^{209}Bi, and ^{238}U; medium resolution mode for ^{23}Na, ^{24}Mg, ^{27}Al, ^{42}Ca, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn,

^{86}Sr, and ^{138}Ba while ^{39}K and ^{75}As were measured in high resolution mode. Indium (1 µg L^{-1}; indium atomic spectroscopy standard solution, Fluka, Germany) was added to all samples as an internal standard. Two separated external calibrations were performed using standard solutions prepared in 1.3% HNO_3 (Rotipuran Supra 69 %, Carl Roth, Germany). Diluted multielement stock standard solution for trace elements (100 mg L^{-1}; Analytika, Czech Republic) with added standard solutions of Cs (1 g L^{-1}; Fluka, Germany), Rb and U (1 g L^{-1}; Aldrich, USA), as well as Sb and Sn (1 g L^{-1}; Analytika, Czech Republic) was used to calibrate the concentrations of trace elements, while calibration for macro elements was performed using multielement standard containing Ca (2.0 g L^{-1}), Mg (0.4 g L^{-1}), Na (1.0 g L^{-1}), and K (2.0 g L^{-1}) (Fluka, Germany). In addition to the river water samples, blank samples were prepared and treated the same way and used for the measurements of elements. To test the accuracy of the measurements, quality control (QC) sample for trace metals (QC trace metals, catalogue no. 8072, lot no. 146142–146143; UNEP GEMS, Burlington, Canada) and QC sample for macroelements (QC minerals, catalogue no. 8052, lot no. 146138–146139; UNEP GEMS) were used. Good agreement was observed between our measurements and certified values with the following recoveries (%): Na 97.6±3.0, Mg 94.7±4.9, Ca 96.9±2.6, K 96.7±3.0, Al 90.6±12.6, As 99.2±5.0, Ba 97.0±0.9, Cd 94.4±0.8, Co 97.6±2.1, Cr 95.1±3.0, Cu 100.1±6.4, Fe 93.8±6.1, Mn 95.5±1.6, Mo 95.4±3.7, Ni 94.6±2.7, Pb 96.8±4.3, Sb 90.5±1.1, Se 98.5±4.9, Sr 98.2±1.6, Tl 95.8±2.3, V 98.2±2.8, and Zn 101.9±11.0. Obtained limits of detection (LOD) for the measured trace elements in the water were (µg L^{-1}): Al 0.4, As 0.03, Ba 0.15, Bi 0.005, Cd 0.002, Co 0.019, Cr 0.06, Cs 0.001, Cu 0.40, Fe 0.6, Mn 0.05, Mo 0.01, Ni 0.10, Pb 0.07, Rb 0.003, Sb 0.001, Se 0.06, Sr 0.2, Tl 0.001, U 0.003, V 0.002 and Zn 7, whereas for the macroelements they were (mg L^{-1}): Ca 0.02, K 0.002, Mg 0.006 and Na 0.006.

The concentrations of all chemical forms present in digested sediments were measured for the same elements, except for Ca and Se with the same instrument in the samples of sediments as well. To ensure the quality control, simultaneous measurements of certified reference materials (CRM) of stream sediment (NCS DC 73309, also known as GBW 07311, China National Analysis Centre for Iron and Steel, Beijing, China) were made. The recoveries of the measured results have already been published in Fiket et al. (2017) in Table 4 and were 91-104%, depending on the element measured. All detailed information on the method and measurements procedures, including LODs were given in Fiket et al. (2017) and can be applied in this study as well, because the measurements were performed in the same analytical laboratory.

We referred to metal concentrations in water and sediments throughout this article having in mind the concentrations of their respective cationic forms.

Data processing and statistics

Basic calculations were made in Microsoft Office Excel 2007. Data on trace and macroelements concentrations are presented as mean ± standard deviation (S.D.). SigmaPlot 11.0 for Windows was used for other statistical analysis and creation of images. Variability of the trace and macroelements levels between the two seasons at each location was tested by applying t-test, while significance of variability in metal concentrations between the three sites was tested by using one-way ANOVAfollowed by the post-hoc Student-Newman-Keuls test. The level of significance was set at 95% ($p<0.05$).

Results and discussion

Physico-chemical parameters of the river-water

According to the classification in Croatian Directive on Water Quality Standards (GRC 2019), water at location of the Maslenjača village can mostly be considered as water of very good and good quality as pH fits the value for water of very good quality, whereas COD and concentrations of nitrates and phosphates were in range of limiting values for waters of good quality for the river type HR-R_4 (Table 1). The only exception at location Maslenjača was concentration of nitrates in spring which slightly exceeded the threshold for waters of good quality. Location of the Ilova village can also be classified as water of good quality because the measured concentrations of nitrates were in the range between 0.7 and 1.3 mg N L^{-1} (Table 1) and the measured concentrations of phosphates were in the range between 0.03 and 0.1 mg P L^{-1} (Table 1). However, the measured value of COD of the water sample from the Ilova village exceeded 5.5 mg O_2 L^{-1} which is the limiting value for waters of good quality (Table 1). Considering Trebež village, values of all classified parameters except pH were above the threshold for the waters of good quality in at least one season (Table 1). COD, which is used to determine the organic pollutant present in the river water, was the highest in the Trebež village, so higher values point to the discharge of greater quantity of organic matter, possibly from the extensive agricultural and industrial activities in the region. COD values were comparable with the values reported for the Ilova River by Radić et al. (2013). Additionally, we have observed high variations in nitrites and phosphates in the Trebež village between autumn and spring (Table 1). The Ilova River watercourse follows a particular regime with high variations in discharge between the seasons (https://hidro.dhz.hr/ e.g. hydrological station Ilova village at the Ilova River (code: 3116) of the Croatian Meteorological and Hydrological Service). This could be the possible explanation for such extremely high concentrations of phosphates and nitrites in the autumn (Table 1) when the water level was low after a long period of summer drought. On the other hand, such extremes could be the consequence of irregular discharge of different types of wastewaters that can possibly occur in the rural areas surrounding the Ilova River.

Although not classified in Croatian Directive on water quality (GRC 2019), dissolved oxygen concentration and saturation also had the same trend as COD and pointed to more disturbed conditions at the Trebež village compared to the Maslenjača and the Ilova villages in autumn, while conductivity was mostly comparable between the sites (Table 1). In connection to that, levels of TDS were also the highest at the Trebež village (Table 1), all as a consequence of the more intense anthropogenic activities (e.g., industry, agriculture) affecting that region.

In the Ilova River, water temperature was moderate and uniform in both studied seasons at two sampling sites - the Ilova and the Trebež village, while the temperature at Maslenjača as the most upstream site was lower for about

7°C and 4°C in autumn and spring, respectively (Table 1). As sites under some anthropogenic disturbances, higher water temperatures at the Ilova and Trebež villages could possibly be associated to "thermal contamination" caused by municipal and industrial wastewater discharges sourcing from the aquacultural, agricultural and industrial activities (Govorushko 2016).

Therefore, water samples from the locations near Maslenjača and Ilova villages were mostly of at least good quality. However, in the downstream part of the river most parameters indicated considerably disturbed conditions, as observed at the location near the Trebež village where parameters exceeded the limits of the good quality waters.

Dissolved trace and macro element concentrations in the river-water

Due to the discharge of municipal and industrial wastewaters into the Ilova River, as well as the influence of fish farming and recreational tourism, we wanted to investigate possible river contamination with trace and macro elements. Dissolved metal fraction obtained by filtration through 0.45μm pore diameter filter contains metals and their complexes which are regarded as more bioavailable to aquatic organisms (Dragun et al. 2009) than the particulate metal fraction which remains on the filter.

Concentrations of Bi, Cr, Pb and Zn in the river water were lower than LOD at all sites (Table 2). Although average concentrations for many elements appeared similar at all three sites of the Ilova River, after the statistical comparison of these values (Table 2), it was observed that most elements were statistically the highest at the Trebež village compared to the other two upstream locations (namely, As, Cd, Cs, Mo, Ni, Rb, Sb, U, V, Ca, K and Na). Smaller differences were also observed between the Ilova village and Maslenjača with more elements being elevated in the Ilova village. Contaminated wastewaters originating from the fertilizer factory near the Town of Kutina are discharged in the river watercourse through the Kutinica River and it could be the cause of the elevated trace and macro element concentrations in the water of the Ilova River at the location of the Trebež village that was observed in our study. Previous study in this area conducted by Durgo et al. (2008) investigated the chemical composition of the phosphoric gypsum transport water collected from the phosphoric gypsum depot near the fertilizer factory. Phosphate rock, which is used in this type of industry, is actually the origin of phosphogypsum and a well-known source of many contaminants, including metals like Pb, V, Cr, Mn, Fe, Ni, Cu and Zn (Carbonell-Barachina et al. 2002). Comparison of factory wastewaters properties based on the maximally allowed element concentrations, according to the Regulation on limit values of parameters of hazardous and other substances in wastewater (OG 40/99; 6/01; 14/01) revealed the greatest enrichment of the following elements: F (308 times higher than maximally allowed), V (35 times higher), Fe (13 times higher) Cr(VI) (7 times higher), Cu and Zn (6 times higher) (Durgo et al. 2008).

In our study, the most pronounced difference between the Trebež village and Maslenjača existed for As, Cd, Cs, Ni, Rb and V. Although Cs concentration was very low, and at the sampling site Maslenjača it was even below LOD (0.001 $\mu g\ L^{-1}$), it was 90 times higher at the Trebež village in autumn. Cadmium had 7 and 26 times higher concentrations, As and Ni 2 and 3 times higher concentrations at the sampling site near the Trebež village site than at Maslenjača in spring 2018 and autumn 2017, whereas the concentration of V was 4 and 7 times higher near the Trebež village in spring and autumn, respectively (Table 2). The opposite trend was observed for Al, Ba, Cu, Fe, Mn, and Sr which were significantly higher at Maslenjača compared to the Trebež village in at least one investigated season with the highest difference observed for Mn in autumn (Table 2). Comparison of the Trebež and Ilova villages revealed similar pattern and highest difference was again observed for Cd, Cs, Ni, Rb and V (Table 2). Caesium concentration was again below LOD at the Ilova village in autumn; Cd, Ni and Rb were 2-6 times higher at the Trebež village depending on the season, while concentrations of Al and V were 12 and 11 times higher at the Trebež than at Ilova village in autumn (Table 2). Ba, Co, Fe, Mn and Se were elevated at the Ilova village in comparison to the Trebež village in at least one season. Between Maslenjača and Ilova villages, the highest differences were observed for Al and Cu with higher levels at Maslenjača, and for Cd, Sb, Se and Tl which were higher at the Ilova village but all these differences were much less pronounced (up to 3 times) than between the Trebež village and the two upstream locations (Table 2).

Considering seasonal differences in Maslenjača and Ilova villages, more elements were significantly higher in spring than in autumn, possibly due to the wash up of agricultural soils and consequential metal leaching. More elements were higher in autumn than spring at the Trebež village probably because of a low water level in that season, as already commented for increased phosphates and nitrites (Table 2).

In the previous study of Radić et al. (2013) covering the similar research area as our, metal concentrations in the Ilova River were measured by energy dispersive X-ray fluorescence method and descending order of metals obtained in studied locations was Fe>Zn>Cu>Ni>Cd. In our study, Zn was below the limit of detection (LOD (Zn) = $7\ \mu g\ L^{-1}$) while other metals had the following order: Fe>Ni>Cu>Cd (Table 2). Similar to Radić et al. (2013) we observed higher concentrations of Cd and Ni at the location of Trebež village that is closest to the Kutinica inflow and located downstream from the fertilizer factory.

To evaluate the water quality of the Ilova River, recorded values were compared with dissolved metal concentrations recorded in two other Croatian rivers: the Sava River–large urban river flowing through the Town of Sisak, City of Zagreb, Town of Velika Gorica, affected by industry contamination (pesticides production facility, ironworks and oil refinery; Dragun et al. 2009), and the Sutla River – medium size river affected by the glass production industry (Dragun et al. 2011; Filipović Marijić et al. 2016). The comparisons were also made with the several European rivers under different anthropogenic impact, as well as with the Environmental Quality Standards (EQS) for few metals provided by the European Union's Water Framework Directive (WFD 2016) (Table 3). Based on the comparison with the other two rivers in Croatia, Ilova River can be considered as moderately contaminated river. Concentrations of Cd, Cs, Cu, Sr, Zn, Ca, K, Mg and Na at all sites of the Ilova River were mostly comparable with the values at the reference site of the Sutla River (Tables 2 and 3). However, reported concentrations of As, Ba, Co, Mn, Ni, Rb, Se, U and V in the Ilova River were mostly higher, even at the less contaminated sites of the Ilova River (Maslenjača and Ilova villages), than at any site of the other two rivers in Croatia. That could be especially emphasized for Mn, with much higher values at the Ilova and Trebež villages in spring than even at the contaminated parts of the Sutla River and the Sava River (Tables 2 and 3). High Mn concentrations in the Ilova River water could be due to more extensive agricultural activities in that area. Manganese is also present as an ingredient in various products such as fertilizers, varnish and fungicides, and in livestock feeding supplements (IPCS 1999; ATSDR 2000). Vanadium was also up to 5 times higher, especially at the Trebež village, than at any other location of the investigated rivers in Croatia (Tables 2 and 3). Vanadium origin is possibly from the phosphogypsum (Durgo et al. 2008) as a waste by-product from the processing of phosphate rock in the production of the phosphoric acid and phosphate fertilizers.

To put our results within a broader perspective, dissolved metal concentrations in water of the Ilova River were further compared with several European rivers (Table 3). The comparison showed that water quality of the Ilova River was still better than of the other investigated rivers, such as the Odra River, where high metal concentrations were mostly the result of the intensive agricultural and industrial activities (petrochemicals, petroleum refining, steel works foundries and non-ferrous metal works) (Tables 2 and 3; Adamiec and Helios-Rybicka 2002). In comparison to the Ilova River, urban/industrial Aire, Wear and Vistula rivers and agriculturally impacted Great Ouse River revealed higher dissolved concentrations of most metals. Metal concentrations from the Ilova River were mostly comparable with the Guadalquivir River in Spain, which was partially impacted by agricultural activities (Mendiguchía et al. 2007). Relatively high concentrations of Co, Ni and Mn recorded in the Ilova River corresponded to the records from the river ecosystems affected by sewage effluents (Tables 2 and 3; Karvelas et al. 2003; Mendiguchía et al. 2007).

Finally, the comparison with European regulations revealed that the concentrations of dissolved Cd, Cu, Ni and Zn were considerably lower than acceptable annual average (AA) values defined by EQS or suggested for priority substances in the inland surface water even at the contaminated site of the Ilova River near the Trebež village (Tables 2 and 3; Crane et al. 2007; EPCEU 2008). On the other hand, Fe concentrations at all sites of the Ilova River (Table 2) were slightly above suggested threshold for this metal, but the proposed value of 16 $\mu g\ L^{-1}$ is considered as very strict and is actually not accepted as recommendable (Table 3; Crane et al. 2007).

It can be concluded that water quality of the Ilova River was still mostly good along its watercourse, and that the impact of fish farms, wastewaters of the Town of Kutina, and nearby factory is moderate. Nevertheless, elements as Al, As, Cd, Cs, Ni, Rb and V should be regularly monitored due to their high toxicity and/or several times higher concentrations observed at the downstream site of the Trebež village in comparison to the less contaminated upstream sites. This undoubtedly indicated the negative influence of the industrial activity on the downstream reaches of the Ilova River, which partly belong to the protected area of the Lonjsko polje Nature Park.

Characteristics of sediments and concentrations and distribution of trace and macroelements

As sediments act like the main sinks for metals in aquatic ecosystem, further evaluation of environmental quality of this region included granulometric description and determination of metal concentration and TOC in sediment samples.

Grain-size distribution of sediments showed several differences between the investigated sites of the Ilova River. In the sediment samples of the uppermost site near the Maslenjača village, sand fraction prevailed (62.2%), and it was followed by silt (34.4%) and minimum contribution of clay (3.4%) (Fig. 2). The mean particle size (Mz) of the sample was 189.6 μm. At the Ilova village, dominant fraction was silt (61.8%) followed by sand (30.9%) and clay (7.3%) (Fig. 2) and Mz was 72.2 μm. Similar patterns as for the sediments from the Ilova village were observed at the Trebež village, but in Trebež, silt was even more prevailing (75.7%), proportion of clay increased to 10.0% and the proportion of sand decreased to 14.3% (Fig. 2). Mz was also much smaller with the average value of 35.5 μm. Therefore, the upper river part (Maslenjača) had 2 to 6 times higher proportions of coarser sediments (sand) compared to the downstream sites (Ilova and Trebež). The water flow rate decreases in downstream way, losing the transmission strength and carrying only the lighter and smaller sediments such as silt and clay that have a higher degree of porosity and permeability which makes them easier to transport (Özkan 2012). Lower water flow enables enough time for sediment suspended in water column to sink and deposit at the riverbed.

Particle size is also one of the dominant factors that could affect TOC content in surface sediments of the Ilova River (Sany et al. 2013). Since smaller particles have larger surface area to volume ratio, they have a larger binding capacity for the adsorption of organic carbon. Further, these organic matter coatings are common in fine sediments, and they bind a variety of trace elements (Nowrouzi and Pourkhabbaz 2014). The highest TOC content in our research was confirmed at the Trebež village, where the smallest grain size and the average TOC value of 2.5% were observed. However, sediments from the Ilova village also contained mostly fine particles, but the average TOC content was only 0.8%. It could suggest the increased input of wastewaters that causes the elevation of TOC (Ouyang et al. 2006) in the sediments sampled near the Trebež village. In Maslenjača, average TOC value was 0.4%. TOC is one of the factors that affects and regulates the presence and behaviour of other chemical components, such as metals, in sediments (Zhang et al. 2001; Lazăr et al. 2012). Consequently, there will be a tendency of higher metal accumulation at sites with fine-grained sediments and increased TOC concentration. It is known that silt and clay fractions have particularly important influence on the transport and storage of heavy metals within fluvial sediments (Saeedi et al. 2013; Zhang et al. 2014).

Considering trace and macroelements levels, statistical analyses showed that almost all elements were present in the highest concentrations in sediments from the Trebež village (Table 4). Most pronounced difference between the Trebež and Maslenjača villages was recorded for Cd with 25 times higher concentration at the Trebež village. This downstream location, which is most extensively impacted by anthropogenic activities, also had 4.6 times higher average levels of Cu and Zn, 2-4 times higher of As, Bi, Co, Cr, Cs, Fe, Mg, Mo, Mn, Ni, Pb, U and V. As already mentioned, Cu, Zn, Cr, Fe and V are among elements characteristic for the phosphoric gypsum depot near the fertilizer factory (Durgo et al. 2008). Other elements (except K) had up to 2 times higher concentration in comparison to the sediments from Maslenjača (Table 4). Comparison of sediments from the Ilova and Trebež villages showed slightly smaller differences, but average level of Cd was about 20 times higher at the Trebež village. Bismuth, Cu, Pb, U and Zn cations had 2-3 times higher average levels at the same site (Table 4). Other elements were either up to 2 times higher at the Trebež village or comparable between the sites (Table 4). Again, sediment enrichment with Cu, Zn, Cr, Fe and V was noticed, suggesting the fertilizer factory as the primary pollution source of these elements in the Trebež village compared to the upstream locations. Considering the sediment samples from Maslenjača and Ilova villages, the most pronounced difference in average levels was observed for Cu and Mn, but Co, Cr, Cs, Fe, Mg, Ni, Sb and V were also significantly elevated at the Ilova village. Opposite trend was observed for K, Na and Sr with higher average levels in the sediment samples from Maslenjača (Table 4). The range of trace and macroelements concentrations in the sediment samples from Maslenjača and Ilova villages were similar (i.e., Al>Fe≥K>Na≥Mg>Mn≥Ba>Sr>Rb>V>Zn>Cr>Ni≥Pb>Cu≥Co>As>Cs>U>Sb>Tl>Mo>Bi>Cd), whereas some more significant differences appeared at the Trebež village with the order: Al>Fe>K>Mg>Na>Mn>Ba>Zn>Sr>V>Rb>Cr>Pb>Ni>Cu>Co>As>Cs>U>Cd>Sb>Tl>Mo>Bi (Table 4).

Furthermore, in order to evaluate the quality of sediments in the Ilova River, average elements concentration of all sites were compared with the globally accepted sediment quality guidelines (SQGs) provided by Canadian Council of Ministers of the Environment (CCME 2002) and study by Long et al. (1995). Regarding CCME recommendations available for several metals, none of them was higher than interim freshwater sediment quality guidelines (ISQG) in Maslenjača (Table 4). Average concentrations of As and Cr were higher than ISQG in the Ilova village, and of As, Cd, Cr, Pb and Zn in the Trebež village (Table 4). As for the probable effects level (PEL), Cd and Cr showed higher values in the Trebež village, while none of the metals exceeded this level at other two locations (Table 4). Arsenic is broadly used as sodium arsenite to control submerged aquatic vegetation in freshwater ponds and lakes (Roy et al. 2006). High Cr concentrations even in the Ilova village could be explained by the presence of fish ponds and farms, because it has been found that poultry and tannery wastes could be added as supplements in the fish feed, whereas $K_2Cr_2O_7$ has an important role in cleaning (Sarker et al. 2016). This trend was not detected in water samples, but suggested the influence of pellet feed that was not eaten by fish and therefore had enough time to settle in sediments.

According to the study of Long et al. (1995), who defined effects range low (ERL) and effects range median (ERM) values for As, Cd, Cr, Cu, Pb, Hg, Ni, Ag and Zn, it can be observed that average levels of As and Ni in the Ilova village, and As, Cd, Cr, Ni and Zn in the Trebež village were higher than ERL, but there were no concentrations higher than ERM criterion (Table 4). Therefore, based on sediment quality according to the SQGs, the Ilova River would mostly be classified as a river with free to moderate pollution impact, with only few concerning elements such as Cd, Cr and Ni being higher than PEL or close to ERM values.

Contamination factor, pollution load index and enrichment factor analysis

In order to gain a deeper insight in the sediment quality and possible contamination with metals, other approaches for environmental and pollution assessment should be considered besides SQGs. Therefore, contamination factors (CFs), pollution load index (PLI) and enrichment factors (EFs) were calculated to estimate and identify anomalous metal concentrations of natural origin or more commonly, to assess the anthropogenic influence on metal levels (Meybeck et al. 2007; Chen et al. 2014; Lučić et al. 2019).

CFs were calculated as:

$$\mathrm{CF(M)} = \frac{c(\mathrm{M})_{\mathrm{sample}}}{c(\mathrm{M})_{\mathrm{control}}}$$

where CF(M) is a contamination factor of the selected metal, $c(\mathrm{M})_{\mathrm{sample}}$ is its concentration in the tested sample and $c(\mathrm{M})_{\mathrm{control}}$ is its concentration in the control sample (Hakanson 1980).

Metal concentrations in sediments from Maslenjača were used as control values since this sampling site was identified as a reference site in the current investigation and because other studies covering this region are limited. Obtained values are shown in Table 5. Out of four distinguished categories for CF values, only CF<1 is considered as low contamination. In the Ilova village, only CFs of Ba, Sr, K and Na were below 1 and in the Trebež village only CFs of K and Na were lower than 1. All other elements from the Ilova village and most elements from the Trebež village were categorized in the category 1≤CF<3, which is considered as moderate degree of contamination. The highest values in the Ilova village were observed for Cu, As and Mn (Table 5). In the Trebež village, CFs of Bi, Cu, Mo, Ni, Zn and Mg were in range 3≤CF<6 which represents considerable contamination, whereas Cd was even highly above the fourth category of CF≥6 representing high contamination degree (Table 5). These values were used to assess the overall PLI of the two investigated sites from the following equation:

$$\mathrm{PLI} = (\mathrm{CF}_1 \times \mathrm{CF}_2 \times \mathrm{CF}_3 \times \ldots \times \mathrm{CF}_\mathrm{N})^{1/\mathrm{N}}$$

where CF is the contamination factor of certain element and N is the number of studied elements.

There are three described categories of PLI: PLI<1 implying unpolluted sediment, PLI =1 showing the presence of only baseline levels of pollutants and PLI>1 indicating deterioration and pollution of the site (Tomlinson 1980). Calculated values at both sites were higher than 1; 1.4 and 2.4 in Ilova and Trebež villages, respectively, showing some anthropogenic pressure in both sites, but considerably higher in the Trebež village, as a most downstream location affected by all pollution sources (Table 5).

To calculate EFs, geochemical normalization of data to conservative element (Al) was applied in our research. Normalization is used to detect and quantify any anthropogenic metal contributions. Therefore, different conservative reference elements or other conservative components, such as Al, Fe, Sc, Ni, Ti, Zr, Li, TOC or granulometric composition, which are not significantly affected by contaminants, are used (Förstner and Wittmann 1981; Loring 1991). EFs were calculated according to the equation:

$$\mathrm{EF(M)} = \frac{(\mathrm{M/Al})_{\mathrm{sample}}}{(\mathrm{M/Al})_{\mathrm{control}}}$$

where EF(M) is an enrichment factor of the selected element, $(\mathrm{M/Al})_{\mathrm{sample}}$ is selected element concentration normalized to Al concentration in the tested sample and $(\mathrm{M/Al})_{\mathrm{control}}$ is its concentration in the control sample.

In our study we used $(\mathrm{M/Al})_{\mathrm{control}}$ values from sediment of Maslenjača. Values of calculated EFs are presented in Table 5. The consensus is that a value 0.5≤EF≤1.5 means that trace metals are originating from crustal contribution, whereas values higher than 1.5 indicate that an important part of metals are provided from non-crustal materials, such as contamination or biota (Zhang and Liu 2002). Sutherland (2000) recognized five contamination categories based on the EF values: EF<2 (no or minimal enrichment and contamination), EF=2–5 (moderate enrichment and contamination), EF=5–20 (significant enrichment and contamination), EF=20–40 (very high enrichment and contamination) and EF>40 (extremely high enrichment and contamination). Considering EFs calculated for the sediments from the Ilova village, only Cu and Mn can be considered as elements of moderate enrichment (Table 5). However, EFs of As, Cr, Fe, Mg, Mo, Ni, V and Zn were greater than 1.5 indicating that there should be another source besides only natural input, which could possibly be attributed to some anthropogenic activities (Zhang and Liu 2002). In Trebež, EF for Cd was significantly higher than any other element and represented the significant enrichment and possible contamination with Cd. Additionally, moderate enrichment and contamination with As, Bi, Cr, Cu, Mg, Mo, Ni, Pb, U and Zn was observed with EFs in descending order: Zn=Cu>Mg>Ni>Mo=Bi>Cr=As>Pb=U (Table 5). Values of Co, Cs, Fe, Mn and V were also higher than 1.5.

Therefore, all parameters of the sediment metal pollution assessment indicated the highest anthropogenic impact near the Trebež village. According to the CFs, most elements appeared concerning at that site, but the results also indicated that the calculation of EFs is more reliable, due to the normalization procedure, which enables division of natural and anthropogenic variations in metal concentrations. Hence, it can be concluded that the sediments of the downstream Ilova River sites were primarily enriched with As, Bi, Cd, Cr, Cu, Mn, Mo, Ni, Pb, U, Zn and Mg in comparison to the Maslenjača reference site. As fertilizers are one of the major sources of trace metals such as As, Cd, Cr, Mn, Ni, Pb and Zn (Thomas et al. 2012), it is likely that their increased values pointed to the influence of the nearby fertilizer factory. Kassir et al. (2012) investigated the effects of fertilizer industry emissions on soil contamination and noticed considerably high levels of Cd and Zn, and elevated levels of Sr, Cr, Ni, U, and Cd, which are almost the same elements as in our research. Very high concentrations and enrichment with Cd in the Trebež village can be to some extent linked with higher TOC content as well, as Cd shows preferential association with organic matter (Dong et al. 2007). Sediment collected from the Ilova village was of higher quality than from the Trebež village, but it also indicated some less pronounced environmental disturbances compared to Maslenjača, which could be connected with the intensity of aquaculture activities in that region (fish farms Poljana and Končanica, Fig. 1). One of the possible issues considering fish farms are feed products that often contain variety of trace metals in different concentrations and chemical forms including Zn, Cu, Cd, Fe, Mn, Co, Ni,Pb, Mg, Se and Hg (Dean et al. 2007), possibly serving as one of the metal sources for the contamination of water and sediments. Other than that, fertilizers, algicides and some other substances that are used in pond management also contain variety of trace metals.

However, as in environmental studies there is always a mixture of contaminants it is hard to detect the exact source for each element. Our study suggests that enrichment, especially of sediments, with Cu, Zn, Cr, Fe and V is probably due to phosphoric gypsum transport water from depot near the fertilizer factory. Furthermore, metals such as Fe, Co, Mn, Cu, and Zn are also often applied as nutritional additives in animal feed for livestock farming and fish production in Europe (Commission Regulation 1831/2003/EC 2003; Seiler and Berendonk 2012). Therefore, this anthropogenic source could have the influence on higher concentrations and observed moderate enrichment with Cu and Mn already at the Ilova village. Extensive agricultural activity also contributes to the elevated Mn and As concentrations in two more downstream locations compared to the Maslenjača village. Trebež village, as the most downstream location, is affected by several anthropogenic contamination sources, and in this case it is difficult to distinguish the impact of exact specific contamination sources.

Conclusions

Evaluated physico-chemical parameters, dissolved trace and macro elementsconcentrations in water, as well as their concentrations in sediments pointed to relatively pristine upstream watercourse of the Ilova River, in contrast to more disturbed conditions in the downstream part affected by anthropogenic activities, mostly aquaculture (fish ponds) and agriculture (farms) in the Ilova village, and industrial activity (fertilizer production) in the Trebež village. Physico-chemical parameters including COD, nitrates and phosphates indicated disturbed environmental conditions in the Trebež village, probably due to the influence of municipal and industrial wastewaters, while water samples from Maslenjača and Ilova villages were of good quality. Majority of measured elements were significantly elevated in water of the Trebež village with Al, As, Cd and Ni as the most concerning elements due to the several times higher concentrations in comparison to the upstream sites.

Sediment samples followed the same pattern as water samples. All parameters linked to the sediment quality assessment indicated deteriorated environmental status in the Trebež village. Cadmium and Cr were among most concerning elements, as their levels in Trebež were above probable effects levels (CCME 2002). CFs of Bi, Cu, Mo, Ni and Zn implied considerable sediment contamination, whereas CF of Cd represented high sediment contamination in Trebež, and it likely contributed to the considerably higher pollution load index at this site in comparison to the Ilova village. EFs were shown to be more reliable, showing at least moderate enrichment with most elements in the Trebež village, and moderate enrichment only with Cu and Mn in the Ilova village. Sediments from Trebež were significantly enriched with Cd, whose EF was almost above the threshold of very high enrichment.

The anthropogenic impact in the Ilova River is mostly still moderate, but the observed negative trends in the downstream part pointed to potential risks for the Ilova River in the future, especially for the protected area of the Lonjsko Polje Nature Park. Therefore, regular and continuous monitoring of this area would be needed for an effective protection. Furthermore, our study confirmed the need of complementary approach in the metal contamination assessment that involves both water and sediment standard criteria, contamination and enrichment factors which should all provide more accurate and reliable estimation of fate and transport of metals from different anthropogenic sources and their impact on the studied ecosystem.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The financial support by the Croatian Science Foundation, within the project "Accumulation, subcellular mapping and effects of trace metals in aquatic organisms" AQUAMAPMET (IP-2014-09-4255) is gratefully acknowledged. Authors are also grateful for the help in the field work to the members of the Laboratory for Aquaculture and Pathology of Aquatic Organisms from the Ruđer Bošković Institute (RBI) and to Dr. Nevenka Mikac from the Laboratory for inorganic environmental geochemistry and chemodynamics of nanoparticles at RBI for the opportunity to use HR ICP-MS.

References

Adamiec E, Helios-Rybicka E (2002) Distribution of pollutants in the Odra River system Part IV. Heavy metal distribution in water of the upper and middle Odra River, 1998–2000. Pol J Environ Stud 11: 669-673

Alvarez-Guerra M, Viguri JR, Casado-Martínez MC, DelValls TÁ (2007) Sediment quality assessment and dredged material management in Spain: Part I. Application of sediment quality guidelines in the Bay of Santander. Integr Environ Assess Manage 3: 529–538

APHA (1985) Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, Washington

ATSDR (2000) Toxicological profile for manganese. Atlanta, GA, United States Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Burton Jr GA (2002) Sediment quality criteria in use around the world. Limnology 3: 65–76

Campbell PGC, Chapman PM, Hale BA (2006) Risk assessment of metals in the environment. In: Hester RE, Harrison RM (eds.) Chemicals in the Environment: Assessing and Managing Risk. RSC Publishing, pp 102–131

Canadian Council of Ministers of the Environment (CCME) (2002) Canadian Sediment Quality Guidelines for the Protection of Aquatic Life

Carbonell-Barrachina A, DeLaune RD, Jugsujinda A (2002) Phosphogypsum chemistry under highly anoxic conditions. Waste Mgmt 22: 657–665.

Chen JB, Gaillardet J, Bouchez J, Louvat P, Wang YN (2014) Anthropophile elements in river sediments: overview from the Seine River, France. Geochem Geophys Geosyst 15: 4526–4546

Chon H-S, Ohandja D-G, Voulvoulis N (2012) The role of sediments as a source of metals in river catchments. Chemosphere 88: 1250-1256

Commission Regulation 1831/2003/ EC (2003). Regulation (EC) No1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Of J Eur Union L268: 29–43

Crane M, Kwok K, Wells C, Whitehouse P, Lui G (2007) Use of field data to support European Water Framework Directive quality standards for dissolved metals. Environ Sci Technol 41: 5014-5021

Dean RJ, Shimmield TM, Black KD (2007) Copper, zinc and cadmium in marine cage fish farm sediments: an extensive survey. Environ Pollut 145: 84–95

Dautović J (2006) Determination of metals in natural waters using high resolution inductively coupled plasma mass spectrometry. Bachelor of Science Thesis. University of Zagreb.

Deutsches Institut für Normung (1986) Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung, 16th ed. Vol II Verlag Chemie, Weinheim

Dong D, Liu L, Hua X, Lu Y (2007) Comparison of lead, cadmium, copper and cobalt adsorption onto metal oxides and organic materials in natural surface coatings. Microchem J 85(2): 270-275

Dragun Z, Roje, V, Mikac N, Raspor B (2009) Preliminary assessment of total dissolved trace metal concentrations in Sava River water. Environ Monit Assess 159: 99–110

Dragun Z, Kapetanović D, Raspor B, Teskeredžić E (2011) Water quality of medium size watercourse under baseflow conditions: the case study of river Sutla in Croatia. Ambio 40(4): 391–407

Durgo K, Oreščanin V, Lulić S, Kopjar N, Želježić D, Franekić Čolić J (2009) The assessment of genotoxic effects of wastewater from a fertilizer factory. J Appl Toxicol 29: 42-51

European Environment Agency (EEA) (2009) Ecoregions for rivers and lakes. Available as a website at: https://www.eea.europa.eu/data-and-maps/figures/ecoregions-for-rivers-and-lakes

European Parliament and the Council of the European Union (EPCEU). Directive 2008/105/EC of the European Parliament and of the Council on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280 EEC, and amending Directive 2000/60/EC of the European Parliament and of the Council. Official J (2008) L 348/84

Fiket Ž, Mikac N, Kniewald G (2017) Mass fractions of forty-six major and trace elements, including rare earth elements, in sediment and soil reference materials used in environmental studies. Geostand Geoanal Res 41: 123–135

Filipović Marijić V, Sertić Perić M, Matoničkin Kepčija R, Dragun Z, Kovarik I, Gulin V, Erk M (2016) Assessment of metal exposure, ecological status and required water quality monitoring strategies in small- to medium-size temperate rivers. J Environ Sci Heal A 51(4): 309–317

Förstner U, Wittmann GTW (1981) Metal Pollution in the Aquatic Environment. Springer-Verlag, Berlin, Heidelberg, New York, 486

Govorushko S (2016) Human Impact on the Environment: an Illustrated World Atlas. Springer International Publishing, Switzerland.

GRC (Government of the Republic of Croatia) (2013) Directive on Water Classification. Official Gazette No. 73. Narodne novine, Zagreb

GRC (Government of the Republic of Croatia) (2019) Directive on Water Quality Standard. Official Gazette No. 96. Narodne novine, Zagreb.

Guéguen C, Dominik J (2003) Partitioning of trace metals between particulate, colloidal and truly dissolved fractions in a polluted river: the Upper Vistula River (Poland). Appl Geochem 18: 457-470

Hakanson L (1980) An ecological risk index for aquatic pollution control. A sedimentological approach. Water Res 14: 975–100

Illies J (1978) Limnofauna Europaea. A checklist of the animals inhabiting European inland waters, with an account of their distribution and ecology, 2nd Edition. Gustav Fischer Verlag, Stuttgart

IPCS (1999) Manganese and its compounds. Geneva, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document 12)

Karvelas M, Katsoyiannis A, Samara C (2003) Occurrence and fate of heavy metals in the wastewater treatment process. Chemosphere 53: 1201–1210

Kassir LN, Lartiges B, Ouaini N (2012) Effects of fertilizer industry emissions on local soil contamination: a case study of a phosphate plant on the east Mediterranean coast. Environ Technol 33: 873-885

Lazăr L, Gomoiu MT, Boicenco L, Vasili D (2012) Total organic carbon (TOC) of the surface layer sediments covering the seafloor of the Romanian black sea. Geo-Eco-Marina 18: 121 – 132

Long ER, MacDonald DD, Smith SL, Calder FD (1995) Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ Manage 19: 81–97

Loring DH (1991) Normalization of heavy-metal data from estuarine and coastal sediments. ICES J Mar Sci 48: 101-115

Loring DH, Rantala RTT (1992) Manual for the geochemical analyses of marine sediments and suspended particulate matter. Earth-Sci Rev 32(4): 235-283

Lučić M, Jurina I, Ščančar J, Mikac N, Vdović N (2019) Sedimentological and geochemical characterization of river suspended particulate matter (SPM) sampled by time-integrated mass flux sampler (TIMS) in the Sava River (Croatia). J Soils Sediments 19**:** 989–1004

Mc Cave IN (1984) Size spectra and aggregation of suspended particles in the deep ocean. Deep-Sea Res 31: 329–352

Mendiguchía C, Moreno C, Garcıía-Vargas M (2007) Evaluation of natural and anthropogenic influences on the Guadalquivir River (Spain) by dissolved heavy metals and nutrients. Chemosphere 69: 1509-1517

Meybeck M, Lestel L, Bonte P, Moilleron R, Colin JL, Rousselot O, Herve D, de Pontevès C, Grosbois C, Thevenot DR (2007) Historical perspective of heavy metals contamination (Cd, Cr, Cu, Hg, Pb, Zn) in the Seine River basin (France) following a DPSIR approach (1950–2005). Sci Total Environ 375: 204–231

Neal C, Robson AJ (2000) A summary of river water quality data collected within the Land Ocean Interaction Study: core data for Eastern UK rivers draining to the North Sea. Sci Total Environ 251/ 252: 587– 668

Nowrouzi M, Pourkhabbaz A (2014) Application of geoaccumulation index and enrichment factor for assessing metal contamination in the sediments of Hara Biosphere Reserve, Iran. Chem Spec Bioavailab 26: 99-105

Official Gazette No. 40 (1999) Regulation on limit values of parameters of hazardous and other substances in wastewater. Narodne novine, Zagreb

Ouyang Y, Zhang JE, Ou L-T (2006) Temporal and spatial distributions of sediment total organic carbon in an estuary river. J Environ Qual 35:93-100

Özkan EY (2012) A new assessment of heavy metal contaminations in an eutrophicated bay (Inner Izmir Bay, Turkey). Turk J Fish AquatSc12: 135-147

Plantak M, Čanjevac I, Vidaković I (2016) Morphological state of rivers in the Ilova River catchment. Croatian Geographical Bulletin 78(1): 5-24

Radić S, Gregorović G, Stipaničev D, Cvjetko P, Šrut M, Vujčić V, Oreščanin V, Klobučar GIV (2013) Assessment of surface water in the vicinity of fertilizer factory using fishand plants. Ecotoxicol Environ Saf 96: 32-40

Roy S, Chattoraj A, Bhattacharya S (2006) Arsenic induced changes in optic tectal histoarchitecture and acetyleholinesterase-acetylcholine profile in *Channa punctatus*: amelioration by selenium. Comp Biochem Physiol 144C: 16-24

Saeedi M, Li LY, Karbassi AR, Zanjani AJ (2013) Sorbed metals fractionation and risk assessment of release in river sediment and particulate matter. Environ Monit Assess 185: 1737-1754

Sany SB, Salleh A, Rezayi M, Saadati N, Narimany L,Tehrani GM (2013) Distribution and contamination of heavy metal in the coastal sediments of Port Klang, Selangor, Malaysia. Water Air Soil Poll 224(4): 1–18

Sarker J, Kanungo I, Tanmay MH, Patwary SA (2016) A study on the determination of heavy metals in sediment of fish farms in Bangladesh. Fish Aquac J 7: 159-163

Seiler C, Berendonk TU (2012) Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front Microbiol 3: 399-408.

Sutherland RA (2000) Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. Environ Geol 39: 611–627

Thomas EY, Omueti JAI, Ogundayomi O (2012) The effect of phosphate fertilizer on heavy metal in soils and *Amaranthuscaudatus*. Agriculture and Biology Journal of North America 3: 145–149

Tomlinson D, Wilson J, Harris C, Jeffrey D (1980) Problems in the assessment of heavy metal levels in estuaries and the formation of a pollution index. Helgoländer Meeresun 33(1-4): 566-575

Water Framework Directive (WFD) - River Basin Management Plans (2016) Reporting Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy as amended by Decision 2455/2001/EC and Directives 2008/32/EC, 2008/105/EC and 2009/31/EC

Wentworth CK (1922) A scale of grade and class terms for clastic sediments. J Geol 30: 377-392

Zhang W, Yu L, Hutchinson SM, Xu S, Chen Z, Gao X (2001) China's Yangtze Estuary: I. Geomorphic influence on heavy metal accumulation in intertidal sediments. Geomorphology 41: 195–205

Zhang J, Liu CL (2002) Riverine composition and estuarine geochemistry of particulate metals in China—weathering features, anthropogenic impact and chemical fluxes. Estuar Coast Shelf Sci 54: 1051–1070

Zhang W, Feng H, Chang J, Qu J, Xie H, Yu L (2009) Heavy metal contamination in surface sediments of Yangtze River intertidal zone: an assessment from different indices. Environ Pollut 157: 1533-1543

Zhang C, Yu ZG, Zeng GM, Jiang M, Yang ZZ, Cui F, Zhu MY, Shen LQ, Hu L (2014) Effects of sediment geochemical properties on heavy metal bioavailability. Environ Int 73: 270-281

https://hidro.dhz.hr/ Croatian Meteorological and Hydrological Service, Hydrological Sector

http://www.pp-lonjsko-polje.hr/new/english/index.html Official web-site of the Lonjsko polje Nature Park

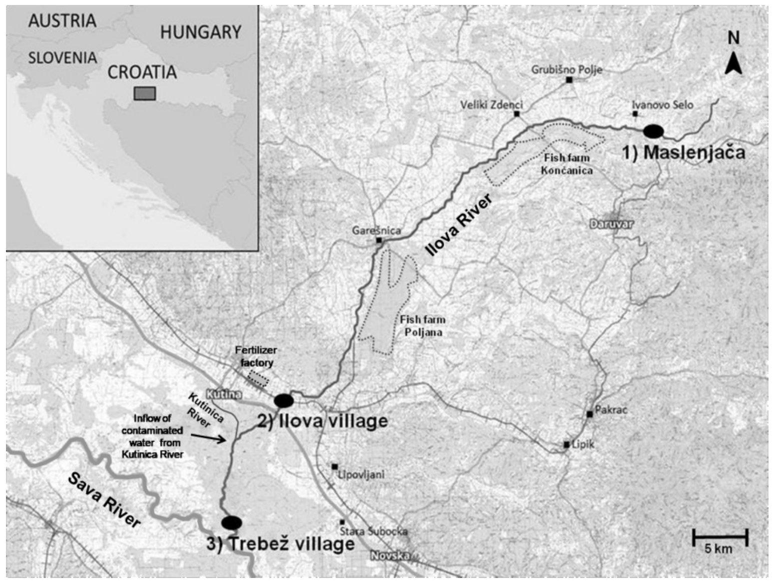

Fig. 1 Map of Croatia with enlargement of the Ilova River watercourse with sampling sites (1 - Maslenjača village, 2 – Ilova village and 3 – Trebež village), freshwater fish farms, fertilizer factory and location of Town of Kutina and Kutinica River

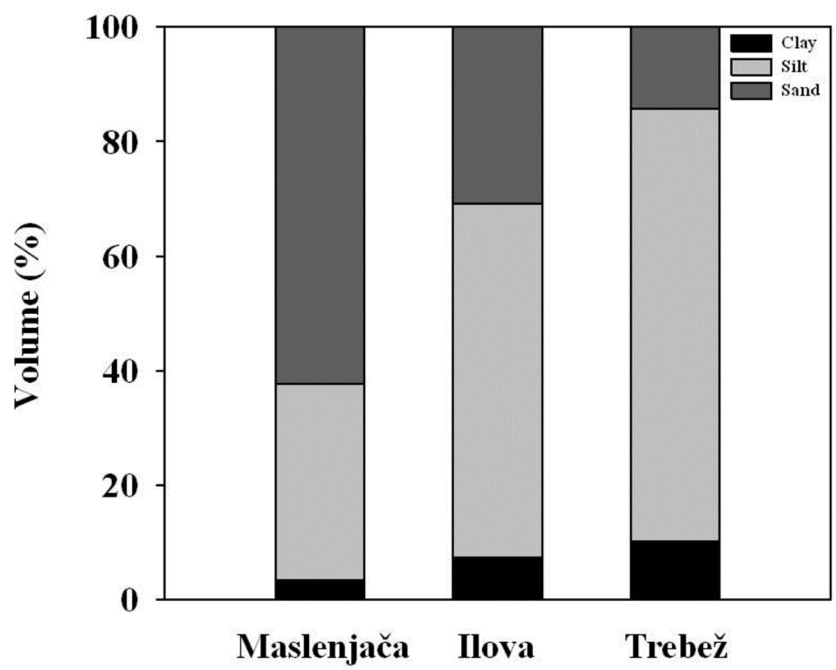

Fig. 2 An average percentage of different fractions (clay, silt and sand) in the sediments of the three investigated sites of the Ilova River

Table 1 Physico-chemical parameters of the Ilova River water sampled at three sampling sites (Maslenjača, Ilova and Trebež villages) in two sampling campaigns (autumn 2017 and spring 2018) and limiting values according to Croatian Directive on Water Quality Standards (GRC 2019). Indicated values (underlined and bolded) are those below good water quality status according to classification in Croatian Directive on Water Quality Standards (GRC 2019).

	Limiting values for type HR-R_4		**Maslenjača village**		**Ilova village**		**Trebež village**	
	very good	**good**	**Autumn 2017**	**Spring 2018**	**Autumn 2017**	**Spring 2018**	**Autumn 2017**	**Spring 2018**
T (°C)			8.1	16.8	14.8	20.3	15.8	20.7
DO (mg O_2 L^{-1})			9.95	7.96	9.98	5.78	5.32	5.53
% O_2			85.5	83.3	101	64.9	54.1	62.7
pH	7.4-8.5	7.0-7.4 8.5-9.0	8.0	8.1	7.7	7.5	7.4	7.4
ORP (mV)			-46.2	-53.2	-27.8	n.m.	-14.8	n.m.
Cond (µS cm^{-1})			328	368	354	411	528	456
TDS (mg L^{-1})			164	184	177	205	262	228
COD $_{KMnO4}$ (mg O_2 L^{-1})	1.8	5.5	3.8	4.2	**<u>5.7</u>**	5.4	**<u>6.5</u>**	**<u>6.3</u>**
Alk (mg $CaCO_3$ L^{-1})			125	195	125	170	205	150
TWH (mg $CaCO_3$ L^{-1})			137	217	134	173	217	185
N-NO_2^- (mg N L^{-1})			0.014	0.090	0.033	0.197	1040	0.721
N-NO_3^- (mg N L^{-1})	0.7	1.3	0.79	**<u>1.44</u>**	0.48	0.83	**<u>4.11</u>**	**<u>4.18</u>**
P-PO_4^{3-} (mg P L^{-1})	0.03	0.1	0.054	0.047	0.064	0.080	**<u>938</u>**	0.072

n.m. - not measured

Table 2 Dissolved trace and macro element concentrations in the water (μgL^{-1} or mg L^{-1}; mean ± S.D.; < LOD values) of the Ilova River at three sampling sites (Maslenjača, Ilova and Trebež villages) from two sampling campaigns (autumn 2017 and spring 2018). Statistically significant differences (ANOVA) at p<0.05 level between three sampling sites within the autumn season are assigned with superscript letters A, B and C, and in spring with a, b and c,while significant differences (t-test) between two seasons at each sampling site are marked with asterisk (*)

	Maslenjača village		**Ilova village**		**Trebež village**	
	Autumn 2017	**Spring 2018**	**Autumn 2017**	**Spring 2018**	**Autumn 2017**	**Spring 2018**
Al (μg L^{-1})	1.4±0.1$^{*, A, C}$	5.9±1.0$^{*, a, c}$	0.5±0.3$^{*, B, C}$	2.6±0.4$^{*, c}$	6.8±0.6$^{*, A, B}$	3.7±0.3$^{*, a}$
As (μg L^{-1})	1.39±0.04$^{*, A}$	2.26±0.06$^{*, a, c}$	2.10±0.13$^{*, B}$	2.72±0.10$^{*, b, c}$	4.5±0.7$^{*, A, B}$	4.87±0.04$^{*, a, b}$
Ba (μg L^{-1})	34.6±1.0^{A}	34.8±0.3$^{a, c}$	36.3±0.4$^{*, B}$	39.3±0.4$^{*, b, c}$	29.1±1.7$^{A, B}$	26.4±0.1$^{a, b}$
Bi (μg L^{-1})	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cd (μg L^{-1})	0.002±0.001$^{*, A, C}$	0.005±0.001$^{*, a}$	0.011±0.006$^{B, C}$	0.006±0.002^{b}	0.053±0.003$^{*, A, B}$	0.035±0.002$^{*, a, b}$
Co (μg L^{-1})	0.110±0.002$^{*, C}$	0.176±0.006$^{*, a, c}$	0.137±0.005$^{*, B, C}$	0.320±0.006$^{*, b, c}$	0.121±0.011$^{*, B}$	0.338±0.009$^{*, a, b}$
Cr (μg L^{-1})	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06
Cs (μg L^{-1})	<0.001^{A}	0.001±0.000^{a}	<0.001^{B}	0.001±0.001^{b}	0.090±0.007$^{*, A, B}$	0.009±0.000$^{*, a, b}$
Cu (μg L^{-1})	0.9±0.5	0.9±0.4	0.40±0.03*,	0.69±0.08*	0.72±0.03	0.68±0.07
Fe (μg L^{-1})	39.7±0.7$^{*, A, C}$	16.4±1.0*	17.9±2.2$^{B, C}$	21.8±2.0	21.6±1.5$^{A, B}$	20.8±5.9
Mn (μg L^{-1})	83.1±1.1$^{A, C}$	121±1$^{a, c}$	93.2±1.1$^{*, B, C}$	317±2$^{*, b, c}$	18.4±0.9$^{*, A, B}$	340±3$^{*, a, b}$
Mo (μg L^{-1})	0.6±0.2^{A}	0.57±0.01^{a}	0.56±0.03$^{*, B}$	0.61±0.01$^{*, b}$	0.98±0.06$^{*, A, B}$	0.72±0.04$^{*, a, b}$
Ni (μg L^{-1})	0.59±0.01$^{*, A}$	0.74±0.02$^{*, a, c}$	0.57±0.02$^{*, B}$	0.94±0.02$^{*, b, c}$	1.80±0.08$^{*, A, B}$	1.63±0.01$^{*, a, b}$
Pb (μg L^{-1})	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07
Rb (μg L^{-1})	0.559±0.007$^{*, A}$	1.27±0.01$^{*, a, c}$	0.644±0.008$^{*, B}$	1.21±0.01$^{*, b, c}$	3.74±0.25$^{*, A, B}$	1.88±0.02$^{*, a, b}$
Sb (μg L^{-1})	0.107±0.002$^{*, A, C}$	0.114±0.001$^{*, a, c}$	0.245±0.006$^{*, B, C}$	0.167±0.003$^{*, b, c}$	0.301±0.017$^{*, A, B}$	0.228±0.002$^{*, a, b}$
Se (μg L^{-1})	0.24±0.03$^{A, C}$	0.203±0.014$^{a, c}$	0.79±0.02$^{*, B, C}$	0.596±0.016$^{*, b, c}$	1.01±0.11$^{*, A, B}$	0.485±0.015$^{*, a, b}$
Sr (μg L^{-1})	171±2$^{*, A, C}$	181±2$^{*, a, c}$	123±1$^{*, B, C}$	154±2$^{*,b, c}$	150±12$^{*, A, B}$	173±1$^{*, a, b}$
Tl (μg L^{-1})	0.002±0.000$^{A, C}$	0.007±0.001^{a}	0.008±0.000$^{B, C}$	0.008±0.001	0.013±0.002$^{*, A, B}$	0.009±0.001$^{*, a}$
U (μg L^{-1})	0.896±0.006^{A}	0.897±0.006$^{a, c}$	0.884±0.001$^{*, B}$	0.695±0.014$^{*, b, c}$	1.57±0.11$^{*, A, B}$	0.923±0.007$^{*, a, b}$

V (μg L^{-1})	0.59±0.01$^{*, A}$	0.98±0.02$^{*, a, c}$	0.35±0.01$^{*, B}$	1.38±0.01$^{*, b, c}$	4.1±0.3$^{*, A, B}$	3.63±0.03$^{*, a, b}$
Zn (μg L^{-1})	< 7	< 7	< 7	< 7	< 7	< 7
Ca (mg L^{-1})	50.8±0.3$^{*, A, C}$	57.6±1.0$^{*, c}$	47.4±1.8$^{*, B, C}$	52.9±0.3$^{*, b, c}$	57.0±0.8$^{*, A, B}$	58.4±0.2$^{*, b}$
K (mg L^{-1})	2.15±0.09$^{*, A, C}$	2.36±0.04*	2.82±0.17$^{*, B, C}$	2.28±0.07$^{*, b}$	4.58±0.07$^{*, A, B}$	2.45±0.06$^{*, b}$
Mg (mg L^{-1})	9.7±0.4$^{A, C}$	11.2±0.1$^{a, c}$	15.1±0.6$^{*, B, C}$	16.4±0.2$^{*, b, c}$	16.9±0.2$^{*, A, B}$	15.4±0.3$^{*, a, b}$
Na (mg L^{-1})	5.0±0.2$^{*, A, C}$	4.47±0.05$^{*, a, c}$	9.9±0.3$^{*, B, C}$	12.2±0.1$^{*, b, c}$	26.5±0.2$^{*, A, B}$	16.6±0.3$^{*, a, b}$

Table 3 The comparison of average trace and macro element dissolved concentrations reported for the Ilova River, two other rivers in Croatia (Sutla, Sava), several European rivers under different anthropogenic impact (Tweed-Teviot, Great Ouse, Wear, Thame, Aire, Marne, Seine, Guadalquivir, Vistula and Odra) and the Environmental quality standards (EQS)

Element/ Rivers and sites	*Ilova River* (Agriculturally and industrially impacted)	*Sutla - reference* (Unpolluted area)	*Sutla - contaminated* (Urban and industrially impacted)	*Sutla - contaminated 2* (Urban and industrially impacted)	*Sava-contaminated* (Urban and industrially impacted)	*Tweed-Teviot* (Rural river)	*Great Ouse* (Agriculturally impacted)	*Wear* (Industrially impacted)	*Aire* (Industrially impacted)	*Thames* (Agriculturally impacted)	*Marne* (Urban and industrially impacted)	*Seine* (Urban and industrially impacted)	*Guadalquivir* (Agriculturally and industrially impacted)	*Vistula* (Mining and industrially impacted)	*Odra* (Agriculturally and industrially impacted)	*EQS* (Environmental quality standards)
	This study	Dragun et al. 2011		Filipović Marijić et al. 2016	Dautović 2006; Dragun et al. 2009	Neal and Robson 2000					Elbaz-Poulichet et al. 2006		Mendiguchía et al. 2007	Guéguen and Dominik 2000	Adamiec and Helios-Rybicka 2002	EPCEU 2008; Crane et al. 2007
Al (µg L^{-1})	0.5-6.8	2.12	7.27	0.47-15.8	-	46.13	8.03	29.20	25.39	12.95	-	-	-	-	-	-
As (µg L^{-1})	1.39-4.87	0.79	3.83	0.45-2.60	-	0.614	1.978	0.680	5.606	2.07	0.64	0.75	-	-	0-376-8.10	-
Ba (µg L^{-1})	29.1-39.3	18.8	37.1	20.0-44.1	-	94.9	24.7	58.7	31.2	13.1	27.0	27.3	-	-	-	-
Bi (µg L^{-1})	<0.005	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cd (µg L^{-1})	0.002-0.053	0.007	0.117	0.007-0.006	0.011	-	-	-	-	-	0.024	0.031	0.015	0.59-0.73	0.02-0.867	0.08-0.15
Co (µg L^{-1})	0.110-0.338	0.068	0.347	0.08-0.29	0.064	0.145	0.620	0.545	1.319	0.527	-	-	0.150	1.34-3.03	-	-
Cr (µg L^{-1})	<0.06	< LOD	0.27	-	0.32	1.394	0.352	0.341	3.748	0.416	-	-	-	0.97-2.44	0.21-12.7	-
Cs (µg L^{-1})	< 0.001-0.090	0.002	0.110	-	-	-	-	-	-	-	-	-	-	-	-	-
Cu (µg L^{-1})	0.40-0.9	0.49	0.93	0.71-3.34	0.54	2.569	4.668	2.437	7.210	4.277	1.80	2.23	2.64	17.8-90.0	0.550-54.6	8.2
Fe (µg L^{-1})	16.4-39.7	36.7	51.8	25.4-68.2	12.6	56.2	27.0	142.1	140.9	29.2	-	-	-	-	16.2-1861	16.0
Mn (µg L^{-1})	18.4-340	17.1	51.5	8.02-266.6	3.44	7.4	4.5	86.7	144.1	6.1	3.92	6.26	20.65	303.2-583.8	5.91-353	-
Mo (µg L^{-1})	0.56-0.98	0.55	11.96	0.49-6.90	0.81	0.39	3.34	1.46	23.48	2.85	0.68	0.97	-	-	-	-
Ni (µg L^{-1})	0.57-1.80	0.45	1.86	0.66-2.73	0.59	1.51	4.73	4.00	9.19	2.88	-	-	2.31	-	0.259-27.2	20.0
Pb(µg L^{-1})	<0.07	< LOD	0.901	0.95	0.055	0.196	0.425	2.741	1.262	0.344	0.208	0.353	0.178	0.24-0.39	0.1-7.84	-
Rb (µg L^{-1})	0.559-3.74	2.38	9.63	0.80-6.24	-	0.96	5.73	16.32	11.70	3.70	1.53	3.15	-	-	-	-
Sb(µg L^{-1})	0.107-0.301	0.082	6.47	2.86	-	0.211	0.704	0.497	2.674	0.365	-	-	-	-	-	-
Se (µg L^{-1})	0.203-1.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sn(µg L^{-1})	-	< LOD	1.60	9.34	-	0.166	0.166	0.098	0.554	0.109	-	-	-	-	-	-
Sr (µg L^{-1})	123-181	216.1	416.8	185.0-344.7	128.0	116.8	493.1	367.1	201.1	358.0	690	540	-	-	-	-
Tl(µg L^{-1})	0.002-0.013	< LOD	0.028	0.008–0.02	-	-	-	-	-	-	-	-	-	-	-	-
U (µg L^{-1})	0.695-1.57	0.55	0.79	0.62	-	0.339	0.750	0.276	0.279	0.794	-	-	-	-	-	-
V (µg L^{-1})	0.35-4.1	0.18	0.72	0.19-0.75	-	-	-	-	-	-	-	-	-	-	-	-
Zn (µg L^{-1})	<7	-	-	4.19-27.4	2.27	4.93	11.09	21.60	31.81	6.49	-	-	1.58	17.3-168.7	12.4-535	7.8
Ca (mg L^{-1})	47.4-58.4	58.8	77.3	48.8-92.8	91.1	34.8	133.2	52.3	61.2	120.8	-	-	-	-	-	-
K (mg L^{-1})	2.15-4.58	3.79	13.4	2.29-9.54	3.20	1.51	11.48	6.43	13.86	6.71	-	-	-	-	-	-

Mg (mg L^{-1})	9.7-16.9	18.6	27.1	12.1-21.4	19.5	10.54	8.48	17.77	13.67	5.1	-	-	-	-	-	-
Na (mg L^{-1})	4.47-26.5	11.3	88.3	9.53-59.8	10.6	10.4	62.4	43.3	110.0	33.0	-	-	-	-	-	-

Table 4 Average concentrations of elements in sediments (mg kg^{-1} or g kg^{-1}) from the Ilova River at three sampling sites (Maslenjača, Ilova and Trebež villages) from autumn 2017. Statistically significant differences (ANOVA) at p<0.05 level between the three sampling sites are assigned with superscript letters A, B and C. Sediment quality guidelines (SQGs) provided by Canadian Council of Ministers of the Environment (CCME 2002) and study by Long et al. (1995) are presented as well

	Maslenjača village	**Ilova village**	**Trebež village**	**CCME**		**Long et al., 1995**	
				ISQG	PEL	ERL	ERM
Al (g kg^{-1})	63.8±1.2^{A}	68±4^{B}	84±2$^{A, B}$				
As (mg kg^{-1})	4.4±0.1^{A}	8.9±1.5	12±4^{A}	5.9	17.0	8.2	70.0
Ba (mg kg^{-1})	406±16^{A}	380±30^{B}	500±50$^{A, B}$				
Bi (mg kg^{-1})	0.14±0.01^{A}	0.22±0.02	0.45±0.06^{A}				
Cd(mg kg^{-1})	0.15±0.08^{A}	0.19±0.01^{B}	3.8±0.7$^{A, B}$	0.6	3.5	1.2	9.6
Co (mg kg^{-1})	6.95±0.02$^{A, C}$	10.9±0.9$^{B, C}$	14.8±0.7$^{A, B}$				
Cr (mg kg^{-1})	33±7$^{A, C}$	60±5$^{B, C}$	96±5$^{A, B}$	37.3	90.0	81	370.0
Cs (mg kg^{-1})	2.82±0.07$^{A, C}$	3.9±0.4$^{B, C}$	6.8±0.5$^{A, B}$				
Cu (mg kg^{-1})	6.9±0.4$^{A, C}$	15±2$^{B, C}$	32±2$^{A, B}$	35.7	197.0	34	270.0
Fe (g kg^{-1})	14.7±0.6$^{A, C}$	27±2$^{B, C}$	35.8±1.8$^{A, B}$				
Mn (mg kg^{-1})	391±27$^{A, C}$	820±120^{C}	960±140^{A}				
Mo (mg kg^{-1})	0.21±0.01^{A}	0.36±0.03^{B}	0.65±0.12$^{A, B}$				
Ni (mg kg^{-1})	13.3±0.1$^{A, C}$	26±3$^{B, C}$	43±3$^{A, B}$			20.9	51.6
Pb(mg kg^{-1})	16.2±0.4^{A}	18±1^{B}	45±7$^{A, B}$	35.0	91.3	46.7	218
Rb (mg kg^{-1})	78.7±0.5^{A}	76±7^{B}	101±3$^{A, B}$				
Sb (mg kg^{-1})	0.80±0.10$^{A, C}$	0.95±0.06$^{B, C}$	1.27±0.07^{A,B}				
Sr (mg kg^{-1})	119±9$^{A, C}$	109±3$^{B, C}$	147±5$^{A, B}$				
Tl (mg kg^{-1})	0.45±0.02^{A}	0.46±0.02^{B}	0.67±0.01$^{A, B}$				
U (mg kg^{-1})	1.68±0.01^{A}	1.98±0.15^{B}	4.6±0.2$^{A, B}$				
V (mg kg^{-1})	39.6±0.3$^{A, C}$	75±5$^{B, C}$	103±3$^{A, B}$				
Zn (mg kg^{-1})	36±4$^{A, C}$	64±5$^{B, C}$	162±10$^{A, B}$	123.0	315.0	150.0	410.0
K (g kg^{-1})	20.4±0.1^{C}	15.6±1.3$^{B, C}$	18.8±1.5^{B}				
Mg (g kg^{-1})	4.2±0.1$^{A, C}$	7.4±1.2$^{B, C}$	14.8±1.6$^{A, B}$				
Na (g kg^{-1})	15.3±0.7$^{A, C}$	10.5±0.3$^{B, C}$	7.7±1.2$^{A, B}$				

Abbreviations: ISQG - Interim Freshwater Sediment Quality Guidelines; PEL - probable effect concentration; ERL - effects range low; ERM - effects range median

Table 5 CF (contamination factor), EF (enrichment factor) and PLI (pollution load index) values for the sediments from the Ilova and Trebež villages calculated in relation to the "reference" sediments from the Maslenjača village

	Ilova village		**Trebež village**	
	CF	***EF***	***CF***	***EF***
As	**2.1**	1.9	**2.9**	**2.2**
Ba	0.9	0.9	**1.2**	0.9
Bi	**1.5**	1.4	**3.1**	**2.4**
Cd	**1.3**	1.2	**25.3**	**19.3**
Co	**1.6**	1.5	**2.1**	1.6
Cr	**1.8**	1.7	**2.9**	**2.2**
Cs	**1.4**	1.3	**2.4**	1.8
Cu	**2.2**	**2.1**	**4.6**	**3.5**
Fe	**1.8**	1.7	**2.4**	1.9
Mn	**2.1**	**2.0**	**2.5**	1.9
Mo	**1.7**	1.6	**3.1**	**2.4**
Ni	**1.9**	1.8	**3.2**	**2.5**
Pb	**1.1**	1.0	**2.8**	**2.1**
Rb	**1.0**	0.9	**1.3**	1.0
Sb	**1.2**	1.1	**1.6**	1.2
Sr	0.9	0.9	**1.2**	0.9
Tl	**1.0**	1.0	**1.5**	1.1
U	**1.2**	1.1	**2.7**	**2.1**
V	**1.9**	1.8	**2.6**	2.0
Zn	**1.8**	1.7	**4.6**	**3.5**
K	0.8	0.7	0.9	0.7
Mg	**1.7**	1.6	**3.5**	**2.7**
Na	0.7	0.6	0.5	0.4
PLI	***1.4***		***2.4***	