BIOREMEDIATION POTENTIAL OF THE SAVA RIVER WATER POLLUTED BY OIL REFINERY WASTEWATER

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ABSTRACT

Microbial enumeration is a screening-level tool which can be used to evaluate *in-situ* response of water microorganisms to petroleum hydrocarbon contamination as well as for evaluating enhanced bioremediation potential of petroleum hydrocarbon contamination. In this investigations the increase between 17- and 44-fold of number of heterotrophs in hydrocarbon contaminated the Sava River water when compared with the no contaminated river water have been recorded. The significant increase of number of facultative oligotrophs in the river Sava water downstream of wastewater discharge (between 70- and almost 100-fold higher number) direct to the conclusion that oligotrophic bacteria (adapted to the environments with low amount of easy-to-degrade nutrients, oligocarbophilic microorganisms) could be better indicator of water bioremediation potential than number of heterotrophic (THR) bacteria. Quantitative composition of heterotrophic, facultative oligotrophic, crude oil degrading, and other physiological groups of bacteria, being, as a rule, higher in samples taken downstream of the waste-water discharge, testify about high biodegradative potential of the River Sava microbial community, if the oil rafinery wastewater is taken into consideration.

Key words: **o**il refinery wastewater, river contamination, bioremediation

INTRODUCTION

According to the methodological approach, presented by Jakšiæ and Matavulj, March, 2001, at the INCO-Copernicus project (Bioremediation Techniques for Detoxication of Hazardous Pollutants in Industrial Waters and Sludges, No IC15-CT-98-0137) workshop in Gent (unpubl.), the indigenous microcosm screening studies have been continued

systematically, with the aim of getting more informations about seasonal properties of bioremediation activity of intrinsic microflora of the contaminated Sava River water.
Microbial enumeration is a screening-level tool which can be used to evaluate *in-situ* response of water microorganisms to petroleum hydrocarbon contamination as well as for evaluating enhanced bioremediation potential of petroleum hydrocarbon contamination. Literature reviews are performed first to assess biodegradability potential. Response of the microbial community, as indicated by significant differences in geometric mean population density in contaminated locations, relative to no contaminated locations (i.e. at least one half an order of magnitude, Schafner et al, 1998), is an indicator of intrinsic bioremediation potential. Because enhanced bioremediation involves stimulating intrinsic bioremediation, the *in-situ* response of the microbial community to PHCs is an important indicator of enhanced bioremediation potential.
Though microbial enumeration studies may be used for assessing microbial activity under oxic as well as anoxic conditions, the focus of this paper is on aerobic biodegradation because bio-oxidation is a relatively energetic pathway for petroleum hydrocarbon contamination.

MATERIAL AND METHODS

The microbial enumeration method identified in this report counts those aquatic micro-organisms capable of colonizing and reproducing, utilizing low-nutrient substrate under aerobic conditions. For water samples, population densities are reported as Total Recoverable Heterotrophs (TRH) which refer to those bacteria recovered from the water or sediment (riverbank mud) sample. TRH include aerobic or facultative anaerobic heterotrophic bacteria, groups that may constitute more than half of total water microbial abundance.
At least 30 genera containing over 100 species of microorganisms are capable of degrading petroleum hydrocarbons (Arthur *et al*., 1991), most of which are bacteria. Besides TRHs, some physiological groups of bacteria that can be responsible for degradation of other: contaminants of oil refinery wastewater effluent have been enumerated, i.e. lipolytic, saccharolytic, and proteolytic bacteria, as well as phenol-oxidizing bacteria. Also, as a specific, and supposed to be the group the most responsible for hydrocarbons degradation, the crude oil-oxidizing bacteria have been enumerated according to Petrovicy *et al*. (1998).
Water and sediment samples for microbial enumeration are collected aseptically from Srpski Brod (Republic of Serbska, B&H) Oil refinery waste-water and the Sava River contaminated water, 200m downstream and no contaminated Sava River water, 200m upstream of the refinery discharge. The water samples were collected on January 23, March 09, April 24 and Mai 28, and sediment sample on January 23, 2001. The standard dilution methods are applied and sediments were treated as water samples considering 1g of sample as 1 cm^3 (1 milliliter) (Matavulj and Molitoris, 1992; Matavulj *et al*., 1993, 1996.).

RESULTS AND DISCUSSION

Changes in bacterial community composition, which typically follow introduction of a new organic carbon source as are petroleum hydrocarbons, are typically accompanied by substantial increases in total bacterial population density following the release, what can be seen at the Figure 1 (THR) and Figure 2 (Facultative oligotrophic bacteria). In this investigations the increase between 17- and 44-fold of number of heterotrophs in

hydrocarbon contaminated the Sava River water when compared with the no contaminated river water have been recorded.

Tan *et al*. (1990) demonstrated a 3,000-fold increase in the indigenous microbial population at a site following a 1,400-gallon diesel fuel spill. The significant increase of number of facultative oligotrophic heterotrophs in the river Sava water downstream of wastewater discharge (between 70- and almost 100-fold higher number) direct to the conclusion that oligotrophic bacteria (adapted to the environments with low amount of easy-to-degrade nutrients) could be better indicator of water bioremediation potential than number of heterotrophic (THR) bacteria.

Though only a small fraction of total biomass is recovered using this method, THR data are meaningful when used comparatively to evaluate microbial activity under different conditions (Zuberer, 1994). The same conclusion arise from our results since comparison of heterotrophic and facultative oligotrophic bacteria number, recorded at different sites (oil refinery waste-water, the river Sava water upstream and downstream of waste-water discharge into the river) enable us to compare and to evaluate microbial activity under conditions of no polluted and polluted aquatic environment.

Microbial enumeration studies include recently enumerations for contaminant-degraders, which count that fraction of recoverable biomass capable of utilizing the contaminant of concern. Methods for enumerating contaminant degraders utilizing the Most Probable Number (MPN) techniques are discussed by Kämpfer and Steiof 1991 and Petrovicy *et al.*, 1998. These methods involve enumeration bacteria on a crude, agarized growth medium containing the contaminant, as well as culturing microorganisms in a liquid growth medium containing contaminant. Enumeration of potential degraders of specific contaminants, such as crude oil oxidizing or phenol-degrading microorganisms in this case, turned to be extremely helpful in evaluating biodegradation potential; in particular where constituents of concern may be targeted (Maksimoviæ *et al.*, 1997; Petroviæ *et al*., 1998; Matavulj *et al*., 2000, Matavulj and Molitoris, 2000).

Our results of enumerating of petroleum degrading bacteria show the significant increase of number of this specific group in the Sava river water downstream of oil refinery wastewater effluent (Figure 3). Such findings are in agreement with the comparative increase of number of lipolytic bacteria (Figure 4), proteolytic bacteria (Figure 5), saccharolytic bacteria (Figure 6) and coliform bacteria (Figure 7) which testify about other kinds of pollutants entering the river Sava water together with hydrocarbon contaminants from the oil refinery waste-water. The same conclusion is supported by comparison of phenol degrading bacteria in the river Sava water upstream and downstream of wastewater effluent (Figure 8).

Investigation of the river Sava sediment was done only in January 2001. Though being not systematic, preliminary results direct to the similar conclusion, as do these based on water bioremediation potential study. Quantitative composition of heterotrophic, facultative oligotrophic and physiological groups of bacteria was, as a rule, higher in samples taken downstream of the waste-water effluent (Figure 9), especially when the most responsible group of bacteria, for hydrocarbon and phenol degradation, were taken into consideration (Figure 10), what testify about increased biodegradative potential of microbial community.

The data about increased biodegradative potential were supported by the evidence of increased rate of heterotrophic biotransformations via biochemical, enzymological analyses. The significant increase of phosphatase activity, shown as Phosphatase Activity Index (PAI) (Matavulj *et al*., 1990, Matavulj and Flint, 1987), testifies about higher biodegradative potential of intrinsic microbial community of the river Sava water (Figure

11) and in the river Sava sediment (Figure 12), as a consequence of higher number of contaminants-degradative bacteria, as well as consequence of increased amount of biodegradable pollutants (Matavulj, 1986).
The informations gained from these studies substantially advance our understanding of microbial hydrocarbons biodegradative potential and will help to predict their response to oil pollution. This prediction will be primarily obtained by the evaluation of the potential of microbial water and sediment population for bioremediation of oil polluted river water and riverbank zones.

CONCLUSION

The primary aim of this research was to evaluate the potential of use of intrinsic microbial community as a means of restoring oil contaminated sites to their original pristine state without the excessive use of dispersants and extensive change in the character of the riverbank features. The ability to exploit and use naturally occurring microbial population such as those present in water and in microbial mats of sediments to degrade pollutants like crude oil is of major significance in the effective management and maintenance of pristine aquatic habitats such as river water, banks, sediments, and beaches.
The significant increase of number of facultative oligotrophs in the river Sava water downstream of wastewater discharge (between 70- and almost 100-fold higher number) direct to the conclusion that number of oligotrophic bacteria could be better indicator of water bioremediation potential than number of heterotrophic (THR) bacteria.
Quantitative composition of heterotrophic, facultative oligotrophic, crude oil degrading, and other physiological groups of bacteria, testify about high biodegradative potential of the River Sava water and sediment microbial community.
The gained technology that arises from these investigations can be employed to develop effective management strategies for the bioremediation of these fragile ecosystems. The development of methods or new techniques and new approaches to microbial population can be transferred to training and educational institutions.

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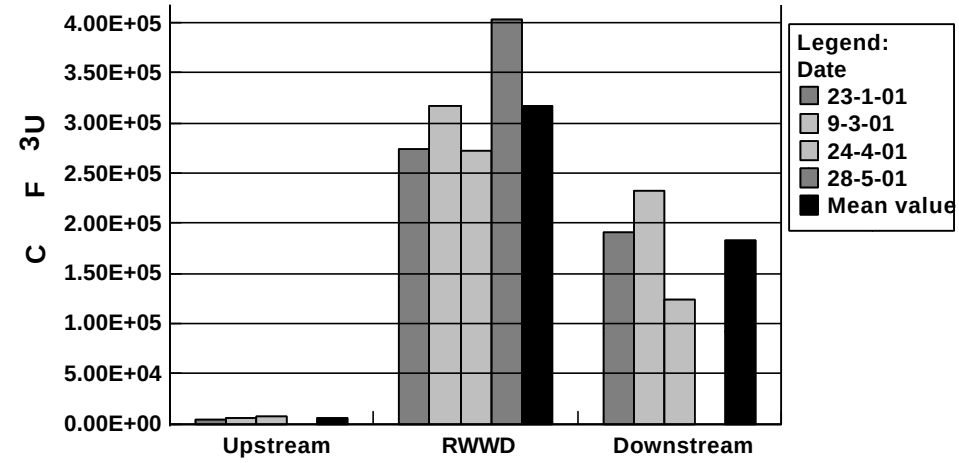

Fig. 1 Quantitative composition of heterotrophic bacteria in the river Sava water and in Srpski Brod oil refinery wastewater discharge (RWWD)

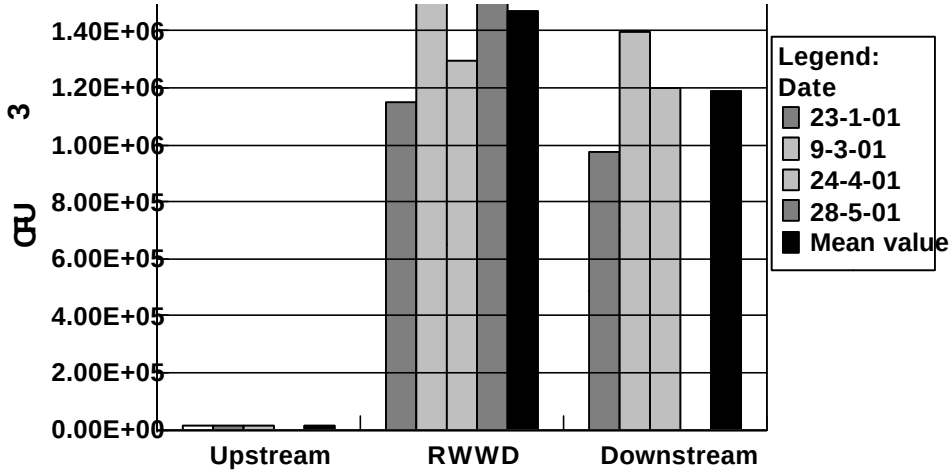

Fig. 2 Quantitative composition of facultative oligotrophic ba in the river Sava water and in Srpski Brod oil refinery wastewater discharge (RWWD)

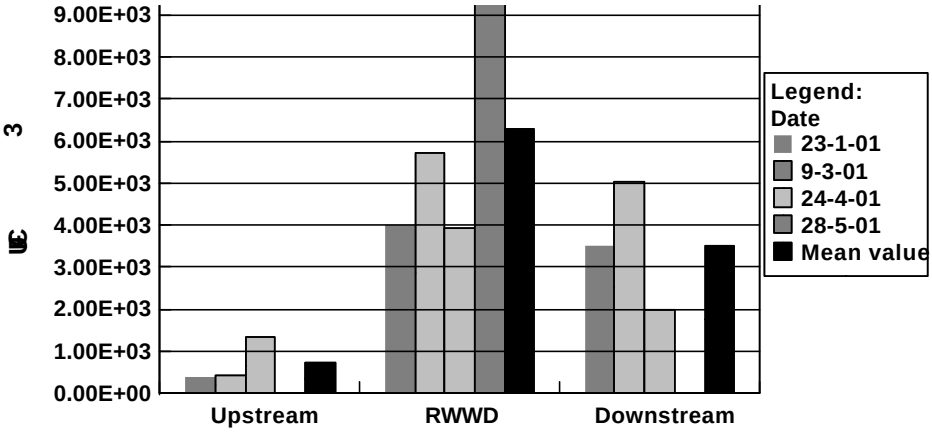

Fig. 3 Quantitative composition of petroleum hydrocarbon degrading bacteria in the river Sava water and in Srpski Brod oil refinery wastewater

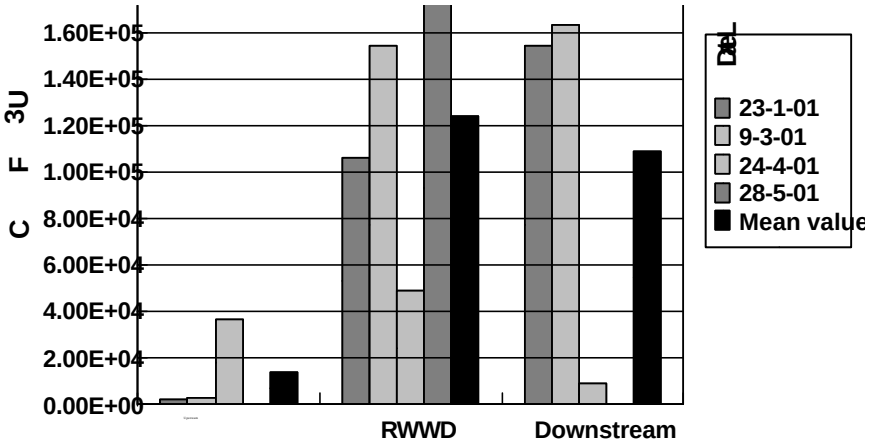

Fig. 4 Quantitative composition of lipolytic bacteria in the river Sava water and in Srpski Brod oil refinery wastewa

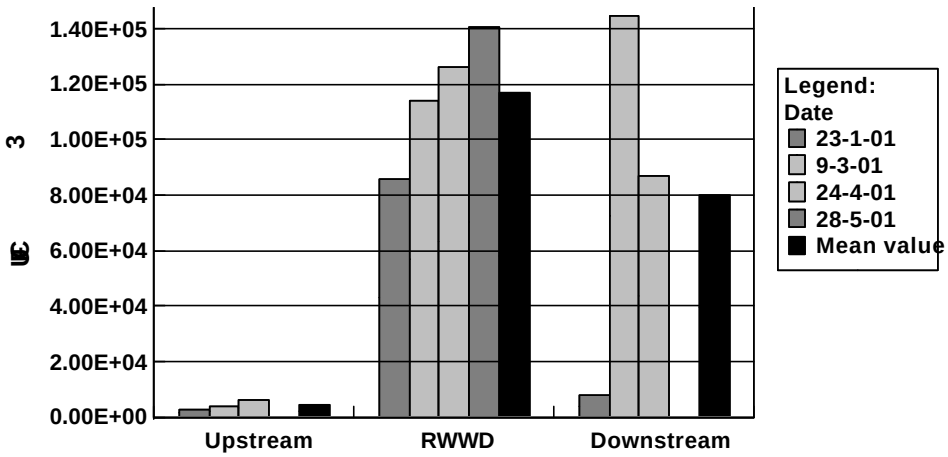

Fig. 5 Quantitative composition of proteolytic bacteria in the river Sava water and in Srpski Brod oil refinery wastewater

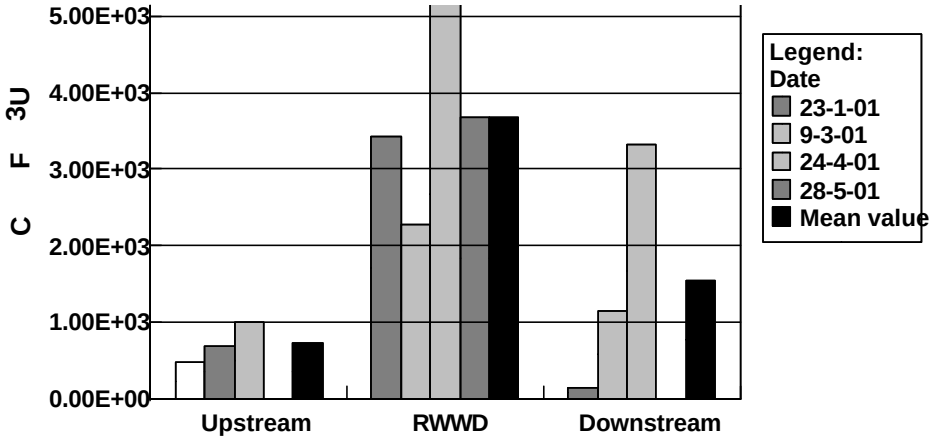

Fig. 6 Quantitative composition of saccharolytic bacteria in the river Sava water and in Srpski Brod oil refinery wastewater

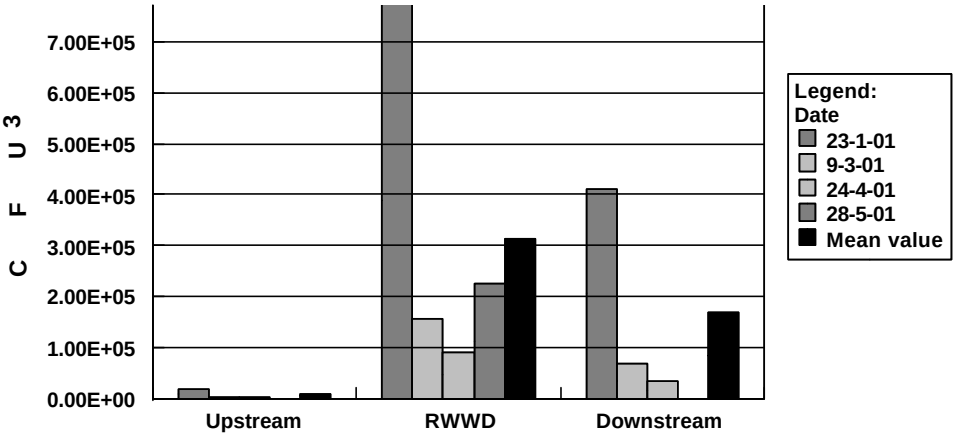

Fig. 7 Quantitative composition of coliform bacteria in the river Sava water and in Srpski Brod oil refinery wastewater

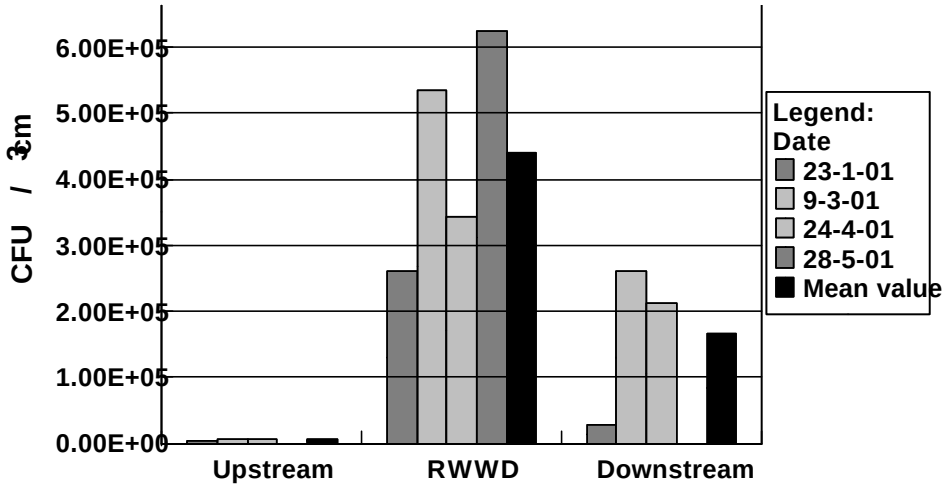

Fig. 8 Quantitative composition of phenol degrading bac in the river Sava water and in Srpski Brod oil refinery was

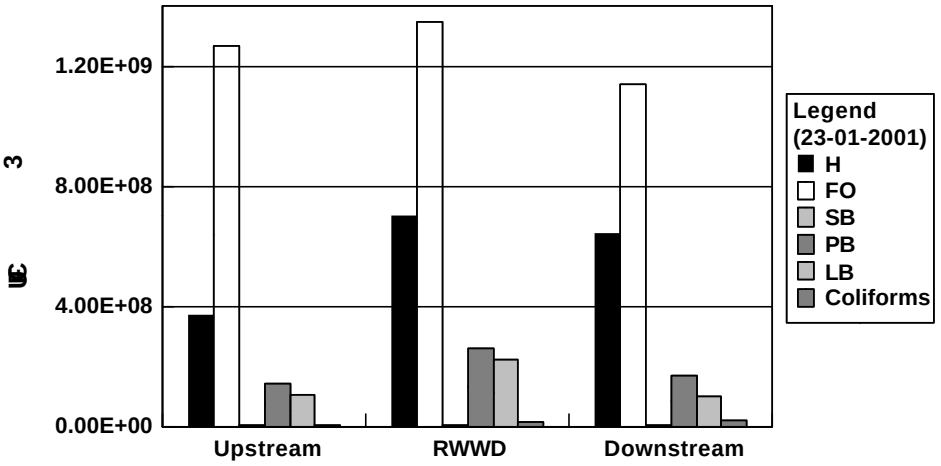

Fig. 9 Quantitative composition of physiological groups of bacteria in the bank mud of the river Sava water and in Srpski Brod oil refinery mud of wastewater

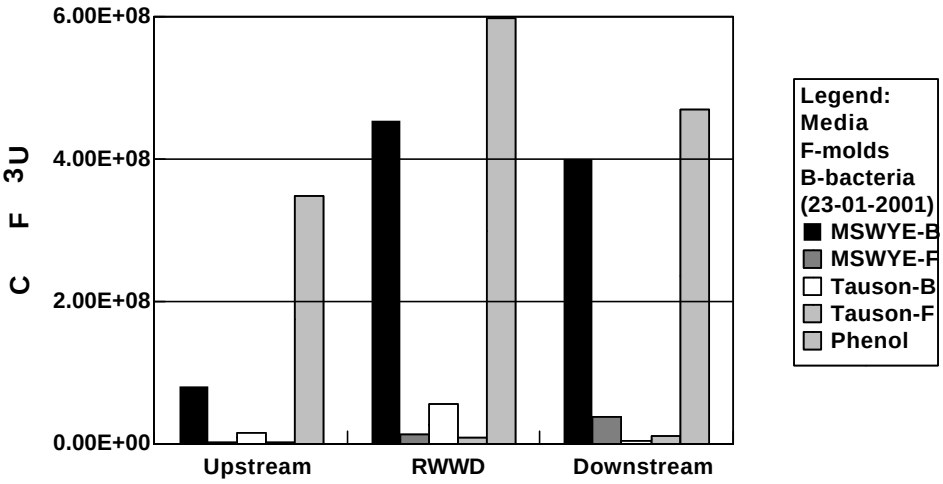

Fig. 10 Quantitative composition of petroleum hydrocarbon and phenol degrading bacteria in the bank mud of the river Sava water and in Srpski Brod oil refinery wastewater mud

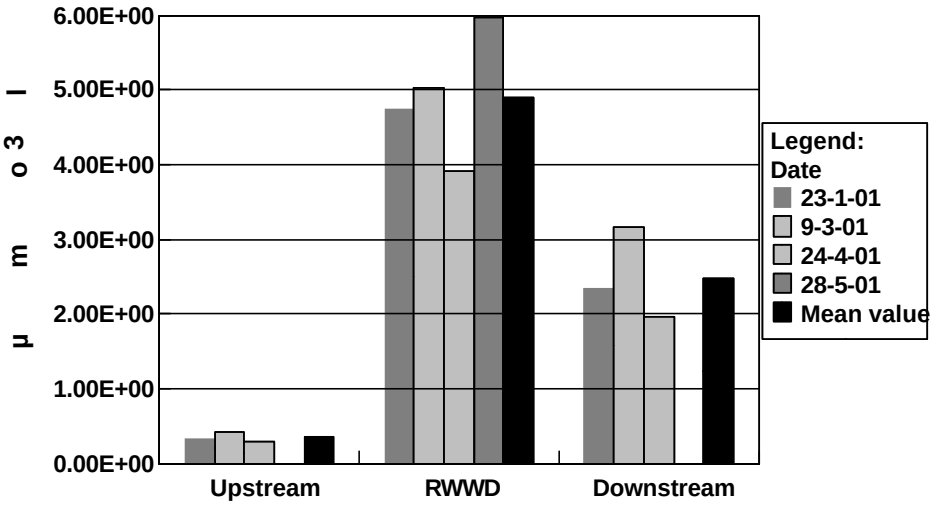

Fig. 11 Phosphathase activity index (PAI) of the river Sava water and of the Srpski Brod oil refinery wastewater

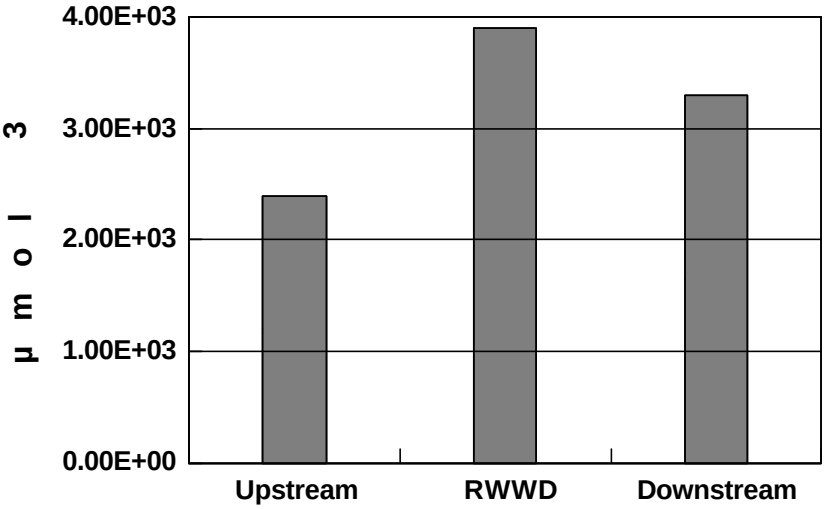

Fig. 12 Phosphathase activity index (PAI) of the river Sava bank mud and of the Srpski Brod oil refinery wastewater mud (23-01-2001)