HEAVY METALS IMPACT ON SEDIMENT MICROBIAL COMMUNITIES IN RIVER-DAM SEQUENCE OF SMALL HYDROPOWER PLANT CASCADE

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Abstract

Heavy metals are widespread pollutants with significant environment risk due to high toxicity and clear tendency for accumulation in different matrices – soils, sediments, biota. The main research objective of this work is to assess the impact of heavy metal pollution on key structural and functional parameters of microbial communities in sediments of river-dam sequence of small hydropower cascade Middle Iskar, Bulgaria. The content of heavy metals (As, Cd, Cu, Hg, Pb, Zn) was measured during the low water summer periods of 2012, 2013 and 2014. The evaluation of site quality and heavy metal pollution was done by use of one integrated index - Pollution Load Index and it was compared to total count of sediment microbiota and count of coliform bacteria, also with total dehydrogenase activity and index of phosphatase activity. The assessment of heavy metal pollution in river-dam sequence of cascade indicates the higher metal concentrations and high Pollution Load Index in dam sediments. At low level of pollution in river sites, the both structural and functional microbial parameters react to local variations of heavy metal concentrations and high negative correlation (r=-0.8÷-0.9) exists between variables. But in dam sites, the microbial community is more resistant to pollution and structural parameters react conservatively with long reaction time. The enzyme activities are more adaptive and sensitive indicators for different level of environmental impact in this case. The complex phosphatase and dehydrogenase activities have a high potential to be used as reliable parameters for precise assessment of hazardous sediment pollution in complicated ecological situations with cumulative impacts.

Keywords: heavy metals, sediments, microbial community, dehydrogenases, phosphatases, small hydropower cascade

Introduction

Sediments are one of the most diverse habitats in freshwaters and have a specific function of sink and source for organics, nutrients and specific pollutants in ecosystems (Torsvik et al., 2002; Santos et al., 2003; Zhu et al., 2012). Sediment biota, especially microbial communities regulates and affects many ecosystem processes in freshwater sediments and is the main contributor for the transformations of different accumulated compounds (Nealson, 1997; Yu et al., 2015). But the hazardous pollutants with high absorbance and retention capacity are considered as serious threat for sediment status, biotic processes and ecological balance due to their high toxicity, persistence and non-degradability in the environment (Pekey, 2006; Sakan et al., 2015; Tang et al., 2014). Heavy metals are classic examples for this type of sediment-associated hazardous pollutants with high risk level, prevalence and serious impact on biota. The high concentrations of heavy metals are not only toxic for alive organisms but they inactivate many of key transformation processes, damage the ecosystem metabolism and functional integrity (Khan et al., 2007; Todorova and Topalova, 2013; Todorova et al., 2015).

The evaluation of heavy metal pollution status of river bed sediments and pollutants interaction with key biotic complexes is a multiplex task with a series of difficulties in its application. The difficulties are complicated additionally from alterations in hydromorphology and disturbance of normal hydrological regime of natural ecosystems – standard consequence from energy utilization of water. The small hydropower plants (SHP) are considered as environmentally sound alternative of fossil fuels but the construction of cascade of several SHPs strongly affects the degree and rate of sedimentation and pollutants accumulation in alternating "river-dam" sequence (ASTAE, 2014). Cascade exploitation dramatically disturbs the balance of sediment inflow and outflow, increases flow depth, decreases flow velocity and causes intensive settling in dam sites (Sumi and Hirose, 2009). The sediment-related problems such as heavy metals pollution are multiplied and intensified since this additional sedimentation and dams are considered as serious pollutants traps. Accumulation of heavy metals magnifies their negative impact on all aquatic organisms and ecosystem processes, including microbial communities and their enzyme activities (Baby et al., 2010). This influences the self-purification processes in the sediments as well as the ecosystem health.

The aim of this work is to assess the interaction between heavy metals pollution and sediment microbial communities (as total count of indicator groups and activities of some key enzymes) in river-dam sequence of SHPs cascade "Middle Iskar". The first element – heavy metals impact on biota is well known and many surveys are published (Yu et la., 2015; Baby et al., 2010; Rial et al., 2011; Kapoor et al., 2015; Neethu et al., 2015). But the study of this impact from the view of cumulative effect of alternating "river-dam" sequence is not well-developped and the gap in management plans of this type modified ecosystems is significant.

Materials and Methods

Study area

The study area is localized in the catchment of Iskar River, Bulgaria - 33 km sector of SHPs cascade "Middle Iskar" between Rebrovo and Gabrovnitza villages (Fig. 1). The Iskar River is situated in the NW part of Bulgaria and belongs to the Danube River Basin. Iskar is the longest

Bulgarian river (368 km) with 8 650 km² basin area and mean annual runoff of 37.57 m^3/s in the study sector (Todorova et al., 2015). The river seasonal flow is typical for continental climate zone with a low run off in summer and winter and a high run off in spring and autumn. In its middle part the river flows through a densely populated and industrialized region and is a long-term receptor of treated or non-treated urban and industrial sewage of Sofia area. The river sector is a subject of intensive hydromorphological impact, too - the construction of Middle Iskar Cascade with barrages and power stations has been started since 2000s and now 5 SHPs are in operation.

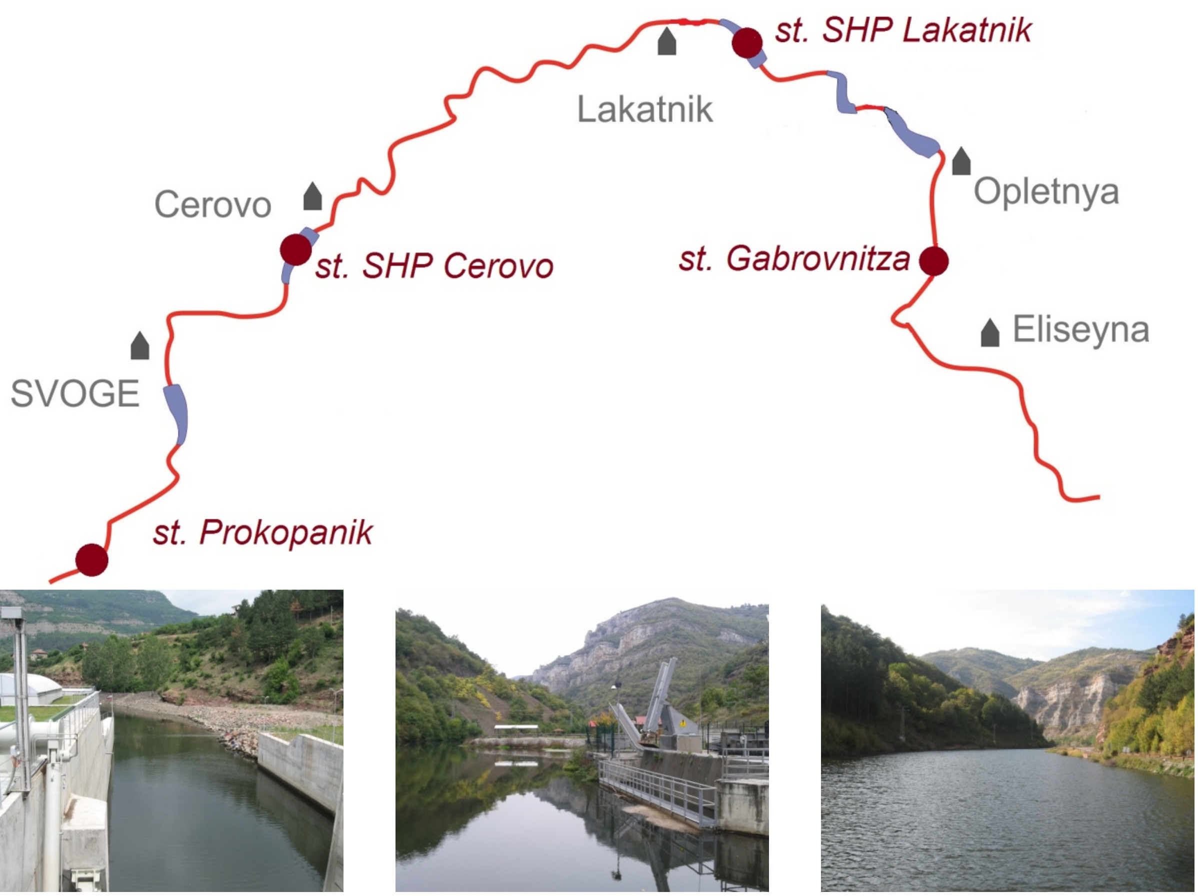


Fig. 1. *Scheme of study area with sampling sites location and pictures of SHPs cascade*

Experimental design and sampling procedure

The present study was conducted during the low water summer periods of 2012, 2013 and 2014 in four sampling sites. The first site was at the beginning of the river sector – Prokopanik (Prok); the second and third sites were at dam walls of SHP Cerovo (Cer) and Lakatnik (Lak); the last was at the end of river sector Gabrovnitza (Gabr) (Fig. 1). The hydrological regime of river sector is strongly modified from the cascade and the study sites can be subdivided into two hydrologically different habitats: "river" sites (Prok and Gabr) and "dam" site (Cer and Lak). In two types of habitats, the content of heavy metals (As, Cd, Cu, Hg, Pb, Zn) was measured; the assessment of site quality and heavy metal pollution was done by use of one integrated index - Pollution Load Index and it was compared to key structural and functional parameters of sediment microbial community.

The sediment samples were collected using Eckman-Berge dredge with an approximated area of 0.04 m^2 for "dam" sediments and by manual dredging for "river" sediments. In site, the stones and plant fragments were removed by passing the samples through a 2 mm sieve. Each collected sample for analyses of heavy metals consisted of at least 1 kg of sediment and was contained in a separate sealed plastic bag. To assess quantitative and functional parameters of sediment microbial communities, individual samples were collected in sterile containers. All of the samples were placed in a cooler at 4◦C and transported to the laboratory immediately. The environmental conditions at the sampling stations were also recorded.

Analytical procedures

For analyses of heavy metals content the sediment samples were air dried, then powdered and finally passed through a 500 µm sieve. The concentrations were determined by atomic absorption spectrophotometry for As, Cd, Cu, Pb and Zn and by cold vapour atomic absorption spectrometry for Hg. The results were presented in mg/kg dry sediments.

Sediment microbial parameters – Total Microbial Count (TMC) and number of coliforms (Colif) were determined by use of count-plate technique on Nutrient agar and Endo-agar for 24-48 h at 35ºC. For enzyme assays the sediment samples were sonicated (UD-20 automatic) 3 x 10 s. Dehydrogenase activity (TDA) was measured according to the method of Lenhard et al., 1964 by the reduction of 2,3,5-triphenyl tetrazolium chloride. Phosphatase activity index (PAI) was determined as an average value of activities of acid, neutral and alkaline phosphatases. The method was based on transformation of p-nitrophenolphosphate (Matavulj et al., 1990). The enzyme activities were presented per mg of total sediment protein. The protein content was determined according to micro-biuret method (Kochetov, 1980).

Calculation of PLI (Pollution Load Index)

For assessment of heavy metals contamination status in sediments of Middle Iskar Cascade, the Pollution Load Index (PLI) was calculated following the method proposed by Tomlinson et al., 1980. This empirical index is a simple, comparative way for assessing the level of heavy metal pollution (Kalender and Çicek Uçar, 2013). This pollution index with simple numeric interpretation is a powerful tool for processing, analyzing, and conveying raw environmental information to decision makers, managers, technicians, and the public (Caeiro et al., 2005). The PLI is based on the contamination factor (CF) of each metal (Hakanson, 1980). The CF is the ratio obtained by dividing the concentration of each metal (C_{sample}) in the sediment by the background value ($C_{background}$ - geochemical background concentration of the metal in Iskar River catchment according Cholakova, 2004) (1). PLI was calculated as the nth root of the product of the n CF (2). Values of PLI<1 or =1 indicate heavy metal loads close to the background level, and values above 1 indicate pollution.

$$CF = C_{sample}/C_{background} \quad (1)$$

$$PLI = \sqrt[n]{(CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n)} \quad (2)$$

Data interpretation and statistical analyses

The presented data were means of three or more repeats. A Pearson correlation with statistical significance set at $p < 0.05$ was used to confirm the relationships between microbial parameters and Pollution Load Index. Statistical analyses were performed using Microsoft Excel 2000/XLSTAT (2014.5.03, Addinsoft, Inc., Brooklyn, NY, USA).

Results and Discussion

Heavy metal content in sediment

Figure 3 presents the results for concentrations of heavy metals in sediments of 4 sampling sites. The data for heavy metal concentrations was interpreted according to local quality standards for soils (Regulation No3, 2008) due to the ongoing procedure for development of the sediment environmental quality standards. The metals content varies between: 4-33 mg/kg for As, 0.2-3 mg/kg for Cd, 39-120 mg/kg for Cu, 0.05-0.44 mg/kg for Hg, 25-110 mg/kg for Pb and 132-441 mg/kg for Zn. Despite that the comparison with previous studied periods (1991-2001) shows the decrease in total metal content in river subcatchment (Cholakova, 2004), some of the metals continue to exceed the standards and this specific type of pollution is still a serious risk for the ecosystem. The exceeding with 10-50% of maximum admissible concentrations was registered for As in 2014 at Prok., for Cd and Zn at dam sites. As a trend, the higher concentrations of heavy metals, excepting the arsenic were detected in the sediments of dam sites – Cerovo and Lakatnik. The alternating conditions of running-standing waters are favorable factor for additional increase of heavy metals concentrations in the impoundments of cascade. The arsenic had a different behavior and increased its content in river sites - high concentration of 33 mg/kg was measured in 2014 at Prokopanik sediments.

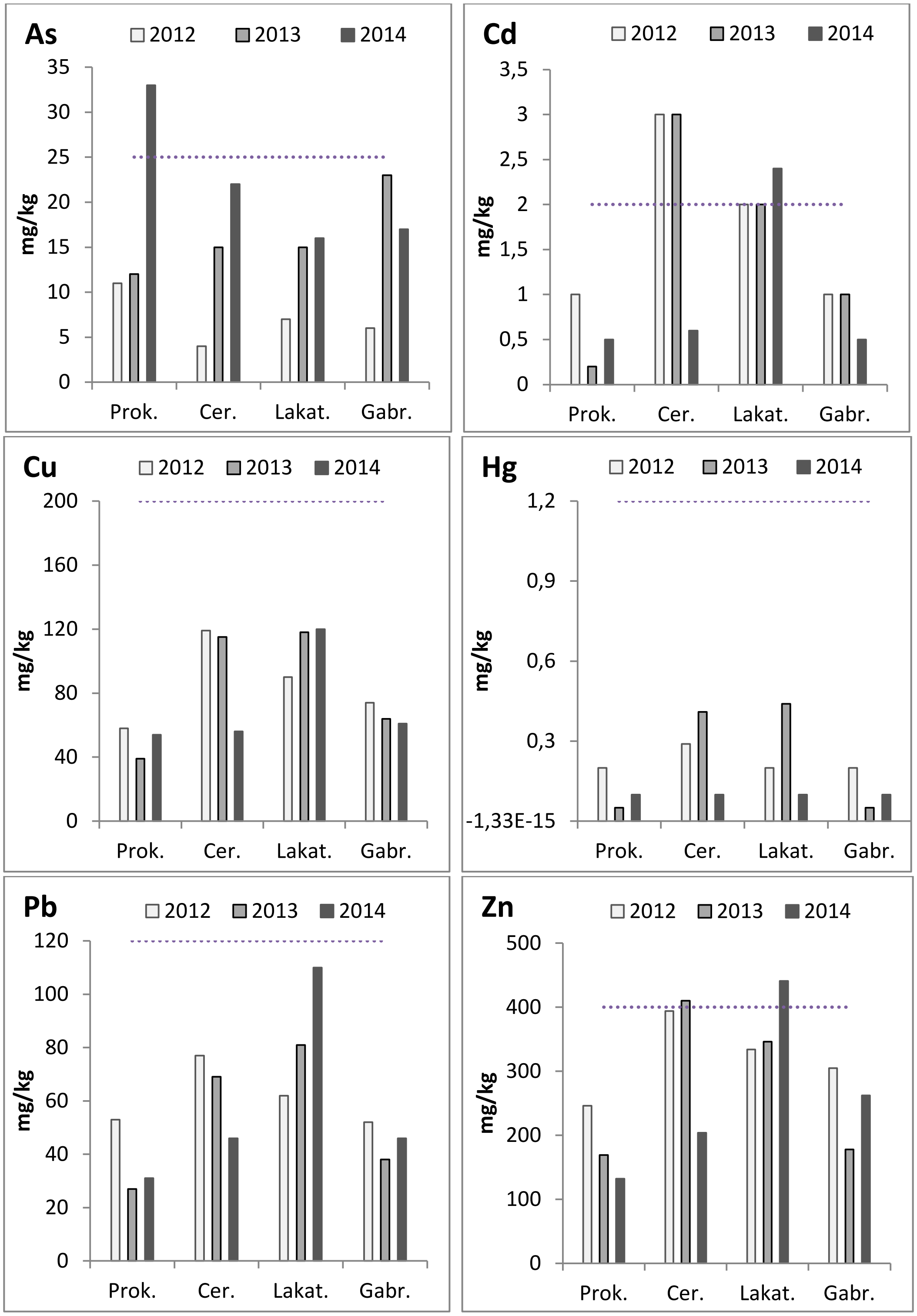


Fig. 2. *Content of heavy metals in sediments of cascade during 2012-2014*

** the dot line is maximum admissible concentrations for each metal according quality standards for soils (Regulation No3, 2008)*

Pollution Load Index (PLI)

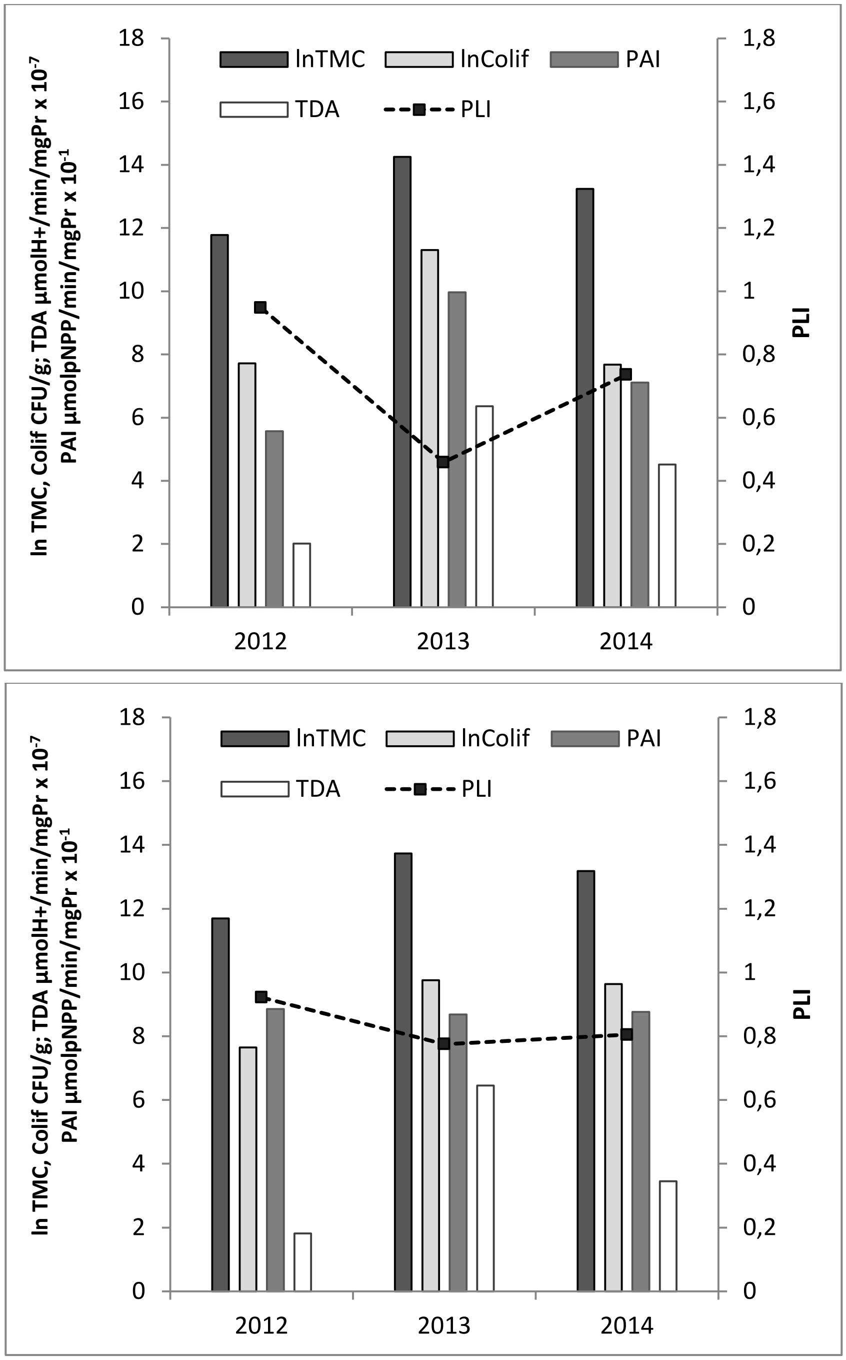
The results for Pollution Load Index are summarized in Table 1. The PLI provides simple but comparative means for assessing a site quality and heavy metals pollution, where a value of PLI < 1 denotes good ecological situation; PLI = 1 presents that only baseline levels of pollutants are presented and PLI > 1 would indicate deterioration of site quality and presence of pollution (Tomlinson et al., 1980). The index analyses showed that the pollution was detected in dam sites of Middle Iskar cascade – values ranged between 1.14÷1.72. Although there is no pollution in river sediments, the index has increased values in the last sampling site – indicator for negative role of SHPs cascade in retention of this type of pollutants.

Table 1. *The PLI (Pollution Load Index) values in the sampling sites (values >1 indicate heavy metal pollution)*

	Prok	Cer	Lak	Gabr
PLI_{2012}	0.942	**1.327**	**1.147**	0.948
PLI_{2013}	0.459	**1.723**	**1.634**	0.775
PLI_{2014}	0.736	0.795	**1.461**	0.805

Sediment microbial parameters and heavy metal impact

The PLI is used to present the integrated heavy metal impact of some structural and functional parameters of sediment microbial community. The selected microbial parameters are total counts of microorganisms and coliforms, and total activities of dehydrogenases and phosphatases. The effect of heavy metals pollution on these microbial parameters in river habitats is presented on Fig. 3. The results show a clear negative impact of high heavy metals content despite the PLI indicates “no pollution” in these sites. The high values of index close to 1 are related to low counts of microbial indicators and low enzyme activities. The inhibition of coliforms and total dehydrogenase activity was more expressed – 10 and 3-fold decrease was registered at Prokopanik in 2012 compared to 2013 when the four studied microbial parameters had the highest values at the lowest index of 0.46. The same trend was observed at other river site Gabrovnitza. Correlation analyses confirm the strong relationships between studied microbial parameters and pollution index – high negative correlation with coefficients -0.8÷-0.9 exist between variables in river sites.



(a)

(b)

Fig. 3. *Effect of heavy metal pollution (as PLI) on sediment microbial parameters in river sites: (a) Prokopanik; (b) Gabrovnitza*

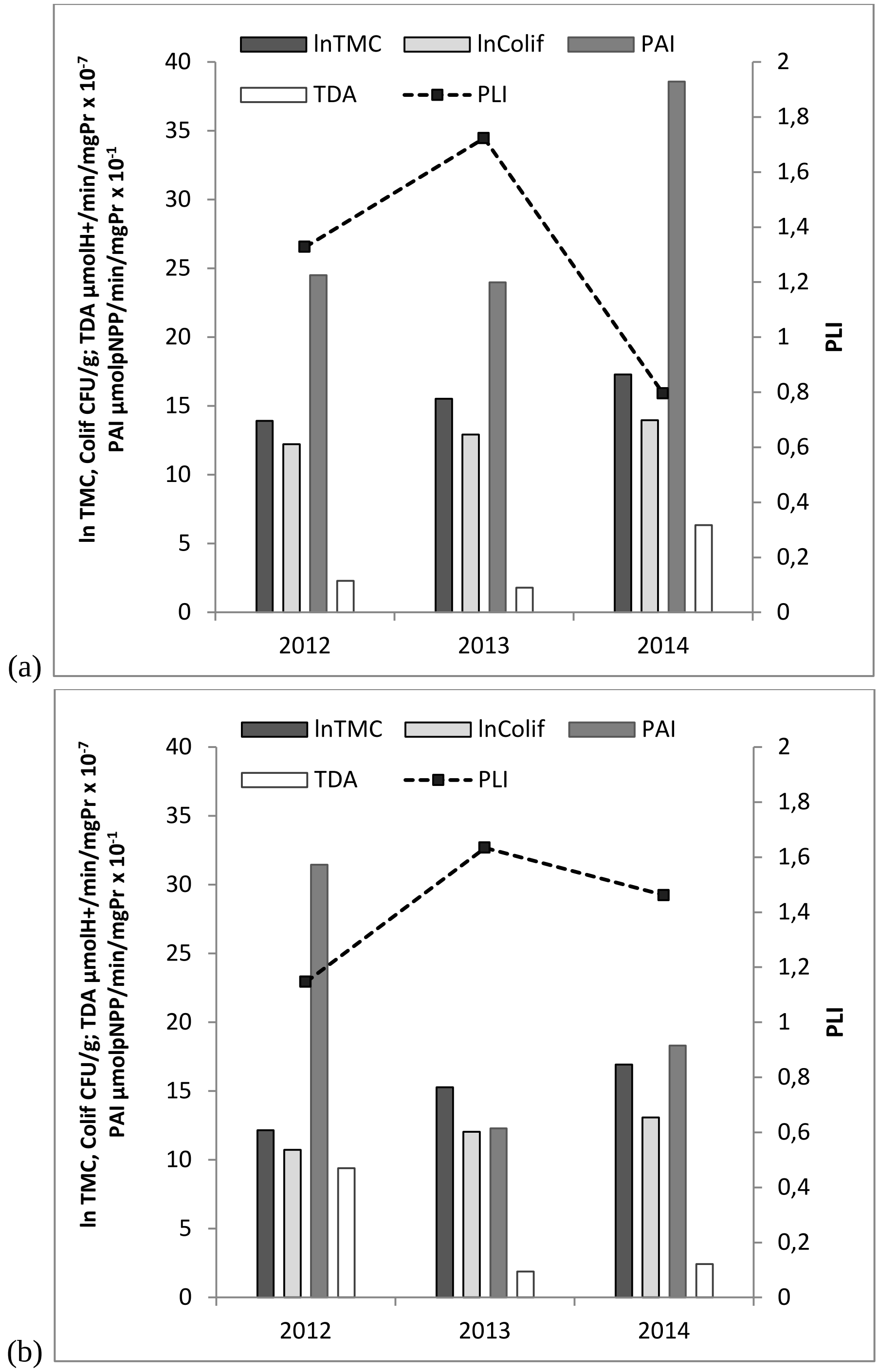


Fig. 4. *Effect of heavy metal pollution (presented as PLI) on sediment microbial parameters in dam sites: (a) Cerovo; (b) Lakatnik*

This strong negative relationship was detected in dam sites too but only for functional parameters (Fig. 4). The total microbial count and coliforms were not affected clearly of high metal content and no correlation was observed with PLI. Probably the permanent higher concentrations of heavy metals in dams' sediments have a role of adaptation factor for bacteria and the sediment microbial community is more resistant on structural level. Their quantitative parameters are less variable and conservative indicators with wide range and long term of reaction. But phosphatase activity index (PAI) and total dehydrogenase activity (TDA) react very sensitively and a decrease of their values was detected at the higher level of pollution in 2013. The two enzyme activities are inhibited by high metal content – the reaction of microbial community is on functional level. The correlation coefficients between PLI and enzyme activities are respectively PAI/PLI= - 0.85 and TDA/PLI= - 0.79.

Conclusion

The construction and operating of SHPs cascade have a negative environmental impact on sediment status by additional accumulation and retention of sediment-related pollutants, especially in dam sites. The assessment of heavy metal pollution in river-dam sequence of SHPs cascade indicates the higher metal concentrations and high Pollution Load Index in dam sediments. The structural and functional parameters of sediment microbial community in river sites of cascade at low level of pollution are indicators with high potential for assessment of heavy metals impact. But in dam sites, the microbial community is more resistant to pollution and structural parameters react conservatively with long reaction time. The enzyme activities are more adaptive and sensitive indicators for different level of environmental impact in this case. The integration of structural and functional response of sediment microbial community is an important tool for the adequate evaluation of the specific pollutants effect. The two selected enzyme indicators have a key biochemical role in the functioning of ecosystem and can provide target information for real heavy metals impact on functional level. Phosphatases and dehydrogenases are an obligatory part of organism enzymatic set, realize the metabolic reactions involved in oxidative energy transfer and are a good indicator of microbial activity. Dehydrogenase enzymes is known to oxidise organic matter by transferring protons and electrons from substrates to acceptors - these processes are part of general respiration pathways and reflect the integral degradation status of soils and sediments (Doi and Ranamukhaarachchi, 2009). The phosphatases catalyzes the hydrolysis of phosphate esters, released phosphate groups bound in complex organic substrates and are involved in the energy metabolism by providing of inorganic phosphates for ATP-synthesis. From this perspective, the enzymatic approach for evaluation of pollutant effects directly assesses the inhibition of key processes in microbial energy metabolism and hence the essential ecosystem functions as an integral productivity and self-purification capacity. The complex enzyme activities – PAI and TDA have a high potential to be used as reliable indicators for precise assessment of hazardous sediment pollution in complicated ecological situations with cumulative impacts.

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