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SHORT-TIME EFFECT OF HEAVY METALS STRESS ON KEY ENZYME INDICATORS IN RIVER SEDIMENTS

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Abstract

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The pollution of aquatic ecosystems made the necessity of new technologies for environmental decontamination more urgent. Bioremediation approaches involving microbial degradative capacities are regarded as effective and environmental friendly alternatives. As mediators in key biodegradation processes, the complex enzyme activities (measured as a total of extracellular and intracellular activity on organism and sub-organism level) have the potential to be used as indicators for mechanisms and rate of transformation processes. However, the presence of some toxic and xenobiotic substrates can modulate (activate or inhibit) the enzyme activities and affects the degree of pollutants transformation. The main objective of this study was to assess the effect of heavy metals on key enzyme indicators (total dehydrogenase activity – DHA and phosphatase activity index – PAI) in sediments of Iskar River middle part. The inoculation with moderate concentrations of cadmium stimulated the DHA but the further increasing inhibited the activity with 80%. The Cu^{2+} had a specific effect by the location of sediments. The Pb^{2+} and Zn^{2+} in all used concentrations inhibit the DHA with 35–95%. The PAI was inhibited by all tested heavy metals but the Zn^{2+}-ions have the higher impact (over 75% inhibition).

Key words: enzyme indicators; Total Dehydrogenase Activity, Phosphatase Activity Index, heavy metal stress, river sediments
Abbreviations: DHA – total dehydrogenase activity; PAI – phosphatase activity index; MHPPs – micro-hydro power plants; HMs – heavy metals

Introduction

The accumulation of pollutants resulting from anthropogenic activities has become a major problem that made the necessity of new technologies for environmental decontamination more urgent. Microorganisms have an enormous catabolic potential and functional biodiversity and could be successfully used in pollutant degradation processes (Watanabe, 2001; Singh et al., 2008). As mediators in biogeochemical processes, namely decomposition of organic matter and xenobiotics detoxification, the enzyme activities have the potential to be used as indicators for functional status of ecosystem.

The new trend in water source management establishes the ecosystem approaches, based on complexity of system. The complex enzyme activities measured as a total of extracellular and intracellular activity on organism and sub-organism level describe the functionality of biological system and give the information for the mechanisms and rate of biotransformation processes on ecosystem level (Todorova, 2009; Topalova, 2009). However, the presence of some chemical compounds (toxic and xenobiotic substrates) can modulate (activate or inhibit) the enzyme activities and affects the level of ecosystem metabolism and effectiveness of pollutants transformations.

The main objective of this study was to assess the effect of heavy metals (HMs) on key enzyme indicators (total dehydrogenase activity – DHA and phosphatase activity index – PAI) in sediments of Iskar River middle part. This river

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sector has a critical meaning because of its strongly affected environmental state with organics and nutrients from Sofia region and with hazardous materials from past industrial activity. In the same time, the river sector has been an object of intensive hydrotechnical activity in last years for construction of cascade of 9 micro-hydro power plants (MHPPs). During the construction and initial exploitation of the cascade, the accumulation and retention of river sediments at dam wall have been a major problem for effective management of this urban hydroecosystem.

Materials and Methods

Sampling sites and experimental design

The sediments were collected from two sampling sites in the area of Middle Iskar MHPPs cascade – 33 km river sector between Svoge and Elisejna. The first site was at the beginning of the cascade – Prokopanik (river sediments) and the second site was at dam wall of the first MHPP Lakatnik (dam sediments). The initial position of these two points has a crucial role for further functioning of the whole cascade. The status of sediments was not corresponded to sediment quality criteria – the threshold levels were exceeded for Cd, Cu, Pb and Zn (Todorova and Topalova, 2010). The collected sediments were treated in lab with additional concentrations (3 working variants of each metal ion) for simulation of intensive sediments disturbance and internal pollution of system with critical amounts of HMs (Table 1). In this study, only the single effect of metals was discussed.

Simulation and analytic procedures

Sediment samples (100 g) of each sampling sites were treated with Cd^{++} ($CdSO_4$), Cu^{++} ($CuSO_4$), Pb^{++} ($Pb(NO_3)_2$) and Zn^{++} ($ZnSO_4$) dissolved in water for achieving of final concentrations of metal ions according to Table 1. The contaminated sediments were shaken to distribute the metal ions homogeneously. After incubation for 1 hour, DHA and PAI were determined by the reduction of triphenyltetrazolium chloride to triphenylformazan and transformation of p-nitrophenol phosphate, respectively (Lenhard et al., 1964; Matavuly et al., 1990). Non-treated sediments were considered as control samples and their measured enzyme activities were regarded as native. The enzyme activities were presented per mg of total sediment protein. The protein content was determined according to micro-biuret method (Kochetov, 1980).

All of data were means of three or more repeats with presented standard deviations.

Table 1

Threshold level values, measured levels and working concentration for Cd, Cu, Pb and Zn in river and dam sediments

	TLV	ML river sed.	ML dam sed.	WCs
Cd	3	1,5	5	4; 6; 8
Cu	200	235	108	400; 600; 800
Pb	120	300	103	140; 210; 280
Zn	400	575	345	500; 750; 1000

* TLV – threshold level value; ML – measured level (according to Todorova and Topalova, 2010); WCs – working concentrations
** All of the concentrations are in mg kg^{-1}

Results and Discussion

PAI of non-treated sediment samples had values of 5.862±1.689 and 3.556±0.593 mkM pNPP $min^{-1}mgPr^{-1}$ x 10^{-2}, respectively for river and dam sediments. The contamination with tested HMs inhibited the PAI both for the two sediment types (Figure 1) but the effect was more strongly presented for the river sediments. The use of different concentrations of HMs had a non-specific effect on enzyme ac-

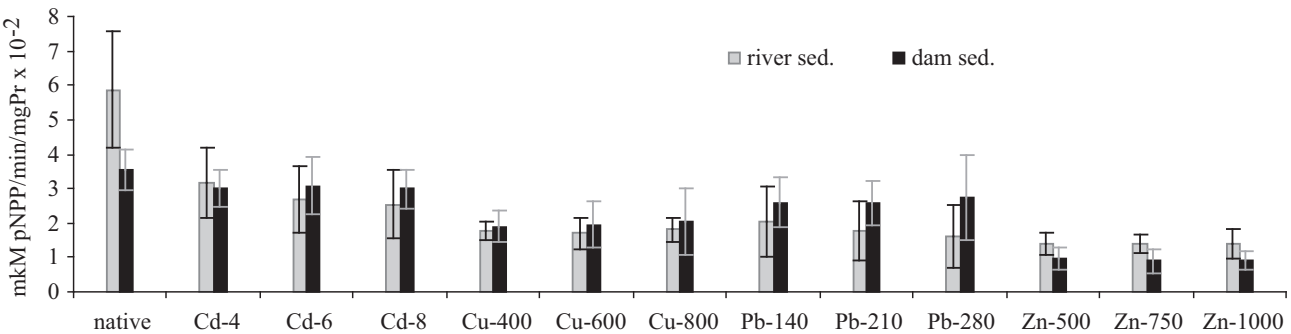

Fig. 1. Effect of heavy metals on PAI in river and dam sediments
***The number after heavy metal symbol is concentration in mg kg^{-1}**

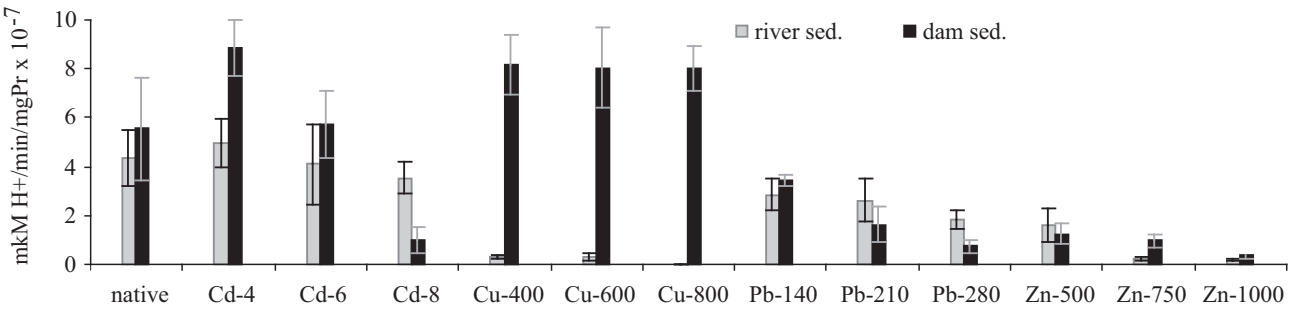

Fig. 2. Effect of heavy metals on DHA in river and dam sediments
***The number after heavy metal symbol is concentration in mg kg^{-1}**

Table 2

Inhibition/activation of PAI (a) and DHA (b) in % at different HMs concentrations

(a)	river sed.	dam sed.	(b)	river sed.	dam sed.
4 mg kg^{-1} Cd	-49.732	-15.551	4 mg kg^{-1} Cd	14.305	59.190
6 mg kg^{-1} Cd	-54.362	-13.214	6 mg kg^{-1} Cd	-5.964	3.260
8 mg kg^{-1} Cd	-56.586	-15.548	8 mg kg^{-1} Cd	-18.694	-81.637
400 mg kg^{-1} Cu	-69.892	-46.498	400 mg kg^{-1} Cu	-93.144	47.204
600 mg kg^{-1} Cu	-70.996	-45.210	600 mg kg^{-1} Cu	-93.017	44.934
800 mg kg^{-1} Cu	-69.230	-42.786	800 mg kg^{-1} Cu	-99.509	44.207
140 mg kg^{-1} Pb	-69.387	-26.959	140 mg kg^{-1} Pb	-34.578	-37.691
210 mg kg^{-1} Pb	-70.081	-27.347	210 mg kg^{-1} Pb	-39.670	-70.651
280 mg kg^{-1} Pb	-72.609	-22.952	280 mg kg^{-1} Pb	-57.494	-86.631
500 mg kg^{-1} Zn	-76.372	-73.195	500 mg kg^{-1} Zn	-63.860	-77.641
750 mg kg^{-1} Zn	-76.241	-74.883	750 mg kg^{-1} Zn	-94.415	-82.636
1000 mg kg^{-1} Zn	-76.221	-74.337	1000 mg kg^{-1} Zn	-95.688	-93.622

tivity – the degree of inhibition is a similar for all working concentrations (Table 2). This result showed that the PAI had a low tolerance to HM impact and the additional HM contamination in studied sediments significantly reduced the activity of enzyme indicator. This was in accordance with studies of Lorenz et al. (2006), who also reported reduced activities for soil enzymes involved in nutrient-cycling due to Cd pollution; and Wang et al. (2007) which results showed a negative effect of Cu and Zn on PAI.

The changes of DHA in all treatments are shown in Figure 2. The DHA of non-treated river sediments was lower than the DHA of dam sediments: 4.358±1.120 and 5.555±2.101 mkM H^+ min^{-1} $mgPr^{-1}$ x 10^{-7} respectively. The effect of different heavy metals was presented heterogeneously. Cadmium in concentration of 4 and 6 mg kg^{-1} activated the DHA with 3÷60% (Table 2). The further increase of Cd-concentration inhibited the enzyme indicator and the effect was more strongly presented for dam sediments with 81.64% inhibition. Contamination with Cu^{++} had a different effect depending on the location of sediments – strong inhibition of DHA in river sediments (more than 93%) and activation in dam sediments (with 44-47%). This result could be explained with different initial (non-treated) level of Cu-contamination in two types of sediments – the concentration was more than twice in river. Pb and Zn had a negative effect on DHA but more clearly presented for Zn. The degree of influence was over 63% for all treatments.

The summary of obtained results was presented in Table 2 as percentage inhibition or activation for each HM and concentration. The native activity was considered as 100%.

Conclusion

The PAI was inhibited by all tested heavy metals but the Zn-ions have the higher impact – the degree of inhibition was over 70%. The Cd and Pb affected in the least degree the phosphatase enzymes in dam sediments. The inhibition was in range of 13÷15% and 23÷27% respectively. Inoculations of sediments with low and moderate concentrations of cadmium stimulated the DHA in wide limits but the further

increasing inhibited the activity over 80% in dam sediments. The Cu^{2+} had a specific effect on the DHA by the location of sediments – a strong inhibition in river sediments (93÷99%) and activation in dam sediments (44÷47%). The Pb^{2+} generally had the least effect on the DHA.

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