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Wastewater canal Vojlovica, industrial complex Pančevo, Serbia – preliminary ecotoxicological assessment of contaminated sediment

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Abstract: Effluents collected from the industrial complex of Pančevo, Serbia (oil refinery, petrochemical plant, and fertilizer factory), are discharged into a wastewater canal entering the Danube River. In this study, which was focused on sediment assessment, a complex triad approach consisting of chemical analysis, sediment toxicity tests and macrozoobenthos community analysis was applied. In toxicity tests on sediment elutriates, the following responses were registered – stimulatory effect in algal bioassay, no effect in acute test with *Daphnia magna*, and low to moderate toxicity in the conventional *Vibrio fischeri* test. Moderate to high toxicities were recorded in solid phase tests on *Myriophyllum aquaticum* and *V. fischeri*. High content of Hg, certain PAHs and non-characterised sediment contaminants accumulated over years contribute not only to the registered toxicity, but also to the complete absence of macrozoobenthos. The obtained results proved that regularly measured conventional and priority pollutants are hardly ever the only toxic contaminants present in sediments. Toxicity tests, in particular the contact test, might guide towards a better selection of parameters to be regularly or occasionally monitored. In addition, complete sediment toxicity tests proved to be an appropriate method for assessing the bioavailability of the chemically detected contaminants. The analysis of the macrozoobenthos composition and structure as inevitable part of se-

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diment risk assessment procedures integrates the effects of multiple stressors and gives a realistic insight into not only sediment contamination by toxic pollutants, but also the sediment status in general.

Keywords: sediment; wastewater canal; toxicity tests; test battery.

INTRODUCTION

Water authorities and regulatory bodies have underestimated sediments as integral components of aquatic ecosystems in the past, due to practical problems as well as to the complex nature of sediments. The European Union Water Framework Directive (WFD), aimed at achieving good ecological and good surface water chemical status, is also focused on the water column and considers contaminants from point and diffuse sources, but underestimates the role of sediments as long-term secondary sources of contaminants and, therefore, a possible cause for deterioration of ecological status.[1] Chapman indicated the need for addressing sediments and sediment quality in addition to water quality since: a) various toxic contaminants found in only trace amounts in the water column accumulate in sediments to elevated levels; b) sediments serve as both a reservoir and a source of contaminants to the water column; c) sediments integrate contaminant concentrations over time whereas water column contaminant concentrations are much more variable; d) sediment contaminants in addition to water column contaminants affect benthic and other sediment-associated organisms; e) sediments are an integral part of the aquatic environment, providing habitat, feeding and rearing areas for many aquatic biota.[2]

To evaluate aquatic sediments with respect to their adverse effects on biota and ecosystems as a whole, neither biotests nor chemical analytical techniques alone are sufficient. For instance, sediment chemistry can provide information on contamination, but even with strong evidence of anthropogenic impacts on the benthic community at many sediment sites, the degree of toxicity (or even its presence or absence) cannot be predicted by contaminant concentrations alone.[3] On the other hand, sediment bioassays can yield data with respect to toxic effects in selected test organisms and test systems; however, the selected tests are conducted under laboratory conditions and usually cannot be applied under *in situ* conditions due to varying environmental conditions.[4]

Thus, integrated approaches are required in order to gain insight into the ecological state of sediments. With the sediment quality triad (SQT), Chapman suggested such an integrated approach, which simultaneously investigates sediment chemistry and sediment toxicity, as well as alternations in the field, for example, modifications of benthic community structure.[5]

Chemical data, including sediment quality guidelines (SQGs), have been used for a number of years by regulatory agencies in different countries to assess and manage contaminated sediments.[6,7] Complex evaluation of sediment quality, based on multiple lines of evidence, including biological effect-based assessment (*in situ* and *ex situ* BEBA) has already found its place in environmental risk assessment of many European countries, such as Belgium, the Netherlands and Germany.[8] In Serbia, the official surface water quality monitoring programmes have relied for years mostly on physico–chemical water quality parameters and to some extent on biomonitoring data (saprobic indices based on plankton communities). Ambient standards, now already rather outdated, are only set for water column, while sediment quality criteria have not been established so far.[9] In addition, WET (whole effluent testing), ambient water, and sediment toxicity evaluation have never been included into official monitoring programmes.[10]

Official monitoring programmes and independent studies performed recently identified several hot spots of severe freshwater pollution and soil and sediment contamination that are attributed to historic pollution, outdated environmental policy, regulations and management practice, heavy pollution caused by insufficient waste water treatment as well as major accidental spills (as the result of the NATO campaign in 1999 or the Baya Mare cyanide accident in 2000, for instance).[11–16] The largest individual hot-spot is the industrial complex near the city of Pančevo – effluents are discharged into the wastewater canal Vojlovica, which enters the Danube River.[9]

The wastewater canal Vojlovica was built in 1962 to collect the wastewater discharges from the industrial complex located at the southern end of the city of Pančevo, the so-called "South zone industrial complex" (SZIC), 20 km to the northeast of Belgrade. The SZIC complex includes a petrochemical factory (HIP Petrohemija), an oil Refinery (NIS Rafinerija, Pančevo) and chemical fertilizers factory (HIP Azotara). The canal is about 2 km long, around 76 m wide and directly connected to the Danube River (Fig. 1). The canal is artificial with no natural flows. For a long time, the environmental conditions of the area surrounding

Fig. 1. Map of the study area – wastewater canal Vojlovica entering the Danube River.

the canal have been strongly affected by the presence of the industrial complex, and the 1999 NATO bombing events exacerbated the already existing vulnerable system.[17]

Since August 1999, several surveys have been performed to evaluate the impact of the Pančevo canal on the Danube River, but they were all realised using chemical analysis of the water column and sediments only, providing no data on ecotoxicity. Hitherto, the resident macrozoobenthos community has never been surveyed. First sediment investigations conducted jointly by the UNEP (The United Nations Environment Programme) and the UNCHS (The United Nations Centre for Human Settlements*)* in 1999 and 2001 showed long-term pollution from the industrial complex, consisting, in particular, of mercury and petroleum hydrocarbons components, as well as free phase dichloroethane (DCE) and a high concentration of mineral oil. The main conclusion was that the water in the canal was not significantly polluted, while the sediments were classified as hazardous wastes (class H-11) due to their high content of mineral oil, mercury, PAHs, DCE and BTEX (benzene, toluene, ethylbenzene and xylene – volatile aromatic compounds typically found in petroleum products). UNOPS (The United Nations Office for Project Services) performed another study of the Pančevo canal in 2002, but again based on chemical analyses only. It was found that the pollutants were strongly bound to sediment particles, while sediment pore-water was not significantly affected.[11] The last of the series of official assessments, which eventually resulted in a feasibility study for remediation of the wastewater canal, was performed in 2005/6 by D'Appolonia S.p.A Genoa, Italy, appointed by the Italian Ministry of the environment and territory (IMET).[18] The results of the chemical analysis were in agreement with the previously reported ones, but no ecological impact assessments have been conducted nor any concrete actions undertaken so far, either in terms of dredging or *in situ* remediation.

According to Wenning and Ingersoll, a sediment ecological risk assessment (ERA) should include lines of evidence derived from several different investigations: nature and extent of contamination; expected or acceptable indices of benthic diversity and abundance in the absence of contamination; estimates of the potential for bioavailability, bioaccumulation and adverse effects posed by chemicals and mixtures of chemicals (the potential for chronic and acute effects) on aquatic organisms; stability (fate and transport) of the sediments and contaminants; and estimates of the potential risks posed by contamination to aquatic biota and associated resources.[19]

Therefore, the purpose of this study was to apply the sediment quality triad, which integrates chemical, ecotoxicological and macrozoobenthos community analysis, to evaluate not only the quality or level of contamination of the Vojlovica canal sediment but also to provide a more realistic assessment of ecological impact that long-term simultaneous contamination by diverse toxic pollutants might pose on the macrozoobenthos and aquatic ecosystems as a whole. The application of several different toxicity tests, using different test species covering all three trophic levels (primary producers – algae *Pseudokirchneriella subcapitata*, aquatic macrophyte *Myriophyllum aquaticum*, consumers – represented by the zooplankton *Daphnia magna* and decomposers – the bacteria *Vibrio fischeri*), test set-ups (solid phase and elutriate) and end-points (primary production, growth, mortality and bioluminescence) was intended to lead to the selection of the most appropriate tests for risk assessment of sediments contaminated by oil and petrochemical industry effluents.

EXPERIMENTAL

Sample collection

Grab sediment samples (surface sediments layer of 5 cm) were taken by a Van Veen sampler for macrozoobenthos community analyses, transported in plastic bags and stored in the laboratory at +4 °C. For comparative purposes, the same types of samples were also taken from the navigation canal flowing parallel to wastewater canal but not receiving any direct discharge of industrial wastewaters. The sediment samples were washed out and sieved through a system of sieves of various mesh size. The identification of macroinvertebrate taxa was performed using standard taxonomical keys.[20]

The sediment samples for chemical analyses and toxicity tests were taken by an Eijkelkamp corer (surface sediments of 40 cm depth) at three sampling sites along the wastewater canal Vojlovica:

V1 sampling site – downstream from the fertilizer factory outlet;

V2 sampling site – downstream from the petrochemical plant and oil refinery outlets;

V3 sampling site – near the confluence of the wastewater canal and the Danube River.

The collected sediment samples were stored at +4 °C in glass jars during transport to the laboratory and then stored at –20 °C. Sediment samples for chemical analyses and toxicity tests on elutriates were freeze-dried and sieved (0.072 mm), while bulk sediment samples were used for solid phase toxicity tests.

Chemical analyses

The total organic carbon (*TOC*) was determined using a high temperature LiquiTOC II analyzer (Elementar Analysensysteme). The heavy metal contents (Cd, Cr, Cu, Hg, Pb, Ni and Zn) in the sediment samples were evaluated based on the aqua regia leaching process according to the ISO 11466 protocol,[21] modified with respect to the employed analytical instrumentation (3 g of dry sediment sample was leached overnight by 7 ml of HNO_3 and 21 ml of HCl and then heated under reflux for 2 h; after cooling, the mixture was filled up in the volumetric flask and diluted before inductively coupled plasma mass spectrometry (ICP–MS) measurement on Agilent 7500ce instrument, Agilent Technologies, Japan. Mercury was determined by the thermo-oxidation method using an AMA-254 analyzer (Altec).

Sediment samples for 16 standard PAHs, and total PCB (standard mixture of 7 PCB congeners: PCB28, PCB52, PCB101, PCB153, PCB180 and PCB 209) analyses were prepared according to the standard methods,[22] and extracted in a Soxhlet apparatus with carbon tetrachloride (100 ml) for 6 h. Those extracts were passed over dry aluminium oxide. Samples were mixed with methanol (5 g wet sediment per ml of methanol) and extracted with a dichloromethane–hexane mixture (1:1) on a magnetic stirrer for 1 h. Elemental sulphur was removed by adding Cu powder. The samples were fractionated over silica gel according to the EPA 3630C procedure.[23] The extracts were analyzed by GC–MS and GC–μECD (HP 5890GC Series II with a 5971 MSD) in the splitless mode. The practical quantisation limit (*PQL*) and method detection limit (*MDL*) for all compounds were 5 and 2 ng g^{-1}, respectively. Recovery for the applied method for PAH analyses was determined for phenanthrene and chrysene using soil samples spiked at the 20 μg kg^{-1} level. Recovery values (mean of 3 measurements) were 64 % for phenanthrene and 78 % for chrysene.

Toxicity tests

Sediment elutriates were prepared according to ISO 14735.[24] Lyophilised sediment samples were mixed with distilled water in a 1:10 ratio, put in closed flasks and shaken for 24 h on an automatic shaker with 5–10 rpm. After the sediment particles had settled, supernatant was used for the following toxicity tests: green algae *P. subcapitata*, invertebrate *D. magna*, and the test with the luminescent bacteria *V. fischeri*. Solid phase toxicity tests were performed with *V. fischeri* and with the aquatic plant *M. aquaticum.*

The algae were cultured under sterile conditions at a temperature of 23±2 °C, constant aeration and illumination of 60–120 μE m^{-2} s^{-1} in Zehnder–Bristol (modified Bold) (ZBB) medium. Three days before the test, sterile ZBB medium was inoculated with algae preculture with a cell density of 5×10^3–1×10^4 cells ml^{-1} in order for the algae to reach the exponential phase at the beginning of the test (approximately 1×10^6 cells ml^{-1}). The test was performed using a modified ISO protocol,[25] in microtitre plates in the following v/v concentrations of elutriate: 1.5, 3, 12.5, 25 and 75 %, in five replicates in three repetitions. The microtitre plates were placed on an automatic shaker in order to keep the algae cells in suspension and to prevent their sedimentation. The temperature and light regimes were the same as for the culturing. After 72 h, the algal optical density was measured at 680 nm using a spectrophotometer BioTek, PowerWave at the start and the end of the test. The results were transformed into number of algal cells using calibration curves and the specific growth rate was calculated for every elutriate concentration. Inhibition of the growth rate was determined in comparison to the control.

D. magna was cultured in standard synthetic water according to the United States Environment Protection Agency (USEPA) method in 10 l aquariums with constant aeration, a temperature of 25±2°C and illumination of 10–20 μE m^{-2} s^{-1} with photoperiod 16 h light/8 h dark.[26] The daphnids were fed daily with algae *P. subcapitata*. The procedure described in ISO 6341 was applied in the toxicity test,[27] *i.e.*, 24 h-old daphnids (neonates) were placed in 50 ml test vessels with 30 ml of test medium. The test was performed with 5 neonates in each vessel, 4 vessels per dilution. The test conditions were the same as for the culturing regarding the temperature and light regime, but the neonates were not fed during the test. Elutriates were tested in the following v/v concentrations: 3.12, 6.25, 12.5, 25 and 50 %. Immobilisation of the neonates was observed after 24 and 48 h and the results were compared to the control.

Toxicity tests on elutriates with freeze-dried *V. fischeri* bacteria, obtained from Macherey-Nagel, Germany, was conducted according to ISO 11348 (1998).[28] The luminescent bacteria were reconstituted in cold 2 % NaCl solution, equilibrated at 0 °C for 30 min, transferred to test tubes and equilibrated for an additional 15 min at 15 °C. In the test with *V. fischeri*, the following v/v concentrations of elutriates were tested: 5, 10, 20, 40 and 80%. In all test treatments, NaCl was added in order to obtain 2 % NaCl in the elutriates. Bioluminescence was measured in time 0 and 30 min using Lumino m90a illuminometer at a working temperature of 15 °C. The decrease in luminescence in each treatment was compared to the control treatment and the percent inhibition was calculated using linear regression. The test was conducted in duplicate in two repetitions.

A sediment suspension for the solid phase test with *V. fischeri*, which was performed according to the method described by the Environment Canada, was made by mixing 7 g of fresh sediment with 35 ml of 2 %NaCl solution.[29] This working suspension was diluted to the defined geometrical range of the test concentrations 30.78–985 mg l^{-1}, with mixing on a vortex between the dilution steps to prevent settlement of the sediment particles. The test was realised in duplicate in two repetitions for a duration of 15 min at 15 °C. The bioluminescence was measured only at the end. For each test concentration, the bioluminescence was compared to the control and the percent inhibition was calculated. In both tests with *V. fischeri*, 2 % NaCl was used as the control.

M. aquaticum was cultured in a modified Steinberg medium,[30] at a constant temperature (24±0.5 ºC) with a 24-h photoperiod. The toxicity test was performed according to Feiler *et al*.,[31] *i.e.*, three whorls were placed in one glass vessel containing 80 g of bulk sediment, three vessels per sediment sample. Artificial sediment, as specified in the OECD Guideline 218, was used as the control,[32] in six replicates, and three whorls in each replicate. In order to minimize evaporation during the test, all vessels were covered with a glass lid and a few ml of Steinberg medium was added to each vessel daily. The plants were incubated for 10 days under constant temperature (24±0.5 °C), and constant light (neutral white 60–75 μE m^{-2} s^{-1}). The weight of each whorl was measured at the start and the end of test and compared to the control. The toxicity of the samples was quantified as % inhibition in comparison to the control.

RESULTS AND DISCUSSION

Chemical analyses

The first step in the complex assessment of the site was to analyse sediments for expected toxic pollutants. Taking into consideration the type of industrial effluents discharged into the canal, as well as the results of previously conducted studies,[11,17,18] the sediment samples were analysed for TOC, heavy metals, PAHs and PCBs.

TABLE I. Concentration of heavy metals (mg per kg of dry weight) in the sediment samples

Sampling site	Cd	Cr	Cu	Hg	Pb	Ni	Zn
V1	1.26	33.4	42.4	32.2	23.5	31.7	146
V2	1.41	38.1	43.8	9.62	26.3	31.8	170
V3	1.17	21.8	37.4	6.31	19.2	25.7	111
ICPDR JDS Danube sediment quality target values	1.2	100	60	0.8	100	50	200
Dutch target value	0.8	100	36	0.3	85	35	140
Rhine target value	1	100	50	0.5	100	50	200

For this artificial waterbody entering the Danube River, ICPDR JDS (Joint Danube Survey of International Commission for the Protection of the River Danube) – sediment quality target values were taken as reference concentrations,[33] while for comparative purposes, Dutch[34] and Rhine[35] target values are also shown. The measured concentrations of Hg (6.31–32.2 mg kg^{-1} dw) were considerably higher than all three reference concentrations and Cd (1.17–1.41 mg kg^{-1} dw) was slightly higher than the reference concentrations for the Danube and Rhine target values, while the content of other metals did not exceed the target values (Table I). The Dutch target values, the most stringent of the three reference values, could be used for comparison only with certain reservation as they are set for sediments normalised to 10 % *TOC* and 25 % clay, while the *TOC* values in the Vojlovica wastewater canal (Table II) ranged from 6.8–7.5 %. Nevertheless, it is obvious that the contents of Cd, Cu, Hg and Zn exceeded the Dutch target values, while the Ni concentration was only slightly below. The two remaining metals, Cr and Pb, were the only two with measured concentrations well below the Dutch target values. However, only in the case of Hg was the Dutch intervention value (10 mg kg^{-1} dw) exceeded in sample V1, while the content in sample V2 was only slightly below.

TABLE II. Content of total organic carbon (*TOC*) in the sediment samples

Sampling site	*TOC* / %
V1	6.78
V2	6.85
V3	7.49

When compared to the USEPA recommendations for sediment quality,[36] which set consensus-based threshold effect concentration (*TEC*) values, below which harmful effects are unlikely to be observed, and consensus-based probable effect concentration (*PEC*) values, above which harmful effects are likely to be observed, only the content of mercury in all three samples exceeds both the *TEC* and *PEC* values (0.18 and 1.06 mg per kg of dry weight (dw), respectively). The chromium and lead contents in all three samples were below the *TEC* values (43.3 and 35.8 mg per kg of dw, respectively). The Canadian sediment quality guidelines,[37] which set values for interim sediment quality guidelines (*ISQG*) and a probable effect level (*PEL*) were also used to evaluate the quality of the Vojlovica sediments. Again, mercury stands out, with the measured concentrations in all three samples being above the *ISQG* and *PEL* values (0.17 and 0.49 mg kg^{-1} dw, respectively), while the measured concentrations of Pb (19.2–26.3 mg kg^{-1} dw) in all three samples were below both the *ISQG* (35.0 mg kg^{-1} dw) and *PEL* (91.3 mg kg^{-1} dw) values. The measured concentrations of Cu (37.4–43.8 mg kg^{-1} dw) and Cd (1.17–1.41 mg kg^{-1} dw) in all samples were above the *ISQG* (35.7 and 0.6 mg kg^{-1} dw, respectively), but below the *PEL* values (197 and 3.5 mg kg^{-1} dw, respectively). The chromium concentration in sample V2 (38.1 mg kg^{-1} dw) and the zinc concentrations in samples V1 and V2 (146 and 170 mg kg^{-1} dw, respectively) were just above the *ISQG* values (37.3 mg kg^{-1} dw for Cr and 123 mg kg^{-1} dw for Zn).

The highest concentrations of total 16 PAHs (35.02 mg kg^{-1}) with a high content of compounds of lower molecular weights was measured in sample V1 (Table III). If compared to the USEPA recommendations,[36] the content of total PAHs in all samples exceeded the *TEC* value (1.61 mg kg^{-1} dw), while only in sample V1 did it exceeds the *PEC* value (22.8 mg kg^{-1} dw). The USEPA also sets *TEC* and *PEC* values for individual PAHs. Values exceeding both the respective *TEC* and *PEC* were recorded for anthracene, fluorine, and naphthalene in sample V1; benzo(*a*)anthracene in sample V2 and pyrene in samples V1 and V2.

TABLE III. Concentration of PAHs (mg kg^{-1} dw) and total PCBs (μg kg^{-1} dw) in the sediment samples

Compound	V1	V2	V3
Naphthalene	5.81	0.21	1.18
Acenaphthylene	4.25	0.96	1.20
Acenaphthene	2.58	0.51	0.64
Fluorene	4.01	0.64	0.70
Phenanthrene	11.06	3.72	2.85
Anthracene	1.17	0.37	0.28
Fluoranthene	0.82	0.36	0.28
Pyrene	3.04	2.41	1.24
Benzo(*a*)anthracene	0.73	1.21	0.49
Chrysene	1.02	1.21	0.49
Benzo(*b*)fluoranthene	0.08	0.12	0.06
Benzo(*k*)fluoranthene	0.08	0.12	0.06
Benzo(*a*)pyrene	0.15	0.25	0.08
Dibenzo(*a*,*h*)anthracene	0.08	0.03	0.02
Benzo(*g*,*h*,*i*)perylene	0.06	0.09	0.04
Endeno(1,2,3-*cd*)pyrene	0.08	0.03	0.02
Total PAH, mg kg^{-1} dw[b]	35.02	12.25	9.61
Sum PAH[a], mg kg^{-1} dw	20.90	7.54	3.68
Total PCBs, μg kg^{-1} dw	116.12	164.57	83.2

[a]Summed PAH: naphthalene, phenanthrene, anthracene, fluoranthene, chrysene, benzo(*a*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, benzo(*g*,*h*,*i*)perylene; [b]dry weight

Dutch sediment quality values are set for Sum PAH (see explanation below Table III); the target value being 1 mg kg^{-1}, while the intervention value is 40 mg kg^{-1}. Obviously, the Sum PAH values in all samples exceeded the target value but remained below the intervention value.

The Canadian sediment quality guidelines are set for several individual PAH compounds.[37] The contents of naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene and pyrene along the whole canal exceeded both the theoretically and empirically probable effect values.

The content of PCBs was far below the Dutch target and intervention values, as well as the Canadian PEL (277 μg kg^{-1} dw) and the USEPA PEC (676 μg kg^{-1} dw) values.

From the presented results, it could be conservatively concluded that among the heavy metals, mercury could be identified as one of the possible key toxic pollutants that might cause or contribute to the severe impairment of the overall ecological conditions in the wastewater canal. The PCBs can be seen as a minor problem, while the high content of certain PAH compounds could be identified as other potential stressors. However, standard chemical analyses say very little about the bioaccessibility and bioavailability of toxic pollutants. Therefore, the second step of the triad approach consisted of toxicity tests of bulk sediments and elutriates – using various test species and test designs to estimate if the obviously present toxic pollutants in potentially toxic concentrations could be identified as the key toxic pollutants that might pose risks to the aquatic ecosystem as a whole.

Toxicity tests

The results of algae growth inhibition tests performed on three elutriates are shown in Fig. 2. Obviously, the tests did not result in typical dose-response curves, on the contrary, statistically significant (one-way ANOVA, $p \leq 0.05$) stimulatory effects in comparison to the control were observed in treatments with higher proportions of elutriate.

Statistically significant growth stimulation in comparison to the control was observed in treatments with ≥12.5 % elutriate (v/v) in the cases of samples V1 and V2 and in the treatment with 75 % elutriate (v/v) in the case of sample V3. Statistically significant growth inhibition in comparison to the control was registered only in the treatments with the lowest elutriate content (1.5 % v/v), *i.e.*, in samples V2 and V3. These findings are not surprising, as different authors have reported many times before similar outcomes of algal toxicity tests of environmental samples. These results can be explained by higher nutrient contents in treatments with higher proportions of elutriates in comparison to the control, indicating, on the other hand, that the aqueous phase does not contain bioavailable toxic pollutants in toxic concentration for the selected species.[38–41]

No negative effects on immobilisation were observed in the 24 and 48 h acute toxicity tests on sediment elutriates. Since *D. magna* is one of the most sensitive test species to heavy metals,[42] the fact that no negative effects were observed suggested, in agreement with the results of the algal test, that the metals present in the bulk sediment, particularly Hg which is, according to chemical analysis, present in concentrations that might cause toxic stress, are not readily bioavailable. Contrary to this, it was reported by Akkanen and Kukkukonen that *D. magna* is relatively insensitive to organic pollutants, PAHs in the first place, due to their ability to biotransform these groups of contaminants.[43] Therefore it should not be a surprising finding that the present PAH compounds did not have any effect on daphnids in the acute test. Moreover, the negative results of this acute test also suggest that other contaminants were present neither in lethal concentrations nor in a bioavailable form.

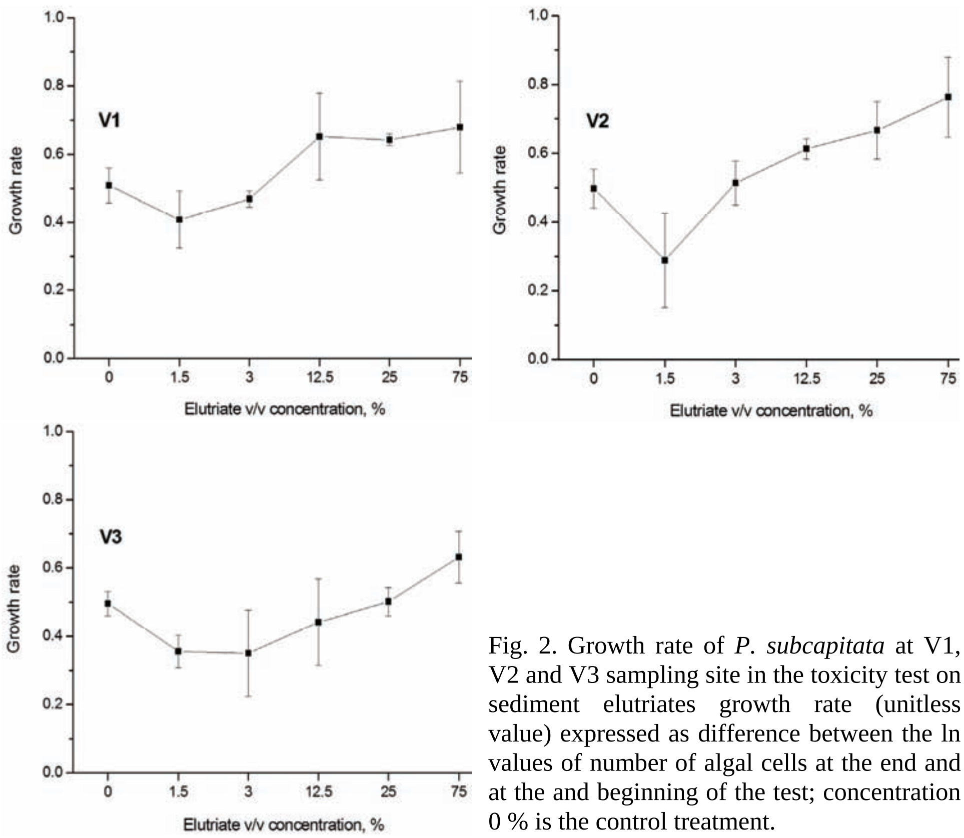

Fig. 2. Growth rate of *P. subcapitata* at V1, V2 and V3 sampling site in the toxicity test on sediment elutriates growth rate (unitless value) expressed as difference between the ln values of number of algal cells at the end and at the and beginning of the test; concentration 0 % is the control treatment.

The results of toxicity tests on sediment elutriate with the bioluminescent bacteria are shown in Fig. 3.

The sample V1 was identified as the most toxic with respect to the selected test species and set-up, EC_{50} 58.73 % and EC_{10} 4.32 %. For samples V2 and V3, only EC_{10} could be estimated at 23.21 and 24.70 %, respectively. Since bacterial species, including *V. fischeri*, were proved rather tolerant to metals,[44–46] it is more likely that the high content of total PAHs and the concentrations of several individual PAH compounds could be identified as potential causes of the observed toxic effect. Sensitivity of *V. fischeri* to certain PAH compounds has been proven earlier.[47–49] However, the testing of samples V2 and V3, which resulted in the hormesis effect in treatments with low elutriate contents, contradicts this hypothesis, as sample V2, that was non-toxic to *V. fischeri* was also characterised by high contents of several PAH compounds as well as total PCBs. Therefore, it could be hypothesised that other, non-characterised contaminants might be the possible cause of the observed toxicity of sample V1 to *V. fischeri*. Stimulatory effect in *V. fischeri* toxicity test in presence of sub-lethal concentrations of toxicant has already been observed and reported.[45,50,51]

Sediment solid phase toxicity tests with *V. fischeri* (Table IV) identified sample V2 as the most toxic (EC_{50} 17.03 mg l^{-1}), while for samples V1 and V3, 50 % inhibition was calculated at considerably higher levels (158.60and 342.11 mg l^{-1}, respectively).

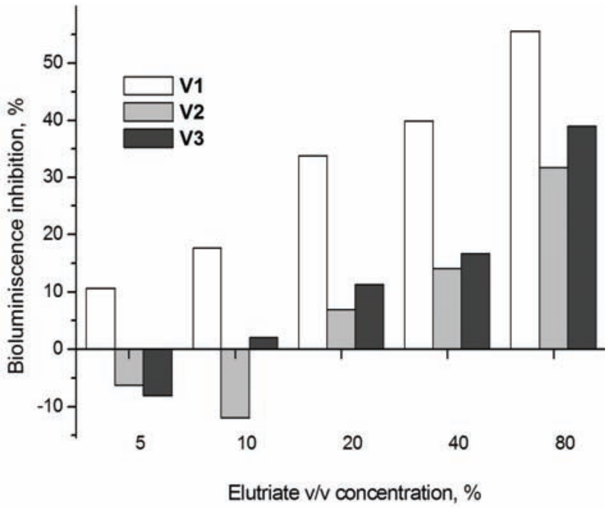

Fig. 3. Inhibition of bioluminescence in the test on sediment elutriates with *V. fischeri*.

TABLE IV. Results of solid phase toxicity tests with *V. fischeri*

Parameter	V1	V2	V3
EC_{50} / mg l^{-1}	158.60	17.03	342.11

According to chemical analysis, sample V2 was rich in several PAH compounds and heavy metals other than Hg. Again, the high content of Hg in sample V1 seems not to cause substantial effects on *V. fischeri*, which was expected due to low sensitivity of the selected species to metals. Thus, metals in sample V2 could not be regarded as the key pollutant group responsible for the high toxicity of sample V2 to *V. fischeri*. Again, it could be speculated that other, not measured contaminants could contribute to the toxicity of sample V2.

The results from sediment solid phase toxicity tests on *M. aquaticum* (Table V) are in agreement with the results from the conventional *V. fischeri* test; sample V1 was characterised as the most toxic – growth inhibition reached 70.35.% in comparison to the control, while with the other two samples, V2 and V3, growth inhibition did not even reach 50 %.

TABLE V. Growth inhibition of *M. aquaticum* in the sediment solid phase test

Parameter	V1	V2	V3
Growth inhibition, %	70.35	33.33	33.90

M. aquaticum is commonly used as test species in toxicity test on pesticides,[52,53] studies on the bioaccumulation of heavy metals,[54] and recently in sediment toxicity tests.[5,55] Stesevic *et al*. found *M. aquaticum* to be highly sensitive to metal burden in sediments of Lake Skadar, which could also explain the growth inhibition in samples rich in metals, observed in the present study.[55]

Analyses of the macrozoobenthos community

Analyses of the macrozoobenthos community were performed on sediment samples in the final tier of the sediment quality triad. The complete absence of a macrozoobenthos community was observed in all three samples taken from the wastewater canal. However, analyses of the macrozoobenthos community of the navigation canal, which flows parallel to the wastewater canal, indicated that the natural conditions are favourable for typical lowland slow-flowing rivers and canals, as it is represented by 4 groups Gastropods (1 species – *Viviparus sp*.), Decapoda (1 species – *Astacus* sp), Diptera (Chironomidae) and the dominant Oligochaeta (represented by three typical species tolerant to muddy substrates, low oxygen concentrations and high organic load – *Branchiura sowerbyi, Limnodrilus hoffmeisteri and L. claparedeanus*). These rather alarming findings on the complete absence of a macrozoobenthos community are not unique, but they are still exceptional in the Vojvodina region. They were reported several years ago for only a few other hot spots along the Danube–Tisa–Danube irrigation network, mainly due to severe organic load resulting in a constant oxygen deficit and the presence of hydrogen sulphide.[56,57] However, the absence of benthic invertebrates in the wastewater canal could not be attributed to similar reasons, as the characterisation of the sediments showed a medium content of organic matter (*TOC* ranging slightly from 6.8 to 7.5 %), which implies that toxic pollution might be seen as the cause, or at least the confounding factor, of the absence of macrozoobenthos. The absence of macroinvertebrates from all samples supports the hypothesis that apart from the measured expected pollutants, the whole sediment toxicity must be also attributed to other, not regularly monitored contaminants, as the overall conditions in the wastewater canal are equally unfavourable to macrozoobenthos despite the fact that the content of the monitored pollutants varies considerably between the different samples, being almost negligible in sample V3.

Integrative assessment of the level of contamination of the Vojlovica canal sediments

For an integrative assessment of the site of the present study, a complex triad approach, which uses multiple lines of evidence of ecosystem disturbances caused by constant contamination, was applied. The results of chemical analyses, restricted to the expected contaminants commonly measured in sediments under the direct pressure of effluents originating from the oil industry, did not, and could not, reveal anything new in comparison to previously conducted studies. Instead, the present results simply confirmed the already existing findings regarding the high content of Hg and PAH compounds in the canal sediments. The authors of the cited studies, however, did not have the possibility to compare their findings with any regionally relevant sediment standards, since those for the Danube River have been suggested only recently. In addition, they refrained from comparing their results with biologically derived standards, such as the Canadian or USEPA. In direct comparison, as shown in Table I, it is obvious that the contents of all the measured metals (except Hg) were still below the Danube River target values. In addition, a comparison of the present findings with other sediment standards, such as the Canadian or USEPA, which are based more on the probable effects of sediment-bound contaminants to aquatic biota, also support the conclusion that among metals Hg really stands out as the potential key toxic pollutant, while contamination with standard PAH compounds, as expected, proved to be rather severe, as it exceeded all biologically derived standards used for comparison, indicating that this group of compounds most probably could cause toxic effects to aquatic biota.

The sampling sites were selected to enable discrimination of the impact of two industrial facilities, which discharge different types of effluents. The content of Hg, as expected, was the highest in sample V1 showing that the long-term discharge of effluents from the vinyl chloride plant has resulted in the accumulation of high quantities of Hg in relatively stable sediments; the concentrations of Hg further downstream (V2 and V3) are considerably lower. However, the highest concentrations of all other metals were recorded in V2. As far as other compounds are concerned, contrary to expectations, the highest value of total PAHs was recorded in sample V1, while V2 was characterised by the highest measured concentrations of PCBs and several individual PAH compounds (Table III). Thus, no clear contamination gradient was observed along the canal, apart from the fact that the V3 sampling site is characterised by the lowest concentrations of the majority of the investigated contaminants. This can be explained by the position of the sampling site in relation to the Danube River; in comparison to the other sampling sites, it is more affected by changes in the Danube water level, which washes out the most downstream sediments.

Toxicity tests followed the chemical analyses with the idea of testing the whole sediment toxicity, which would aid the assessment of the bioaccessibility and bioavailability of all the contaminants present, analysed or non-analysed. Another objective of the toxicity testing was to attempt to establish links between the content of the measured contaminants and their possible effect on biotic systems and the ecosystem in general in the search for an answer to whether the measured contaminants are really the key toxic pollutants. Sample V3 proved to be the least toxic to all selected test species, regardless of the test set-up and sediment phase tested. This was to have been expected, due to the lowest content of contaminants recorded, as just explained above. However, the toxicity testing of samples V1 and V2 resulted with rather inconsistent outcomes. Sample V1 was the most toxic of the 3 samples tested using *V. fischeri* in the aqueous phase, while sample V2 was most toxic to *V. fischeri* in the solid phase.

The differences in the test results with aqueous and solid phase can be explained by different properties of the test phases – in tests on the aqueous phase, only soluble the fraction of the contaminants present in the samples contributed to the overall toxicity, while in the direct contact test, the effects of contaminant bound to the sediment particles are taken into account as well. However, taking into consideration all the differences between the test set-ups and relative sensitivity of the chosen species towards respective environmental contaminants, it could be speculated that the measured contaminants are not the only toxic pollutants present in the Vojlovica canal sediments that contribute to the observed sediment toxicity. This speculation is supported by recently published results on an assessment of Vojlovica sediment performed by Effect Directed Analyses (EDA) using micro-EROD and cell proliferation tests in different fractions obtained from the same samples tested in the study.[58] Chemical analysis of the most active fractions revealed high concentrations of methylated PAHs and other alkyl-substituted PAHs. Only minor portions of biologically derived TCDD-EQs could be attributed to the monitored PAHs with known relative potencies (*REP*s). Therefore, it is hypothesised that a major part of the activity was due to non-monitored alkylated and heterocyclic PAHs. The results of the cell cytotoxicity/proliferation assay on rat hepatoma H4IIE cell line also suggested the presence of uncharacterised sediment pollutants with pronounced potency to disturb cell growth. All these speculations are supported by the results of macrozoobenthos community analysis. Its complete absence along the whole canal, even from sample V3 characterised by the lowest content of the measured pollutants as well as the lowest toxicity to all selected species, indicate not only extreme contamination of the canal sediment, but also unfavourable overall conditions in the canal.

Selection of the appropriate tests for sediments contaminated with oil refinery and petrochemical effluents

In the tests on elutriates, inhibition was observed only in the toxicity test on *V. fischeri*. Since no toxic or inhibitory effects were observed in the algal and crustacean tests, *V. fischeri* proved to be the most sensitive species in the selected test battery. Davoren *et al.*, in their assessment of contaminated estuarine sediments, found *V. fischeri* as the most sensitive species in comparison to algal tests and even to the survival test with *Artemia salina*.[40]

As test species, algae are predominately sensitive to herbicides and to some extent to heavy metals,[59] while their relative tolerance towards organic contaminants apart from pesticides is well documented.[60] On the other hand, both solid phase tests were sensitive enough to detect the toxic effects of the investigated sediments, proving that contact tests have by far a greater potential in risk assessment of contaminated sediments than aqueous phase tests. It has been proven repeatedly, including this study, that sediment-bound contaminants are neither readily bioavailable nor, consequently, easily extractable by overlying water. Therefore, a more realistic estimate of the whole sediment toxicity, as a part of a complex sediment risk assessment procedure, could be obtained using sediment contact tests. The *V. fischeri* solid phase test has already shown some of advantages, but care should be taken with the interpretation of the results obtained by its application, as it has been well documented that the species is highly sensitive to hydrogen sulphide, which is often present in contaminated sediments.[61,62]

Aquatic organisms of the three trophic levels (producers, consumers, reducers) have been used for a long time for ecotoxicological assessments of water quality. Algae, daphnia, and luminescent bacteria are often used as test organisms in biotest systems that have become established standards. However, the results of the present study, as well as comparative studies, have shown that algae are not sufficiently sensitive to a large number of contaminants and thus cannot be the exclusive indicator organism of any toxic effects on autotrophic life. Moreover, the EU Water Framework Directive (EU-WFD) sets equally, side-by-side, macrozoobenthos, fish, phytoplankton, and macrophytes as indicators of the status of waters.[63] Nevertheless, biotest systems relying on higher plants have rarely been used in assessments of aquatic sediments to date. Against this background, the aquatic duckweed test was developed and standardised,[30] and the sediment contact test was developed with *M. aquaticum*.[31] Compared to tests with algae, some of its advantages is that it is more representative of higher plants, since target organism and test organism are closer related in terms of evolutionary history. The results of this study, as well as of the study of Feiler *et al*., demonstrated that the application of biotests with higher plants in analyses of contaminated sediments yields valuable results that may contribute to a concept for the integrative risk assessment of contaminated sediment much more efficiently than algal tests.[64]

CONCLUSIONS

In this study, we applied a complex triad approach was applied in an assessment of sediments of an industrial wastewater canal. According to the obtained results, the selected study site justified its well-known status as a contamination hot spot. The high contents of not only Hg and certain PAHs but also of non-characterised sediment contaminants accumulated over years of severe pollution by partly treated or untreated effluents contribute not only to the observed toxicity to some of the selected species (namely, *M. aquaticum* and *V. fischeri*), but also to the complete absence of macrozoobenthos, which indicates extremely unfavourable overall conditions in the canal and can be regarded as a serious signal for immediate remediation action.

Sediment risk assessment cannot be imagined without chemical analysis, but obviously, regularly measured conventional and priority pollutants are hardly ever the only toxic contaminants present in sediments. Toxicity tests, particularly contact tests, along with targeted bioanalyses might guide, more precisely and efficiently, towards a better selection of parameters to be regularly or occasionally monitored. In addition, if put in reverse order, whole sediment toxicity tests proved to be an appropriate method for assessing bioavailability of chemically detected contaminants.

Finally, macrozoobenthos community analysis, as an unavoidable part of sediment risk assessment procedures, integrates the effects of multiple stressors and gives realistic insight not only into sediment contamination by toxic pollutants, but also into the sediment status in general, is necessary and valuable not only in sediment ERA, but also for an accurate evaluation of the ecological status of every waterbody as well.

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ИЗВОД

ПРЕЛИМИНАРНА ЕКОТОКСИКОЛОШКА ПРОЦЕНА КОНТАМИНИРАНОГ СЕДИМЕНТА – КАНАЛ ОТПАДНИХ ВОДА ВОЈЛОВИЦА, ИНДУСТРИЈСКИ КОМПЛЕКС ПАНЧЕВО, СРБИЈА

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У нашем истраживању које се фокусира на процену седимента, примењен је тријажни приступ који се састоји од: хемијских анализа, тестова токсичности на узорцима седимента и анализе бентосне фауне на узорцима седимента из канала отпадних вода Војловица. У тестовима тоскичности на елуатима седимента забележени су следећи одговори: стимулација раста у алгалном тесту, изостанак токсичноcг ефекта у тесту са *Daphnia magna* и слаба до умерена токсичност у конвенционалном тесту са *Vibrio fischeri*. Умерена до изражена токсичност је

забележена у тесту чврсте фазе седимента са тест врстама *Myriophyllum aquaticum* и *Vibrio fischeri.* Високе концентрације живе, појединих PAH једињења, али такође и контаминаната који нису анализирани, акумулирани у седименту дуги низ година, доприносе не само забележеној токсичности него и потпуном одсуству бентосне фауне. Наши резултати потврђују да редовно праћени и мерени конвенционални и приоритетни полутанти у већини случајева не представљају једине токсичне контаминанте присутне у седименту. Тестови токсичности, нарочито контактни тестови, могу дате смернице за бољи одабир параметара за редовно или повремено праћење. Такође, тестови токсичности чврсте фазе седимента су се показали као одговарајући метод за процену биодоступности анализираних контаминаната. Анализа састава и структуре бентосне фауне, као саставног дела процене ризика седимента, интегрише ефекте мултиплих стресора и даје реалну слику не само о контаминираности седимента токсичним полутатнима, него и о статусу седимента уопште.

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