

**IJS Delovno poročilo**  
**DP-9579**

**Establishment of the methodology for the estimation of extent of pollution with metals  
and organic pollutants of the Sava River sediments**

Janez Ščančar, Simona Murko, Tea Zuliani, Milena Horvat, David Kocman, Ester Heath,  
Radmila Milačič

**Department of Environmental Sciences, “Jožef Stefan” Institute**  
**Jamova 39, 1000 Ljubljana, Slovenia**

**Ljubljana, marec 2007**

Naročnik: SARIB - Sava River Basin: Sustainable Use, Management and Protection of Resources

Izvajalci: Institut Jožef Stefan  
Odsek za znanosti o okolju  
Jamova 39, 1000 Ljubljana

Pogodba št.: INCO-CT-2004-509160

Naslov poročila: Establishment of the methodology for the estimation of extent of pollution with metals and organic pollutants of the Sava River sediments

Št. Delovnega poročila: IJS DP-9579

Kopije: naročnik - SARIB (1x)  
IJS knjižnica (1x)  
arhiv Odseka za znanosti o okolju (1x + original)

Ljubljana, marec 2007

## **Establishment of the methodology for the estimation of extent of pollution with metals and organic pollutants of the Sava River sediments**

Janez Ščančar, Simona Murko, Tea Zuliani, Milena Horvat, David Kocman, Ester Heath, Radmila Milačič

Department of Environmental Sciences, "Jožef Stefan" Institute, Jamova 39, 1000 Ljubljana, Slovenia

Objectives: To propose analytical methodology for the estimation of the extent of pollution in river sediments.

### **SAMPLING OCTOBER 2004**

#### Sampling:

**Sample 1** - polluted sediment from the hydroelectric reservoir Moste (26.10.2004)

**Sample 2** - 12 km up stream the Sava dolinka River – unpolluted (reference sediment at Mojstrana) 26.10.2004

**Sample 3** – Krupa River polluted site (Mlini) (27.10.2004)

**Sample 4** – unpolluted site (reference sediment) taken on the Krupa River source, 2 km upstream from the polluted site (27.10.2004)

Sediments were taken from the first 5 cm layer together with the corresponding water, homogenized and divided to aliquots of 1 L – in polyethylene bottles and put on dry ice – aliquots (2 L from each sampling location) were sent to NIVA and BOKU Wiena. Other aliquot samples were stored at -20°C at JSI until being analysed.

### **ANALYTICAL PROCEDURES:**

#### Total metal concentrations:

Sediments were dried at 40°C for three days (until constant weight) in dark, homogenised and sieved through 200 µm sieve. Before analysis sampling flasks were shaken vigorously for one minute. Approx. 0.25 g of sediment was weighed into Teflon vessel and 4 mL of conc. nitric

acid s.p., 2 mL of hydrofluoric acid s.p. and 1 mL of hydrochloric acid s.p. were added, and samples gently mixed until all the sample has been wetted with acid. The Teflon vessels were then covered by vessel cups and submitted to closed vessel microwave digestion on a CEM Corporation (Matthews, NC, USA) CEM MARS 5 Microwave Acceleration Reaction System using the following programme:

Ramp to temperature 30 min, T = 210 °C, hold 60 min (T = 210 °C), cool 30 min. After that the Teflon vessels were vented and vessel caps removed. 12.5 mL of boric acid s.p. (4% water solution) were added to each Teflon vessel in order to dissolve fluorides. Teflon vessels were covered by vessel cups and submitted to closed vessel microwave digestion again using the following programme: Ramp to temperature 15 min, T = 200 °C, hold 30 min (T = 200 °C), cool 30 min. After the digestion clear solution was obtained. The contents were quantitatively transferred to 30 mL graduated polypropylene tubes and filled to mark with water doubly distilled in quartz. These samples were used for ETAAS or FAAS determinations and diluted, if necessary before measurements.

The same procedure, with exception that no sample was added, was applied to determine blanks.

Sample preparation and ETAAS determinations were done under the clean room conditions (class 10000).

#### BCR three-step sequential extraction procedure:

The BCR three-step sequential extraction procedure developed by Quevauviller *et al.*<sup>1</sup> was slightly modified. For practical reasons, the weight of sample and volume of extractant was reduced by half, so that the weight to volume ratio was the same as in the original BCR extraction scheme. It was demonstrated previously in our group that such a reduction in the weight of sample and volume of extractant gave the same results as when the original BCR scheme was applied<sup>2</sup>.

In step 1 (extraction in **0.11 mol dm<sup>-3</sup> acetic acid, pH 2.8**) of the BCR scheme, metals present in ionic form, bound to carbonates and the exchangeable fraction were released. In step 2 (extraction in **0.1 mol dm<sup>-3</sup> hydroxylamine hydrochloride, pH 2**), metals bound to amorphous Fe and Mn oxides and hydroxides were leached. In step 3 (oxidation in acid-stabilised 30% hydrogen peroxide and extraction in **1 mol dm<sup>-3</sup> ammonium acetate, pH 2** adjusted with nitric acid) metals bound to organic matter and sulphides were separated. For an internal check on the sequential extraction procedure, the residue from step 3 was digested in nitric, perchloric and hydrofluoric acid (step 4). The concentrations of elements in steps 1-4 were summed and compared to their concentration after total digestion of the original sample.

In each analytical step standards were prepared in the extracting solutions and the corresponding blank samples were also analysed. Analyses were made in duplicate. Results of extraction steps were expressed on a dry mass basis (105° C).

References:

1. Ph. Quevauviller, G. Rauret, J. F. López-Sanchez, R. Rubio, A. Ure, H. Muntau, *Sci. Total Environ.*, 1997, **205**, 223.
2. N. Pustišek, R. Mialčič, M. Veber, *J. Soils & Sediments*, 2001, **1**, 25.

Extraction in 25% acetic acid:

The acetic acid extractable metal concentration was determined according to the United Nations Environment Programme procedure Loring and Rantala<sup>1</sup>. 2.000 ± 0.001 g of air-dried sample was weighed into a 100 cm<sup>3</sup> Erlenmeyer flask. 25 cm<sup>3</sup> of 25% v/v acetic acid was carefully added, the flask capped with a glass stopper and shaken slowly in a mechanical shaker for 6 hours. After that the contents were quantitatively transferred in a polyethylene centrifuge tube and centrifuged at 2500 RPM for 10 minutes. The supernatant was decanted into a 50 cm<sup>3</sup> volumetric flask. The residual sediment in the centrifuge tube was washed with 10 cm<sup>3</sup> of water doubly distilled in quartz, shaken and centrifuged again. The supernatant was added to the volumetric flask and made up with acetic acid solution to a volume of 50 cm<sup>3</sup>. Acetic acid extractable metal concentrations were determined using standard solutions prepared in 25% v/v acetic acid.

References:

1. Loring, D.H., Rantala, R.T.T.: 1992, Manual for the geochemical analyses of marine sediments and suspended particulate matter. *Earth-Science Reviews*, **32**, 235-283.

Sediment pore water:

Sediment samples were transferred to polyethylene tubes. The water that covered sediments was carefully removed by a pipette. After that sediments were centrifuged for 10 min at 7000 rpm. Pore water was collected with a pipette and allowed the particles to settle for half an hour. An aliquot was acidified with concentrated nitric acid (10 µL / 1 mL) for the determination of total metal concentrations in pore water. Another aliquot was first filtered through 45 µm membrane filter and after that acidified (concentrated nitric acid: 10 µL / 1 mL) to determined soluble metal concentrations in pore water.

Table 1: Total metal concentration<sup>a</sup> of Pb, Zn, Cu, Ni, Cr, Cd and Hg in river sediments determined by FAAS\*, ETAAS and CV-AAS#

Sample No.	Pb (mg kg <sup>-1</sup> )	Zn* (mg kg <sup>-1</sup> )	Cu* (mg kg <sup>-1</sup> )	Ni (mg kg <sup>-1</sup> )	Cr (mg kg <sup>-1</sup> )	Cd (mg kg <sup>-1</sup> )	Hg# (µg kg <sup>-1</sup> )
1	30.7±0.4	97±1	30.7±0.2	89±4	207±2	0.45±0.01	138 ± 12
2	2.4±0.6	22.0±0.5	8.6±0.1	6.6±0.2	15.6±0.2	0.17±0.01	13.2 ± 0.9
3	39.1±0.4	210±2	48.7±1.0	45.0±0.6	125±1	0.76±0.01	149 ± 9

4	41.4±0.2	215±2	49.9±1.1	63±1	157±2	1.01±0.02	169 ± 12
---	----------	-------	----------	------	-------	-----------	----------

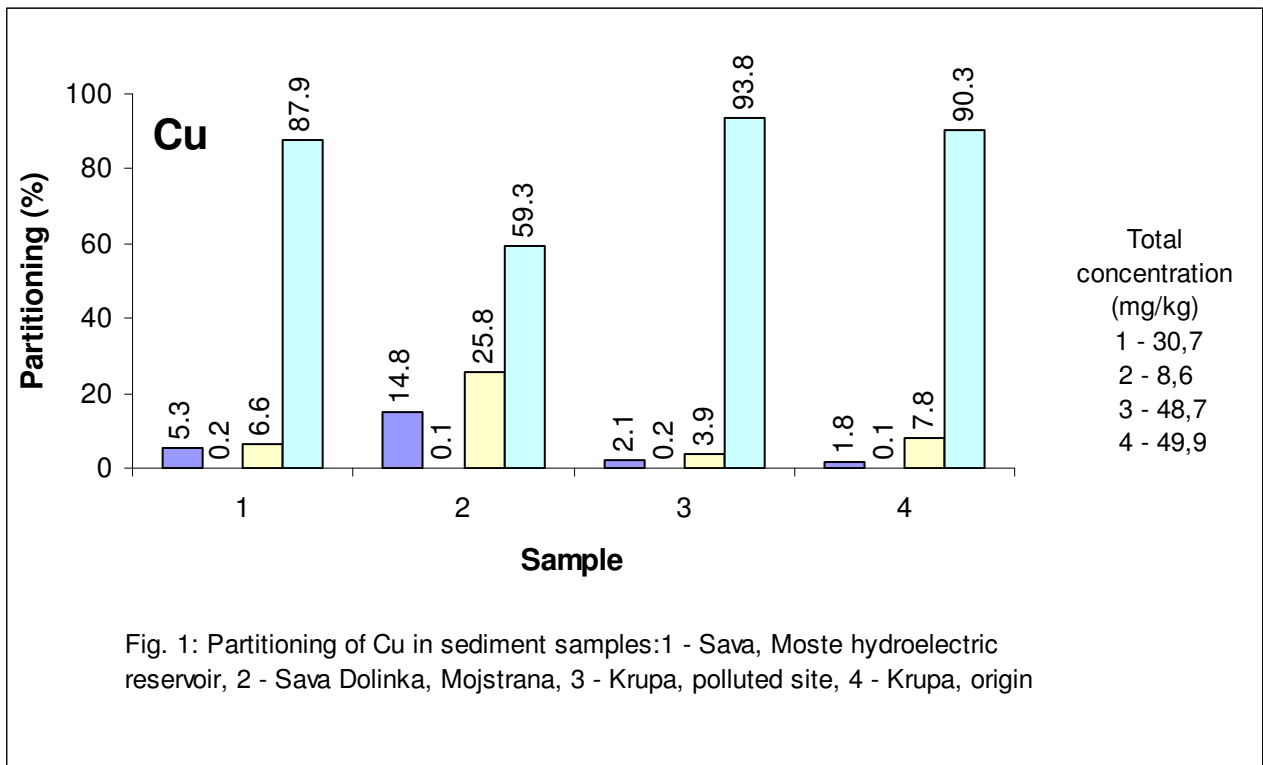
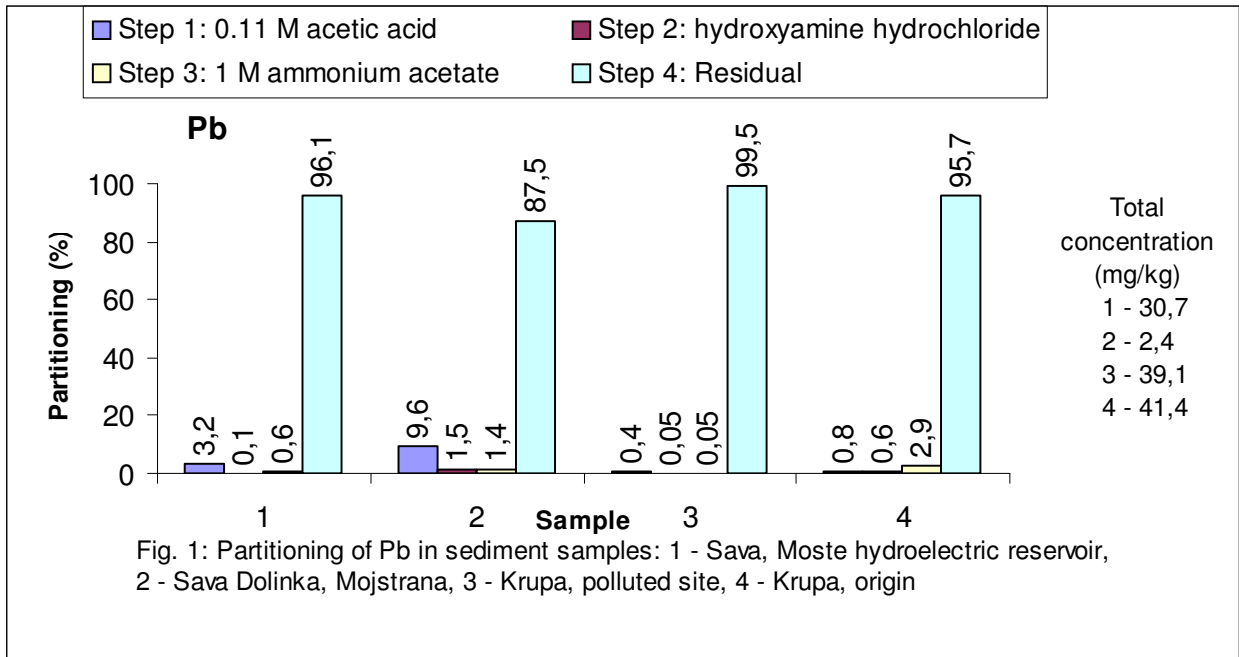
<sup>a</sup>Results are expressed as the mean of two parallel sample determinations ± standard deviation.

Table 2: Comparison of metal concentrations in river sediments leachable in 25 % acetic acid and in 0.11 mol dm<sup>-3</sup> (0.61%) acetic acid.

Element	Concentration	Sample 1 (mg kg <sup>-1</sup> )	Sample 2 (mg kg <sup>-1</sup> )	Sample 3 (mg kg <sup>-1</sup> )	Sample 4 (mg kg <sup>-1</sup> )
Pb	25 % CH <sub>3</sub> COOH	10.7±0.1	1.80±0.04	7.1 ± 0.1	6.5 ±0.1
	0.11 M CH <sub>3</sub> COOH	0.99±0.02	0.23±0.01	0.16±0.01	0.35±0.01
Zn	25 % CH <sub>3</sub> COOH	14.0±0.1	1.70±0.01	18.7±0.2	28.7±0.3
	0.11 M CH <sub>3</sub> COOH	6.4±0.1	1.44±0.03	17.7±0.3	13.5±0.1
Cu	25% CH <sub>3</sub> COOH	1.9±0.1	0.50±0.01	1.40±0.02	1.04±0.01
	0.11 M CH <sub>3</sub> COOH	1.63±0.03	1.27±0.02	1.04±0.02	0.95±0.04
Ni	25% CH <sub>3</sub> COOH	5.9±0.1	0.44±0.01	1.12±0.01	1.27±0.02
	0.11 M CH <sub>3</sub> COOH	5.0±0.2	0.48±0.01	0.96±0.02	1.42±0.01
Cr	25% CH <sub>3</sub> COOH	4.3±0.1	0.31±0.01	1.12±0.01	0.89±0.01
	0.11 M CH <sub>3</sub> COOH	0.41±0.01	0.15±0.01	0.09±0.01	0.10±0.01
Cd	25 % CH <sub>3</sub> COOH	0.05±0.01	0.06±0.01	0.21±0.01	0.22±0.01
	0.11 M CH <sub>3</sub> COOH	0.03±0.01	0.02±0.01	0.04±0.01	0.06±0.02

Table 3: Concentrations of Pb, Zn, Cu, Ni, Cr and Cd (ng cm<sup>-3</sup>) in the pore water of the river sediments

Element	Concentration	Sample 1 (ng cm <sup>-3</sup> )	Sample 2 (ng cm <sup>-3</sup> )	Sample 3 (ng cm <sup>-3</sup> )	Sample 4 (ng cm <sup>-3</sup> )
Pb	total	7.4 ± 0.3	12.4 ± 0.4	< 1.0	< 1.0
	soluble	2.1 ± 0.1	< 1.0	< 1.0	< 1.0
Zn	total	< 5.0	< 5.0	< 5.0	< 5.0
	soluble	< 5.0	< 5.0	< 5.0	< 5.0
Cu	total	4.5 ± 0.2	7.8 ± 0.3	2.5 ± 0.1	1.7 ± 0.1
	soluble	1.4 ± 0.1	< 0.4	2.3 ± 0.1	1.6 ± 0.1
Ni	total	20.7 ± 0.6	5.0 ± 0.2	1.1 ± 0.1	1.9 ± 0.1
	soluble	17.9 ± 0.5	2.3 ± 0.1	< 1.0	< 1.0
Cr	total	1.1 ± 0.1	0.80 ± 0.06	< 0.2	< 0.2
	soluble	0.50 ± 0.05	< 0.2	< 0.2	< 0.2
Cd	total	< 0.2	< 0.2	< 0.2	< 0.2
	soluble	< 0.2	< 0.2	< 0.2	< 0.2



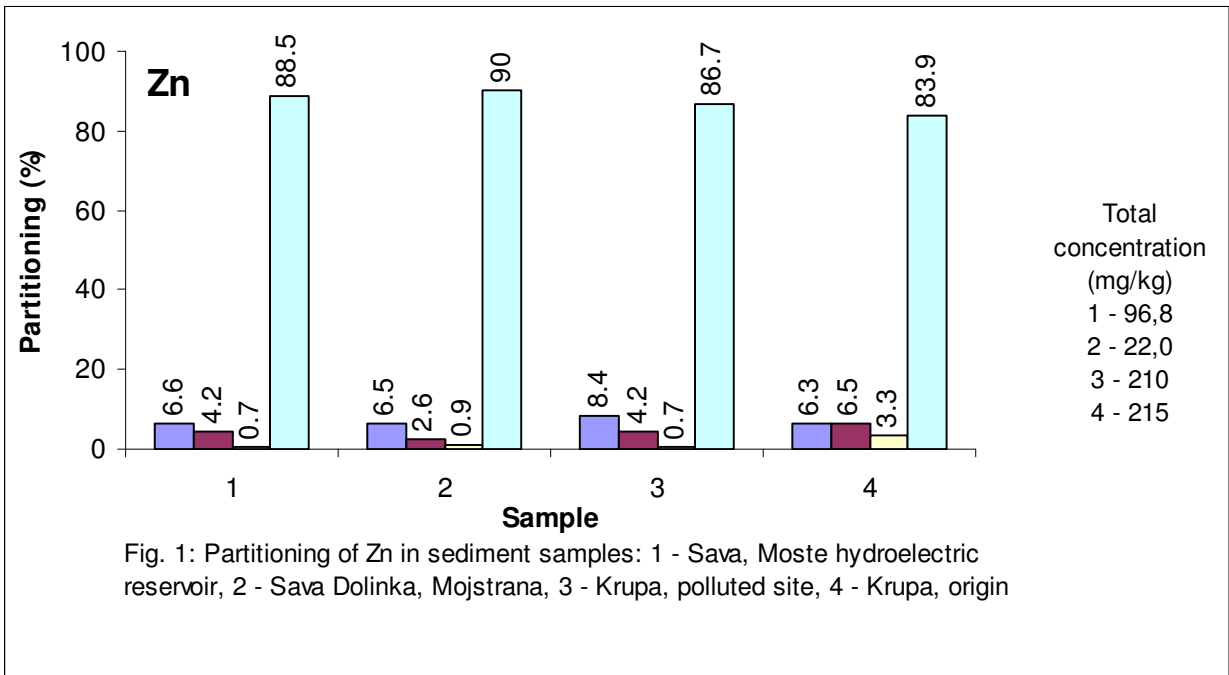


Fig. 1: Partitioning of Zn in sediment samples: 1 - Sava, Moste hydroelectric reservoir, 2 - Sava Dolinka, Mojstrana, 3 - Krupa, polluted site, 4 - Krupa, origin

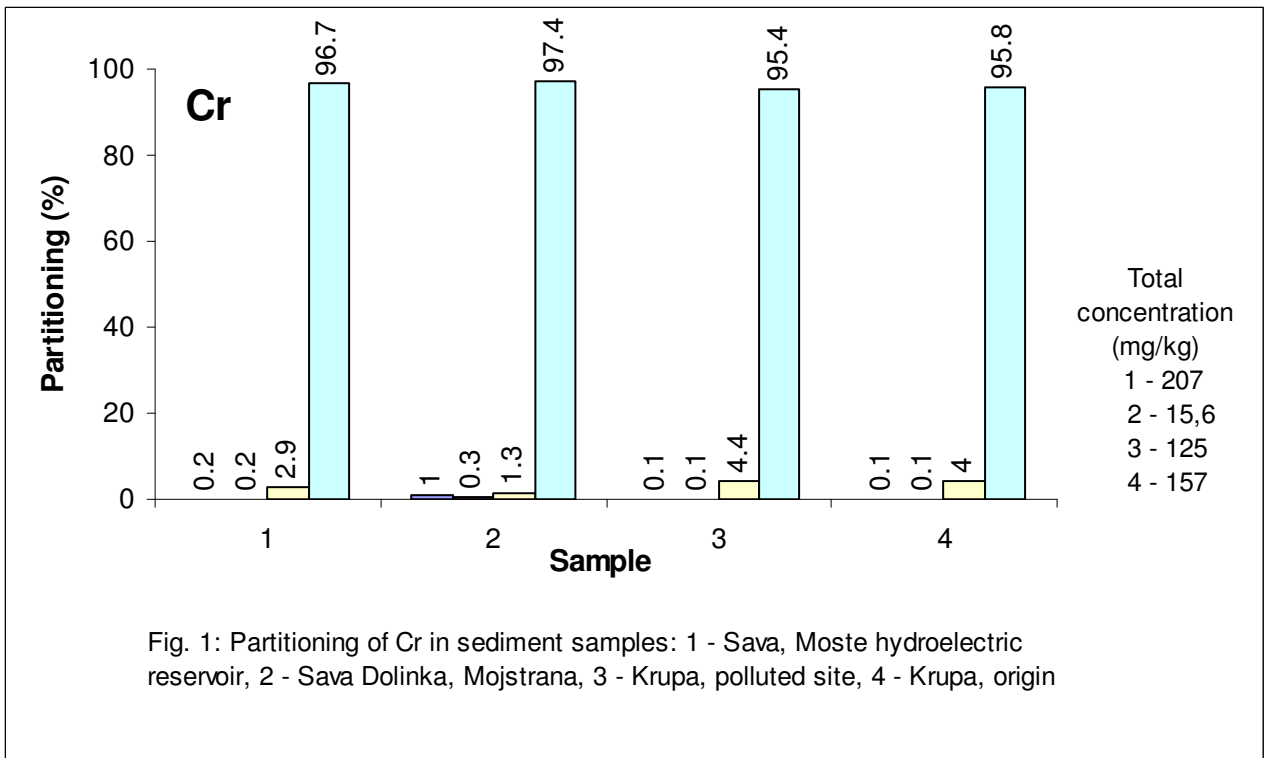
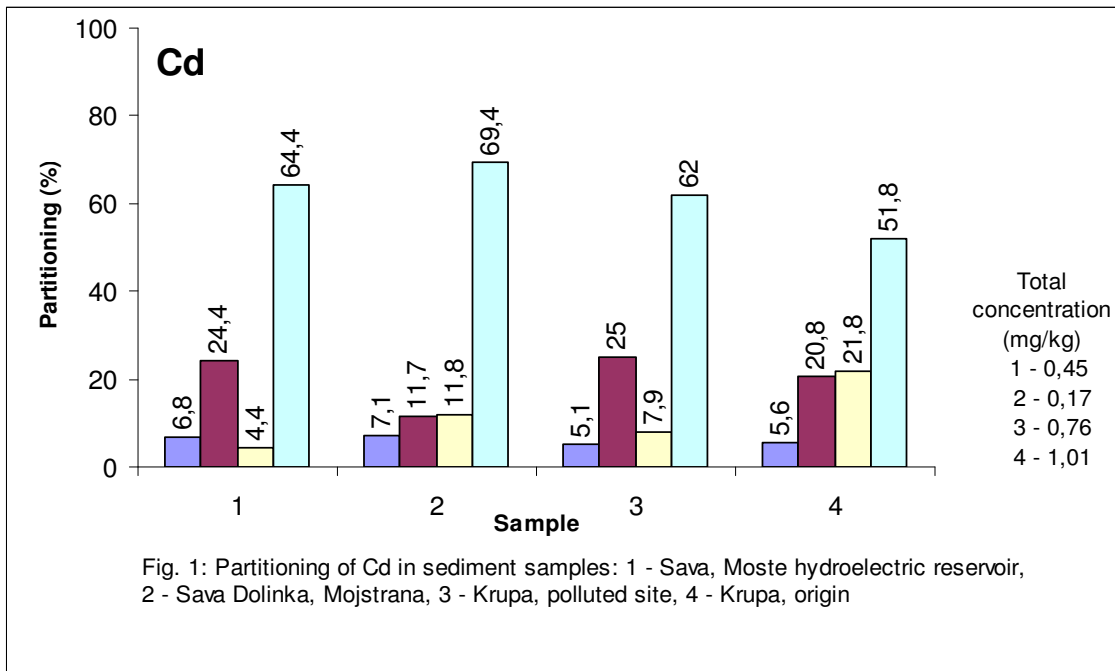
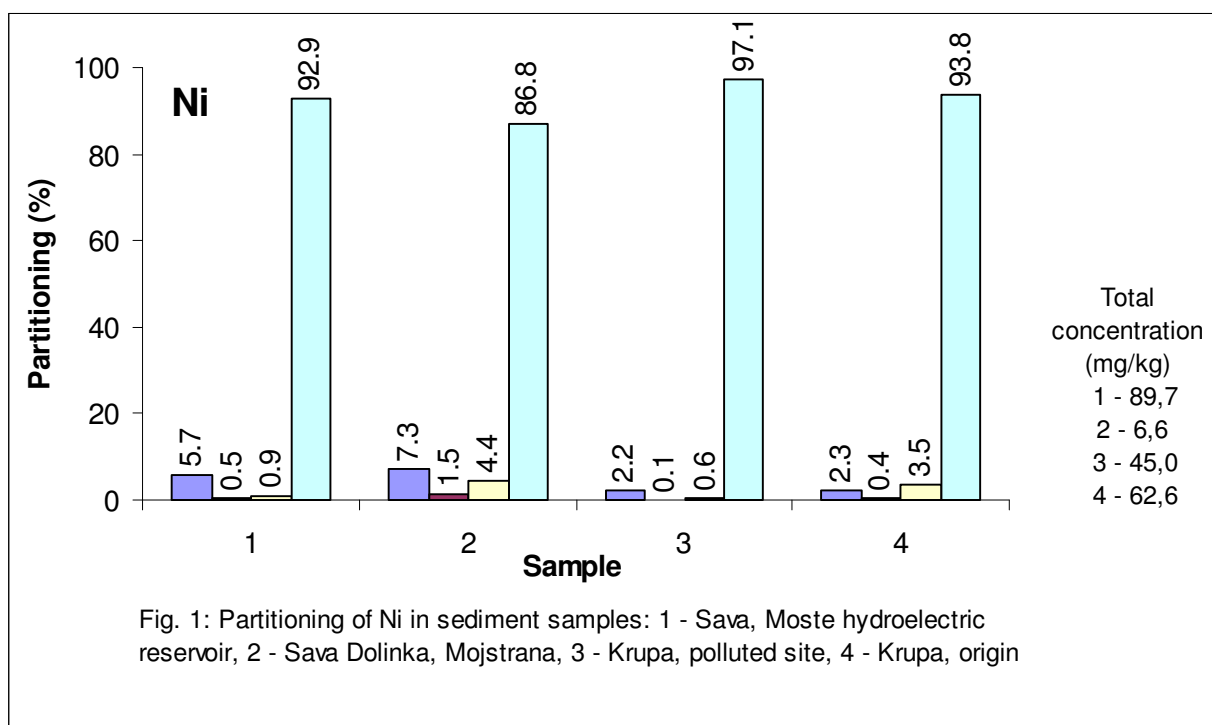


Fig. 1: Partitioning of Cr in sediment samples: 1 - Sava, Moste hydroelectric reservoir, 2 - Sava Dolinka, Mojstrana, 3 - Krupa, polluted site, 4 - Krupa, origin





### On the basis of the present experiments it was decided:

- Total element concentrations (Zn, Cu, Cd, Pb, As, Ni, Cr, Cu and Hg) will be determined in the Sava River sediments. Sediments will be wet sieved through 63 µm plastic sieve and dried to constant weight at ambient temperature at dark.
- 0.11 M acetic acid will be apply to estimate the easily soluble sediment fraction
- Normalisation to Al or Fe will be applied to estimate the anthropogenic inputs of elements to the sediments of the Sava River.
- Sediment pore water will be investigated at experimental sampling locations – slow and fast river flow. Sediment pore water will be centrifuged at RCF 6082 g for 45 min. After centrifugation pore water will be decanted and leave to settle for next 30 min. The same protocol will be applied at NIVA and BOKU Wien.
- Organotin concentrations in sediments will be determined when all sediments from the Sava River will be collected.

These steps will enable COMPARABILITY OF DATA to other River basin (Danube...) and comparability of data to bioassay analysis performed by partners NIVA and Boku Wien.

## POLLUTION OF SEDIMENT: ORGANIC POLLUTANTS

In the first period of SARIB project we analysed two sets of sediments (Sava river near Jesenice and Krupa river – Sava tributary) for the presence of PAHs (expected in Jesenice area from pirogenic sources) and PCBs (expected from PCB pollution history in Krupa river area). In both cases samples were taken at two locations - from contaminated and uncontaminated sites.

Sediments were extracted with accelerated solvent extraction coupled with supercritical fluid extraction (ASE-SFE). Approximately 4g of dry sediment spiked separately with mixture of 4 deuterated PAHs (internal standards for PAH determination) and PCBs congener 30 and 209 (internal standards for PCB determination). ASE was performed with dichloromethane in static and dynamic mode, while SFE was performed with CO<sub>2</sub>. All extracts were treated with activated copper for sulphur removal. In case of PAH determination, GC-MSD was applied, while PCBs were determined after cleaning on Florisil column with GC-ECD.

Results unexpectedly showed higher contamination with PAHs in Krupa area known to be historically contaminated with PCBs (1663 ng g<sup>-1</sup>) than North Sava river (711 ng g<sup>-1</sup>). In both cases predicted uncontaminated sites had lower amounts of PAHs (Sava: 32 ng g<sup>-1</sup> and Krupa: 179 ng g<sup>-1</sup>). In the future we will try to determine the source of PAH contamination in Krupa area from ratio of PAH and methylated PAHs. PCB contamination of Krupa river was at both points of sampling lower (4-6 ng g<sup>-1</sup>) than in the past decades (1982-1994: 10-800 mg kg<sup>-1</sup>) what shows the successful remediation of polluted site. Results are shown in Table 1.

**Table 1:** PAH and PCB content in Sava Mojstrana (uncontaminated and contaminated sediment sample) and Sava tributary Krupa (uncontaminated and contaminated sediment sample) sediment (\*MDL for single PAHs: 0,1 - 0,002 µg kg<sup>-1</sup> (GC-MSD), MDL for single PCBs: 2-0,06 ng kg<sup>-1</sup> (GC-ECD)).

SEDIMENT	Σ PAHs (ng g <sup>-1</sup> )	Σ PCBs (ng g <sup>-1</sup> )
Sava Mojstrana	32,36	UDL*
Sava Moste (cont.)	711,09	UDL*
Krupa Source	179,06	6,24
Krupa Mill Per (cont.)	1663,50	4,21

Pore water was extracted from all 4 sediment samples. In the first stage, water was eliminated from sediments, afterwards sediments were centrifuged for 10 minutes at 7000rpm. In general, from 130g of sediment sample, 2,5 – 16,1 mL pore water was extracted. Pore water samples were extracted with liquid-liquid extraction with dichloromethane. In case of PAH determination, samples were simultaneously analysed by GC-MSD in SIM (Selected Ion Monitoring) mode. For PCB determination, solvent was exchanged to hexane and extracts were cleaned on Florisil column. PCB content was determined with GC-ECD.

Results (Table 2) show the presence of PAHs and PCBs in pore water. All sediment samples showed the same level of PAH contamination ( $0,6 - 3 \text{ ng mL}^{-1}$ ) while PCBs were determined only in Krupa river sediment pore water ( $2-8 \text{ ng mL}^{-1}$ ).

**Table 2:** PAH and PCB content in Sava Mojstrana (uncontaminated and contaminated sediment sample) and Sava tributary Krupa (uncontaminated and contaminated sediment sample) sediment pore water (\*MDL for single PAHs:  $0,1 - 0,002 \text{ } \mu\text{g kg}^{-1}$  (GC-MSD), MDL for single PCBs:  $2-0,06 \text{ ng kg}^{-1}$  (GC-ECD))

<b>PORE WATER</b>	<b><math>\Sigma</math> PAHs (<math>\text{ng mL}^{-1}</math>) / V (mL)</b>	<b><math>\Sigma</math> PCBs (<math>\text{ng mL}^{-1}</math>)</b>
<b>Sava Mojstrana (uncont.)</b>	4,55 / 2,5	UDL*
<b>Sava Moste (cont.)</b>	3,08 / 15,0	UDL*
<b>Krupa Source (uncont.)</b>	0,83 / 15,0	7,90
<b>Krupa Mill Per (cont.)</b>	0,64 / 16,1	2,23