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Short Communication

HEAVY METAL AND BACTERIAL POLLUTION OF THE SAVA RIVER IN SERBIA

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The aim of this study was to establish microbial and heavy metal pollution of the Sava River at three locations close to industry and urban areas (Šabac, Obrenovac, Beograd) in Serbia. Heavy metal analysis included Cu, Zn, Pb, and Cd in the river water and sediment samples. Using the microbiological analysis we tried to establish the effectiveness of total coliforms, faecal coliforms and Escherichia coli in detecting pollution of surface waters. We found that *E. coli* levels steadily increased downstream from Šabac (location 1; 2100 MPN per 100 mL) to Belgrade (location 3; 10000 MPN per 100 mL). To prevent bacterial contamination, it is necessary to reduce the discharge of wastewater with faecal matters near highly populated towns. Heavy metal levels in sediments correlated with those in the river water. Fluctuations attributed mainly to anthropogenic sources were not high. These results point to acceptable anthropogenic contribution to heavy metal content in the Sava River and to low environmental risk.

KEY WORDS: *Cd, Cu, distribution coefficient,* E. coli*, microbiological indicators, Pb, sediment, Zn*

Industrial and urban growth have been accompanied by growing pollution of the aquatic environment (1). The 940-km long Sava River flows through four countries. While the upper stretch of the river in Slovenia and Croatia has recently been studied for heavy metal and radionuclide pollution (2), downstream in Serbia the river has received little attention so far.

Microbial indicators have been used for many years to establish the sanitary condition of food and water, but bacteria have also been used to evaluate environmental pollution. Microbiological analysis of river water quality involves testing for bacterial pathogens. The most indicative of bacterial pollution and therefore used in many studies are the total coliform bacteria (3).

Heavy metals are among the most common environmental pollutants in water and have mostly been studied in river sediments due to their accumulation in sediments. Natural and anthropogenic sources are usually determined using biota. However, effects from anthropogenic sources can also be calculated using the heavy metal enrichment factor (EF) (4), which refers to concentrations above the background level in a studied area.

The enrichment factor (EF) method normalises the measured heavy metal content with respect to a sample reference metal, such as iron, scandium or aluminium (5). EF is usually calculated according to the equation:

$$EF = \frac{\left(\frac{Cx}{Cref}\right)_{sample}}{\left(\frac{Cx}{Cref}\right)_{averageshale}} \quad [1]$$

where C_x is the concentration of a metal and C_{ref} a normalising element. Usually an average shale or non-contaminated crust is used as a reference material

to which a sample is compared. Aluminium is often used as the normalising element, provided that it is not enriched due to local contamination. The baseline values for metal content in non-contaminated crust are usually used from literature.

This approach however is not appropriate for determining heavy metal contamination in a river basin. In this study we therefore suggest a more appropriate way to assess the toxic effects of accumulated heavy metals by analysing their distribution between sediment and river water, presuming that heavy metals originating from anthropogenic sources might be in trace concentrations.

MATERIALS AND METHODS

For the experiment we took water and sediment samples from three sampling locations along the Sava River (Figure 1). Location 1 (L1) is near an industrial area of the town of Šabac. Location 2 (L2, Obrenovac) is downstream of the coal-fired power plant "Nikola Tesla", where the Kolubara River flows into the Sava River. Location 3 (L3) is at the mouth of the Sava into the Danube, in a wider metropolitan area of Belgrade.

Water and sediment samples were collected between 2005 and 2009 at six-month intervals to cover both the dry (autumn) and wet (spring) seasons. Water samples intended for microbiological analysis were taken early in the morning at about 50 cm below the water surface and placed into sterile 250-mL bottles. The bottles were kept cold in ice-packed cooler boxes and returned to the laboratory for analysis on the same day they were collected. For the analysis we used the spread-plate method. All samples were tested for total bacteria count, total coliforms, faecal coliforms, and *E. coli*. Bacterial colonies were counted after incubation on the surface of plastic plates filled with nutrient agar and directly inoculated in serial diluted river water onto each plate. Total coliforms (TC) and *E. coli* were scored using the most probable number (MPN) method (6, 7) and inoculated into a series of five tubes containing Lauryl tryptose broth (Torlak Institute, Belgrade, Serbia) and Endo agar at 37 °C for 24 h. Gram-negative bacteria were scored as faecal coliforms (FC). Samples of total FC were inoculated onto incubation plates with MacConkey agar (Difco, Detroit, USA) at 37 °C for 24 h. The plates for thermo tolerant FC were incubated at 44 °C for 24 h (7).

Figure 1 *The three sampling locations along the Sava River: Šabac (L1); Obrenovac (L2); Beograd (L3)*

Sediment samples were collected from the top 10 cm of sediment surface. All samples were heated at 105 °C until they reached constant weight. The caked sediment was then finely ground to grains below 1.0 mm in diameter. Samples of sediment (2.5 g) were dissolved in 25 cm^3 of HNO_3 (Merck, Germany) to desorb mobile species of heavy metals.

To measure the concentration of heavy metals we used the flame atomic absorption spectrometry (US Perkin Elmer AAnelist200 spectrometer with air/acetylene flow (USA.) Cadmium concentration was determined using the graphite furnace technique (Perkin Elmer AA 600 with transversely-heated graphite atomiser, THGA (USA) and Zeeman-effect background correction. The THGA provides uniform temperature distribution over the entire tube length, rapid heating and an integrated Lvov platform, resulting in an improved signal/interference ratio and high analytical sensitivity (8). Analytical injection (20 µL) and atomisation were done in five steps, controlled by appropriate software and auto-sampler.

For both techniques, adequate hollow cathode lamps were used for irradiation, and reference standard solutions were prepared for analysis by mixing Merck certified atomic absorption stock standards (1000 µg mL^{-1}) and Millipore purified water. Modifiers were not added.

Statistical evaluation of the results was performed using the correlation analysis. The correlations were primarily associated with spring and autumn data sets.

RESULTS AND DISCUSSION

Information on physico-chemical and microbiological indicators may help to predict the level and trends of river pollution. Since such data for the Sava River in Serbia are lacking, in this study we focused on three sampling locations, situated upstream

and downstream from a pollution source, the coal-fired power plant, and investigated the levels of bacterial and heavy metal pollution.

Analysis of microbiological parameters

To ensure effective detection of point pollution sources, we collected all water samples early in the morning. It was observed earlier (9) that bacterial numbers might significantly vary between early morning and the afternoon samplings, usually due to variations in water temperature and levels of ultraviolet radiation.

Figure 2 shows the results of the microbiological analysis of the Sava River water, expressed as mean total coliform scores by sampling location. We observed that in both seasons microbiological contamination increased downstream. The highest total coliform and *E. coli* levels were found at Location 3, near the Sava mouth into the Danube (Figure 1). At this location, total coliforms during the wet season were still above 10000 MPN per 100 mL. These findings are consistent with available literature (10).

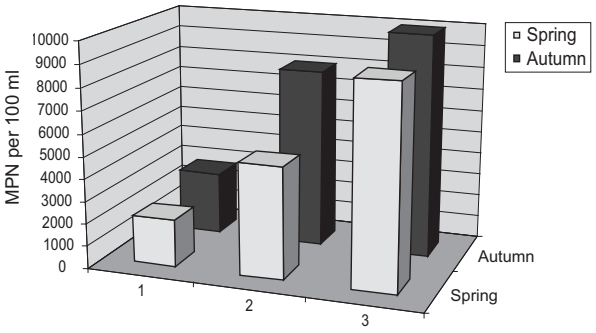

Figure 2 *Mean total coliforms in the Sava River water samples by location and season.*
Sampling locations were located upstream (1) and downstream (2, 3) from the pollution source, the coal-fired power plant
1 – Šabac, 2 – Obrenovac, 3 – Beograd

In the tested samples we found *Klebsiella* spp. and *Citrobacter,* which were also detected in similar studies (6, 7, 11), but did not find other pathogenic bacteria.

Statistical evaluation shows the association between microbiological contamination and different factors of natural and anthropogenic origin. Factors of natural origin include hydrology, weather conditions, and physico-chemical parameters of river water, while anthropogenic factors include agricultural and industrial influence and population density. We found a statistically significant correlation between natural sources and the season of sampling (Figure 2), with r_s values that ranged from 0.45 to 0.55. In contrast, r_s values for anthropogenic influences ranged from 0.33 to 0.40.

Our results showed that the Sava River water was microbiologically polluted, with values that exceeded acceptable freshwater bacterial counts (12).

Distribution of heavy metals

Mean heavy metal mass fractions in sediments for all three locations and four sampling years were: 56.0 mg kg^{-1} for Zn; 39 mg kg^{-1} for Cu; 27.0 mg kg^{-1} for Pb, and 4.6 mg kg^{-1} for Cd. These levels are similar to findings reported by Oreščanin et al. (2) for the upper stretch of the Sava River, about 450 km from its mouth into the Danube River, except for Zn.

Fluctuations in sediment heavy metal mass fractions were not high. However, we observed seasonal differences for Pb and Cd, whose levels were higher in the autumn for more than 20 %. Differences for Cu and Zn were negligible compared to the spring.

Heavy metal levels in both solid and liquid phases were relatively low. Concentration of Cd in river water did not exceed the 5 μg L^{-1} threshold for the A class of surface waters and neither did Pb exceed the 50 μg L^{-1} limit (13).

Heavy metal levels in sediment correlate with water levels. Partitioning of heavy metals between suspended matter and water is described in terms of distribution coefficient K_d (L kg^{-1}), which is a concentration ratio between the solid and liquid phase under equilibrium conditions (14). The distribution coefficient evidences the capability of sediment to accumulate a heavy metal. The distribution coefficients (K_d) for heavy metals in our study decreased in the following order Cd>Pb>Cu~Zn. They ranged as follows: (0.86 to 3.6)x10^3 L kg^{-1} for Zn; (1.1 to 6.9)x10^3 L kg^{-1} for Cu; (2.6 to 5.6)x10^3 L kg^{-1} for Pb; and (5.6 to 19.6)x10^3 L kg^{-1} for Cd (Table 1).

Over the past two decades, Serbia has not expanded its industrial activity, which resulted in barely noticeable anthropogenic input of heavy metals in the Sava river from close environment. In spite of that, high K_d (in 10^3 L kg^{-1}) evidences that heavy metals have been accumulating in the sediment.

Some authors such as Krishna et al. (15) used a different, multivariate approach to interpret surface water monitoring results. The idea is to distinguish between the geogenic and anthropogenic sources, to identify possible nonpoint sources of contamination, and to estimate each separate source contribution.

Table 1 *Distribution coefficients (K_d) of heavy metals for 3 locations in different seasons*

Season	K_d / x10^3 L kg^{-1} L1 - Šabac				L2 - Obrenovac				L3 - Belgrade			
	Zn	Cu	Pb	Cd	Zn	Cu	Pb	Cd	Zn	Cu	Pb	Cd
Aut.05	2.3	1.6	3.2	9.1	1.9	2.2	4.1	13.2	3.5	2.1	3.2	11.8
Spr.06	1.6	1.4	2.6	6.8	1.6	2.1	3.6	11.6	3.3	1.7	3.1	10.7
Aut.06	1.8	1.9	3.2	11.6	3.0	1.8	3.1	11.7	2.9	2.2	5.6	16.9
Spr.07	0.9	1.0	3.1	9.1	1.7	1.1	2.8	10.5	2.8	1.9	4.7	11.3
Aut.07	1.2	2.0	3.5	16.1	1.6	1.4	5.1	9.6	2.6	2.7	3.9	19.6
Spr.08	1.2	1.1	2.9	10.6	1.2	1.3	4.3	8.4	3.7	2.4	3.4	17.1
Aut.08	1.6	1.7	4.0	16.8	1.3	2.0	3.8	7.3	2.9	2.3	4.0	16.5
Spr.09	1.3	1.0	2.8	11.2	0.9	1.6	3.2	5.6	1.6	1.9	3.6	12.4

Anthropogenic input into sediment is usually assessed with the help of the enrichment factors (EF).

In this study EF were calculated as follows (16):

$$EF = (Me/Al)_{sample} / (Me/Al)_{crust}, \quad [2]$$

EF is the ratio between mass fractions of particular metal and aluminium (Al) in the sample and their mass fractions in non-contaminated crust minerals. We used reference Me_{crust} values from Olivares et al. (17), as follows: 127.0 mg kg^{-1} for Zn, 32.0 mg kg^{-1} for Cu, 16.0 mg kg^{-1} for Pb, 0.2 mg kg^{-1} for Cd, and 6.9 % for Al.

Heavy metal level higher than twice the background implies anthropogenic pollution, while EF higher than 2 indicates contamination (4, 18-20). The enrichment of heavy metals in sediment is mainly due to surface adsorption and ionic attraction (21). For a reliable assessment of human risk by anthropogenic sources, not only does one have to know the background level of heavy metals in the sediment and the enrichment factors, but also has to keep in mind the dynamic equilibrium between the river sediment and water (22).

The environmental impact of heavy metals in river systems is better evaluated by investigating the distribution of heavy metals between the solid and liquid phases, because the input of heavy metals from anthropogenic sources is usually in trace concentrations. The behaviour of heavy metals depends on hydrological and geo-chemical processes. To assess the risk, it is important to determine their flux from the river water into the sediment and *vice versa*, and their flux from sediment surface to deeper layers. The fraction in the sediment is not expected to pose a direct threat, provided that metal ions are immobilised due to encapsulation. Distribution coefficients between the sediment and the river water point out exchangeable and mobile fractions of heavy metals that may be toxic for the environment, and thus are responsible for their ecotoxicological potential.

Our data show statistically significant correlations (r_s) between contamination from natural sources and seasons (Table 1), and they range from 0.8 to 0.9. At the same time, we have not observed any statistically significant correlation with anthropogenic sources of contamination.

Almost constant K_d for respective heavy metals in our study suggests that the sorption capacity of the Sava River sediment is not even near saturation. In other words, instead of total metal concentration, changes in K_d could be used as an indicator of potential sediment heavy metal effects (21).

CONCLUSION

This study has confirmed the role of distribution coefficients that might be used in assessing the potential effects of the heavy metals in sediment, instead of using total metal concentration.

To reduce the existing bacterial contamination of the Sava River it is necessary to control faecal discharge near cities like Belgrade. Heavy metals measured at the same time in the Sava River sediment and water are not posing a risk.

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Sažetak

ONEČIŠĆENJE RIJEKE SAVE U SRBIJI TEŠKIM METALIMA I BAKTERIJAMA

Ispitivani su mikrobiološki parametri i teški metali u rijeci Savi na tri lokacije u blizini industrijskih i urbanih centara (Šabac, Obrenovac, Beograd). Analiza je obuhvatila parametre kvalitete: teške metale – Cu, Zn, Pb i Cd u riječnoj vodi i sedimentu te bakterije i patogene bakterije u riječnoj vodi. Radi utvrđivanja bakterijske kontaminacije površinskih voda testirani su koliform *Escherichia coli* i fekalni koliformi. Brojnost *E. coli* povećava se od lokacije 1 prema ušću Save od 2100 do 10000 u 100 mL NVB (najvjerojatniji broj). Nađen je velik broj biološki aktivnih mikroorganizama i bakterija. Koncentracija teških metala u sedimentu u korelaciji je s njihovom koncentracijom u riječnoj vodi ako se izrazi s pomoću koeficijenta distribucije K_d ($dm^3\ kg^{-1}$) između čvrste i tekuće faze. Predložen je postupak za procjenu toksičnosti teških metala.

KLJUČNE RIJEČI: *bakterije,* E. coli, *koeficijent distribucije, teški metali*

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