

## IMPACT OF OIL SHALE OPENCAST MINING AND COMBUSTION ON NARVA RIVER AND ITS TRIBUTARIES: CHEMICAL AND ECOTOXICOLOGICAL CHARACTERISATION

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**Abstract.** *The influence of oil shale extraction in Narva opencast and combustion in Eesti and Balti power plants on the quality of surface waters and sediments of the Narva River left-bank tributaries was studied. Altogether, 22 sites were sampled for water and 21 for sediments.*

*The concentrations of Cd, Pb, Cu and Cr in the surface water and sediment samples remained below the respective limit values for surface water and residential soils, respectively. Cd/Zn/Pb and Cr in the sediments were below the detection limits of the respective sensor bacteria (mg metal/kg sediment):  $<0.006 \text{ Cr}^{3+}$ ;  $<0.05 \text{ Cr}^{6+}$ ;  $<0.16 \text{ Zn}^{2+}$ ;  $<0.11 \text{ Cd}^{2+}$ ;  $<0.15 \text{ Pb}^{2+}$ . As-specific sensor bacteria showed bioavailable sub-toxic levels of As in two sediments (up to  $0.26 \text{ mg As}^{3+}/\text{kg}$  or, alternatively, up to  $0.1 \text{ mg As}^{5+}/\text{kg}$ ). Comparative ecotoxicity analysis of the suspensions and aqueous extracts of sediments using *Vibrio fischeri* kinetic luminescence inhibition test (Flash Assay) showed particle-bound acute toxicity in suspensions of four sediment samples out of 20 analysed. However, there was no acute toxicity of the respective aqueous extracts of sediments in the Flash Assay. The obtained results show the importance of combining both chemical and biological methods for environmental monitoring.*

**Keywords:** *Oil shale, river sediments, bioavailable heavy metals, ecotoxicity, luminescent sensor bacteria, *Vibrio fischeri**

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## 1. Introduction

Oil-shale mining and processing in Estonia date back to 1916 and this 90-year exploiting has left an impact on the environment [1, 2]. Currently the opencast mining is performed in North-East Estonia nearby the main water resources of the region: the Narva River and the Narva Reservoir. Mine water containing of 64–80% of precipitation water and 20–34% of groundwater [3] is discharged into the Mustajõgi and Narva rivers [4]. Mine water contains large amount of sulphates, up to 500–600 mg/l [3, 5], formed by pyrite oxidation in the rocks upon exposure to atmospheric oxygen. In anaerobic conditions *e.g.* anoxic sediments, sulphates may transform into remarkably more toxic hydrogen sulphide that according to Kahru et al. [6] is remarkably more toxic than sulphate if analysed with luminescent bacteria bioassay.

Oil shale processing for energy production in Eesti and Balti power plants uses surface water from the surrounding water bodies (the Mustajõgi and Narva rivers and the Narva Reservoir) for cooling / heat carrying in the combustion process of thermal power plants (TPPs). Since the closed circuit is used, the chemical composition of received and discharged water is the same. However, the elevated temperature of discharged water increases the temperature of receiving surface waters, causing “thermal” pollution [7].

Water bodies nearby TPPs are influenced also by solid wastes of combustion process: long-term deposits of ash and particulate emissions *via* air (fly ash). The volume of ash stored on the ash plateaus is approximately 120 million m<sup>3</sup> [8], and TPPs generate annually over 5 million tons of ash that forms 86% of total solid waste (84% of hazardous waste) annually produced in Estonia [9]. When ash comes into contact with circulation water, transporting ash to ash plateaus and rainwater, alkaline leachates (pH>12) are formed [7, 10]. In general, the concentration of heavy metals and metalloids (Cd, Cu, Zn, Ni, Cr, Pb, As) in oil shale combustion ashes is low [8, 11, 12], remaining below the respective Estonian permitted limit values for residential area soils [1]. It can therefore be presumed that these low levels of heavy metals in oil shale ashes pose also no acute water-extractable hazard. However, heavy metals may bioaccumulate in biota: for mosses it has been shown that airborne emissions of oil shale combustion highly affected their Cr, Fe, Ni, V and moderately Cd, Cu, Pb, Zn content [13]. In addition, atmospheric pollution by fly ash reported by Pets [14] for North-East Estonia in 1997 was characterized by specifically high content of sulphur and calcium [15]. The sulphur emissions were remarkably reduced after introduction of the fluidized-bed combustion (FBC) technology in 2004–2005 [16] that had step-wise replaced the pulverised firing (PF) technology. The FBC technology also yields lower concentration of PAH in combustion ashes [17], but leaching of PAHs from FBC ashes has been shown to be about two times higher than from PF ashes [18]. In a recent study [19] bioavailable amounts of As, Cu, Cd, Cr in two different FBC and

PF ashes from Narva PPs were analysed using recombinant luminescent bacterial sensors. There was no bioavailable Cu (to Cu-sensor bacteria) nor As (to As-sensor bacteria) in any of the four ashes studied. The sensor bacteria analysis, however, showed that there was bioavailable Cr in aqueous eluates of all these ash samples and bioavailable Zn or Pb in eluates of PF combustion technology ashes [19].

The airborne emissions of PAHs (2003) have been shown to be comparable to PAH concentrations in the urban air, and the dioxine emissions (2007) have been close to the detection limit  $4 \text{ pg/m}^3$  [20, 21]. However, there is a possible risk for accumulation of contaminants in soils and sediments [1] that may lead to bioconcentration.

Ecotoxicity studies of oil shale-related influences on the environment have shown sulphuric compounds [1, 6], phenols [6], alkalinity [1, 12] and heavy metals [22, 23] to be potential causes of toxicity. High concentrations of heavy metals (e.g. Cd up to  $920 \text{ mg/kg}$ ; Cu up to  $3400 \text{ mg/kg}$ ) and PAH (up to  $278.4 \text{ mg/kg}$ ) have been recorded in 1998 in the Narva River sediments (near the Mustajõgi River, the Balti Power Plant and downstream from the town of Narva) by Huuskonen et al. [22]. Differently from this study, further monitorings performed during 2005–2007 [24–26] have shown that concentrations of heavy metals and persistent organic pollutants in water and sediments of the Narva River were low.

The aim of the current research was to study the quality of surface waters and sediments of the Narva River and its tributaries on the left bank which have been influenced by oil shale extraction in the Narva opencast since 1970 and by the oil shale combustion in Eesti and Balti power plants since the 1960s. Focus was put on the analysis of sediments, as sediments are sinks for persistent pollutants (heavy metals, PAHs) and therefore the composition of contaminants in sediments is likely to reflect the pollution 'pattern' formed during the past 40–50 years of intensive oil-shale industry.

The chemical and ecotoxicological test methods were combined: (i) chemical methods were used for the quantification of Cd, Pb, Cu and Cr in sediments and in the water column; (ii) general ecotoxicity of the sediments was evaluated with *Vibrio fischeri* kinetic bioluminescence inhibition test (Flash Assay) [27]; (iii) bioavailability of heavy metals in sediments was analysed using recombinant luminescent sensor bacteria. These biosensors have previously been used for environmental analysis to quantify bioavailable concentrations of target metals in complex environmental samples [28–30] but they have not yet been used for analysis of heavy metals in sediments influenced by oil-shale industry in Estonia. Recombinant bacterial sensors developed so far include sensors for Cd, Pb, Hg, Cr, Ni, Co, Zn, Cu and As [31]. In the current study three different sensors were used: sensors responding to As, to Cd/Zn/Pb and to Cr, respectively.

The results of the current study are very timely and relevant since, according to the press release of Eesti Energia (January 14<sup>th</sup>, 2011), a new oil-shale operated power plant with at least two FBC blocks ( $2 \times 300 \text{ MW}$ )

will be built next to the Eesti Power Plant. The first block will be ready by the year 2015; building of the second block will be decided in 2012.

## 2. Materials and methods

### 2.1. Water and sediment sampling and sample preparation for analysis

In August 2007 surface water (22 samples) and corresponding sediments (26 samples from 21 sites) were collected from the Poruni River (a presumably clean reference area), drainage ditches and sedimentation pond on the Narva opencast territory, the Konso channel, the Mustajõgi River and the Narva River. Coordinates and locations of the sampling points are given in Table 1 and Fig. 1.

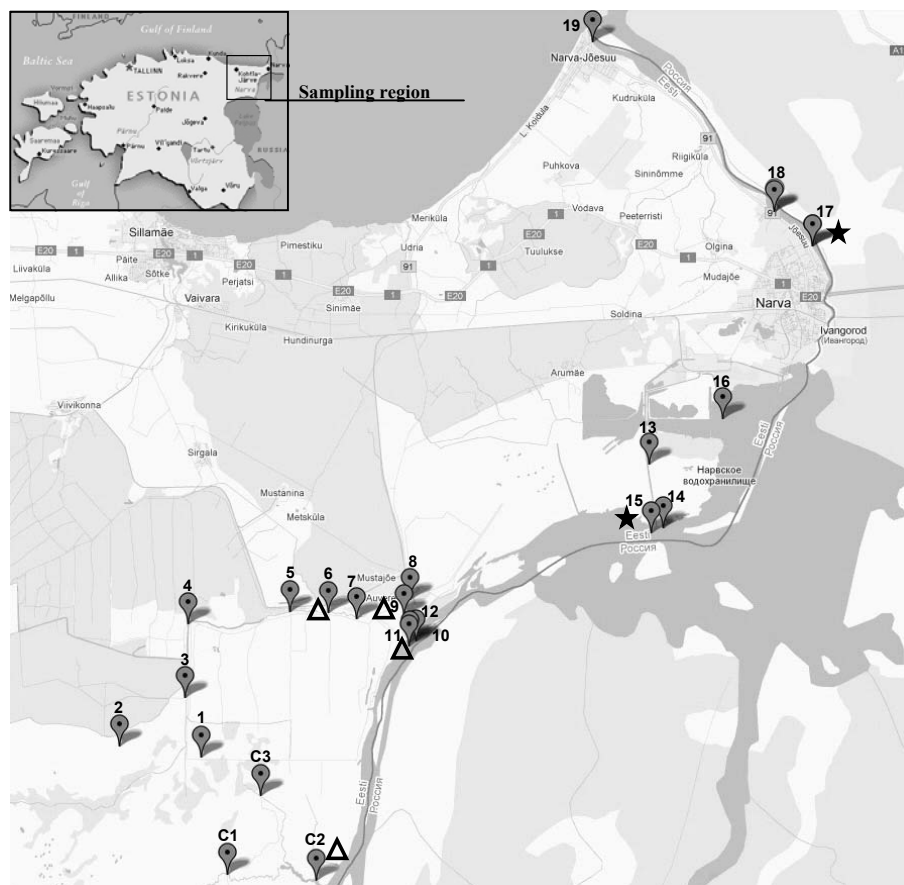


Fig. 1. Location of sampling sites. The sampling sites where ecotoxicity of sediments (toxic units  $TU > 1$  in Flash Assay) was detected are marked with  $\triangle$ , bio-available As in sediments is marked with  $\star$ .

**Table 1. Description of the sampling sites of waters and sediments**

Presumably clean references	Sample	Description of sampling site	Coordinates
	C1	Poruni River	59°10.530 N / 027° 47.802 E
	C2	Poruni River (from Narva River side)	59°10.398 N / 027°51.415 E
	C3	Drainage ditch at the beginning of Narva quarry	59°12.187 N / 027° 49.183 E
	1*	Sediment pool in Narva opencast	59°12.982 N / 027° 46.750 E
	2	Konso main channel	59°13.207 N / 027° 43.418 E
	3	Channel (mining water+natural water)	59°14.222 N / 027° 46.080 E
	4	Mustajõgi River (observation tower)	59°15.754 N / 027° 46.219 E
	5	Mustajõgi River	59°16.010 N / 027° 50.359 E
	6	Mustajõgi River	59°15.995 N / 027° 51.940 E
	7	Mustajõgi River	59°15.866 N / 027° 53.085 E
	8	Mustajõgi River	59°16.276 N / 027° 55.261 E
	9	Mustajõgi River	59°15.931 N / 027° 55.003 E
	10	Mustajõgi River (confluence to Narva River)	59°15.391 N / 027° 55.263 E
	11	Narva River (upstream the confluence of Mustajõgi River)	59°15.308 N / 027° 55.227 E
	12	Narva River (downstream the confluence of Mustajõgi River)	59°15.409 N / 027° 55.499 E
	13	Balti PP – channel	59°19.085 N / 028°05.021 E
	14	Balti PP – channel (confluence to Narva River)	59°17.763 N / 028°05.624 E
	15	Narva Reservoir (confluence of Balti PP channel)	59°17.652 N / 028°05.111 E
	16	Narva Reservoir (near the ash plateaus and water taking channel of Balti PP)	59°20.040 N / 028°08.052 E
	17	Narva River (Narva town)	59°23.631 N / 028°11.715 E
	18	Narva River (after WWTP outflow)	59°24.333 N / 028°10.142 E
	19	Narva River (Narva-Jõesuu)	59°27.846 N / 028°02.734 E

\* the sampling site where only water sample was taken  
 WWTP – wastewater treatment plant; PP – Power Plant

For location of the sampling sites see the map in Fig. 1.

Approximately 15–20 cm of surface sediments were taken with bottom grab sampler for sediment samples. Some of the collected samples were cut into 3–10 cm layers for chemical analysis, ecotoxicological tests were performed with samples from the upper layer of the sediments. Sediment samples were closed air-tight and stored in glass vials at +4 °C in the dark for ecotoxicological testing. Sediment samples for chemical analysis and metal bioavailability tests were collected into polyethylene bags and stored at –18 °C until analysis. Water samples were also stored at –18 °C until analysis. *Prior* to analysis, a part of each sediment sample was dried at 40 °C, ground in agate mortar for homogenization for chemical analysis and sieved (d = 2 mm) for ecotoxicity and heavy metal bioavailability tests.

## 2.2. Chemical analysis

Chemical analysis was performed, according to standard procedures, from filtered water samples (glass microfiber filter, grade GF/C 1.2  $\mu\text{m}$ , Whatman). Temperature, pH, electrical conductivity and dissolved oxygen content in water were measured *in situ* by a portable multi-parameter instrument (WTW, Germany). Total and inorganic carbon were measured with Carbon/Nitrogen analyzer (TOC-V CPH, TNM-1, Shimadzu); sulphate ions by turbidimetric method [32]. Total carbon, sulphur and nitrogen in sediments were analyzed by Elemental Analyzer (Elementar Analysensysteme GmbH). For selected heavy metals Cd, Pb, Cu and Cr microwave-assisted extractions of sediment samples were carried out according to US-EPA method 3051A in Anton Paar Multiwave 3000 digestion system with  $\text{HNO}_3$ . Quantification of the heavy metal content in water and the digested sediment samples was performed using atomic absorption spectrophotometer (AA-6800, Shimadzu) equipped with graphite furnace atomizer (GFA-EX7). The quantification limits for water samples were 0.1  $\mu\text{g/l}$  for Cd and 1  $\mu\text{g/l}$  for Pb, Cu and Cr, and for sediment samples 0.1 mg/kg for Cd and 1 mg/kg for other tested metals, respectively. Relative standard deviations in metal analyses were less than 10%. Operational conditions were in accordance with the manufacturer's recommendations. All the used standard solutions and reagents were of analytical grade.

## 2.3. Analysis of bioavailable heavy metals using recombinant luminescent sensor bacteria

Bioavailability of As and selected heavy metals (Cd/Zn/Pb, Cr) was determined with heavy-metal specific recombinant sensor bacteria: *Escherichia coli* MC1061(parsluxCDABE) was used for As quantification, *E. coli* MC1061(pSLzntR/pDNPzntAlux) for Cd/Zn/Pb, and *Ralstonia eutropha* AE104(pchrBPchrAlux) for Cr essentially as described by Ivask et al. [31].

Bioluminescence of these sensor bacteria increases proportionally with the concentration of the respective bioavailable heavy metal(s). Respective luminescent control strains (not inducible by heavy metal) were used to take into account potential toxicity/stimulation/luminescence quenching effects of the sample to the sensor bacteria. The control strains were *E. coli* MC1061(pSLlux) for As, *E. coli* MC1061(pDNlux) for Cd/Zn/Pb and *R. eutropha* AE104(pDNlux) for Cr. Freshly grown bacteria (both sensors and controls) in Luria-Bertani (LB) medium were harvested from exponential phase ( $\text{OD}_{600} = 0.6$  for all strains except *Ralstonia* pDNlux for which exponential phase cell density was  $\text{OD}_{600} = 0.39$ ), washed with the test medium (0.9% NaCl, 0.1% casAA, 0.1% glucose) and used immediately for testing. 100  $\mu\text{l}$  of the bacterial inoculum was pipetted onto equal volume of the sample (on 96-well microplates) and incubated for 2 h at 30  $^{\circ}\text{C}$  in the dark. Luminescence was measured with a plate luminometer (Berthold Orion II). Sediments were analyzed as aqueous slurries (liquid-to-solid-ratio;

LSR = 10) that were prepared in MilliQ water, rotated for 24 h on a Labinco BV rotator at room temperature and tested in 2 independent trials (2 parallels in both) within 2 days after preparation. Before the analysis the slurries were stored at +4 °C in the dark. The amount of bioavailable heavy metals was calculated, assuming that the respective metal salts (analysed in parallel as NaAsO<sub>2</sub>, Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O, CrCl<sub>3</sub>, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, ZnSO<sub>4</sub>·7H<sub>2</sub>O, CdCl<sub>2</sub> and Pb(NO<sub>3</sub>)<sub>2</sub> solutions in MilliQ water) were 100% bioavailable for the respective sensor bacteria. For calculation details, see Ivask et al. [28].

To verify the performance of the sensor bacteria, heavy-metal polluted soil sampled from the vicinity of Zn/Cd metal smelting plants in Northern France [29] and analysed earlier [1, 30, 33] was included as a positive control. The pH of this 'positive control' soil for sensor bacteria analysis was 7.9 and composition (per kg of dry wt) was as follows: 236 g sand, 209 g clay, 555 g silt, 32.4 g organic matter, 3 g CaCO<sub>3</sub>, 20.1 mg Cd, 1050 mg Pb, and 1390 mg Zn [33]. Formerly we have shown that less than 1.6% of the total Cd in this soil was water-extractable. However, 4.5% of the total Cd in this soil was bioavailable to Cd sensor bacteria *Bacillus subtilis* BR151(pTOO24) in a contact assay [30]. In the current study, sensor bacteria *E. coli* MC1061(pDNlux) that sense Cd/Zn/Pb quantified 4.06±2.37 mg metals per kg soil that is very similar to that reported by Ivask et al. [30]. Thus the bioluminescent signal of *E. coli* Cd/Zn/Pb sensor, observed upon exposure to this heavily polluted control soil, was probably due to bioavailable Cd. In addition, 2.1±0.75 mg As<sup>3+</sup>/kg (0.84±0.24 mg As<sup>5+</sup>/kg) was quantified in this positive control soil using *E. coli* As sensor. As<sup>5+</sup> is the prevailing species of arsenic in the environment, and As sensor bacteria are slightly more sensitive to As<sup>5+</sup> than As<sup>3+</sup>.

#### 2.4. Ecotoxicological analysis

Ecotoxicity of the sediments was evaluated by *Vibrio fischeri* Flash Assay [34, 35] that was recently standardized by ISO (Water quality – Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri*) [27]. In Flash Assay the test endpoint, bacterial bioluminescence, is reduced if the sample contains compounds that are bioavailable at harmful concentrations to the test bacteria. If the luminescence is reduced by at least 20%, the sample is considered toxic. Every sample acts as its own control, thus no turbidity/colour correction is needed. This test can be used e.g., for turbid sediment suspensions as well as for the 'clear' particle-free aqueous extracts of the same sediment sample. The difference in toxicity of suspensions and the respective aqueous extracts shows the presence of particle-bound toxicants. The test was conducted as follows: lyophilised test bacteria *V. fischeri* NRRL-B 11177 from Aboatox (Turku, Finland) were reconstituted according to the standard [27] procedure with the solution containing 20 g/l NaCl, 2.035 g/l MgCl<sub>2</sub>·6H<sub>2</sub>O and 0.3 g/l KCl. The assay was conducted at 20 °C using an automated tube luminometer 1251 (ThermoLabsystems, Finland) connected to a computer operated

by Multiuse software (BioOrbit, Finland) as described by Põllumaa et al. [35]. The suspension of test bacteria *V. fischeri* (100 µl) was automatically dispensed into the sample (100 µl), and the kinetics of the luminescence in each sample was measured during 30 sec under constant mixing. EC<sub>50</sub> values (concentration of the sample that reduces the luminescence of bacteria by 50% compared to the control sample – 2% NaCl) were calculated for 30 min exposure time. Sediment-water slurries (LSR = 3) and the respective aqueous extracts (suspensions centrifuged 13 000 rpm for 10 min and filtered d = 0.45 µm) were prepared from pre-dried and sieved (d = 2 mm) sediments using the approach previously used by us for analysis of contaminated soils [36]. All the samples were prepared in 2% NaCl (optimal salinity for *V. fischeri*) and 2% NaCl served also as a control. Testing was performed in at least 3 replicates in 3 separate runs on the same day. Inhibitory effects on test bacteria luminescence were calculated as described in [37] and ecotoxicity expressed in toxic units (TU = 100/EC<sub>50</sub>), according to toxicity classification systems as described in [38, 39].

### 3. Results and discussion

#### 3.1. The quality of water samples

The chemical characteristics of water samples measured at each site are summarized in Table 2. Water pH was between 6.80 and 8.55. The concentration of dissolved oxygen (DO) varied between 7.6 and 12.3 mg/l with the lowest value recorded in the Narva River water in the town of Narva. Clear impact of oil shale industry on water quality was indicated by high electrical conductivity (Table 2) in the Mustjõgi River which can be explained by high concentration of sulphates in water due to the mining of oil shale in Narva opencast. According to Erg and Punning [5], natural background concentration of sulphate ions is 2–10 mg/l, and in mines the corresponding range in water is 43–620 mg/l. Elevated concentrations of sulphates (up to 620 mg/l) in the current study that indicated the impact of mining were recorded in the water of the Mustajõgi River (water samples 3–7). Higher concentrations of sulphate in water were accompanied by higher values of inorganic carbon (IC) (Table 2). It confirms that mine water also increases the level of HCO<sub>3</sub><sup>-</sup> ion in the water bodies. The total organic carbon (TOC) content in waters varied from 4.6 to 24 mg/l. In contrast to the IC, TOC values were low in samples 3–7 due to the impact of mine waters indicating lower concentrations of TOC in mine water compared to natural surface water.

Concentrations of the studied heavy metals in water samples (Table 2) were mostly below the detection limit of the analytical method, <1 µg/l for Cr and Pb and <0.1 µg/l for Cd, respectively. Concentrations of copper in water samples remained below 10 µg/l. The results are similar to those obtained during monitorings in 2003 and 2006 [25, 40]. Concentrations of Cr, Pb, Cd and Cu in the studied water samples were below the environ-



mental quality standards for inland surface water [41] as well as below the limit values of the quality of water intended for human consumption [42]. It was possible to observe negative moderate correlation between sulphate and Cu concentrations in water indicating lower concentrations of Cu in mine waters compared to water from natural water bodies (data not shown).

**Table 2. Results of water analysis**

Sample	Temp., °C	pH	EC µS/cm	DO mg/l	SO <sub>4</sub> <sup>2-</sup> mg/l	TOC mg/l	TC mg/l	IC mg/l	Cu µg/l	Cr µg/l	Cd µg/l	Pb µg/l
C1	18.5	6.80	297	nd	9.6	18.1	40.7	22.6	1.9	<1	<0.1	<1
C2	25.1	8.55	270	10.8	14.6	12.1	32.8	20.8	4.0	<1	<0.1	<1
C3	22.2	6.90	170	nd	1.5	24.1	40.4	16.3	5.2	<1	<0.1	<1
1	23.9	7.70	1989	nd	1042	5.4	52.8	47.3	<1	<1	<0.1	<1
2	23.7	7.85	435	nd	61.0	18.0	45.6	27.6	8.2	<1	<0.1	<1
3	21.5	7.94	1440	nd	603	4.6	49.0	44.5	<1	<1	<0.1	<1
4	22.3	8.14	1400	nd	575	4.9	45.6	40.7	3.2	<1	0.13	<1
5	21.6	8.10	1434	nd	620	6.9	43.1	36.2	1.4	<1	<0.1	<1
6	21.8	8.11	1390	nd	575	7.1	45.0	37.9	2.1	<1	<0.1	<1
7	22.6	8.07	1388	nd	620	8.9	44.2	35.3	4.5	<1	<0.1	<1
8	30.0	8.4	nd	nd	33.0	12.7	32.4	19.8	6.9	<1	<0.1	<1
9	29.1	nd	nd	nd	33.9	10.3	33.8	23.5	6.4	<1	<0.1	<1
10	nd	nd	nd	nd	33.8	12.2	35.7	23.6	4.3	<1	<0.1	<1
11	23.5	8.47	326	nd	16.4	11.8	32.8	21.0	4.2	<1	<0.1	<1
12	29.7	8.49	327	8.6	29.3	11.7	34.3	22.6	9.8	<1	<0.1	<1
13	30.6	7.71	310	8.2	21.2	11.6	34.6	22.9	4.3	<1	<0.1	<1
14	29.3	7.62	308	10.2	18.8	11.9	34.4	22.5	4.2	<1	<0.1	<1
15	25.8	8.22	262	11.1	17.4	12.2	32.6	20.3	5.1	<1	<0.1	<1
16	24.5	8.42	244	12.3	17.4	10.4	34.9	24.5	2.6	<1	<0.1	<1
17	23.5	8.25	314	7.6	18.8	11.5	35.0	23.4	3.4	<1	<0.1	<1
18	23.9	8.30	307	8.9	19.3	11.5	35.7	24.2	5.4	<1	<0.1	<1
19	23.8	8.40	354	8.2	20.5	11.7	38.3	26.6	4.4	1.0	<0.1	<1

EC – electrical conductivity; DO – dissolved oxygen; TOC – total organic carbon; TC – total carbon; IC – inorganic carbon; nd-not determined.

Environmental quality standards for inland surface water: Cu = 15 µg/l; Cr = 5 µg/l; Cd = 0.08–0.25 µg/l; Pb = 7.2 µg/l [43].

### 3.2. Chemical and ecotoxicological characterisation of sediment samples

Sediments of water bodies are ultimate sinks for persistent and accumulating chemicals. The concentrations of contaminants in aquatic sediments are typically higher than in the overlying water column, therefore it is essential to study the sediments for the potential hazard they pose for the aquatic biota. The quantified heavy metals (Cu, Cd, Cr, Pb) were selected according to the earlier (1998) results of Huuskonen et al. [22]. In the current study, concentrations of nitric-acid extractable Cu, Cd, Cr and Pb in sediments were low (Table 3), remaining not only below the Estonian permitted reference values for residential area soils (mg/kg) (Cu 150, Cd 5, Cr 300, Pb 300) but even below the respective target values (mg/kg) (Cu 100, Cd 1, Cr 100, Pb 50) [44]. However, the results in Table 3 still indicate a slight

increase in the concentration of studied metals downstream the Mustajõgi River and in the Narva River in the town of Narva (samples 5, 6, 7, 9 and 17, 18 respectively, Table 3). In addition, higher concentrations of studied metals were detected in the sediment sample from the inlet of the Balti PP channel (sample 13, Table 3).

The study showed that mine water had an impact on the water quality of the Mustajõgi River. In addition, the water from the Mustajõgi River and also water from the Narva Reservoir is used as cooling water in the Eesti Power Plant so that all the water from the Mustajõgi River flows through Eesti PP [4]. Heavy metal analysis of sediments (Table 3) indicated that higher concentrations of Cd were accompanied by higher concentrations of Pb (correlation coefficient 0.92). In the presumably clean reference sediment C2 the level of analysed heavy metals was higher than in other clean sediment samples C1 and C3, being comparable rather to the presumably contaminated samples from the research area (Table 3). This discrepancy has to be further analysed.

For some sediment samples (8, 9, 12 and 15) chemical analysis was performed for bottom and upper layers separately. The results from different sampling sites did not indicate similar distribution of metals between different layers of the sediments (Table 3). However, clear dissimilarity between the studied metal concentrations in different layers of sediments were seen for sampling sites 11 and 15 (the Narva River and the Narva Reservoir, respectively), showing that content of heavy metals in the upper layer is higher than in the bottom layer for sample 11 and *vice versa* for the sample 15.

In samples 6 and 7 (the Mustajõgi River) the content of total sulphur was high, about 1% of the dry weight of the sample (Table 3). Comparison of the results on sulphate ions in water (Table 2) with sulphur content in sediment samples (Table 3) from the same sampling sites were carried out. No positive correlation between concentration of sulphates in water column and total sulphur in sediments was found.

Bioavailability evaluation of As and selected heavy metals (Cd/Zn/Pb, Cr) in sediments with recombinant metal-specific sensor bacteria detected bioavailable As in two sediment samples out of 21:  $0.16 \pm 0.03$  mg As<sup>3+</sup>/kg dwt ( $0.07 \pm 0.004$  mg As<sup>5+</sup>/kg) in sample 15 (the Narva Reservoir) and  $0.26 \pm 0.12$  mg As<sup>3+</sup>/kg ( $0.1 \pm 0.04$  mg As<sup>5+</sup>/kg) in sample 17 (the town of Narva) (Fig. 1, Table 3). In sediments 16 and 18 the concentrations of bioavailable As remained just below the detection limit:  $0.04$  mg As<sup>3+</sup>/kg and  $0.02$  mg As<sup>5+</sup>/kg. The sediments 15–18 were sampled from the Narva Reservoir and the Narva River in the vicinity of the town of Narva. The results are in agreement with Käkinen [19] who showed bioavailable As (2.3–7% from the total concentration) in soil samples from the same region, probably indicating the contamination by particulates from solid wastes or fly-ash. Bioavailable Cd, Zn, Pb and Cr in all the analysed sediment suspensions remained below the detection limits of the sensor bacteria (mg metal/kg sediment):  $<0.11$  Cd<sup>2+</sup>;  $<0.16$  Zn<sup>2+</sup>;  $<0.15$  Pb<sup>2+</sup>;  $<0.006$  Cr<sup>3+</sup>;  $<0.05$  Cr<sup>6+</sup>.

**Table 3. Results of chemical and ecotoxicological analysis of sediments**

Description of sampling site	Sample	Sub-sampling layer	C	S	N	Cd	Pb	Cu	Cr	Acute toxicity, TU*	Bioavailable As <sup>3+</sup> /As <sup>5+</sup> mg/kg**
			%			mg/kg					
Presumably clean references	C1		0.31	0.01	0.02	<0.1	1.6	1.8	1.4	<1	<dl
	C2		10.61	0.64	0.87	0.62	25.1	13.5	39.3	<b>1.29</b>	<dl
	C3		13.93	0.18	0.6	<0.1	5.7	2.1	3.9	<1	<dl
Konso channel	2		0.22	0.01	0.02	<0.1	1.5	0.9	<1	<1	<dl
Channel	3		0.37	0.02	0.02	<0.1	2.5	0.7	2.5	<1	<dl
Mustajõgi River	4		2.89	0.11	0.12	0.11	4.3	2.6	8.0	<1	<dl
Mustajõgi River	5		5.27	0.16	0.21	0.16	9.5	5.3	16.7	<1	<dl
Mustajõgi River	6		15.91	1.03	0.40	0.29	15.2	7.7	33.6	<b>1.39</b>	<dl
Mustajõgi River	7		11.94	0.99	0.45	0.31	11.8	23.6	33.5	<1	<dl
Mustajõgi River	8	0–2 cm	0.99	0.02	0.06	<0.1	3.3	8.9	3.2	<1	<dl
		2–6 cm	1.02	0.03	0.06	<0.1	3.1	5.4	2.0	nd	nd
Mustajõgi River	9	0–3 cm	8.70	0.52	0.67	0.37	18.0	46.2	26.4	<b>2.44</b>	<dl
		3–20 cm	8.88	0.33	0.56	0.39	19.1	43.0	33.9	nd	nd
Mustajõgi River	10		3.11	0.08	0.20	<0.1	11.2	13.0	27.0	<1	<dl
Narva River	11	0–3 cm	7.72	0.14	0.85	0.52	19.9	19.0	42.1	<b>1.72</b>	<dl
		3–10 cm	4.28	0.08	0.35	0.19	13.7	6.3	20.0	nd	nd
Narva River	12	0–2 cm	0.44	0.03	0.05	<0.1	2.4	3.5	2.9	<1	<dl
		2–6 cm	2.51	0.08	0.14	<0.1	2.4	2.4	1.8	nd	nd

Table 3. Continued

Description of sampling site	Sample	Sub-sampling layer	C	S	N	Cd	Pb	Cu	Cr	Acute toxicity, TU *	Bioavailable As <sup>3+</sup> /As <sup>5+</sup> mg/kg**
			%			mg/kg					
Balti PP channel	13		23.12	0.54	1.14	0.47	28.0	67.8	38.8	<1	<dl
Balti PP channel	14		23.72	0.24	0.76	<0.1	8.6	2.7	1.9	<1	<dl
Narva Reservoir	15	0–5 cm	5.78	0.18	0.31	0.13	7.1	5.9	8.0	<1	<b>0.16±0.03/</b> 0.07±0.004
		5–10 cm	7.42	0.49	0.42	0.32	15.7	17.8	27.4	nd	nd
Narva Reservoir	16		1.74	0.06	0.10	<0.1	4.1	1.3	1.9	<1	<dl
Narva River	17		2.40	0.08	0.18	0.42	29.5	12.6	18.0	<1	<b>0.26±0.12/</b> 0.1±0.04
Narva River	18		4.39	0.19	0.26	0.20	13.2	6.6	35.8	<1	<dl
Narva River	19		1.14	0.04	0.06	0.10	7.6	2.1	10.4	<1	<dl

\* acute toxicity of the sediment suspension sample analysed by Flash Assay. TU (toxic unit) = 100/EC<sub>50</sub> mg dry sediment/l, bold values indicate toxic samples (i.e. TU>1).

\*\* analysed with As-sensing recombinant bacteria; bold values indicate samples that contained bioavailable As  
dl – detection limit, <0.04 mg/kg As<sup>3+</sup> or alternatively <0.02 mg/kg As<sup>5+</sup>; nd – not determined.

When the recombinant heavy metal sensor bacteria ‘register’ subtoxic amounts of metals that in higher concentrations can cause also acute toxicity, the *Vibrio fischeri* Flash Assay detects acute toxic effects despite the type of the contaminant present. Flash Assay has been successfully used in general toxicity assessment of oil shale waste and contaminated soil [1, 45] as well as particulate chemicals such as nanosized metal oxides [46]. In several ecotoxicity studies of contaminated particulate environmental media [1, 36], Flash Assay has proven to be the most sensitive test and is especially relevant for assessing the hazards to biota exposed *via* direct contact. In the current study, the ecotoxicity analysis with Flash Assay showed that the aqueous extracts of sediments were not toxic to the test bacteria (data not shown). However, out of the 20 sediment suspensions that were analysed in Flash Assay, the presence of particle-bound toxicants in 3 sediment samples and in one presumably clean reference sediment (C2) was registered. Apparently, these 4 sediment samples contained toxicants that were not water extractable but upon direct contact with test bacteria during the test became bioavailable and exerted toxicity (Table 3). According to the toxicity classification system by Persoone et al. [38, 39], the 4 sediment samples (C2, 6, 9 and 11) analysed by *V. fischeri* Flash Assay could be considered harmful ( $EC_{50} < 100 \text{ mg/L} = TU > 1$ ). Observed acute toxic effects of sediments to *V. fischeri* may also be due to sulphate reduction to sulphide in anaerobic conditions, as well as due to some other contaminants not determined during this study. Therefore, further investigation is needed.

#### 4. Conclusions

Chemical analysis of water samples in the Mustajõgi River showed environmental impact of opencast mining and thermal power plants. Electrical conductivity due to high concentration of sulphates and elevated concentrations of  $\text{HCO}_3^-$  ions was 3–8 times above the natural background. The concentrations of analysed heavy metals (Cu, Cr, Cd, Pb) in both water column and sediments were low, not exceeding the established environmental quality standards for inland surface waters and target values for soils, respectively. Analysis with sensor bacteria showed bioavailable As (up to  $0.26 \text{ mg As}^{3+}/\text{kg}$  or, alternatively, up to  $0.1 \text{ mg As}^{5+}/\text{kg}$ ) in two sediment samples from the vicinity of Narva town. Bioavailable Cd, Zn, Pb and Cr in all the analysed sediment suspensions remained below the detection limits of the sensor bacteria ( $\text{mg metal/kg dry sediment}$ ):  $<0.11 \text{ Cd}^{2+}$ ;  $<0.16 \text{ Zn}^{2+}$ ;  $<0.15 \text{ Pb}^{2+}$ ;  $<0.006 \text{ Cr}^{3+}$ ;  $<0.05 \text{ Cr}^{6+}$ .

*Vibrio fischeri* acute toxicity test – Flash Assay – detected the presence of particle-bound bioavailable toxicants in 4 sediment samples out of 20 but these were not coinciding with the samples showing the presence of bioavailable As. The current study shows that biotests give additional information to chemical analysis.

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