Actual contamination of the Danube and Sava Rivers at Belgrade (2013)

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Abstract: This study was focussed on a comprehensive investigation on the state of pollution of the Danube and Sava Rivers in the region of Belgrade. Different complementary analytical approaches were employed covering both *i*) organic contaminants in the river water by target analyses of hormones and neonicotinoids as well as non-target screening analyses and *ii*) heavy metals in the sediments. Finally, some common water quality parameters were analysed. The overall state of pollution is on a moderate level. Bulk parameters did not reveal any unusual observations. Moreover, quantification of preselected organic contaminants did not indicate to elevated pollution. More significant contaminations were registered for chromium, nickel, zinc and partially copper in sediments with values above the target values according to Serbian regulations. Lastly, non-target screening analysis revealed a wider spectrum of organic contaminants comprising pharmaceuticals, technical additives, personal care products and pesticides. The study presented a comprehensive view on the state of pollution of the Sava and Danube Rivers and is the base for setting up further monitoring programs. As a superior outcome, it was illustrated how different chemical analyses can result in different assessments of the river quality. A

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comparison of target and non-target analyses pointed to potential misinterpretation of the real state of pollution.

Keywords: river systems; state of pollution; organic pollutants; heavy metals; screening analyses; non-target screening.

INTRODUCTION

Large rivers are severely contaminated water bodies in aquatic ecosystems, which have become increasing serious problems for decades. Riverine contaminants are derived from both anthropogenic and biogenic sources.[1–4] Anthropogenic inorganic, organic and organometallic pollutants are emitted into rivers by for example runoffs and leaching from agricultural fields, direct discharge of waste water, by atmospheric deposition and shipping activities. In more detail, the contamination is caused by heavy metals, nutrients and many low-molecular weight organic compounds, such as pesticides, plasticizers, pharmaceuticals, ingredients of personal care products, *etc*. Many of them are known to have, or are suspected to have, toxic, ecotoxic or endocrinic effects at trace levels. Noteworthy, surface water is becoming more and more relevant as a source of drinking water. Therefore, an appropriate water quality control is needed as a base for a sustainable usage of river water.

Pollution of the aquatic environment caused by inorganic chemicals is considered a major threat to aquatic organisms including fish. The most common anthropogenic sources of metals are industrial plants, petroleum contamination, waste water treatment plants and sewage disposal.[5] Metal ions can be incurporated into the food chains and concentrated in aquatic organisms to a level that affects their physiological state. Trace metals such as Zn, Cu and Fe play a biochemical role in the life processes of all aquatic plants and animals; therefore, they are essential in the aquatic environment in trace amounts.[6] One of the major problems that heavy metals cause with respect to their effects on aquatic organisms is their long biological half-life. Therefore, they are among the most frequently monitored micropollutants, and reliable techniques have been established for their extraction and quantification, since sediment contamination by heavy metals in rivers and estuaries has become an issue of increasing environmental concern.[7–10] However, in most cases only total amounts are determined instead of the more relevant bioavailable fractions.

River sediments act as a fundamental environmental compartment and hold many functions, *e.g*., providing foodstuff for living organisms. They also serve as a sink and reservoir for a variety of environmental contaminants in accumulation or deposition areas. It has been recognized that aquatic sediments accumulate persistent and toxic chemicals.[11] In the past years, tremendous efforts have been made to characterize the fate, loading and distribution of heavy metals in sediments.[12]

The River Danube is the second longest river in Europe with a length of about 2 800 km.[13] Its catchment area covers 801 500 km^2, with approximately 81 million inhabitants in 19 countries.[14] The Danube flows through four capitals (Vienna, Budapest, Bratislava and Belgrade) with 0.5 million to 2.5 million inhabitants contributing to extensive water use and pollution.

Many organic compounds have been identified in the Danube River, *i.e.*, to date: hydrophobic alkylphenolic, lipid series (*n*-alkanoic acids, *n*-alkanes, *n*-alkanols and sterols), pharmaceuticals (such as ibuprofen, diclofenac, sulphamethoxazole and carbamazepine), pesticides and their degradation products (*e.g*., bentazone, 2,4-D, mecoprop, atrazine, terbuthylazine and desethylterbutylazine), perfluorinated acids, and endocrine disrupting compounds (nonylphenol, NPE1C, bisphenol A and estrone).[15–17] Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs), such as dichlorodiphenyltrichloroethane (DDT) and analogues, hexachlorocyclohexanes (HCHs) and hexachlorobenzene (HCB), were measured in sediments and biota.[18] Lipids, amino acids and carbohydrates are reported to be present in particulate and dissolved organic matter as constituents of the organic matrix.[16]

The Sava River represents the largest tributary of the River Danube. It is 945 km long and it flows through Slovenia, Croatia, Bosnia and Herzegovina and Serbia. The Sava River Basin covers a catchment area of 95 719 km^2, which is approximately 40 % of the countries' combined total surface area and it is the source of more than 80 % of the total available freshwater in the area. Only a few publications have considered contamination of the Sava River water. The following persistent organic pollutants were investigated: polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), selected chlorinated pesticides and organo-tin compounds.[19] It has been reported that many organic compounds were found in Sava groundwaters: hydrocarbons, petroleum hydrocarbons, aromatic hydrocarbons, PAHs, fatty acids, phenolic compounds, alkylsulphides, indoles, benzophenone, detergent-derived organic compounds, biodegradation products of nonylphenol polyethoxylates surfactants and EDTA.[20] On the other hand, dissolved metals were observed in grab water samples - Fe, Mn, Zn, Ni, Cu, Cr, Co, Pb and Cd. Inorganic pollutants were investigated in sediments as well - Cd, Pb, Ni, Hg, Cu, Zn, Cr, As and P were determined.[21]

The present study focused on a comprehensive investigation on the state of pollution of the Danube and Sava Rivers in the area of their confluence, the Belgrade region. For these purposes, different complementary analytical approaches were used at different laboratories in Europe (Czech Republic, Slovenia, United Kingdom and Germany). The analytical approaches covered on the one hand organic contaminants in the river water by target analyses of hormones and neonicotinoids as well as non-target screening analyses. On the other hand inorganic contaminants were investigated in sediments, particularly heavy metals.

Furthermore, some common water quality parameters were analysed, including Total Organic Carbon (TOC), Total Nitrogen (*TN*) and major ions.

The overall goal of this case study was to illustrate how complex and complementary can be a more comprehensive analytical view on an aquatic system. To the best of our knowledge this approach has been ignored as far as possible in environmental geochemical studies in the Danube Basin catchment area.

EXPERIMENTAL

Sampling

Water samples. River water samples were taken during two different sampling campaigns in January and February 2013 at five sampling locations along the Danube and the Sava Rivers in Serbia (Fig. 1, Table I). Water samples of 2.5 L were taken from approximately 20 cm below the water surface nearby the river bank and bottled in pre-cleaned flasks. The sample material was stored in the dark at a temperature of approximately 4 °C. Before extraction and *TOC* determination, all water samples were filtered through pre-cleaned Whatman GF/F filters (0.45 mm) in order to remove suspended particulate matter from the aqueous phase.

Sediment samples. Surface sediment samples were taken at the same five sampling locations along the Danube and the Sava Rivers in Serbia (see Table S-I, of the Supplementary material to this paper). Sediment samples from the first two locations on the Sava River (Zabran and Makiš) were taken by means of a Van Veen grab from a depth of 1.20 m and at the third location (Kapetanija), from a depth of 3.50 m. Sediment samples from the two locations on the Danube River were taken from depths of 0.90 m (Batajnica) and 1.20 m (Vinča). The samples were kept in pre-cleaned vessels and stored at a temperature of approximately 4 °C.
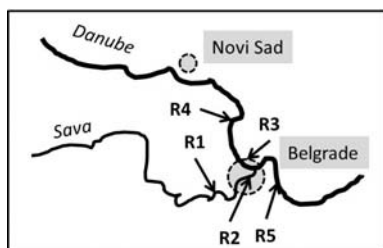

Fig. 1. Map of the sampling locations on the Danube and Sava Rivers.

Water quality parameters

TOC *analyses.* Total non-purgeable organic carbon (*TOC*) concentration were analyzed by a TOC Analytik Jena AG multi N/C 3100 instrument, calibrated with potassium hydrogen phthalate. Prior to the measurements, the samples were acidified to pH 2–3 with hydrochloric acid.

Major ion analysis by ion chromatography

The anions were determined on a Dionex IonPac AS4A separation column (250 mm×4 mm) using a Shimadzu LC-10Ai liquid chromatograph with a CDD-6A conductivity detector. A mixture of sodium carbonate and sodium bicarbonate in water (1.8:1.7 mole ratio) was used as the mobile phase at a flow rate of 1.5 mL min^{-1}. The injection volume was 100 µL. For

quantification purposes, linear calibration curves were prepared and the r^2 values of the regression lines were around 0.99.

The cations were determined on a Shimatzu LC 10Ai ion Chromatograph using a DIONEX ION PAC SCS1 separation column (250 mm×4 mm). As a mobile phase 3 mM sulphuric acid was used at a flow rate of 1.0 mL min^{-1}. The injection volume was 50 µL.

Determination of ammonium

Aliquots of 2 mL of ammonium standard solutions or water samples were separately mixed with 200 µL manganese sulphate solution (2.5 mM), 0.5 mL sodium salicylate solution (1.5 M), 0.5 mL potassium sodium tartrate solution (30 mg mL^{-1}), 0.5 mL 5 % aqueous hypochlorite solution and 0.5 mL sodium hydroxide solution (0.5 M). The mixture was incubated 10 min for Indophenol Blue formation. After 10 min, the samples were diluted by adding a mixture of acetonitrile:water (1:1, *V/V*) to obtain a final volume of 10 mL. The mixtures were sonicated in an ultrasonic bath for 20 s before 3 mL of the mixture was introduced into a 10 mm optical path length quartz cell. All analyses were performed on the same day and each sample was analyzed in triplicate.

Ammonium determinations based on the formation of Indophenol Blue and thermal lens spectrometric (TLS) detection were performed on a dual beam, mode mismatched TLS spectrometer using the TLS experimental setup consisting of a krypton laser (Coherent, Innova 300C, Santa Clara, CA, USA) with excitation beam source tuned at 647 nm providing 100 mW power. An He–Ne laser (Melles Griot, Uniphase, model 1103P, Carlsbad, CA, USA) provided the probe beam at 632.8 nm with 2 mW of power.

Non-target screening

Extraction. Sequential liquid/liquid extraction was preformed on approximately 2000 mL aliquots of the water samples with two kinds of solvents – *n*-pentane and dichloromethane.[22] Three extraction steps were performed in a separating funnel with 50 mL of the solvent. The first one with *n*-pentane, the second one with dichloromethane and the third one with dichloromethane after addition of 2 mL of concentrated hydrochloric acid that was pre-cleaned by intense extraction with *n*-hexane. Subsequently, the organic layers were separately dried by filtration over approximately 1 g of anhydrous granulated sodium sulphate (Merck, Germany) and 50 µL of an internal standard solution containing d$_{34}$ *n*-hexadecane, fluoroacetophenone and decafluorobenzophenone in *n*-hexane (the concentrations were 6.0, 7.2 and 6.9 ng µL^{-1}, respectively) was added to the first and second extracts. Acidic compounds in the third extract were methylated by addition of a diazomethane solution. The methylated extract was purified by fractionation with dichloromethane and methanol (50:50, *V/V*) through silica-gel in order to remove high polar derivatisation artefacts. In addition, 50 µL of the same internal standard was added to the third fraction as well. Before injection into the gas chromatograph (GC), all of the extracts were reduced to a volume of 50 µL at room temperature. For the gas chromatographic–mass spectrometric (GC–MS) analyses, the extracts were reduced to a volume of 20 µL at room temperature.

GC–MS analyses. GC–MS analysis was performed on a Finnigan Trace MS, ThermoQuest (Egelsbach, Germany) linked to a Carlo Erba, HRGC 5160 gas chromatograph equipped with a 25 m×0.22 mm ID×0.25 µm film BPX5 (SGE, Germany). The GC oven heating programme was 3 min hold at 60 °C, 60 °C to 300 °C at a rate of 3 °C min^{-1} and a 20 min hold at 300 °C. The injection was performed via a split/splitless injector at 270 °C; the splitless time was 60 s. The carrier gas was helium at a velocity was 2 cm s^{-1} and a source temperature of 200 °C. The

MS was operated in the electron impact ionization mode (EI$^+$, 70 eV) scanning from 35 to 500 Da at a rate of 1 s dec^{-1} and an inter-scan time of 0.1 s.

Identification of the organic compounds. The EI$^+$-mass spectra were compared with spectra of reference compounds from mass spectral databases (NIST/EPA/NIH Mass Spectral Library NIST05, Wiley/NBS Registry of Mass Spectral Data, 7th electronic version) and gas chromatographic retention times. The retention times of the internal standard compound were used for correction of inaccuracies in the injection time.

Hormone analyses

Extraction. The target compounds were isolated from the river water using SPE. SUPELCLEAN ENVI-18 SPE Tubes (6 mL, 1 g) that had been conditioned with 5 mL of *n*-hexane, 5 mL of ethyl acetate and 5 mL of methanol and washed with 10 mL of Milli-Q water. Then 400 mL of river water was loaded under moderate flow (3 mL min^{-1}). SPE cartridge was subsequently dried under vacuum for 20 min and then the hormones were eluted with 6 mL of a mixture of ethyl acetate and methanol (5:1). Extract was evaporated to dryness under gentle stream of nitrogen and reconstituted in small volume (100 μL) of methanol. This solution was filtered using LUT Syringe Filters PTFE, 13 mm, 0.45 μm and transferred to low-volume autosampler vials.

HPLC–ESI-MS analyses. An Agilent 1100 HPLC system with an Agilent 6320 spherical ion trap mass spectrometer and electrospray ionization was used. The hormones were separated on a Phenomenex Kinetex C$_{18}$ column, 100 Å, 150×3 mm, 2.6 μm particle size (Core-shell type) at a temperature of 25 °C using an acetonitrile (ACN)/water mixture at a flow of 0.25 mL min^{-1} (t_0: 40 % of ACN, t_{20}: 90 % of ACN). Nitrogen was used as the nebulising gas at a pressure of 25 psi, drying gas (N$_2$) temperature and flow were 350 °C and 10 L min^{-1}, respectively. The ion trap scanning range was set from 50 to 800 u. Estradiol (*α*- and *β*-), estriol, estrone, diethylstilbestrol and ethinyl estradiol were detected in the negative ESI-mode, and norethindrone and progesterone in the positive ESI-mode. The limit of quantification (*LOQ*) ranged from 0.03 to 0.5 ng L^{-1} and the limit of detection (*LOD*) was between 0.01 and 0.15 ng L^{-1}. The recoveries obtained for all target compounds, evaluated by using spiked real samples, were higher than 70 %.

Neonicotinoid pesticides analyses

Extraction. Water samples of 1 L in volume were divided into two replicates and extracted using Strata C18-E columns polymeric sorbent (200 mg, 6 mL). Prior to extraction, the cartridges were activated with 5 mL of methanol and 5 mL of distilled water. After extraction, the samples were eluted with 5 mL of methanol. After evaporation of the solvent, the samples were dissolved in 0.5 mL of mobile phase and analyzed using HPLC-DAD.

HPLC-DAD analysis. Neonicotinoids were analyzed by HPLC-DAD (UV–Vis) consisting of a Hewlett Packard 1100 Series chromatograph coupled with a DAD detector operating in the UV–Vis range. The separation was achieved using a C8 column (250 mm×4.6 mm) with Chromasil 100 (5 μm) as the stationary phase. The column thermostat was maintained at 25 °C. The injection volume was 75 μL. The eluents consisted of 30 % acetonitrile and 70 % acetic acid; the flow rate was 1 mL min^{-1} and the wavelength for thiamethoxam was 247 nm, for imidacloprid 250 nm and for clothianidin 260 nm. The retention time for thiamethoxam was 5.7 min, for imidacloprid 8.5 min and for clothianidin 7.4 min. For quantification purposes, calibration curves in the range from 0.1 to 100 mg L^{-1} were prepared. The *LOD* value for all the analysed neonicotinoids was 0.5 mg L^{-1} mg.

Ecotoxicological tests

The ecotoxicity of samples was assessed using liquid-dried luminescent bacteria *Vibrio fischeri* NRRL B-11177 with system LUMIStox, Dr. Lange. The toxicity endpoint was determined as the reduced luminescence emission after incubation in the presence of selected chemicals or mixtures. Before analyzing the samples, the pH was adjusted to 7±0.2 with hydrochloric acid or sodium hydroxide and sodium chloride salt was added to obtain a 2 % concentration in order to avoid possible adverse effects due to an incorrect pH value or an inappropriate sodium chloride concentration. The liquid-dried luminescent bacteria were reactivated before the test was started. An aliquot containing *V. fischeri* was added to each vial in two parallels and luminescence was measured immediately. Afterwards the selected sample was added to the vial with bacteria and thermostated to 15±1 °C for 30 min. Luminescence was measured with a photomultiplier LUMIStox 300 luminometer and thermostated at 15±1 °C. The luminescence of the bacteria within the sample was again measured after 30 min of exposure and the inhibition of luminescence with 95 % confidence limits, according to ISO 11348-2, was calculated, using a model supported by computer software. The whole process was completed in accurate time intervals provided by machine and computer signalization. The blank test was performed with 2 % sodium chloride solution.

Heavy metal determination in sediments

Prior to heavy metal analysis, the sediment samples were dried at 110 °C for 24 h. After drying, the sediments were subjected to mechanical homogenization by grounding to a powder. Then, aliquots of 0.5 g of dry sediments were digested in 10 mL of concentrated nitric acid and the mixture was heated to 190 °C in a microwave oven at 400 W until total dissolution. The solution was filtered through a Whatman No 41 filter, diluted with water to 100 mL and analysed for heavy metal concentration using inductively coupled plasma optical emission spectrometry (ICP-OES). A Perkin Elmer, Optima 4300 DV spectrometer equipped with an AS-93 plus autosampler was used with a sample flow 1.5 mL min^{-1} and a wet plasma aerosol.

RESULTS AND DISCUSSION

Since a major aim of this study was to provide a comprehensive view on the state of pollution of the Danube and Sava Rivers in the region of Belgrade, an extensive analytical investigation was performed. In the following, results from bulk parameter analysis, heavy metal determination as well as target and non-target screening analyses on organic compounds are presented and discussed in detail.

General characterisation of the water quality

The water samples investigated in this study were first characterized by some general parameters in order to reflect the general state of the water quality.

As a relevant parameter, the *TOC* was determined and the data are given in Table S-I. For a preliminary assessment, a comparison with literature data is useful. The average *TOC* values for natural water systems were reported as follows: in common ground waters – around 0.7 mg L^{-1}; in ground waters with high amounts of humic substances – 6 to 15 mg L^{-1}; in sea water – around 2 mg L^{-1}; and in rivers and lakes – up to 10 mg L^{-1}. The data for Save and Danube

River water ranged from 2 to 4 mg L^{-1} and hence none exceeded those registered under natural conditions.[23]

Quite similar and low ammonium concentrations (0.12–0.14 mg L^{-1}) were found in waters from three locations, while for the Sava at Zabran and the Danube at Vinča, the values were below the limit of detection (*LOD* = 0.1 mg L^{-1}, Table S-II). These concentrations comply with the reference values for the highest quality class of surface waters according to the Water Framework Directive (European Environment Agency, 1975, Council Directive 75/440/EEC).

Furthermore, the results of ion chromatography pointed dominantly to the environmentally relevant presence of nitrate and sulphate, with the highest values at the Dunav Batajnica sampling point. However, all the concentrations in samples were below 1 mg L^{-1} (Table S-II) and hence the quantities of the ions were not harmful for freshwater organisms.

Lastly, a brief ecotoxicity screening performed with *V. fischeri* showed none of the river water samples had a toxic effect on this kind of bacteria.

Non-target screening analyses of river water

Non-target screening analyses based on GC/MS techniques were applied to all five water samples. The overall aim of this part of investigation was to identify specific organic contaminants in the two rivers and to obtain an overview on the emission sources affecting the river systems. All compounds indentified in the Danube and Sava Rivers are presented in Table S-III. They are grouped according to their technical application, unless they occur naturally in the environment (group: Natural products). Abbreviations are given for certain compounds.

The majority of the identified contaminants were of anthropogenic origin and many of them have been reported formerly as river pollutants. This is the case for, *e.g*., of carbamazepine, which is frequently used as an antiepileptic drug.[24] It was identified in both samples from the Danube, but only in one from the Sava (the one closest to the confluence – location Kapetanija). A higher diversity as compared to the pharmaceuticals was evident for personal care products with their several subgroups, such as: fragrances, washing agents, or ingredients in cosmetics. Some of them may have both anthropogenic and natural origins; therefore, it is partially not possible to determine their source accurately. However, galaxolide (4,6,6,7,8,8-hexamethyl-1,3,4,6,7,8-hexahydrocyclopenta-[*g*]isochromene) and tonalide (1-(3,5,5,6,8,8-hexamethyl-6,7-dihydronaphthalen-2-yl)ethanone) are known synthetic fragrances and have been frequently detected in the environment, since they are emitted from domestic sewage.[25] These two compounds were detected in large quantities in all five samples. Direct exposure to these compounds in fragrances or indirect exposure from their presence in the environment pose no significant risk to human health, but if they

diffuse through the skin from alcoholic solutions, they can be stored in human fat tissues and human milk because of their lipophilicity.[22] In addition lilial (butyl-phenylmethylpropanal) and methyl dihydrojasmonate frequently appear in fragrances. They were detected in both rivers, but methyl dihydrojasmonate was more abundant than lilial, particularly in the Danube. *N,N,N′,N′*-Tetraacetylethylenediamine (TAED) is used as bleaching activator in laundry detergents.[24] Its presence was significant in all of the samples. 4-Methoxy-2-ethylhexylcinnamate is a constituent of sunscreens where it acts as a UV-protector.[26] It was identified in small quantities in all samples. Selected personal care products are presented in Fig. 2.

A further group of specific contaminants comprises the technical additives. [2,2,4-Trimethyl-3-(2-methylpropanoyloxy)pentyl]-2-methylpropanoate (TXIB) is added as a plasticiser to polymers such as poly(vinyl chloride), which is used in drinking water pipes, and hence TXIB, albeit of low solubility (1–2 mg L^{-1}), can be liberated into the water. No information on the environmental fate and occurrence of TXIB is available, but it is reported to have low toxicity.[24] Both Rivers contained large quantities of this compound. Tri-*n*-butylphosphate (TBP) is used in the manufacture of dyestuffs, lacquers, resins and plasticizers. It has moderate toxicity for aquatic organisms.[27] TBP was found in small amounts in all samples, except in the sample from near Vinča where higher concentrations were detected. Tris(2-chloroethyl)phosphate (TCEP) and tris(2-chloro-*iso*-propyl)-phosphate (TCPP) are flame retardants and weak plasticizers that are frequently used as plastic additives as well as flame inhibitors in carpets, and casings of electric devices. They are well known contaminants in river water, sewage effluents and river sediments.[28,29] It was reported that the industrial usage of TCEP ceased some years ago.[24,27] Nevertheless, both TCEP and TCPP were found in the samples, except in the sample from the location nearby Obrenovac. TMDD (2,4,7,9-tetramethyl-5-decyne-4,7-diol) is used as a dispersing additive in pigment paints and as a surfactant in printing inks.[24] Its occurrence in the aquatic environment is rarely reported, but it has recently been discussed in detail as river water contaminant.[25,26] This compound is found in both the Sava and Danube Rivers, with the concentrations being higher in the Danube than in the Sava. Benzothiazole is the major leachate compound of rubber, but is also directly used as a flavouring agent and as an additive in fungicides.[30] It could be associated with the residues of automobile tires. Therefore, its occurrence may be partially attributed to street runoff or rubber waste discharge from the national road of the area.[31] Quantities of this contaminant are larger in the Danube than in the Sava. Irgacure 184 (1-hydroxycyclohexyl phenyl ketone) is used as a UV photoinitiator in coatings, inks and adhesives. It may be harmful to human organism by inhalation, ingestion or skin absorption.[32] Large quantities of Irgacure 184 were found in both rivers. *N*-Butylbenzenesulphonamide (NBBS) is a known neurotoxic

pollutant of, *e.g.*, surface water, groundwater, wastewater and also landfill leach-ates.[33–36] 2,6-Di-*tert*-butyl-1,4-benzoquinone acts as an antioxidant and was present in significant quantities in both river waters.[37] Uvazols are used as UV stabilizer for polyolefines, polyester resins and coatings.[37] Large quantities of Uvazol 236 (2-(5-chloro-2*H*-benzotriazole-2-yl)-6-(1,1-dimethylethyl)-4-methyl-phenol) was found in the samples from the Sava River. Selected technical additives found in the water sample are presented in Fig. 2.
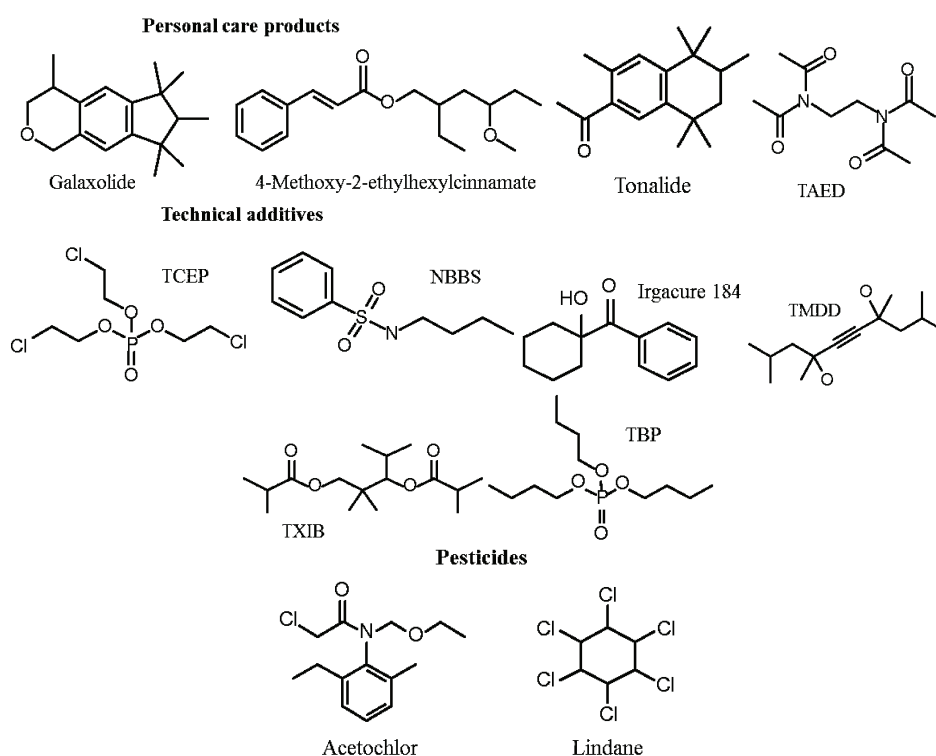

Fig. 2. Chemical structures of selected compounds identified in the waters of the Danube and Sava Rivers.

A very well known group of water pollutants are pesticides. Acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)-acetamide) is a constituent of a variety of commercial herbicides (*e.g.*, TripleFlex®), but its use is restricted because of its high toxicity.[39,40] In the US, acetochlor is the third most frequently detected herbicide in natural waters.[41] Acute toxicology data submitted to the EPA place technical acetochlor in the toxicity category II for eye irritation and toxicity category III for acute oral, acute dermal, and acute inhalation.[39,42] Acetochlor can accelerate metamorphosis in amphibians and can also affect the grow rate of fish.[43] This compound was detected in small amounts in

three samples (the Sava – Makiš, the Sava – near confluence and the Danube – Vinča). Lindane (the γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane) was produced industrially until the late 1980s when it was banned, but it can still be detected in the environment, as was case of one of the samples from the Danube River. Besides being found dissolved in water, it can also be associated with particulate matter.[44] It poses a threat to human health because of its suspected carcinogenicity.[44] The chemical structures of lindane and acetochlor are given in Fig. 2.

Furthermore, one food constituent has been detected. Caffeine is a component of several medicines and beverages. It is frequent component of sewage effluents, therefore, its higher concentrations are not unusual. It is found in all five samples.[24]

Finally, dipropyldisuphide and dipropyltrisulphide are known to be formed by blue–green algae (*microcystis flos aquae*) in fresh waters and their industrial applications are unknown.[24] Hence, they likely represent natural products. Both compounds were detected in all of the samples.

Quantitative target screening analyses of river water

Quantitative target analyses were applied to all water samples on the pre-selected substance classes of hormones and neonicotinoids. The HPLC measurements revealed no occurrence of the selected neonicotinoid insecticides clothianidin, thiacloprid, imidacloprid and tiametoxam in any of the samples. Hence, a contamination with this type of emerging pollutants seemed to present no environmental problem for the Danube and Sava Rivers in this region in spite of the fact that this group of insecticides in becoming increasingly more important in agricultural practice. It should be stressed as well that neonicotinoid insecticides are applied in much lower concentrations than organophosphorus or other insecticides were in the past and are used in a different way (*e.g*., seed treatment instead of spraying).

On the other hand, quantitative analyses showed the presence of all eight hormones in the Sava River. Ethinyl estradiol was found only in the Makiš sample (0.16 ng L^{-1}) while estrone, estriol, progesterone and norethindrone were detected in concentration ranges of 0.15–0.19, 0.37–0.57, 0.02–0.03 and 0.05––0.13 ng L^{-1}, respectively. A lower level of contamination was determined in the samples from the Danube River. The compounds β-estradiol, diethylstilbestrol, and estriol were not detected in the Danube River samples. Norethindrone was only detected in the Vinča sample at low concentration of 0.08 ng L^{-1}, while ethinyl estradiole was only present in the Batajnica sample. α-Estradiol, estrone and progesterone were determined in the concentration ranges of 0.11–0.23, 0.10–0.20 and 0.03–0.06 ng L^{-1}, respectively. The levels of α-estradiol showed increasing tendency downstream of the rivers, while the levels of estrone and

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progesterone were more or less stable. This observation points to an increasing introduction of estradiol along the river course and persistence of estrone and progesterone. The levels of hormones found in the water of the Danube River water were quite similar to those found in German rivers, where estrone, 17α--estradiol, 17β-estradiol and 17α-ethinylestradiol were determined at the 20–200 pg L^{-1} level.[46]

Heavy metals in the sediments

To exemplify the distribution and levels of sediment contamination by heavy metals in the Sava and Danube Rivers, the metals As, Cd, Cr, Cu, V, Pb, Ni and Zn were selected for analyses due to their abundance and toxic effects in the environment of highly industrialized and urbanized areas. The determination of total content of heavy metals in sediments is particularly useful to collect information on the genesis of the soil and on the level of contamination. The trace metal levels in the examined sediments are presented in Table S-IV. For a preliminary risk assessment, the contents of metals were compared with Dutch recommendations and Serbian regulations of limit values of pollutant substances in surface, groundwater and sediment (Official Gazette of RS, 50/2012, in Serbian). These indicative levels for serious soil contamination and the accompanying target values for soil/sediment are also presented in Table S-IV. The given concentrations are in a standard type of sediment with 10 % organic matter and 25 % clay. Since the *TOC* and clay content of the investigated sediments may vary and differ significantly, the employment of these values is partially restricted. The soil remediation intervention values indicate when the functional properties of a soil for humans, plant and animal life are seriously impaired or threatened. They are representative of the level of contamination above which there is a serious case of soil contamination. The Dutch system has disadvantages being based on the total concentrations of contaminants, and in spite of a correction, the estimated risk often exceeds the actual risk.

In more details, the content of Cd was below detection limit in all samples. Furthermore, the levels of sediment contamination by As and Pb were below the target values, indicating a low risk level. The same was the case for the contents of Cr and Ni in the sediments of the Danube River at the location Batajnica, where the data were below the target values indicating low or no risk. At all other locations, the content of Cr did not exceed the intervention value but were above the target values and indicated contamination. Hence, a risk for the aquatic environment has to be assumed, but an intervention is not required.

With respect to the Cu content, the Danube sediments at the location Vinča and the Sava sediments at the location Kapetanija slightly exceeded the target values (36 mg kg^{-1}), but all other locations were characterized by insignificant contamination. Zn was detected below target values in the river sediments at the

sampling locations Batajnica and Makiš but with higher concentrations above the target values, however, not exceeding the intervention values. Hence, similar to Cr an environmental risk has to be stated, but intervention is not suggested.

In summary, a low to moderate contamination level with some location exceeding target values was evident. Noteworthy, the lowest level of contamination with all heavy metal concentrations below the target values was observed at the location Batajnica in the Danube River.

CONCLUSIONS

Water and sediment samples from the Danube and Sava Rivers in the area of Belgrade were investigated with complementary analytical approaches. Bulk parameters, such as the *TOC* values, and the concentrations of major ions did not reveal any unusual observation. Moreover, quantification of preselected organic contaminants (neonicotinoid pesticides and hormones) did not indicate to elevated pollution of the river waters. A more significant contamination was measured for the heavy metal contents in the sediments. For chromium, nickel, zinc and partially copper, values above the target values according to Serbian regulations were measured, but in no case did they reach Intervention Values in. Hence, an environmental risk was evidenced but intervention is not suggested for the investigated areas. Lastly, non-target screening analysis revealed a high diversity in chemical composition and a wide spectrum of organic contaminants comprising pharmaceuticals, technical additives, personal care products and pesticides. Some of the identified compounds are known pollutants whereas some other substances are so far unregistered contaminants, particularly in Serbian rivers. Although it was reported that the application of some of the identified compounds has been banned or restricted (*e.g*., TCEP, TCPP, lindane and acetochlor), this investigation showed that they can still be found in the environment.

First, in general, it could be claimed that the overall state of pollution of the Sava and Danube Rivers in the area of Belgrade is on a moderate level. Secondly, this study presented not only a comprehensive view on the state of pollution but might also act as a base for further well adapted monitoring measures for the Sava and Danube Rivers. However, as a more relevant and superior outcome, it has been illustrated how different chemical analyses for preselected pollutants could lead to totally different assessments of the quality of a river. In particular, the confrontation of target and non-target analyses indicates to potential misinterpretation of the real state of pollution by restricted analytical approaches.

SUPPLEMENTARY MATERIAL

Tables S-I–S-IV are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

ТРЕНУТНА ЗАГАЂЕНОСТ ДУНАВА И САВЕ КОД БЕОГРАДА (2013. Г.)

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Ова студија је усмерена на свеобухватно испитивање стања загађености Дунава и Саве у пределу Београда. Различити комплементарни аналитички приступи су употребљени за испитивање: *и*) органских загађујућих материја у речној води циљаном анализом хормона и неоникотиноида, као и скрининг анализом и *ии*) тешких метала у седиментима. Коначно, анализирани су и неки уобичајени параметри квалитета воде. Укупно стање загађености је на осредњем нивоу. Укупни параметри нису открили било шта неуобичајено. Ни квантификација унапред одабраних органских загађујућих материја није указала на повишено загађење. Нешто значајнија загађеност је измерена за хром, никл, цинк и делимично бакар у седиментима, са вредностима изнад циљаних вредности према српским законима. На крају, скрининг анализа је открила шири спектар органских загађујућих материја као што су фармацеутски производи, технички адитиви, средства за личну хигијену и пестициди. Студија даје широки поглед на стање загађености Саве и Дунава и представља основу за постављање даљих програма мониторинга. Значајан резултат је да је илустровано како различите хемијске анализе могу да укажу на различите оцене квалитета речне воде. Поређење циљане и нециљане анализе указало је на потенцијално погрешно тумачење стварног стања загађености.

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