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UNESCO Conference on
EMERGING POLLUTANTS IN WATER

BOOK OF ABSTRACTS

9-11 July 2013, Belgrade, Serbia

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CONFERENCE OVERVIEW

Surface water and groundwater contaminated by municipal and industrial sources, as well as diffuse sources from urban and agricultural areas, continue to build up pollution levels in the environment. Environmental legislation, even in highly developed countries, mostly deals with conventional pollutants and the so-called priority pollutants (POPs, pesticides, toxic metals, radionuclides, etc.). Only recently, attention has started to shift towards emerging pollutants.

THEMES

1. Analytical methods for the detection of emerging pollutants and their transformation products
2. Toxicity of emerging pollutants and their water-related properties (degradability, solubility, sorption)
3. Emissions and treatment of emerging pollutants
4. Occurrence and fate of emerging pollutants in surface water and groundwater, and mathematical modeling.

INTRODUCTION

Emerging pollutants are not regulated by international or national environmental legislation, and conventional methods of treatment often do not remove them. The elimination of these chemicals requires a multidisciplinary approach. Water and wastewater treatment is not the sole solution to removing these pollutants from the environment. Certain treatment techniques can remove these chemicals; however, such systems tend to be very costly. Also, most of the countries have either limited or no water purification and wastewater treatment infrastructure, rendering the implementation of new technologies infeasible.

For most of the emerging pollutants, risk assessment and ecotoxicological data are not available and it is, therefore, difficult to predict which health effects they may have on humans, terrestrial and aquatic organisms, and ecosystems. Concern has been growing, especially in the past 20 years, over the increasing incidence of human organ disorders, chemical accumulation in human tissue, and the like. Fertility problems and neurological issues are also on the rise.

Therefore, it is very important to know the occurrence, distribution and fate of emerging pollutants in surface water and groundwater. Degradation and sorption are the major factors affecting attenuation of emerging pollutants in river and groundwater systems.

The impact of climate change (declining low flows, etc.) might aggravate emerging pollutant challenges in certain regions of the world.

Having this in mind, the UNESCO International Hydrological Programme (UNESCO-IHP), UNESCO Category II Center: Water for Sustainable Development and Adaptation to Climate Change (WSDAC), Jaroslav Černi Institute for the Development of Water Resources, and Serbian Water Pollution Control Society are organizing a UNESCO Conference “Emerging Pollutants in Water”, to offer the needed technical guidance.

TENTATIVE LIST OF EMERGING POLLUTANTS

Category	Subcategory	Examples
Pharmaceuticals	Veterinary and human antibiotics	Trimethoprim, Erythromycin, Lincomycin, Sulfamethoxazole, Ampicillin, Azithromycin, Doxycycline, Amoxicillin
	Analgetics, anti-inflammatory drugs	Codeine, Ibuprofen, Acetaminophen, Acetylsalicylic acid, Diclofenac, Fenoprofen, Metamizole sodium metabolites (4 FAA = 4-formylaminoantipyrine (FAA), 4 AAA = N-Acetyl-4-aminoantipyrin (AAA))
	Psychiatric drugs	Diazepam, Carbamazepine, Lorazepam, Bromazepam
	Lipid regulators	Bezafibrate, Clofibrac acid, Fenofibrac acid, Atorvastatin, Amlodipine, Cilazapril, Simvastatin, Enalapril
	β -blockers	Metoprolol, Propranolol, Timolol, Bisoprolol
	X-ray contrast agents	Lopromide, Lopamidol, Diatrizoate
	Steroids and hormones	Estradiol, Estrone, Estriol, Diethylstilbestrol
Personal care products	Sun-screen agents Insect repellents	Benzophenone, 4-Methylbenzylidene camphor N,N-diethyltoluamide
Fragrances		Nitro, polycyclic and macrocyclic musks
Biocides		Triclosan, Chlorophene
Detergents		4-Nonylphenol di-ethoxylate , 4-Octylphenol di-ethoxylate
Flame retardants		Polybrominated diphenyl ethers (PBDEs), Tetrabromo bisphenol A, C10-C13 Polychlorinated alkanes Tris(2-chloroethyl)phosphate
Industrial chemicals		Benzothiazol-2-sulfonic acid
Gasoline additives		Methyl-tert-butyl ether (MTBE)
Others		Dialkyl ethers
Algal toxins		Microcystin
Anticorrosives		1,2,3-Benzotriazole, Hydrazine
Antifoaming agents		Surfinol-104
Antifouling compounds		Dibutyl tin ion, Irgarol
Antioxidants		2,6-Di-tert-butylphenol
Bio-terrorism / sabotage agents		Chloropicrin
Disinfection by-products (drinking water)		Trichloroacetamide, Chloroacetaldehyde
Drugs of abuse		Morphine, Dihydrocodeine
Food additives		Sucralose, Triacetin
Nanoparticles		Limestone (nanoparticles), Titanium dioxide (nanoparticles)
Perfluoroalkylated substances and their transformation products		Perfluorooctane sulfonate
Pesticides		2-Aminobenzimidazole
Plasticisers		Bisphenol A
Wood preservatives		2,4-Dinitrophenol

EMERGING POLLUTANTS IN WATER RESOURCES - DETERMINATION, OCCURRENCE AND FATE, CHALLENGES FOR WATER UTILITIES

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Introduction

The presence of emerging pollutants (organic micropollutants) in water resources is a major problem worldwide. Reasons for their occurrence in the water cycle are the increasing production and use of man-made chemicals as well as the inadequate waste disposal and removal in waste water treatment plants. Moreover, the enormous progress of analytical equipment and methods led to findings of a huge number of organic micropollutants in extremely low concentrations (ng/L) in water resources.

Emerging micropollutants can be found not only in raw and treated waste water and surface water but also in groundwater, spring water and drinking water as well. People are afraid on the fact that drinking water could be polluted with chemicals. Water supply and drinking water utilities in many countries have to face the problem that organic micropollutants are present in drinking water resources although there is no visible hazard or risk for the consumers. It will be a major challenge and communication task to explain the consumers that emerging pollutants can be found in drinking water but nevertheless drinking water is safe and wholesome and without any chemical risk.

Trace-level determination of emerging pollutants in water

Within the past 20 - 30 years analytical equipment and methods for the trace-level determination of chemicals in water have been improved and optimized very significantly. That means that analytical techniques are more sensitive and selective, more robust and user-friendly than in decades before. In detail sample pre-treatment and pre-concentration are done automatically or via online-techniques and chromatographic separation was improved by new materials, higher temperature and pressure which led to better performance including faster separation. Detection and identification were optimized by high performance and high resolution tandem mass spectrometry which enabled lower limits of quantification.

Moreover, very efficient and highly productive analytical systems are available in research institutes, water laboratories as well as in agencies and commercial enterprises. Problems with safety and handling with chemicals have been overcome in chemical laboratories and contamination problems don't exist anymore. Within the last decades standardization of analytical methods was significantly improved and quality criteria as accuracy, reproducibility and comparability of analytical results have been adopted in general.

A big challenge for the trace-level determination of emerging pollutants is the huge number of chemicals known (www.cas.org). At present more than 72 Mio. chemicals are listed. Rough estimation results that about 100.000 chemicals are produced and applied in Europe and 5.000 - 10.000 could be found in water resources. Additionally "not yet known" or "unknown" organic micropollutants have been and will be found in future so that the question arises, how many emerging pollutants have to be analyzed and how many of them are relevant in water resources?

Within the last decades highly volatile organic compounds (VOC) like chlorinated solvents, pesticides, polynuclear aromatic hydrocarbons (PAH), perfluoro compounds (PFC), nitrosamines, flame retardants, artificial sweeteners etc. and their transformation products have been found in water, generally in very low concentrations. The formation of metabolites by microbiological processes as well as of transformation products by chemical/ electrochemical oxidation will increase the number of chemicals found in the aquatic environment. One example: The chemical oxidation of the pesticide metabolite methyldephenyl-chloridazon with ozone leads to the formation of many oxidation products (OP or TP) which could be persistent or not in water. In most cases only a limited number of more or less persistent metabolites are left over and can be found in real life samples finally.

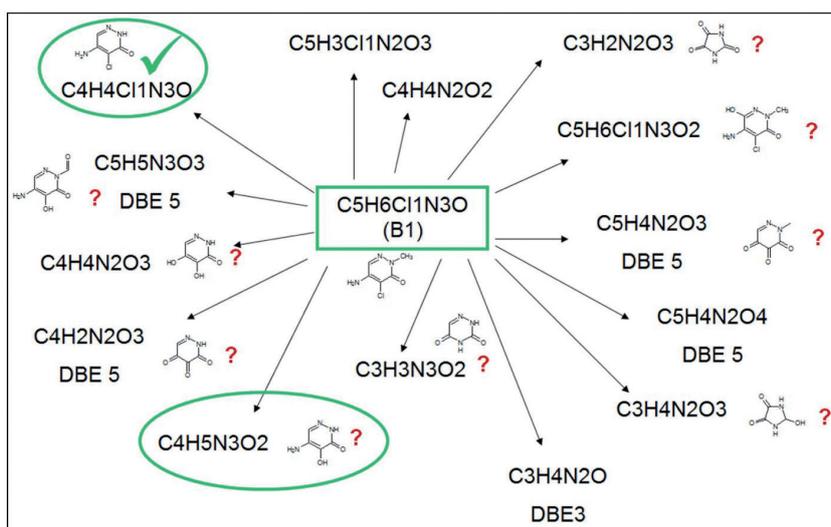


Figure 1: Identified and suspected transformation products (TP) formed by the reaction of methyldesphenyl-chloridazon with ozone

Occurrence and Fate

The number of organic micropollutants found in water samples is dependent on the analytical techniques and methods used.

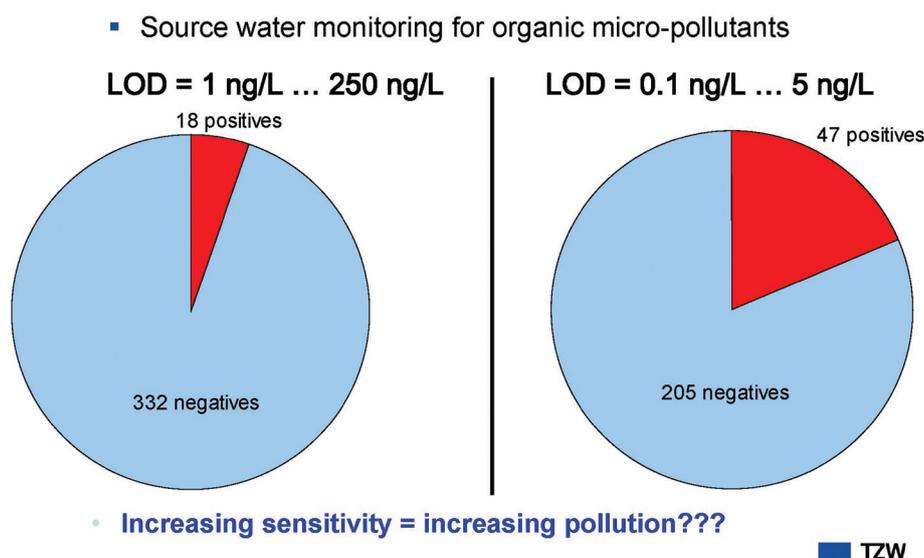


Figure 2: Higher sensitivity of analytical equipment enables more findings of emerging pollutants in water resources

With a limit of detection (LOD) of 1 ng/L for monitoring purposes, 18 emerging micropollutants have been found out of 350 chemicals analyzed. The concentration of every 332 residual compounds was below 1 ng/L, respectively. In a second monitoring campaign 252 organic substances have been analyzed with a limit of detection of 0,1 ng/L. 47 chemicals were found above 0.1 ng/L and 205 below 0.1 ng/L so far. What does it mean finally, when water quality has to be assessed? In the first sample only 18 positive results were found out of 350 compounds, in the second sample 47 positive results out of 252. Is the conclusion correct, that increasing sensitivity of analytical techniques may lead to increasing pollution of water resources?

Higher concentrations of emerging pollutants are regularly found in raw and treated waste water. The removal rate for emerging pollutants in waste water treatment plants is often between 0 and 99 %. A limited number of organic substances are not removed by biological degradation, adsorption on sewage sludge or by abiotic processes. The effluent concentrations of emerging micropollutants are expected to be in the range between

1.000 and 10.000 ng/L (1 - 10 µg/L). That means there is a good chance to detect many of them in surface water, dependent on the dilution factor (1:10 - 1:100).

Figure 3 and 4 show mean concentrations of various organic micropollutants in the river Rhine (2012). A significant increase of concentrations from upstream location Karlsruhe to downstream monitoring station Düsseldorf is evident due to major treated waste water effluents.

PHARMACEUTICAL RESIDUES IN RIVER RHINE (Annual average 2012)

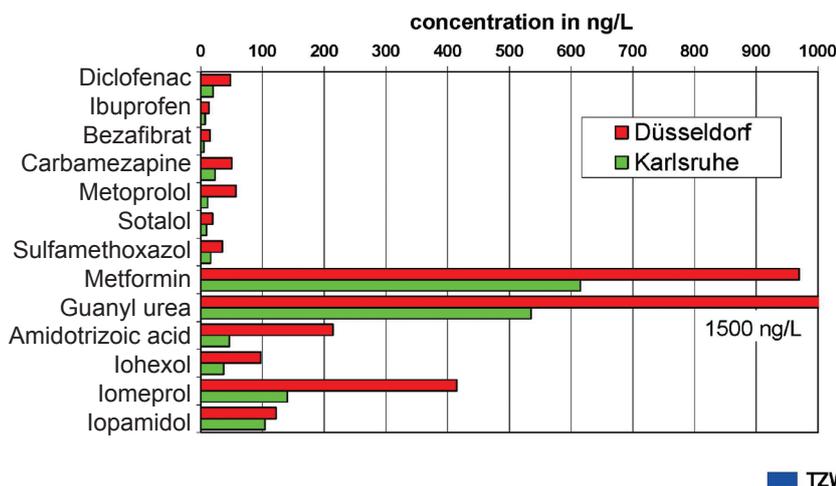


Figure 3: Mean concentrations of pharmaceutical residues in river Rhine (2012)

TRACE POLLUTANTS IN RIVER RHINE (Annual average 2012)

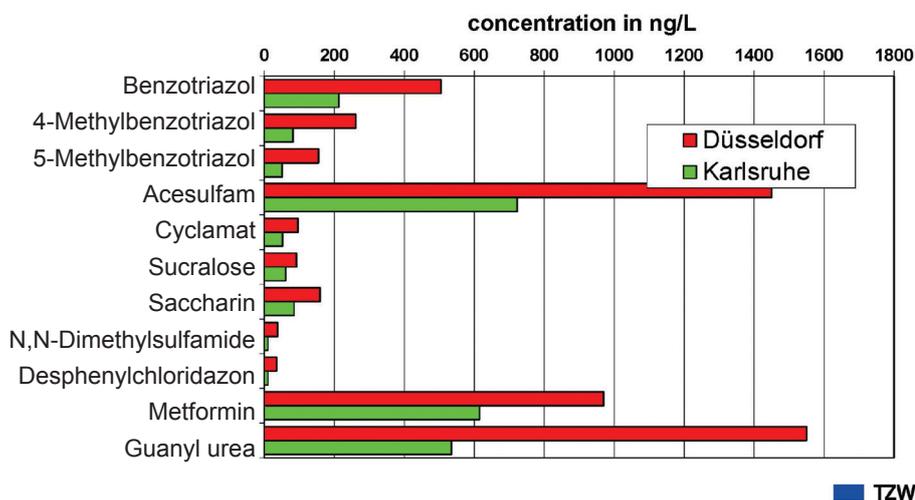


Figure 4: Mean concentrations of trace pollutants in river Rhine (2012)

Evaluation and Assessment

From the perspective of water suppliers it has to be distinguished if emerging pollutants are present in surface and ground water or in drinking water as well. According to the European drinking water directive (DWD) limit values have been set for some emerging pollutants as benzene, polynuclear aromatic hydrocarbons (PAH), trichloroethylene and tetrachloroethylene and pesticides as well. Individual guide or limit values for pharmaceuticals, perfluoro compounds, flame retardants, etc. doesn't exist.

Within the past years health-related indication values (HRIV) were proposed by the federal environmental agency in Germany as a basis for non-regulated microcontaminants in drinking water. Dependent on the physical, chemical, biological and toxic properties of individual emerging pollutants HRIV between 0,1 and 10 µg/L have been derived. These HRIV are generally accepted by health agencies, water suppliers and industry as well and serve as substitute limit values in drinking water. If HRIV are kept, drinking water is wholesome and safe and without any chemical risk. For “not yet known” or “unknown” emerging pollutants a precautionary value of 0,1 µg/L was proposed. That means concentrations below 0,1 µg/L of emerging pollutants in drinking water are without any health-related risk for consumers on a life-long basis (excluded for genotoxic substances). The deriving of HRIV are consistent with the TTC (threshold of toxicological concern) concept.

As a conclusion, health-based risks caused by emerging pollutants in water resources and in drinking water cannot be recognized at present. For precautionary reasons and for the protection of the aquatic environment, inputs of organic micropollutants into surface and groundwater have to be reduced and minimized by appropriate measures. Water suppliers and their associations advocate for the need of comprehensive monitoring programs, precautionary measures in water catchment zones and the up-grade of waste water treatment plants.

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THE ENVIRONMENTAL FATE OF THREE EMERGING POLLUTANTS IN AN AGRICULTURAL IRRIGATION DISTRICT REUSING UNTREATED WASTEWATER

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Abstract:

In this study, the environmental fate of naproxen, carbamazepine and triclosan was determined in an irrigation system reusing wastewater through lab-scale photodegradation, biodegradation and soil column transport experiments that simulate the conditions of the field. Samples of wastewater used for irrigation, a long-term irrigated soil as well as surface and groundwater were taken in the irrigated area. Results showed that the three compounds are susceptible of being photo and biodegraded in the matrices tested; the order at which compounds were degraded by both processes was triclosan>naproxen>carbamazepine. Compounds showed higher rate of photodegradation in surface water than in wastewater and soil, while biodegradation was more rapid in wastewater and soil than in natural waters. Carbamazepine was more susceptible to be photodegraded than biodegraded in all of the tested matrices. In the transport experiments, naproxen resulted as the most mobile compound, while carbamazepine and triclosan were more strongly retained onto the soil, presumably by the organic domain in the solid matrix. Triclosan resulted to be retained and degraded in the soil matrix before it passes through the first 60 cm depth of the soil profile, therefore it is considered as a important topic to evaluate the occurrence and environmental fate of the by-products produced by this and other compounds.

Keywords: Degradation; transport; sorption; pharmaceutical compounds; wastewater reuse

Introduction

Reuse of untreated wastewater in agriculture is a practice widespread in México and in other developing countries within arid and semiarid areas (Jiménez and Asano, 2008). In spite of the advantages of reusing wastewater, such as the recycling of water and nutrients, the savings of fresh water, the economical benefits for farmers due to the increase of crop yields and the money and energy savings for not treating wastewater, the potential of pollute the soils, the water sources nearby and the crops with a wide variety of contaminants is of concern. Based on the aforementioned, wastewater reuse should be carried out with caution and knowing the nature and fate of the pollutants being discharged into the environment.

In the last two decades, particular attention has been put to the so-called emerging pollutants. An important number of studies have been aimed to determine the occurrence, concentration and distribution of these pollutants in aquatic systems and, in a lower extent in solid matrices, such as soils, sediments and sludge. Fate of emerging pollutants is commonly studied in waste and drinking water treatment systems and little is currently known about their fate in soil and natural waters. Such information is relevant in order to achieve more accurate ecological and health risk assessments in the different zones where untreated wastewater is being reused. The aim of this work was to determine the environmental fate of three emerging pollutants, namely naproxen, carbamazepine and triclosan in an agricultural irrigation district where untreated wastewater has been used for almost a century, this by lab-scale photodegradation, biodegradation and soil columns transport experiments, taking care of simulating the conditions observed in the field.

Methods

Grab samples of untreated wastewater, surface and groundwater as well as of bulk soil and unaltered soil columns (at 10 and 40 cm depth) were taken from an agricultural zone from central México which has been irrigated with wastewater for the last 85 years (Figure 1).

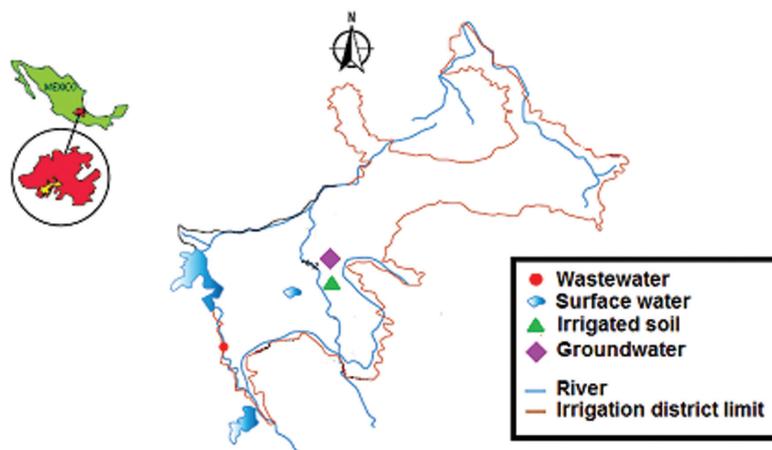


Figure 1. Sampling points of the tested matrices

In the laboratory, relevant physicochemical properties of the liquid and solid samples were determined through standard methodologies (APHA–AWWA–WEF, 1998; Carter and Gregorich, 2008). Results of the characterization of samples are shown in Tables 1 and 2.

Table 1. Relevant physical and chemical properties of water samples taken in the study area

Parameter	Wastewater	Surface water	Groundwater
pH	7.4	7.3	7.9
Turbidity (UNT)	68	0.18	0.1
Total suspended solids (mg/L)	117.3	2.33	2.8
Total organic carbon (mg/L)	192	0.6	1.4
Absorbance at 254 nm	0.39	0.052	n.d.
Electrical conductivity ($\mu\text{S}/\text{cm}$) _i	1011	1319	1616
Dissolved oxygen (mg/L)	0.2	4.7	n.d.
Redox potential (mV)	-152	63	-169
Nitrate (mg/L)	n.d.	63	34
Nitrite (mg/L)	n.d.	n.d.	n.d.
Sulfate (mg/L)	2200	352	367
Naproxen (ng/L)	6359	1.0	5.2
Carbamazepine (ng/L)	193	17.2	0.7
Triclosan (ng/L)	11401	1.8	1.2

Photo and biodegradation as well as sorption experiments were carried out as batch assays, while the transport of the target compounds through soil was done by both transient and steady–state column tests. Photodegradation of pollutants was evaluated in wastewater, surface water and soil; the tested matrices were spiked with the mixture of the pollutants to reach a concentration of 100 $\mu\text{g}/\text{L}$ of water and 100 $\mu\text{g}/\text{kg}$ of soil. Irradiation of samples was performed in a SUNTEST CPS+ apparatus for 2h to achieve a dose of 3600 KJ/m^2 .

Biodegradation experiments were carried out under aerobic conditions for soil and surface water, while anaerobic conditions, favoring either sulfate-reducing or denitrifying media, were maintained in the assays using groundwater. For the aforementioned matrices, experiments were performed for 70 days. For wastewater, biodegradation tests were carried out for 11 days; in the first three days anoxic conditions were maintained (dissolved oxygen concentration was 0.2 mg/L), while for the other 9 days, oxygen was supplied by bubbling wastewater to achieve a concentration of 3 mg/L. For the biodegradation experiments, matrices were fortified with the cocktail of the target pollutants to achieve concentrations of 10 µg/L for wastewater, 20 ng/L for natural waters (i.e. ground and surface water) and 10 µg/kg for soil.

Table 2. Relevant physical and chemical properties of the tested soil taken at two depths

Parameter	Soil depth	
	10 cm	40 cm
pH	8.01	8.14
Total organic carbon (mg/g)	26	16
Electrical conductivity (µS/cm)	1792	1601
Texture	Clay loam	
Sand (%)	13	12
Silt (%)	38	43
Clay (%)	49	45
Specific surface area (m ² /g)	66	115
Hydraulic conductivity (cm/h)	1.3	1.0
Naproxen	3.2	0.7
Carbamazepine	4.8	1.4
Triclosan	7.7	3.1

Sorption tests were conducted by weighing 3 g of soil in glass tubes and adding 15 mL of 10 mM CaCl₂ solution. Six concentrations of the target pollutants were tested 0.5, 1, 5, 10, 15 and 20 µg/L. The glass tubes were agitated under controlled temperature conditions for 24h, and then centrifuged in order to separate the liquid and solid phases. The concentration of the compounds was determined in both the supernatant and soil. For the transport experiments, soil columns were obtained directly in the field using the methodology showed in Figure 2. In brief, a 40 cm depth pit was dug; then, soil monoliths were carved and shaped with a knife to achieve a cylindrical shape of 15 cm height and 9 cm of diameter. The soil monoliths were put into stainless steel cases and then liquid paraffin (chemically pure, at 60°C) was poured in the free space between the soil monolith and the stainless steel case, in order to both fixing the monolith to the column and avoiding the border effect during the displacement experiments. For the transport assays, soil columns were treated by a series of 8 simulated irrigation events. Irrigation consisted on supplying the irrigation water at the top of the soil column and then recovering the leachate at the bottom of the soil column. Each irrigation event took an average of 6 h, followed by a dry period of 4 days. The first irrigation event consisted on the application of CaCl₂ solution, in order to achieve the same humidity conditions in all the soil columns; in the second irrigation event 0.1 M KBr solution was added as a conservative tracer, as well as the mix of the target pollutants at a concentration of 10 µg/L, each compound. The following irrigation events were performed by applying only the 10 mM CaCl₂ solution, in order to displace the compounds through the soil column.

In all the experiments the response variable was the concentration of the target compounds. For the degradation assays, the removal efficiency as well as the degradation rate constants were determined, while for the sorption and transport experiments the sorption coefficients and the retardation factor for each compound was evaluated for each soil depth.

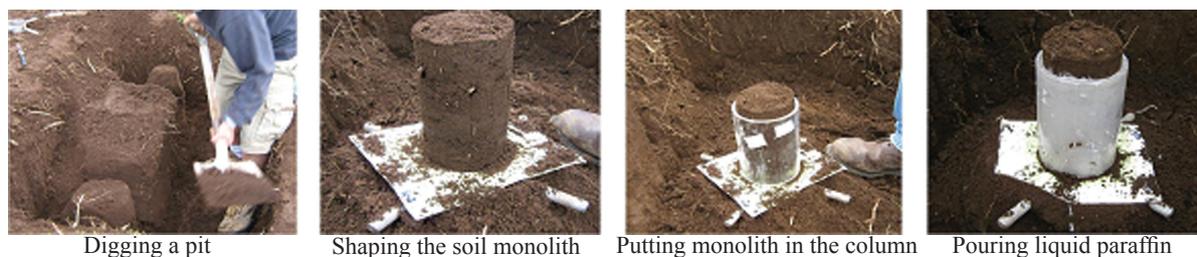


Figure 2. Methodology used to obtain the unaltered soil columns

The concentration of the target compounds in the liquid samples was determined following the procedure developed and validated by Gibson et al. (2007). In brief, the pH of samples was adjusted to 2; then, samples were passed through Oasis HLB cartridges, previously conditioned twice with 5 mL of acetone and once with 5 mL of 5% acetic acid solution. After this, naproxen and carbamazepine were eluted using 5.5 mL of a mixture 40:60 of acetone:sodium bicarbonate 0.1 M, adjusted to pH 10. Following this, triclosan was eluted with 5 mL of acetone. Samples were concentrated using a gentle atmosphere of ultra high purity nitrogen and derivatization was done before the chromatographic analysis by the production of methylsilyl derivatives using the agents MTBSTFA and BSTFA.

Analysis of the emerging pollutants in soils was performed using the method proposed and validated by Duran–Alvarez et al. (2009). The extraction of the target compounds was done using the pressurized liquid extraction technique. For this, 5 g of soil were accurately weighed in 22 mL–ASE stainless steel cells and extracted using a hexane:acetone:acetic acid (49:49:2 v/v/v) mixture. The extraction conditions were as follows: two cycles, 100°C, 10.34 MPa, 0 min of pre–heat, 5 min of heating time, 5 min of static time and flush at 60%. After extraction, evaporation of extracts was done to reach a volume of ~3 mL. After evaporation, 20 mL of HPLC–grade water were added to samples. The resulting solutions were passed through the OASIS HLB cartridges, previously conditioned as stated above. Naproxen and carbamazepine were eluted from cartridges using the pH 10 40:60 acetone:sodium bicarbonate 0.1 M mixture, while triclosan was eluted using 5 mL of a 50:50 acetone:dichloromethane solvent mixture. After elution, the sample preparation procedure was the same as described for liquid samples.

Separation and quantification was carried out using a HP 6890 gas chromatograph in tandem with a HP 5397 mass selective detector. The chromatographic column was a fused silica capillary column (30 m × 0.25 mm, 0.25 µm of film thickness). The carrier gas was Helium at a constant flow of 1 mL/min, and 1 µL of sample was injected in the splitless mode. The oven temperature program was as follows: 100°C for 1 min, ramp of 20°C/min to 280°C, and 280°C for 10 min. The detector was used in the selected ion monitoring (SIM) mode. The electron impact source temperature was 230°C with electron energy of 70 eV. Calculation of the analytes' concentration was done through the internal standard method. Quality assurance was guaranteed by the use of surrogate standards. The recoveries obtained were between 90% and 96% for the tested compounds.

Results and discussion

Results obtained in the photo and biodegradation tests are shown in Table 3. Naproxen and triclosan were found as susceptible of undergo degradation by both light irradiation and microbial metabolism; conversely, carbamazepine showed to be recalcitrant. The highest removal efficiencies in the photodegradation tests were obtained in surface water samples; which was attributed to the high penetrability of light in the clear liquid matrix, in contrast to what happens in wastewater and soil. For biodegradation, removals of the target pollutants were higher in aerobic media than when anaerobic conditions were used. In the case of groundwater, for which anaerobic biodegradation was tested, higher removals were obtained when sulfate–reducing conditions were used. Results obtained in the degradation tests indicate that carbamazepine is more likely to be degraded by photolysis than by the microbial metabolism.

In the sorption tests, the order at which compounds were retained by soil was: triclosan>carbamazepine>naproxen. The values of the sorption coefficient (K_d) for the tested pollutants in the topsoil were 2.39, 5.81 and 64.9 L/kg for naproxen, carbamazepine and triclosan; while in sub–surface soil, these values were 4.41, 11.4 and 19.2 L/kg, respectively.

Table 3. Average removals of the target emerging pollutants in the degradation tests.

Compound	Average removal (%)						
	Photodegradation			Biodegradation			
	Wastewater	Surface water	Soil	Wastewater ¹	Surface water	Groundwater ²	Soil
Naproxen	92	98	35	50 [12]	96	12 [20]	94
Carbamazepine	34	60	8	17 [4]	5	8 [10]	22
Triclosan	98	99	20	48 [10]	92	20 [22]	76

¹Values in brackets correspond to the biodegradation performance during the anaerobic step of experiment; ²Biotransformation achieved in experiments under sulfate-reducing conditions

Results of the sorption tests indicate higher sorption of naproxen and carbamazepine in the topsoil than in the subsurface soil, and the contrary behavior for triclosan. This may be attributed to the differences in the nature of the soil organic matter at both depths. Triclosan was as strongly retained by the two tested soils that desorption of this compound showed to be negligible.

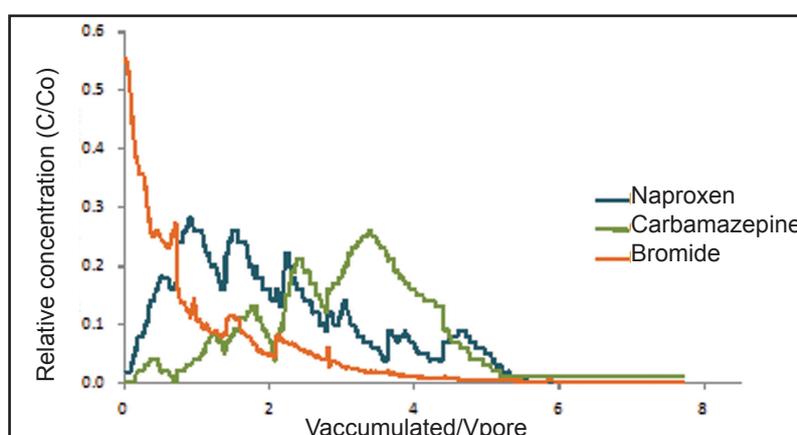


Figure 3. Breakthrough curves of the bromide tracer and the target pollutants in the soil columns experiments

Results of the transport experiments confirmed the strong retention of triclosan in the tested soils, since this compound was not detected in any of the leachate samples analyzed. On the other hand, naproxen showed a rapid movement through the soil columns, demonstrating its high potentiality to reach groundwater. Carbamazepine was less mobile than naproxen but more mobile than triclosan. The analysis of soil after the transport experiments (upon this analysis a mass balance was obtained) evidenced that triclosan, additionally to be notably mobile, is readily biodegradable in the soil. Carbamazepine was recalcitrant to biodegradation, thus even though its lower mobility, the compound could eventually reach groundwater.

Concluding remarks

The environmental fate of three emerging pollutants in water and soil of a wastewater-irrigation district was explored by lab-scale experiments. Naproxen and triclosan showed to be susceptible to degradation while carbamazepine displayed a considerable extent of recalcitrance.

Polar compounds, such as naproxen can easily migrate into the aquifer due to their low affinity to soil, while the transport of carbamazepine is retarded by its sorption onto the organic domain of the soil; although its recalcitrance may lead to carbamazepine reach groundwater in a long-term scenario.

It is necessary to use the results obtained in this study to scale the determination of the environmental fate of this class of pollutants toward the field scale (e.g. in a valley or in the piedmont) as well as launch studies aimed to determine the occurrence and environmental fate of the by-products produced by the degradation of

the target emerging pollutants.

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EMISSIONS AND TREATMENT OF EMERGING POLLUTANTS

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Abstract:

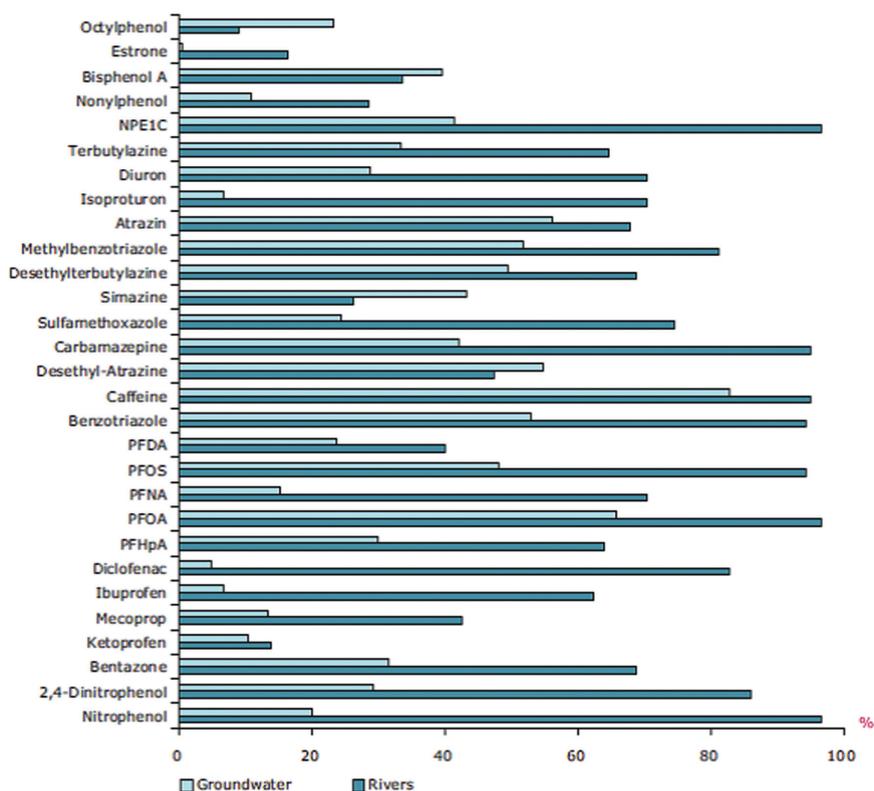
The best available technology (BAT) in wastewater treatment nowadays implements nutrient removal (C N P) by nitrification, denitrification and phosphorous precipitation (or enhanced phosphorous uptake). Despite the success of BAT in carbon and nutrient removal and resulting benefits for the ecological status of receiving waters new concerns regarding organic micropollutants found in the aquatic environment in the low $\mu\text{g/l}$ or even ng/l range arose. Concerns involve implication of micropollutants for drinking water supply as well as their potential effect on aquatic life and biomagnification. As wastewater treatment plants (wwtp) are not the single input path for micropollutants, a first step involves the identification of relevant pathways through the environment and specifically through wwtp in order to specify substances that are targeted for further removal. Endocrine disruptors (EDCs) as well as pharmaceuticals and personal care products (PPCPs) were found to be the most significant substances released from even tertiary treated wastewater treatment plants. Technologies investigated as advanced treatment steps include activated carbon application (AC) and further chemical oxidation by ozone (O_3). This contribution will give an overview on the occurrence of organic trace pollutants in wwtp effluents presenting results from a survey conducted in 90 European waste water treatment plants (WWTPs) and published by the European Commission's Joint Research Centre (JRC) in Ispra (EU-JRC 2012). After giving that overview, the two most promising technologies for removal of organic trace pollutants that are discussed intensively are presented. Those technologies are the application of activated carbon (AC) and ozonation (O_3 application for advanced chemical oxidation). Their application as well as related specific aspects and technical applicability is presented.

Introduction

Due to the implementation of nutrient removal in wastewater treatment plants as the best available technology (BAT) the effluent and subsequently the water quality in receiving waters significantly could be improved with regard to traditional parameters such as carbon, nitrogen and phosphorus. Those parameters induce direct – acute effects in aquatic ecosystems (oxygen depletion, ammonia toxicity, eutrophication, ...), represented the major pressures for ecosystem integrity over decades and still are of major concern on a global scale. Based on analytical findings in the aquatic environment, the occurrence of endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products (PPCPs) got into the focus of scientists since the early 2000s. The amount of substances discussed steadily is increasing implementing a broad spectrum of use pattern and even considering transformation products of parent substances. Legislation and administration is dealing with the topic on international level to discuss environmental quality norms and implementation of management strategies. Pharmaceutically active compound and estrogenic hormones even found their way into the recent proposed watch list for priority pollutants in the European Water Framework Directive.

Occurance

An extensive review and survey on the occurrence of organic micropollutants in European surface- and ground-water recently was published by the European Environmental Agency (EEA 2011). Figure 1 gives an overview on the water samples analyzed positively for selected organic trace pollutants. Without giving information on the concentrations measured, Figure 1 shows a symptomatic picture on the waterbodies investigated.



Source: Loos et al., 2009; Loos et al., 2010.

Figure 1: Occurrence of organic trace pollutants in surface waters and groundwater (Loos et.al., 2009 & 2010 in EEA 2011))

In that connex it has to be stated, that a chemical analytical detection of a substance does not give information on the relevance of the substance for consumer safety or biosystem integrity. Only eco-/toxicological tests based on bioassays and other approaches similar to the testing of chemical safety (eg. as suggested in REACH - Registration, Evaluation, Authorization and Restriction of Chemicals) that correlate NOEL (no observable effect level) with PEC (predicted environmental concentration) are able to address the significance of a measured value. As in a traditional approach the death of 50% of the test organisms exposed to a defined substance exposure (LC_{50} – lethal concentration of 50% of test organisms) was the base of a toxicological assessment, nowadays long term chronic effects and new modes of actions are discussed as an evaluation base that implement mutagenity, cytotoxicity, cancerogenity and others. Whereas for the estrogenic potential nowadays robust test systems are available, for most of the analyzed substances no final agreement on the selection of relevant tests is found.

Before addressing mitigation and removal technologies a thorough assessment of relevant emission pathways has to be undertaken in order to identify the major pathways of a substance in the environment as a base for targeted and resource effective risk management measures. Based on use pattern (location of use, origin or emission; amounts) and chemical properties (hydrophilic substances tend to be found in water phase whereas hydrophobic substances tend to adsorption on particles or soil) substances usually do not occur even distributed in all possible pathways, sinks and compartments. E.g. in the Guidance Document No. 28: Technical Guidance on the Preparation of an Inventory of Emissions, Discharges and Losses of Priority and Priority Hazardous Substances (EC 2012) a general working scheme for assessing emissions into surface waters is defined (see Figure 2 as in EC 2012).

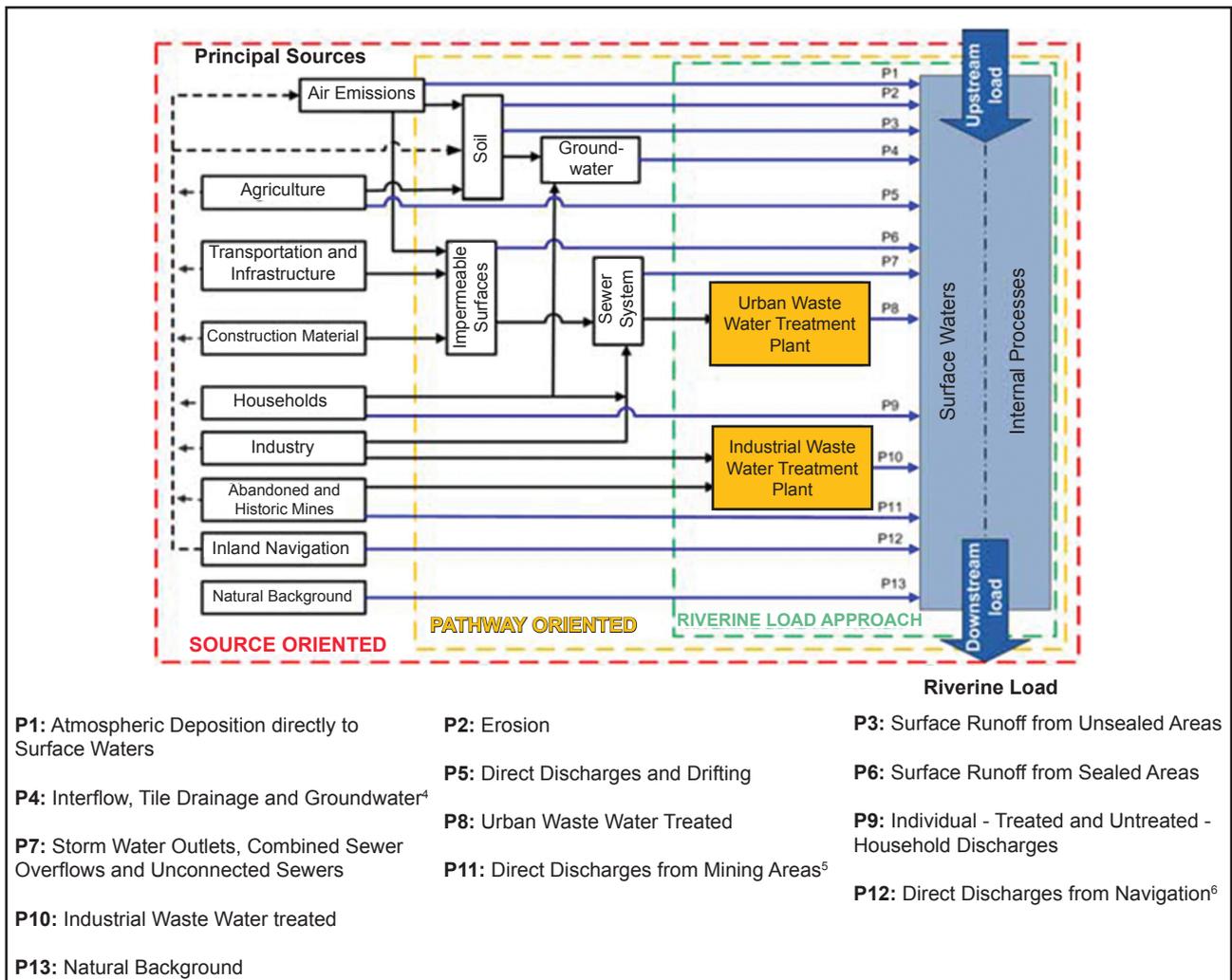


Figure 2: General working scheme of inventory (EC 2012).

For a range of organic trace substances emissions via wwtps into water bodies were identified as the major pathways for surface water immissions. Basically that applies for substances used in the households (eg. pharmaceutical active compounds, substances in personal care products, cloths, material preservation, ...) collected via sewer systems but that are (more or less) resisting removal steps implemented in existing wwtps. As a consequence, even the application of BAT in wastewater treatment at the present level does not provide complete elimination of all micropollutants and subsequently, residues of organic micropollutants enter the aquatic ecosystem through wastewater.

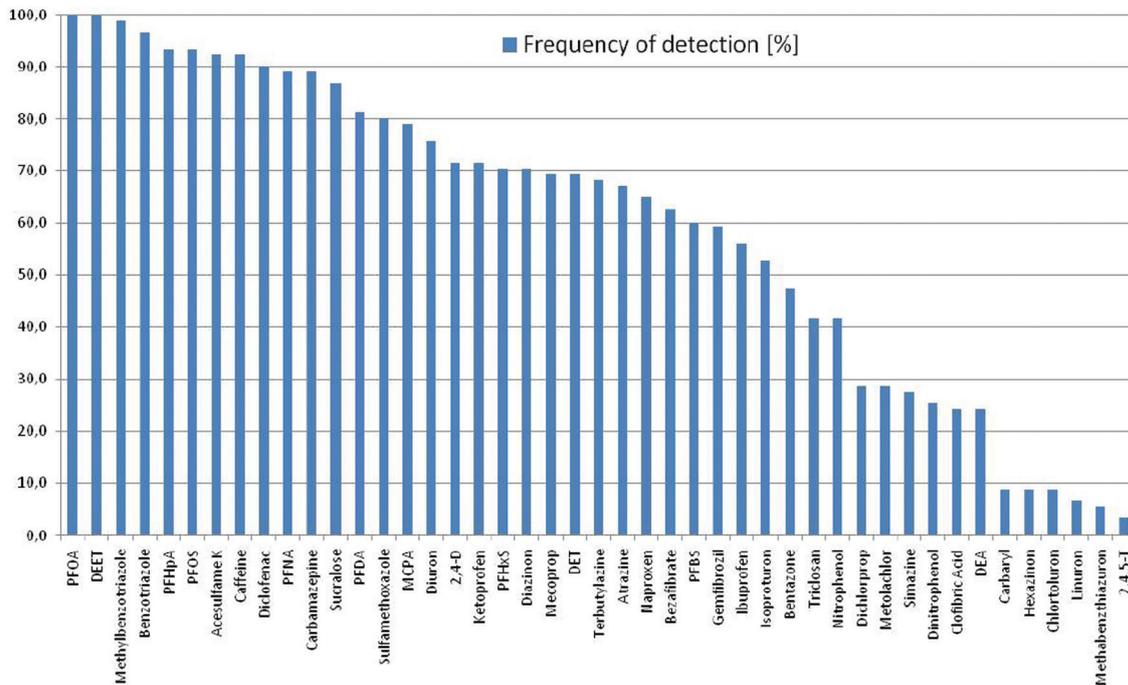


Figure 3: Occurrence of trace organic pollutants in the effluent of 90 wastewater treatment plants (EU JRC 2012) Occurrence in % of samples analyzed

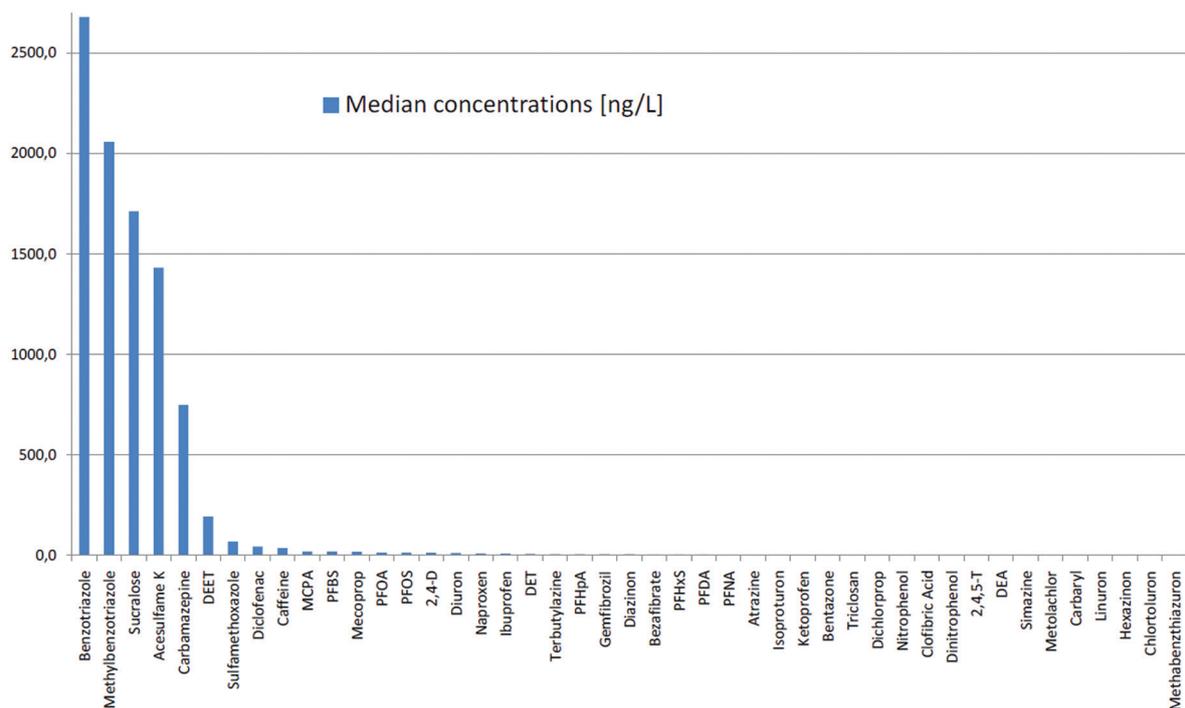


Figure 4: Occurrence of trace organic pollutants in the effluent of 90 wastewater treatment plants (EU JRC 2012) Median concentrations in samples analyzed

In a report by the Joint Research Centre Institute for Environment and Sustainability of the EU in Ispra, the results of a pan-European survey on organic pollutants in the effluents of 90 European wwtps was published (EU JRC 2012). In Figure 3 the percentage of samples tested positively for the indicated substance is shown. Some of the substances (eg. PFOA - Perfluorooctanoic acid or Diethyltoluamide) were found in all 90 wwtp samples, whereas other substances only seem to play a minor and local role. 5 Substances (Benzotriazole &

a metabolite, Sucralose, Acesulfame and Carbamazepine) were found in mean concentrations above $0.5 \mu\text{L}^{-1}$ (Figure 4). Acesulfame and Sucralose both are artificial sweeteners that are approved as food additive, so human toxicity should not be an issue. As they are intended to be “no”-calorie sweeteners, they are not only not metabolized in the human body but consequently even recalcitrant to microbial, chemical and physical removal in wwtp. Via the wwtp effluent they end up in surface waters that may be used as a raw water source for drinking water supply. An occurrence in the treated drinking water may be possible in low concentrations that pose no toxicological but an esthetical problem to consumers.

This situation poses new challenges for wastewater treatment. Since most of the micropollutants are of anthropogenic origin and even tertiary treated wastewater is not able to remove a variety of persistent substances to a high extend, efforts were made to develop advanced technologies to further remove relevant micropollutants from wastewater. The application of chemical oxidation by ozonation and adsorption by activated carbon are two of the most promising technologies investigated in the past year. Both technologies already are applied successfully for drinking water treatment, but are rather new for the application on waste water treatment. The state of technological implementation of those two options nowadays already is at the level of first full scale pilots. This contribution will give an overview on the potential of both technologies and focus on first detailed results for the application of ozone.

Treatment options

General remarks

Today's WWTPs designed and operated according to BAT (best available technology) are very effective in removing macropollutants (Carbon, Nitrogen, Phosphorous) from wastewater and mostly are realized as low loaded activated sludge plants. Despite the fact, that they are not designed for the removal of organic trace pollutants, some of them are removed to an high extend. Even high loaded systems for Carbon removal only (without nitrification and denitrification) as realized for non-sensitive receiving waters can remove unpolar substances by adsorption from the bulk on activated sludge flocks to some extend (dark bars in Figure 5). Low loaded BAT plants with high solid retention times realizing nitrification show a significant higher removal potential for a broader spectrum of substances (grey bars in Figure 5). For some substances as Diclofenace (DCF) or Carbamazepine (CBZ) no removal can be observed and only advanced treatment steps as eg. ozonation (white bars in Figure 5) are able to eliminate those substances.

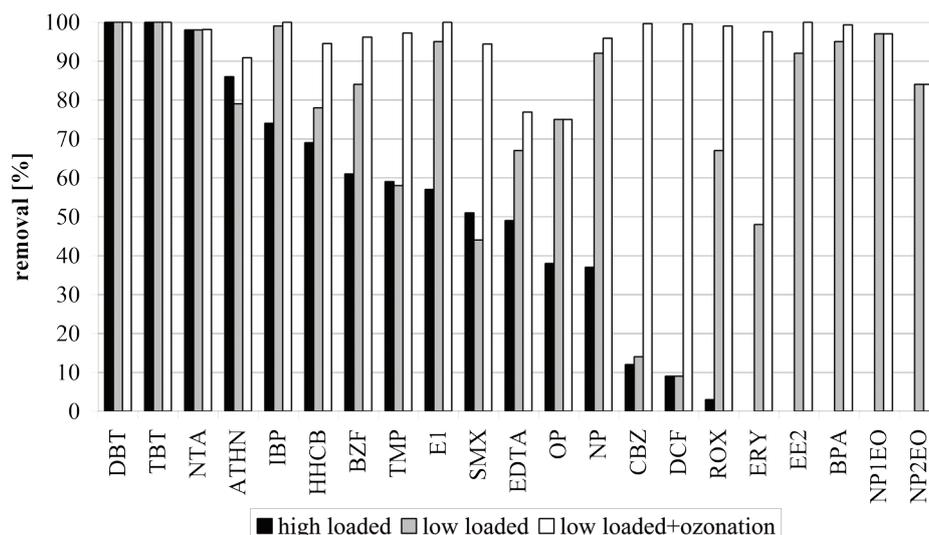


Figure 5: removal efficiency for selected organic trace pollutants in conventional wastewater treatment (high loaded: C-removal only; low loaded: nitrification & denitrification) and a subsequent ozonation step (Schaar et.al. 2010)

Those findings indicate, that nitrification plants not only remove ammonia toxicity in the effluent, but are able to remove even hormones as e.g. Ethinylestradiol (EE2 in Figure 5) to a much higher extent compared to high loaded wwtps. Area wide implementation of nitrification on wwtps therefore can significantly reduce emissions of organic trace pollutants (and ecotoxicological effects) to the aquatic environment. Nevertheless relevant substance emissions as e.g. DFC will be not affected by that improvement and subsequent advanced treatment steps have to be considered in the case no other mitigation strategies are suitable. In any case advanced treatment steps as ozonation or application of activated carbon due to technological and economic reasons only make sense after low loaded biological treatment.

Compared to the application for drinking water treatment, the application of activated carbon (AC) and ozone (O₃) for advanced treatment of (municipal) wastewater faces some conceptual challenges that have to be overcome:

- **Wastewater matrix**
in contrast to raw water for drinking water supply, wastewater comprises a complex matrix of a broad variety of substances that are non-target substances in regard to treatment. Especially humic acids, products from cell hydrolysis and the EPS of activated sludge flocs primary are not the target substances for the advanced treatment, but due to their higher concentrations compete with the micropollutants for the processes of advanced treatment. Due to that competition it is, why significant higher dose of activated carbon or ozone have to be used in waste water treatment to remove the same amount of micropollutants compared to the treatment of raw water for drinking water with its lower background DOC.
- **Fluctuations in wastewater inflow**
The inflow to wastewater treatment plants is not constant neither in quantity nor in matrix composition. Depending on the size of the sewer network, the type of the sewer (mixed vrs. Combined), precipitation, the demography in the catchment and other relevant aspects, there is a seasonal and diurnal fluctuation in the inflow quantity and quality. These fluctuations require a more sophisticated and complex process control compared to drinking water treatment.
- **Treatment targets**
Before applying any new technologies their potential end effectiveness has to be evaluated in regard to the treatment targets. Traditional targets for conventional parameters in wastewater treatment implement defined effluent concentrations for C, N and P compounds or percentage of load reduction combined with statistical probabilities of occurrence. As chemical analysis of the micropollutants is costly and complex there is an ongoing discussion whether substance specific chemical analysis or effect based integrating biotests should be applied to evaluate the treatment goals. Effect based biotests have the advantage to integrate the effects of multiple substances without the risk to “forget” a substance that may have a significant effect, but that is not known to exist in the particular waters. A selection of accepted tests has to be defined and agreed upon prior to a broad application of the new technologies. Otherwise the applied technology may not be able to perform as requested and the implementation is stranded investment.

The aspects mentioned above are posing a technological as well as conceptual challenge and are a core matter of scientific and technical research. Whereas the first two (Matrix and Fluctuation) can be dealt with on a pure technological level, the last aspect involves a broad range of research fields as toxicology, ecology or chemical analysis. In addition there has to be political agreement for the targets.

Activated carbon

AC is produced by thermal activation of various raw materials as these can be brown coal, mineral coal, coconut shells or others that significantly influence the behavior as adsorbent. The basic principle is the high surface of the AC (1000-1500 m²g⁻¹) due to its micro- and macropores that provide high adsorption areas. AC is used as powder (PAC – powdered AC; ~ 1-200 µm particle size) or granules (GAC – granular activated carbon; ~0.5-1.5 mm size). PAC is mixed with the activated sludge or the effluent and abstracted from the system by excess sludge abstraction therefore increasing the excess sludge production. Various systems implement recycling of a post treatment AC application into the biological step. Usually an additional filter is required to

retain smallest AC particles loaded with the adsorbed micropollutants from leaving the plant via effluent. GAC usually is applied as replacement of filter material in sand filters.

The amount of PAC to be applied very much depends on the background DOC where e.g. humic acids compete for adsorption places with the target EDCs and PPCPs. Durability is not the issue for PAC, as it is dosed according to the matrix and amount of water treated. Durability for GAC still is a matter of research but very much depends on the DOC background matrix (natural organic matter, NOM). GAC rather is sensitive to competitive adsorption, where substances with high adsorption potential can displace others with lower adsorption, even when they already are adsorbed.

Depending on the chemical structure of the target substance it adsorbes better or to the worse. Polar hydrophilic substances have a low adsorption coefficient and show only little adsorption. The worse the adsorption behavior the more AC has to be added to achieve a certain removal rate. Additionally adsorption depends on the type of the coal itself. Considering the specific aspects of AC adsorption it is possible to eliminate 80% of most of the target substances making the AC application a suitable technology for advanced treatment.

Ozonation

Ozone (O_3) is a strong oxidant ($E_0 = 2,07$ V) and produced on site from oxygen feed gas by cold high frequency electric discharge. The oxygen (O_2) necessary is provided by a liquid oxygen storage tank or by air separators that use molecular sieves to separate the oxygen out of the gas mixture in the air. In the ozone generator the oxygen is split up and three of the resulting single atom Oxygen radicals react to form the ozone (O_3) molecule. The resulting ozone is transferred into a reaction chamber with the to be treated wastewater by bubble aerators or high concentrated in a water feed stream by an injector. In the reactor the O_3 decays and the resulting oxygen radicals target specifically electron dense chemical structures as double bonds, aromatic ring structures or deprotonated amines. Substances with corresponding chemical structures as e.g. sexual hormones show a high reaction rate whereas substances with rather linear structures as e.g. fatty acids hardly are targeted. Excess ozone finding its way into the air over the reaction chamber has to be collected and destroyed in a specific residual ozone destruction unit (closed system).

Comparable to the AC application, the efficiency of the ozone reaction for target substances is depending on the content of non target organic matter as NOM (natural organic matter as humic substances) due to the competition for the oxygen radicals. In order to avoid that competition the application of ozone is a post treatment step after tertiary treatment with low loaded activated sludge plants. The effluents of those plants shows minimum background DOC and therefore requires less oxygen radicals for the target substances. Because of the competition with NOM / DOC the amount of ozone used for the process is not given as mg/l O_3 but as specific ozone dose Z_{spec} with the unit mg O_3 / mg DOC. The typical ozone dosage applied for post treatment of municipal wastewater is between 0.7 and 1 mg O_3 /mg DOC. That dose does not lead to complete mineralization of the DOC, but breaks down biologically stable hardly degradable substances resulting in an increase of bioavailability and therefore the BOD. Due to the fluctuation in the effluent matrix (e.g. stormwater) the amount of ozone introduced has to be controlled thoroughly in order to avoid residual ozone in the effluent.

One aspect intensively discussed together with ozonation is the formation of unspecific byproducts that potentially could be more harmful compared to their parent substances. Applying standardized effect based biotests as applied in chemical safety approaches there is no direct evidence for an increase of the toxicity due to ozonation. Considering the specific aspects of ozone application it is possible to eliminate 80% of most of the target substances making it a suitable technology for advanced treatment.

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SELF PURIFICATION PROCESSES IN ALLUVIAL GROUNDWATER AND THEIR SIGNIFICANCE FOR ELIMINATING THE EMERGING MICROPOLUTANTS

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Abstract

The groundwater is extremely important part of the planet Earth system. It is dominant source of drinking water supply, and during the low flow periods it significantly contributes to river flow and perseverance of wetlands, as well as oasis in arid areas. Self purification characteristics of groundwater are very important factors and they have to be taken into account, and used adequately. In the case of water quality disturbance, these characteristics lead groundwater towards its basic quality. This is extremely important in the case of design of technical/technological line and groundwater protection zones.

The speed of groundwater flow is several (mostly 4-6) orders of magnitude lower than the speed of water flow in rivers. Dissolved matter in groundwater is continually exposed to various physical and biochemical processes happening in water, aquifer skeleton and on the border between various phases (water, skeleton, air). Utilization of alluvial groundwater is extensive all around the world. It is deemed that more than half of the world's water supply comes from the groundwater, of which alluvial groundwater is, again, approximately more than half.

Methods of utilization of groundwater in alluvia are mostly bank filtrations and artificial recharge. In these methods transformation of the raw water (mostly river water) is very significant, most often aimed at elimination of the organic pollution. This is very important, since river water (especially the water from large rivers) in spite of successful efforts of the developed countries to protect its quality, contains man-made organic matter and micro-pollutants. Of course, this also goes for the emerging substances, that cannot be fully eliminated in the devices for wastewater treatment.

Basic quality of the groundwater

Considering the speed of flow and wide-spread process, groundwater has more local features than surface water. One can speak of specific differences in physical and biochemical parameters of solutions of surface and groundwater (oxic and anoxic).

Table 1. Main differences in basic quality of surface and groundwater

Parameter	Surface water	Groundwater	
		oxic groundwater	anoxic groundwater
Total roganic substances (COD, TOC, BOD, and KMnO_4 demand)	occurrence frequently due to anthropogenic contribution	usually lower concentrations that in surface water, occur in natural, baseline concentrations; if increased by human activity, self-purification mechanisms strive to achieve baseline concentrations. Degradation of organic pollutants generally better in aerobic than in anaerobic conditions	
Iron and manganese	generally very low concentrations, except in eutrophic waters	low concentrations possible, usually of dissolved Fe^{3+}	frequent occurrence in concentrations higher than in surface water or aerobic groundwater. Iron occurs as insoluble Fe^{2+}
Dissolved oxygen	frequently close to saturation (8-9 mg/l)	present in concentrations lower than the concentration of saturation	virtually absent $\text{O}_2 < 0.2-0.5 \text{ mg/l}$
Nitrates	generally low concentrations (5-10 mg/l), except in polluted water	generally low concentrations; elevated concentrations are caused by anthropogenic impact	generally absent
H_2S	generally absent	generally absent	temporary present

Processes of purification (physical, chemical and biochemical) aim to bring the quality of groundwater, possibly disturbed by some activity, to basic quality. The basic quality itself depends, most of all, of mineralogical content of the aquifer skeleton, oxicity and dynamics of the groundwater movement.

Issue of oxicity of the alluvial aquifers is extremely important, for several reasons. The first set of reasons pertains to purifying potential of the aquifer, which is generally more intensive in oxic conditions (with certain exceptions). The second set is consisted of the processes of biochemical colmation of the wells in conditions of decreased oxicity (anoxic conditions in aquifer) [5].

The issue of oxicity depends largely of the consumption of oxygen. Here it should be noted that oxygen consumption cannot be explained only by oxidation of the organic matter in water, but also there is a significant role of the mineralogical oxygen consumption (for example, transformation of Fe^{2+} from mineral to Fe^{3+}). Figure 1 shows a diagram for the influence of the alluvial aquifers genesis to the level of oxygenation.

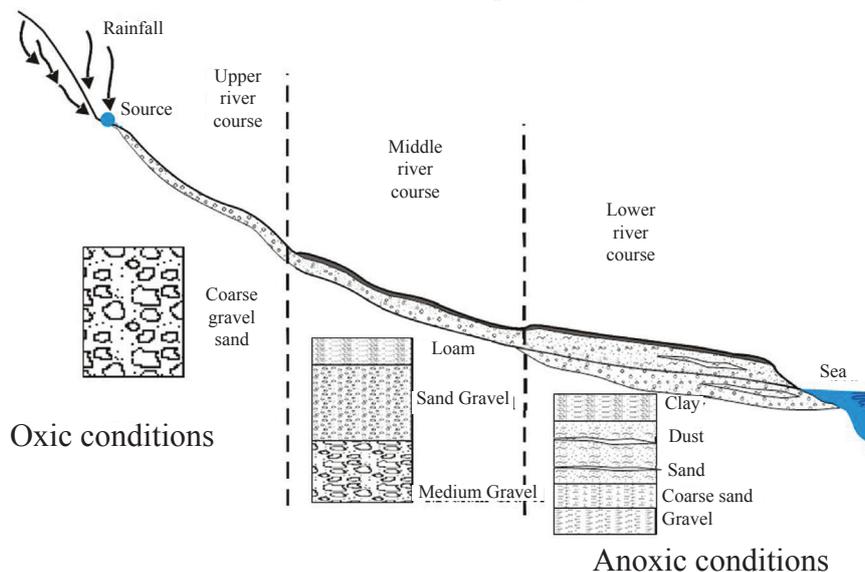


Figure 1. Typical scheme of an alluvial aquifer

Transformation of the groundwater quality

Groundwater quality changes in response to two basic mechanisms:

- In the first case, the quality of the inflowing water is stable and shows small to moderate variations over time. In this case the quality of groundwater in the aquifer basically stays unchanged;
- In the second case, there is a rapid and significant change in the quality of infiltrating water (e.g. accidental pollution, Dimkić et al., 2008b). In such a case the quality of groundwater can also change significantly.

In both cases there is a sequence of processes and associated parameters which lead to groundwater quality transformation (Table 2):

Table 2. Purification processes in an aquifer (Dimkic, 2007)

Transport via liquid phase	Convective transport of solute	1.1 Convection
		1.2 Diffusion
	Dispersion/diffusion of solute	1.3 Dispersion
Transfer from liquid to solid phase	Transfer of solute from solid phase into solution	2.1 Desorption
		2.2 Rock dissolution
		2.3 Transfer of solid into solution
	Transfer of substance from solution to solid phase	2.4 Physical sorption
		2.5 Chemical sorption
		2.6 Precipitation
Loss of a particular substance from the aquifer	Degradation processes	3.1 Biodegradation
		3.2 Chemical oxidation-reduction processes
		3.3 Radioactive decay
	Other processes	3.4 Evaporation

When the baseline quality of groundwater is altered by pollution, the processes shown in Table 2 lead, over time, to the restoration of baseline groundwater quality. In such cases, the aquifer medium acts like a physical and biochemical reactor with respect to the groundwater.

Regardless of the fact that for significant number of emerging substances limited capabilities have been established for purification of the wastewater (insufficient sorption at filters, insufficient kinetics of the dissolution) one must have in mind that sorption and biodegradation are very important processes in groundwater filtration, which are very widely spread and thus most often cause significant or total elimination of the organic micro-pollutants.

Under oxic conditions, oxidation takes place through the oxygen dissolved in water. Under anoxic conditions, biochemical oxidation is based mainly on nitrates, compounds of ferric iron and tetravalent manganese, as well as sulphates.

Oxic environments are generally more favourable for self-purification processes. However, some substances can also degrade in anoxic conditions. Knowledge of the effects that the degree of groundwater oxicity has on the baseline quality of groundwater and understanding of the process of purification of river water to the baseline quality level are fundamental for the design, use and protection of groundwater sources.

In utilization of the bank filtration method four phases of water filtration can be distinguished (Figure 2).

Each of these phases is specific either by processes of transformation of the groundwater quality or/and by influence to water intake wells.

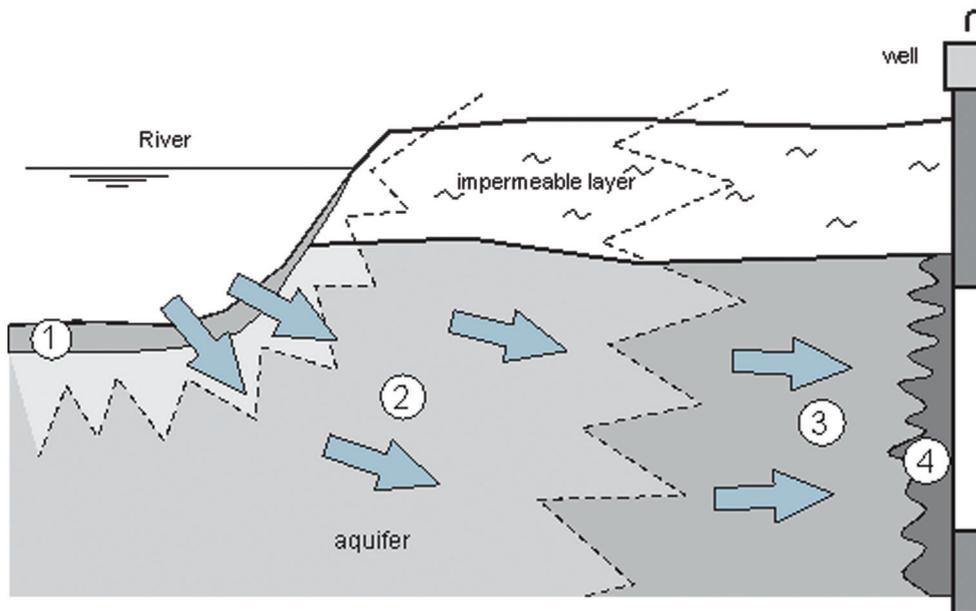


Figure 2. Phases of water filtration from the river to the well

LEGEND: Phase 1 – strong sorption and biochemical activity, Phase 2 – filtration in aerobic conditions, Phase 3 – reduced oxic or anoxic filtration, Phase 4 – changes around well's screen

Each of these phases requires different conditions for water filtration and potentially different processes and effects of the ground water quality transformation.

Some elements of the water quality transformation

There are many examples for purification in groundwater filtration. Herebelow we shall mention some very interesting examples from practice in Serbia.

- **Belgrade groundwater source** includes around 100 radial wells along the left and the right banks of the river Sava, capping its alluvial aquifer. Figures 3 and 4 shows changes in consumption of the potassium permanganate and organic carbon between the river Sava and raw water from the well in congregative shafts of the wellspring.

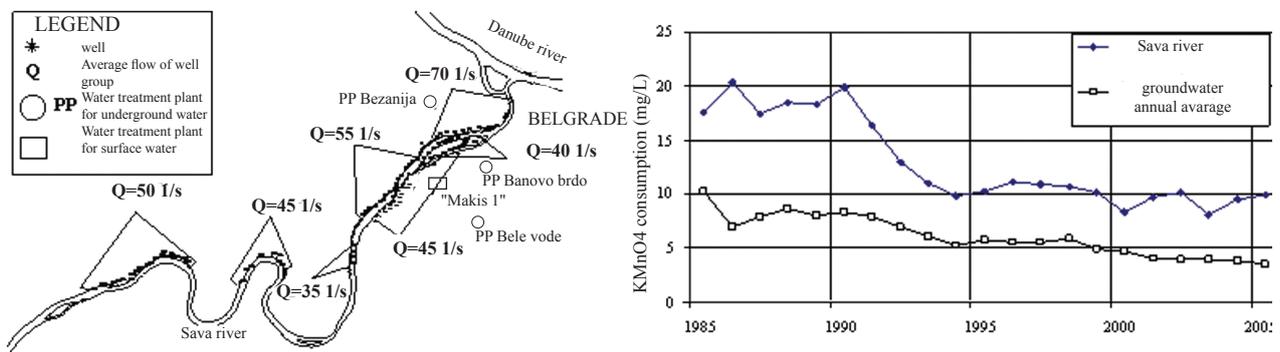


Figure 3. Consumption of the potassium permanganate on Belgrade groundwater source

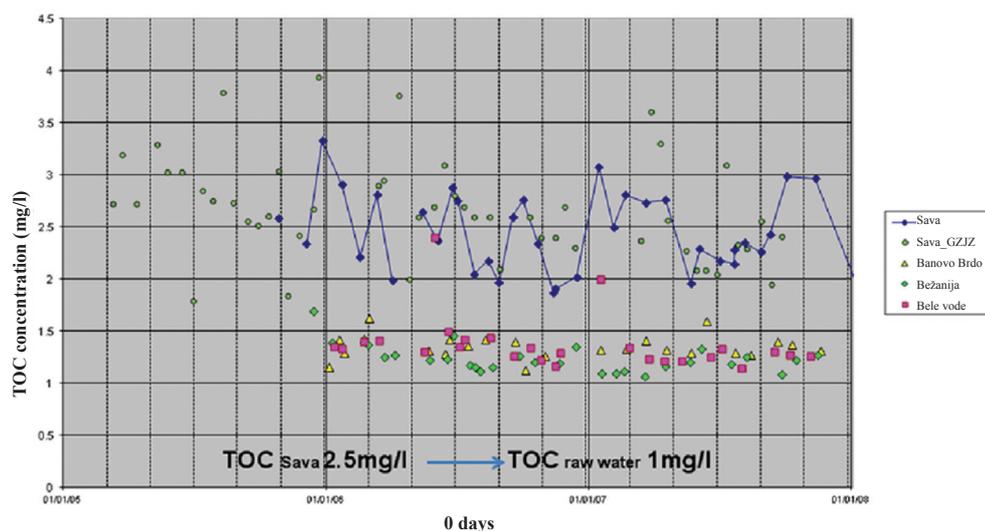


Figure 4. Total Organic Carbon (TOC) in river water and raw water from the wells on Belgrade groundwater source

- Between 2009 and 2012, total of 122 samples of the surface and groundwater have been analyzed in the Republic of Serbia. Groundwater was scooped from the wells mostly taking water from the alluvia of the pertinent river (the Sava, the Danube, the Tisa, the Morava).

Approximate results are shown in Tables 3 and 4. and in Fig. 5

Substance	Carbamazepine	Trimetropin	4-FAA	4-AAA
Total analysis for all rivers	49	49	49	49
Total detection for all analyzed rivers	23	7	26	29
Percentage of positive detection	47%	14%	53%	59%
Concentration (ngl-1)	6-68	4-223	9-213	75-520

Table 3. Overview of results of detection for the chosen pharmaceuticals in larger rivers of the Republic of Serbia

Substance	Carbamazepine	Trimetropin	4-FAA	4-AAA
Total analysis for all profiles	73	73	73	73
Total positive findings	7	0	14	12
Positive detection percentage	9%	0%	19%	16%
Concentration (ngl-1)	5-23	0	31-150	25-128

Table 4. Overview of results of detection for pharmaceuticals in chosen wellsprings of the Republic of Serbia

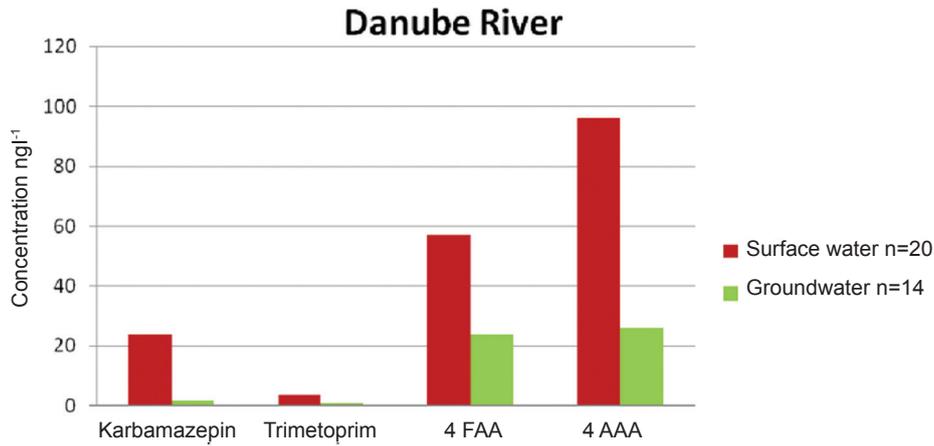


Figure 5. Diagram of average concentration recorded in the Danube and pertinent concentrations in the groundwater for 4 chosen pharmaceuticals.

Calculation method

The system of at least 3 equations is used:

- hydrodynamic equation (1) defining water streaming in the aquifer;
- equation defining matter transport by groundwater (2)
- equation defining the ratio of matter in the liquid and solid stage of the aquifer skeleton (3)

$$\frac{\partial}{\partial x} \left(T \frac{\partial \Pi}{\partial x} \right) + \frac{\partial}{\partial y} \left(T \frac{\partial \Pi}{\partial y} \right) + \frac{\partial}{\partial z} \left(T \frac{\partial \Pi}{\partial z} \right) = \varepsilon \frac{\partial \Pi}{\partial t} - q \quad (1)$$

$$(1) \quad (2) \quad (3) \quad (4) \quad (5) \quad (6)$$

$$\nabla D' \nabla C - \nabla C U - n W_1 C - W_1 (1-n) S = n \frac{\partial C}{\partial t} + (1-n) \frac{\partial S}{\partial t} \quad (2)$$

$$S = S(C) \quad (3)$$

$\nabla D' \nabla C$	- Diffusion and dispersion	$n W_1 C$	- Degradation of substance in liquid phase
$\nabla C U$	- Convection	$(1-n) W_2 S$	- Biochemical or chemical degradation on solid phase
$n \frac{\partial C}{\partial t}$	- Concentration change in liquid phase	$(1-n) \frac{\partial S}{\partial t}$	- Joint outcome of processes 1-5

Solution of the equation (1) defines the field of the groundwater potential. Simultaneous solution of equations 1, 2 and 3 is giving us the field of concentration for materials monitored in the aquifer.

By benchmarking of the system of equations (1), (2) and (3) we need to get the land subsidence of measured values of the groundwater potential and concentration of matter transported by the groundwater. Herewith there is a very attractive issue of defining the kinetics of degradation by groundwater. This issue is a subject matter in the paper by Kovačević, et al. also presented within this Conference.

Equation (3) is written for the stationary relation between the matter concentration in the solid and liquid stages of the aquifer. This simplification can be accepted for the cases when filtration process of the groundwater lasts much longer than the kinetics of transfer of the matter from solid to liquid stage. For small concentrations in water this relation can most often be regarded to be linear.

Establishing the parameters of sorption (by defining the sorption isotherms), as well as degradation (by benchmarking the math model, or by experience) allows the possibility of defining the elements of source by the river (bank filtration) or the source to which method of artificial recharge is applied. Configuration of the source is defined in such a way that during filtration and through the aquifer the unwanted matter in water is eliminated in sufficient quantity and with sufficient efficiency. Figure 6. shows diagram of elimination of various substances on their way between the water and the water intake wells placed on different distances from the river.

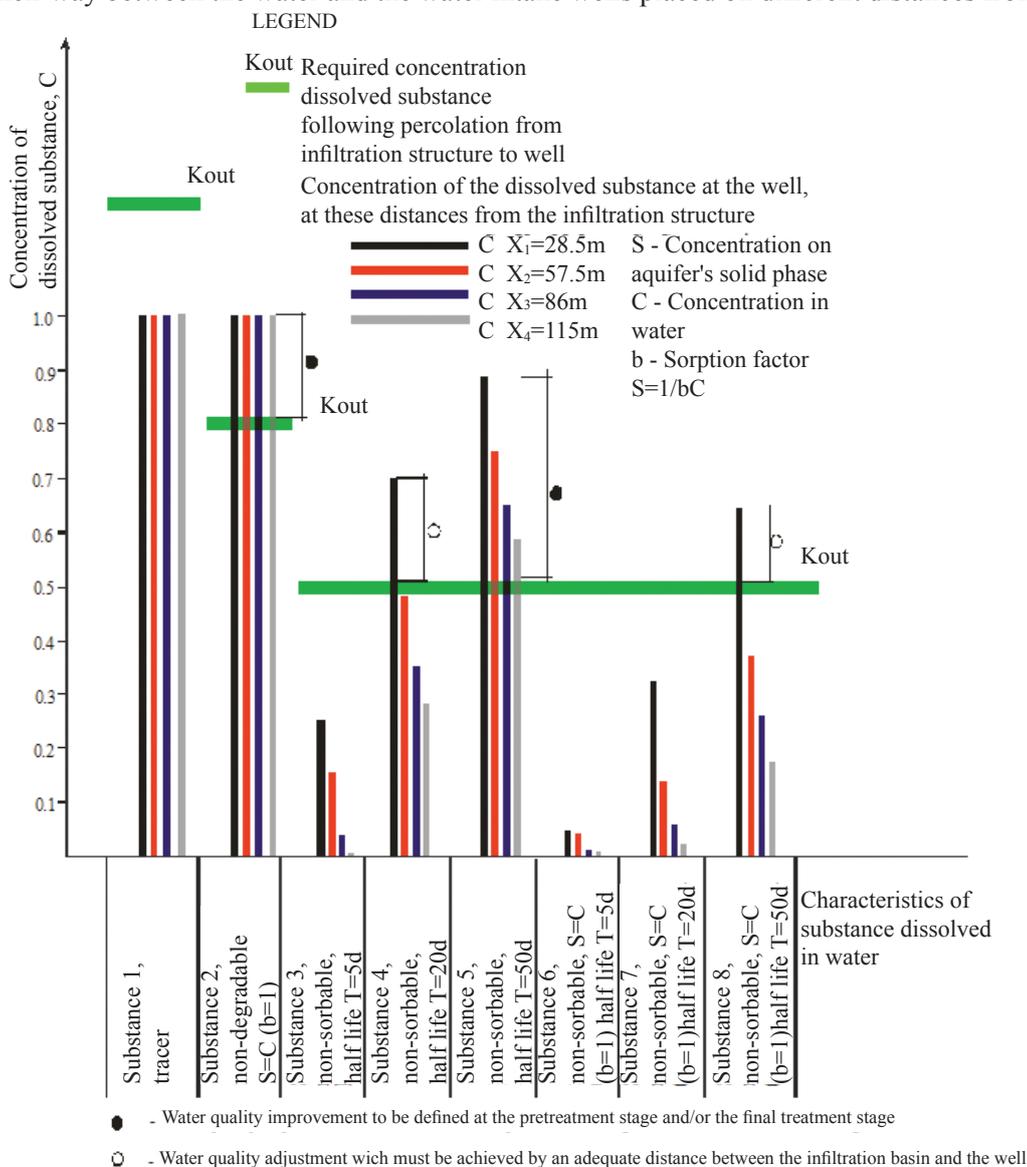


Figure 6. Elimination of various substances on their way between the water and the water intake wells placed on different distances from the river.

The issue of the implemented software features is also very significant. For problems where the groundwater spreading is mostly horizontal, we are using 2D or eventually quasi 3D math models (with adequate software). For more prominent three-dimensional flows we are using the 3D model. This is especially important if we know that in alluvial aquifer there is a very frequent case of horizontal layering and thus anisotropy. This is especially important when we consider, for example, flow from the river towards the radial well (horizontal draining well). For more efficient solution of this type of cases a software has been developed in the Jaroslav Černí Institute in cooperation with the R&D Center for Bioengineering "BIOIRC" for flow and transportation of the matter in groundwater – presented at this Conference) by Vidović et al.

Finally, we would like to stress out once more the importance of this approach for solving various tasks within defining the fate of the matter in groundwater and defining the protection and technological and technical constellation of the groundwater source.

Acknowledgments

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EMERGING ENVIRONMENTAL POLLUTANTS IN THE CONTEXT OF RISK ASSESSMENT OF EUROPEAN RIVER BASINS

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The need to look beyond the conventional target pollutants when assessing the hazards of chemicals to human health and to ecosystems is now generally recognised as a priority issue in all environmental policy areas at both the European level and national level in the various countries. It has also become clear that it is not possible for individual countries alone, to develop the knowledge and methodologies needed for measuring and evaluating the effects and associated risks of a vast number of emerging pollutants. In spite of this, the vicious cycle “not regulated because not monitored/not monitored because not regulated” is still not broken and policy makers do not get the information they need to make informed decisions. The information on the occurrence, fate, (eco)toxicity and use of majority of emerging substances, their metabolites and mixtures is either not available or, if available, primary data are often of such a poor quality they cannot be trusted.

All of the above challenges are currently being addressed by the NORMAN network (www.norman-network.net). It was created in 2005 in response to an EU DG Research 6th Framework Programme call for a project establishing a permanent network of reference laboratories, research centres and related organisations dealing with emerging substances. In November 2008, NORMAN was set up as a permanent, self-sustaining network in the form of a non-profit association. The NORMAN Association today has almost 60 members [1] from 19 countries, including two members from Canada. The network is internationally well recognised, thanks mainly to successful synergies between research teams and their numerous national/international projects, in-kind contributions and membership fee used as ‘seed’ money for starting up new activities and financing on-going ones.

The main mission of the NORMAN network is to enhance the exchange of information and collection of data on emerging environmental substances and to encourage validation and harmonisation of measurement methods and monitoring tools so that the demands of risk assessors can be better met. The activities address emerging contaminants in all environmental compartments, covering all steps, from first identification of a potential new emerging substance based on evidence of occurrence in the environment and/or identification of effects on the ecosystems and human health, down to confirmation of its status as a future regulated priority pollutant. In this context, the network strongly supports an integrated approach, including chemical and biological tools, for the identification of substances that should be considered for further investigation, either at the local scale or more widely.

NORMAN plays a significant role as an interface organisation between science and policy, with the advantage of speaking with a ‘bigger voice’ to the European Commission and other public institutions. The activities of the network range from a scientific watch and the feeding of data on emerging substances into the NORMAN databases to the organisation of working groups and workshops (producing position papers on research priorities), the setting-up of interlaboratory studies and the organisation of measurement campaigns.

The ‘overarching’ activity of the network is prioritisation of relevant environmental contaminants. The Working Group (WG) on Prioritisation of Emerging Substances identifies emerging substances for priority attention, including priority needs for improving existing data, analytical methods, biological tests, etc. With regard to the specific needs for emerging substances, NORMAN has developed an advanced prioritisation methodology [2], whose key principles have already been published and applied to several independent datasets [3, 4, 5]. In the recent study by von der Ohe et al. and the prioritisation WG of the NORMAN network, a simplified approach was used to select Water Framework Directive (WFD) river basin specific pollutants for evaluation of the ecological status of surface water bodies in four European river basins [3]. In general, the added value of the NORMAN methodology, compared to the previous prioritisation approaches, is that it is a global scheme which also considers compounds for which not enough data are available and prioritises them by action needed,

taking into account the current knowledge gaps. A general scheme of NORMAN activities leading towards identification of relevant pollutants is in Figure 1.

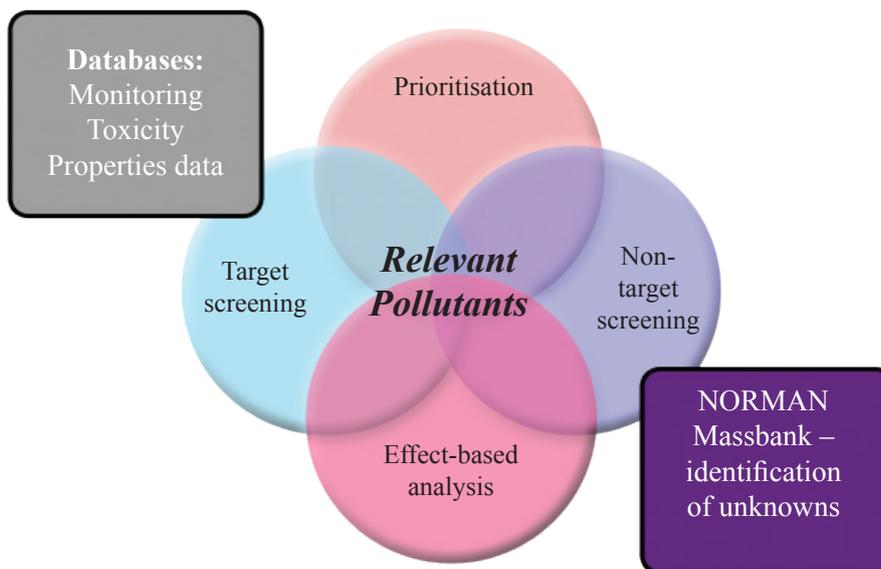


Figure 1 Overview of NORMAN activities needed for prioritisation of relevant pollutants.

The prioritisation process is supported by systematic data collection of new occurrence data (EMPODAT database) on the NORMAN substances (list of ‘frequently discussed emerging substances’ identified by the NORMAN experts based on citations in the scientific literature), their ‘lowest’ PNEC (the lowest value among acute-based ($PNEC_{acute}$) and chronic-based ($PNEC_{chronic}$) thresholds derived from experimental or modelled data [11]), Kow, Koc, fugacity (distribution in soil/water/air environment), PBT, vPvB, CMR and ED data for the final ranking.

An approach based on individual target compounds has well-known limitations, as it cannot take into account the complexity of environmental contamination and its effects. NORMAN therefore also attempts to trigger screening and effect-based approaches for identification of the toxicants responsible for adverse effects in the environment, even if they do not appear as targets on priority monitoring lists. Site-specific effect-directed analysis (EDA) and multivariate approaches to correlate chemical screening data with effect patterns are promising tools to address these challenges. The development and promotion of these tools were the reasons for supplementing the existing WGs on prioritisation (WG1) and bioassays (WG2) with a new working group on EDA in NORMAN (WG3) [7]. Since a full analysis and assessment of these compounds will not be possible, EDA is regarded as a potential tool to isolate, identify and prioritise those chemicals that cause measurable effects on biological systems (for a description of the process, see Figure 2).

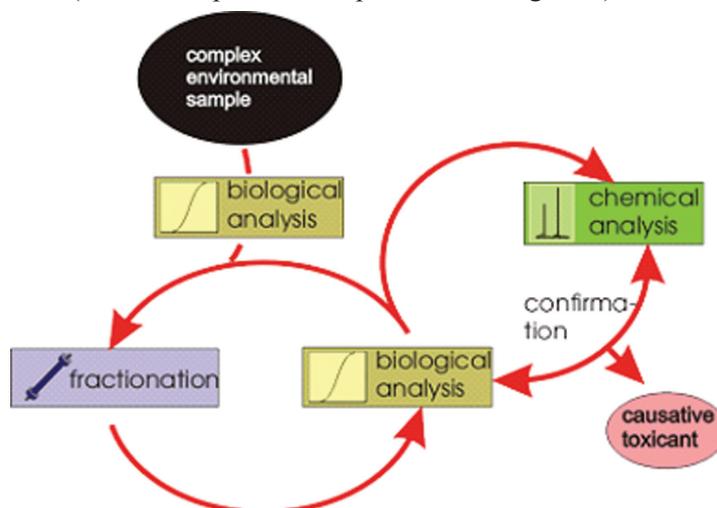


Figure 2 Effect-directed analysis process including the chemical analysis and identification phase of the toxicants [8].

The mission of the WG is to bring together European experts in EDA to exchange knowledge, methods and data via workshops, data sharing and scientific collaboration, to compile existing EDA methodologies for scientists and end-users and to promote scientific progress in, and application of, EDA tools for a more realistic quality assessment of complex contaminated environments.

Systematic collection of raw monitoring data as well as effect data on emerging substances and their exploitation via automated tools is the basis for linking predicted and field data and is the key to identification of gaps and priority actions for emerging substances. Since its creation as an EU-funded project, NORMAN has been maintaining and regularly feeding three web-based databases: (i) EMPOMAP [10]: a database of leading experts, organisations and projects dealing with emerging substances; (ii) EMPODAT [11]: a database of geo-referenced monitoring and occurrence data accompanied by ecotoxicological information from bioassays; and (iii) NORMAN MassBank (former EMPOMASS): a database of mass spectrometric information on provisionally identified and unknown substances. As to the latter, the former EMPOMASS database was transferred in 2011 to the new MassBank portal [9] hosted by UFZ (Leipzig, Germany). It is now available on the NORMAN public site as a platform for the collection and exchange of mass spectral data among NORMAN members and between NORMAN and the scientific and regulatory community worldwide. With this initiative the NORMAN network intends to contribute to motivating the environmental chemistry community to share their mass spectra of analytical standards and environmental samples with others in order to improve the identification of environmental suspects and unknowns.

A WG on bioassays and biomarkers linked to the WG EDA will be part of the network's activities in 2013 to look at current applications of biological test tools in water quality monitoring. Efforts to define a battery of bioassays for Europe-wide toxicity profiling started in 2012. An activity aimed at promoting Europe-wide harmonisation of non-target screening methodologies will also be organised by the NORMAN network in 2013. First steps towards developing of an approach for prioritisation of unknown pollutants were made recently [12] and a special sub-group of the WG on Prioritisation was formed to deal with the issue in detail.

As regards the methods validation aspects, particularly in respect of emerging pollutants, there is concern about the reliability of analytical data, due to a lack of harmonisation in method validation and QA/QC requirements. A major achievement of the NORMAN experts was the publication in 2009 of a common European protocol for the validation of methods [13] for the monitoring (occurrence) and bio-monitoring (effects) of emerging pollutants in environmental matrices. The protocol was tested in three case studies as part of the NORMAN project, and negotiations are now under way at CEN for the adoption of this protocol as a CEN Technical Specification. Moreover, on the basis of the identification of needs for specific emerging substances or groups of substances, NORMAN encourages Proficiency Testing (PT) providers and / or partners of the network to organise and conduct interlaboratory studies. NORMAN ensures dissemination of information about interlaboratory studies organised in the framework of research projects and encourages participation of NORMAN laboratories in these exercises. These examples show the role of the NORMAN network in encouraging harmonisation of practices among research and routine laboratories dealing with emerging compounds and promoting synergies with nationally- and internationally-funded initiatives to speed up the progress of research in this field.

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OCCURRENCE OF EMERGING POLLUTANTS IN SPANISH URBAN WASTEWATER AND THEIR REMOVAL

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Introduction

So far two decades ago, hazardous chemical substances like POP (persistent organic pollutants) and heavy metals were focus of awareness and intensive monitoring programs were developed to manage these substances. A drastic reduction of this type of wastes has been reached in industrialized countries due to appropriate technical and legal measures. Since then, the emerging pollutants have emerged as unregulated substances. These compounds and their bioactive metabolites can be continually introduced to the aquatic environment as complex mixtures via a number of routes but primarily by both untreated and treated sewage. Aquatic pollution is particularly troublesome because aquatic organisms are captive to continual life-cycle, multigenerational exposure. The possibility for continual but undetectable or unnoticed effects on aquatic organisms is particularly worrisome because effects could accumulate so slowly that major change goes undetected until the cumulative level of these effects finally cascades to irreversible change--change that would otherwise be attributed to natural adaptation or ecologic succession. As opposed to the conventional, persistent priority pollutants, Pharmaceutical and Personal Care Products (PPCPs) need not be persistent if they are continually introduced to surface waters, even at low parts-per-trillion/parts-per-billion concentrations (ng-microg/L). Even though some PPCPs are extremely persistent and introduced to the environment in very high quantities and perhaps have already gained ubiquity worldwide, others could act as if they were persistent, simply because their continual infusion into the aquatic environment serves to sustain perpetual life-cycle exposures for aquatic organisms (1).

The relatively recent awareness of PPCPs impact on environment is reflected in literature since the 1990s through the exponentially increasing number of studies concerning this emergent class of water pollutants. This rising interest is not only concomitant with the widespread and growing use of human and veterinary pharmaceutical products consumption, but also with the analytical techniques improvement, that allows detecting traces of substances (ng/L or less) in any type of water (2).

In Europe, around 3000 different pharmaceutical active compounds are used as human and veterinary drugs and are susceptible of reaching the natural media. From the quantitative point of view, a report published by the British Government compared the consumption of 14 groups of drugs in 14 countries, revealing that the Spanish people are Europeans that consume more drugs, only surpassed by the Americans, and followed by France, Denmark and Australia. Although some countries emerge as generally high or low users, there is no uniform pattern across disease areas and categories of drugs. France, Spain, USA and Denmark have high levels of usage generally, but not across all disease areas. Low levels of usage are also observed for all four countries in some groups of drugs. Generally lower than average levels of usage were observed in Norway (3).

In general, pharmaceuticals and their byproducts are spread into 24 therapeutic classes, among which 4 have been so far specially studied. About 40% of studies concern non steroidal anti-inflammatory drugs (NSAIDs), the three others being anticonvulsants, antibiotics, and lipid regulators. NSAIDs are represented by ibuprofen and diclofenac, the most studied lipid regulator is gemfibrozil. Carbamazepine is targeted in many studies as anticonvulsant, and antibiotics are represented by a great variety of substances (e.g. macrolides, sulfonamides, β -lactams, quinolones, etc.).

This paper will present the results of three studies conducted in Spanish sewage. Two of them belong to a large water reuse project that took place from 2006-2011. 24 research groups in Spain participated in the project. One of the objectives was to monitor emerging contaminants. This study was conducted for about two years in five sewage treatment plants spread in different geographical areas of Spain. A part of the results of this study are presented here. The other study, also conducted over two years, three treatment plants in the region of Catalonia by researchers in that region.

Removal of pollutants in Spanish WWTPs

To protect the environment from the potential chemical pollution of urban wastewater discharges and emissions from the industry, a considerable progress has been made following the implementation of the Directive 91/271 (Urban Waste Water Directive). In Spain, progress in addressing waste water-related challenges resulting from this regulation, has ensured notable improvements, in quantity and quality of water. More than 3000 WWTPs have been build in the last two decades to treat more than 90% of the Spanish urban waters. Around a 10% of this treatment plants incorporate advanced treatment. However, in many cases, the effectiveness of waste water treatment plants (WWTPs) for removing chemicals is limited. Therefore, a large number of organic pollutants escape to treatments becoming ubiquitous contaminants in the environment. The ubiquity of drugs is related to specific sales and practices in each country. For example, antibiotics antihistamines, analgesics, and antidepressants are the families of drugs with major consumption in Spain, according to the National Health System.

Case Study 1

A two-years monitoring programme was carried out in five municipal sewage treatment plants (WWTPs) located in the north, centre and south-east of Spain (4). The study evaluated the occurrence and persistence of a group of 100 organic compounds belonging to several chemical groups (pharmaceuticals, personal care products, pesticides and metabolites).

In terms of frequency of detection, most chemicals were detected at least once over the campaign developed from the 100 contaminants included in the study, 90 were detected in at least one occasion in influent samples and 88 in the effluents. Mean concentrations detected ranged from 7 ng/L to 59 µg/L in influent samples and from 5 ng/L to 32 µg/L in effluents. Given this wide range of concentrations, it is noteworthy that a group of only 20 compounds is responsible for 83% of the total load of pollutants in the effluent. These compounds presented not only a high concentration but also a high detection frequencies, being discarded those compounds that had high concentrations occasionally. In this group are included 13 pharmaceutical, mainly analgesics/anti-inflammatories (diclofenac, codeine, naproxen, ibuprofen), antibiotics (ofloxacin, ciprofloxacin, erythromycin), diuretics (furosemide, hydrochlorothiazide), lipid regulators (gemfibrozil), β-blockers (atenolol), ulcer healings (ranitidine) and the stimulant caffeine.

It is also remarkable the presence at very high concentrations of a group of metabolites such as fenofibric acid (metabolite of fenofibrate), paraxanthine (metabolite of caffeine) and four of the main metabolites of the antipyretic drug dypirone, 4-MAA, 4-AAA, 4-FAA and 4-AA, which represent a very important amount of the total load. This finding highlights that for certain chemicals such drugs, probably is of higher concern to monitor metabolites generated in the human body or during the water treatment, specially when drug metabolism rates are high and whose presence in the environment can be the cause, in some cases, of a higher risk than that expected from the original compounds.

The sole representative of the PCPs group, included among the most abundantly detected contaminants, is the synthetic fragrance galaxolide. However, the contribution of these compounds can vary depending on the characteristics of the WWTPs. Moreover, there are pollutants such as atenolol, caffeine and its metabolite or all the main metabolites of the antipyretic drug dypirone which although ubiquitous in all effluent treatment plants studied, at relatively high concentrations, their contribution in the total load of pollutants in the effluent vary depending of the WWTP characteristics also.

From the analysis, data obtained over a two year period of monitoring in the selected WWTPs, activated sludge biological treatment was more effective, comparatively among the different chemical families studied, in the removal of stimulant compounds (>80%), UV filters (>86%) and some synthetic fragrances, analgesics/anti-inflammatories or disinfectants (>70%). Most of lipids regulators, diuretics and β -blockers showed medium removal rates, with mean values around 50%. Macrolide antibiotics and the anti-epileptic carbamazepine presented poor or no elimination. Thus, despite the secondary treatments applied, effluents from the WWTPs can still contain a large number of emerging pollutants that continuously are being discharged into bodies of water.

It has been observed compounds with lower removal rates but also some compounds highly degraded, such as analgesics, stimulants or anti-inflammatory drugs. This fact could be explained due to all of them remained relatively high concentrations in the effluent samples analysed, implying the continuous input into the aquatic environment of these compounds and thereby confirming that the discharges of effluent from WWTPs are among the main pathway responsible for pollution of surface water.

Case Study 2

43 pharmaceutical compounds were studied in three different WWTPs in order to obtain more information on their fate during conventional wastewater treatment (5). The selected pharmaceuticals belong to different therapeutic groups (i.e. non-steroidal antiinflammatory drugs (NSAIDs), lipid modifying agents, psychiatric drugs, histamine H₂-receptor antagonists, antibacterials for systemic use, beta blocking agents, beta-agonists, diuretics, angiotensin converting enzyme). The samples were provided from three conventional full-scale activated sludge sewage treatment plants with anaerobic digestion of sludge, from the region of Catalonia (Spain).

Out of 43 analyzed pharmaceutical compounds, 32 were detected in influent, 29 in effluent and 21 in sludge samples. The analysis of wastewater and sludge showed huge variation in concentration levels from campaign to campaign of a given plant. This can be due to changes of the composition of influent waters in different seasons, weather conditions and operational conditions of the plant, as well as due to the amount of the drug that is used.

According to the daily loads and population served by each plant, the amount of the selected pharmaceuticals disposed in these plants is estimated to be 5.6, 2.0 and 0.4 g/day/1000 equivalent inhabitants for the three plants. The highest levels at the influent of all three WWTPs were observed for NSAIDs that were expected due to their high consumption. At the influent of the plants, this group accounts for 65% of all the therapeutic groups analyzed.

Lower but still significant levels of lipid modifying agents (including fibrates and statins) (7-12%), diuretics (8-10%), and beta-blockers (5-9%) were detected entering these WWTPs.

The amount found in effluent or sludge depended on the removal efficiency of plant and/or the physicochemical properties of the compounds. In the effluent waters, NSAIDs were present in the highest percentage (35-44%), followed by the lipid modifying agents (8-29%) and psychiatric drugs (both antiepileptic and benzodiazepine derivative drugs) (17-30%).

Analysis of sludge samples showed the presence of 21 out of 43 pharmaceuticals, covering a wide range of physicochemical properties, as in the case of wastewater effluent. Diuretics accounted for 19%, antibacterials for 16-21% and lipid modifying agents 15-20% of all the pharmaceuticals analyzed (depending on the plant). On the other hand, beta-blockers, beta-agonist and histamine H₂-receptor antagonists were found in very low concentrations in sludge.

The total loads pharmaceuticals that leave the plants unmodified (including sludge and effluent water) were calculated to equal 1.1, 0.9 and 0.1 g/day/1000 equivalent inhabitants for the three plants of which only 3-9% (depending on the plant) was retained by sludge.

Removal of pollutants and sorption processes

A third study (6) show the regular presence of over seventy anthropogenic individual pollutants in Madrid rivers, some of which are encountered in relatively high amounts. In raw sewage, 25 compounds were detected in the mg/L range. Acetaminophen (paracetamol) and caffeine were persistently detected over 1 ppb in untreated wastewater. Galaxolide was also encountered in high concentration, but its occurrence showed a significant variability. Two metabolites, paraxanthine from caffeine and 4-FAA from metamizol were also found in high amounts in almost all samples. Other metabolites detected were 4-AA and 4-MAA from dipyrone, fenofibric acid from fenofibrate and 4-AAA also from metamizol. This findings stress the need for exploring not only the pattern PPCP, but also their metabolic or photodegradation intermediates.

We could assume that a drug that is highly metabolized in humans will be subjected to extensive degradations in the environment, however, a high metabolic rate in humans does not necessarily mean that the lifetime of the pharmaceutical in the environment will be short. For some compounds, this assumption is correct (e.g., ibuprofen, diclofenac, propranolol, metoprolol, and carbamazepine), and they were found to be easily dissipated in the environment. On the other side, atenolol, trimethoprim, and naproxen are substances with a low metabolic rate in humans, and they are excreted mainly unchanged or as acyl-glucuronide (naproxen), whereas their half-lives range from 10 days to 1 year. Commonly, glucuronide and sulphate conjugates are the major metabolites that leave the biologically active group of the parent drug intact. Although more polar metabolites are presumed to be less hazardous to aquatic organisms, the European Medicines Agency (EMA) guideline suggests environmental risk assessment of all human metabolites that constitute more than 10% of the total excretion of drug.

There is experimental evidence that the removal of organic pollutants in WWTP is largely controlled by sorption process with solid–water distribution coefficients being a function of the octanol–water distribution coefficient D_{ow} ; a parameter that takes into account the octanol–water partition coefficient, K_{ow} , as well as the dissociation constant of acidic or basic compounds. The efficiency of removal of PPCP is roughly dependent on its hydrophobicity expressed as apparent D_{ow} . For most compounds, the removal efficiency during biological treatment increased with hydrophobicity.

As an exception, a small group of compounds were almost completely removed in the WWTP even they present relatively low D_{ow} values. They are the metabolite of caffeine paraxanthine, caffeine itself, acetaminophen (paracetamol), ibuprofen and nicotine, compounds otherwise usually found in high concentrations in raw urban wastewater.

Another exceptions is a group formed by 14 compounds. They exhibit removal efficiencies below 20%, even their octanol–water distribution coefficient was not particularly low. They are all important pharmaceuticals prescribed and delivered to sewage in high amounts and include the beta-blockers atenolol, metoprolol and propranolol; the lipid regulator bezafibrate, fenofibric acid; the antibiotics erythromycin, sulfamethoxazole and trimethoprim; the anti-inflammatories diclofenac, indomethacin, ketoprofen and mefenamic acid; the antiepileptic carbamazepine and the antiacid omeprazole.

Conclusions

The evolution of micropollutants in wastewater that reaches the Spanish WWTPs are under great variability, both temporally (across the day and seasonal) and geographical, there is some regional variation.

The large number of emerging pollutantns under study makes it difficult to systematize. To carry out the study by gathering the micropollutants according to the use for which they were created, for example, for its therapeutic features, the diversity of chemical structure of substances makes it difficult to reach general conclusions. When grouped in response to the chemical structure not very general conclusions are reached because of the large number of variables involved.

It seems necessary to optimize treatment systems to prevent future environmental problems with emerging pollutants.

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PHARMACEUTICALS AND PESTICIDES IN SEDIMENTS, SURFACE AND GROUNDWATER

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Contamination of surface water by different pharmaceuticals and pesticides has been reported in the last decade in a number of studies. Due to the high water solubility and often poor degradability polar organic compounds such as residues from pharmaceuticals and polar pesticides may, under unfavorable conditions, pass through all natural filtrations and leach into the groundwater aquifers. Standard water treatment plants are not equipped to remove pharmaceuticals as well as some pesticides from water and ultimately they can reach drinking water. Recently, pharmaceuticals have been detected at trace levels in drinking water in United States, Canada as well as in Europe. Despite of growing concern, drugs have not yet been included in the environmental regulations. In 2009, in collaboration with Jaroslav Černi Institute, we have started the extensive task of monitoring pharmaceuticals and pesticides in surface and groundwater of Danube river basin in Serbia. At the beginning of this project we have developed the multiresidual methods for preconcentration and quantitative analysis of multiple classes of pesticides [1] and pharmaceuticals [2] based on solid phase extraction and HPLC-MS/MS analysis using ion trap mass spectrometer with electrospray ionization source. The SPE method consisted of the loading of the water sample (100 ml, pH 6) on the Oasis HLB cartridge followed by 10 min drying by vacuum suction, cartridge elution with 15 ml of methanol, and, finally, extract evaporation to dryness and reconstitution with 1 ml of methanol. The chromatographic separation of compounds was performed on a reverse-phase Zorbax Eclipse® XDB-C18 column.

Residues of 14 pesticides, belonging to the different chemical classes, were monitored. Pesticides were selected based on the existing regulations and the extent of their use in Serbia. Eighteen most frequently used pharmaceuticals in Serbia that belong to major groups of antibiotics, sedatives, antiepileptics, analgesics/antipyretics and cardiovascular drugs were chosen for the study. In addition two metamizole metabolites (4-FAA and 4-AAA) were analyzed and used as indicators of sewage contamination and markers for natural water pollution with wastewater.

The surface water samples were collected from 11 sampling sites of the river Danube in different cities, and four sampling sites of its tributaries Tisa, Sava, Morava and Pek near their confluence with the Danube. The groundwater samples were collected from observation or operation wells in the vicinity of the surface water sampling sites. More than 100 samples were analyzed in last 4 years.

The list of trace level pharmaceuticals and pesticides detected in surface and groundwater is presented in table 1. During the spring campaign 2011 we have analyzed river sediments from the Danube and its tributaries Tisa, Sava, Morava and the results are also included in table 1.

In the surface water samples, carbamazepine and metamizole metabolites (4-FAA and 4-AAA) were the most frequently detected drugs [3]. The residues of carbamazepine were detected in the concentration range below 100 ng l⁻¹. The frequency of its detection can be explained by low sorption, resistance to biodegradation and low removal rate in waste water purification plants, as well as high administered doses of this antiepileptic drug. Carbamazepine residues detected in the Danube in Serbia are lower than those found in the surface waters in Germany, USA and Switzerland but higher than those reported for Finland and Canada. Carbamazepine is also detected in river sediments where it is partially removed. However, this drug is passing through natural filtrations, reaching the groundwater.

Table 1. Pharmaceuticals and pesticides detected in sediments and water samples

Sample	Pharmaceuticals	Pesticides
River water	4-FAA, 4-AAA, carbamazepine, azithromycin, lorazepam, metoprolol, bisoprolol, trimethoprim	carbendazim, atrazine, dimethoate, propazine, carbofuran,
Groundwater	4-FAA, 4-AAA, carbamazepine,	carbendazim, atrazine
River sediment	carbamazepine, diazepam, clopidogrel, erythromycin	dimethoate, atrazine, propazine, malathion, carbofuran

The pesticides found in the highest concentrations in the water of the Danube River Basin are atrazine and carbendazim [4]. Among monitored pesticides the most frequently found was carbendazim. However, a concentration of atrazine, one of the pesticides which is on the list of the priority substances, was present at the level lower than those established in the legislation as maximum allowable concentrations. Both pesticides were also found in groundwater.

In order to establish the sorption efficacy of the selected analytes on the sediments, sediments were sampled from the different depths of the Danube riverbed near the water and groundwater sampling sights. The most of the pharmaceuticals and pesticides studied were mainly adsorbed on the riverbed and therefore not found in groundwater. However, the adsorption of atrazine, carbamazepine and metamizole metabolites 4-AAA and 4-FAA on riverbed is moderate or poor.

The adsorption of atrazine, carbamazepine and metamizole metabolites 4-AAA and 4-FAA is illustrated in table 2. The sediments sampled from the riverbed, and from the depths of 5-14, 10-16 and 14-18 m were kept in contact with the mixture containing 500 ppb of each analyte for 48h. The amount of each analyte in table 2 is expressed as the percentage adsorbed on soil samples. Adsorption of metamizole metabolites 4-AAA and 4-FAA is in general very poor, but better on the riverbed than on the clay or sand. Therefore the river sediments are not the efficient barrier and 4-AAA and 4-FAA are leaching into the groundwater.

Adsorption of carbamazepine and atrazine is also poor, but more efficient on sand and clay than on the riverbed, but they are also penetrating the groundwater, where they were found very often.

Table 2. Adsorbed amount (%) of selected analytes on sediments sampled from different depths in contact with 500 ppb solution for 48h

	Danube riverbed	5-14 m	10-16 m	14-18 m
Pharmaceuticals %	%	%	%	%
4-AAA	26.8	3	14.7	3.7
4-FAA	34.5	2.1	0	0
carbamazepine	16.3	25.5	52.6	36.6
Pesticides %	%	%	%	%
atrazine	16.5	76.2	71.4	68.3

Acknowledgment

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OCCURRENCE AND FATE OF EMERGING POLLUTANTS IN SURFACE WATER OF TWO REGIONS WITH CONTRASTED SOCIO-ECONOMIC CONDITIONS: FRANCE AND UKRAINE

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Pharmaceuticals are emerging pollutants what have been detected in surface and ground waters of different countries. Some of them became an object of the long term monitoring, ecotoxicology and regulation. The presence of pharmaceuticals in the aqueous environment is generally related to anthropogenic processes: pharmaceuticals production, its consumption, water treatment and discharge. Pharmaceuticals are produced with known physical and chemical properties what can help to classify them and even predict the behaviour in the environment. The consumption of human and veterinary drugs varies from country to country, that depends on the current pharmaceutical market, its regulation and control, but also on the impact of local climate and social conditions. For example, compare to economically developed EU-countries, Ukraine, as country with a transitional economy, has the lowest official level of human drugs consumption in monetary and DDD (defined daily doze) values and the lowest level of access of population to the health care (Table).

Table. Basic indicators of health market in some European countries (2007)

Country	Total population, mln.inh	Access to medical care, beds/10,000 persons	Access to pharmaceutical shops, persons/shop	Human drugs use, USD/person (DDD)	Expenses on health care, % of GDP
Ukraine	46.2	95.2	5500	18(11.84)	3.8
France	63.2	na	2000	299(32.2)	11.1
Spain	44.3	347.5	2100	210(na)	8.2
Germany	82.2	846.4	3800	260(na)	10.7
Italy	59.1	400.9	3600	212(na)	8.9

na - data were not available

From one side, a weak access of population to health services can mean the lower level of pharmaceuticals use in the country, from other side – the “black market” of pharmaceuticals and inappropriate use of medicaments. For example, just a half of medicaments which are sold on the Ukrainian market are prescribed. The low developed system of medical care and pharmaceuticals prescription can effect in the wrong and chaotic use of medicaments by pollution. Such conditions result in the accumulation of date expired or unused pharmaceuticals what can be through away in the sewage or waste systems and come in the environment in non-metabolized forms. The wastewaters can be the main source of pharmaceuticals in the aquatic environment. The most contaminated is hospital wastewaters as they content highly concentrated mixture of various medicaments. The removal of pharmaceuticals on treatment plants is depends on physical chemical properties of compounds and used treatment processes, biological degradation, presence of other chemicals, for instant nitrates, in water, climate conditions, seasonal variations and even geographical location. A lot of countries have very limited facilities to remove pharmaceuticals from raw waters. Recently the processes of degradation and occurrence of pharmaceuticals during various treatment processes are little known with a lot of uncertainties and assumptions, e.g. appearance of drugs metabolites and synthesis of different molecules, impact of environmental factors and its combination. Thereby the portion of pharmaceuticals, what are able to get into water from different sources depends on various factors: (i) management and control of pharmaceuticals manufacturing; (ii)

regulation of medicaments consumption, i.e. quantity of prescription and sold drugs, (iii) metabolism of drugs in organisms, (iv) property of molecules, e.g. solubility, degradation; (v) environmental parameters, i.e. climate conditions, dilution and (vi) treatment processes. Some of these factors are predictable, e.g. metabolism, degradation, some are unpredictable, e.g. improper use and disposal.

Our study was focused on processes of occurrence and fate of emerging pollutants in surface water of two regions with contrasted socio-economic conditions: France and Ukraine. At the first step, pharmaceuticals with contrasting hydro-chemical properties of molecules (water solubility, bioaccumulation, persistence during wastewater treatment processes) were discriminated on conservative, labile and with combined properties in order to provide the information on wastewater treatment plant efficiency and the potential behaviour in the environment. Further, 21 pharmaceuticals of different therapeutic groups (antidepressants, analgesics, psychiatric drugs, stimulants, sedatives, bronchodilators, non-steroidal anti-inflammatory and lipid regulators) were monitored along the studied Ukrainian and French rivers. Among them 18 medicaments (alprazolam, aspirin, amitriptyline, bromazepam, caffeine, carbamazepine, clenbuterol, diazepam, diclofenac, fluoxetine, gemfibrozil, ibuprofen, ketoprofen, naproxen, nordiazepam, paracetamol, salbutamol and theophylline) have been found in the Jalle River in France and 15 of these targeted compounds (alprazolam, aspirin, amitriptyline, caffeine, carbamazepine, diazepam, diclofenac, fluoxetine, ibuprofen, ketoprofen, naproxen, nordiazepam, paracetamol, salbutamol and theophylline) were detected in the Lopan River, Ukraine. The distribution of pharmaceuticals in streams showed the different patterns, with the domination of the stimulants and non-prescription drugs in upstream sites and prescription medicaments in the sites downstream of municipal wastewaters discharges and urban areas. According to the maximum concentration values, the Ukrainian river has been found more contaminated by targeted organics compare to the French river that can be linked to disparity in the medicament consumption, efficiency of wastewater treatment and natural dilution of anthropogenic inputs. At the last step, applying mass balance modelling, medicaments were described as relevant socio-economic indicators, which can give a picture of main social aspects of the region. Here, the data on pharmaceuticals were treated and modelled for the description of potential socio-economic factors such as the consumption patterns, which can impact on the presence of these organic contaminants in natural streams. However, in order to understand the influence of the consumption patterns, environmental data have been used for the estimation of intake of selected medicaments (diclofenac, caffeine and carbamazepine) in studied regions. For this commitment, the mass balanced modelling using the data on the drug excretion, efficiency of treatment, water flow and environmental concentrations has been realized:

$$M_C = (Q_W C_W - Q_U C_U) / (K_1 (1 - K_2))$$

M_C - drug consumption rate in a studied settlement, which is served by sewage system ($\text{g}\cdot\text{d}^{-1}$);

K_1 – drug excretion rate (a part of a pharmaceutical component which enters a sewage system as unchanged form by human excretion), ($\text{g}\cdot\text{g}^{-1}$). Pharmacokinetics represents a very complex process and depends on the metabolism, age, activity etc. We used previously reported data on the parent drugs excretion.

As results, the comparison of pharmaceuticals in two rivers of Ukraine and France approved that consumption patterns of medicaments influenced the accumulation of these micro-pollutants together with the another parameters, such as the wastewater treatment efficiency and management, natural instream hydrological and hydrochemical processes.

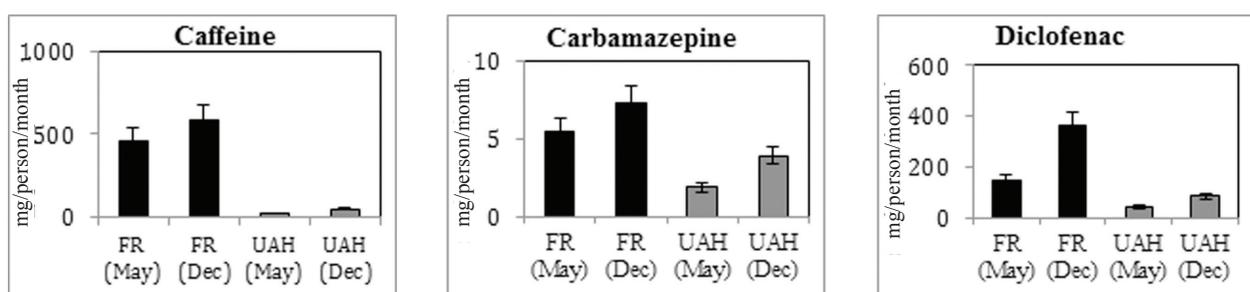


Figure. The estimated consumption rates in Ukraine and France

Outcomes of mass balance modelling suggest that water monitoring can be used for the estimation of particular social indicators as medical and illicit drug consumption rate.

Results of the research demonstrate the first data on the presence of pharmaceuticals in the Ukrainian watercourse and the application of the water monitoring data for the identification and comparison of the socio-economic determinants of water pollution in different regions (Ukraine vs France), i.e.:

- (i) *the regional medicaments consumption patterns* –using both socio-economic (i.e. number of population, wastewater treatment efficiency) and hydrological (i.e. concentration of the chemicals in the watercourses, water flow rates) indicators;
- (ii) *negative activities what influenced water pollution*, i.e. illegal and uncontrolled discharges – these processes can be discriminated using the pharmaceuticals as molecular markers.

Proposed methods of the data collection and interpretation can be applied to the rivers with similar hydrological and geochemical characteristics and provide the pilot information on occurrence of emerging pollutants, but also to give the preliminary socio-economic characteristics for particular regions in term of the drug market and medicament consumption, presence of the illegal and uncontrolled discharges.

EMERGING POLLUTANTS IN THE DANUBE RIVER (OUTCOMES OF JOINT DANUBE SURVEYS)

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Pollution of water bodies by chemical substances belongs to key problems of the Danube River Basin. To assess the magnitude of this issue a basin-wide monitoring network (Transnational Monitoring Network - TNMN), has been established under the Inter-national Commission for the Protection of the Danube River (ICPDR) in 1996. This monitoring activity provided the necessary basis for harmonized water quality assessment throughout the whole basin, which not only gave an overview of water quality trends in the basin and of loads of substances discharged into the Black Sea but it fostered achieving of compatibility between water quality assessment approaches in the Danube countries.

While the annual monitoring activities focus on EU WFD priority substances, the occurrence of the emerging chemical substances is being checked in the frame of ICPDR Joint Danube Surveys. These periodic investigative surveys are carried out every six years in synchronicity with the river basin management planning period according to the EU Water Framework Directive (WFD). The first Joint Danube Survey was carried out in 2001. For the first time comparable data about the entire course of the river have been provided covering over 140 different biological, chemical and bacteriological parameters. These data were used as an essential information source for the first analysis of the Danube River Basin District according to WFD Article 5. Six years later the second Joint Danube Survey has created a comprehensive and homogeneous database on the status of the aquatic ecosystem of the Danube and its major tributaries, which was used for the preparation of the first Danube River Basin Management Plan.

JDS1

Among other chemical parameters JDS 1 monitored of para-tert-octylphenol, 4-iso-nonylphenol, di[2-ethylhexyl]phthalate, pentachlorophenol, pentabromodiphenyl ether and tributyltin in suspended particulate matter and sediment samples as well as selected pharmaceuticals in water from the river Danube and its major tributaries.

No pentabromodiphenyl ether or pentachlorophenol was detected in any of the JDS1 samples, while tributyltin was found in relatively low concentrations. Para-tert-octylphenol was detected exclusively in bottom sediments, while 4-iso-nonylphenol and di[2-ethylhexyl]phthalate were found in bottom sediments as well as in suspended solids in significant concentrations (from a few $\mu\text{g}/\text{kg}$ up to more than 100 mg/kg), indicating the relevance of these compounds as markers for industrial pollution of solids in the river Danube. The results for pharmaceuticals showed that only a few of the analytes extracted at neutral pH were detectable. The only substances present in nearly every sample were isopropylphenazone, which was found in low concentrations of 0.003-0.004 $\mu\text{g}/\text{l}$, and N-acetyl-4-aminoantipyrine (metabolite of metamizol), which concentrations showed certain variations. From Budapest downstream, the concentrations of N-acetyl-4-aminoantipyrine showed a relatively constant level of around 0.1 $\mu\text{g}/\text{l}$ only in three tributaries this level was significantly exceeded (Iskar influenced by Sofia wastewaters, Jantra and Arges influenced by Bucharest wastewaters).

Concentration profile of another metabolite of metamizol, N-formyl-4-aminoantipyrine, showed the same pattern but it could not be detected in some cases due to a too high limit of detection. The three tributaries mentioned above also contained the analgesic phenazone in concentration range 0.029 - 0.46 $\mu\text{g}/\text{l}$.

In addition to the target analyses of organic substances, a GC-MS screening of water and sediment samples was performed during JDS1. Altogether 98 water samples were analyzed by GC-MS and in each sample a number of organic compounds were identified. Based on the spectral information collected, the chemical structures of 96 compounds occurring in the Danube have been suggested. The most ubiquitous compounds in water were phthalates, fatty acids, aliphatic chlorohydrocarbons and sterols.

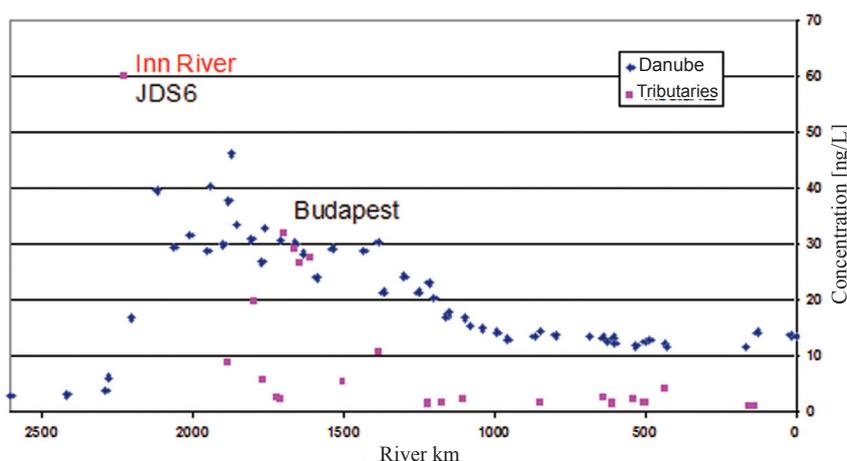
JDS2

The second survey strived not only to obtain homogeneous information on the occurrence of all Water Framework Directive (WFD) priority substances in the Danube and its major tributaries but the attention was given to a number of emerging substances as well. Polar water-soluble organic contaminants were analyzed in the water by solid-phase extraction (SPE) followed by triple-quadrupole LC-MS-MS analysis. Focus was given on pharmaceutical compounds (such as ibuprofen, diclofenac, sulfamethoxazole, carbamazepine), pesticides and their degradation products (e.g. bentazone, 2,4-D, mecoprop, atrazine, terbutylazine, desethylterbutylazine), perfluorinated acids (PFOS; PFOA), and endocrine disrupting compounds (such as nonylphenol, NPE1C, bisphenol A, estrone).

In general, concentrations of the target organic compounds were relatively low in the Danube River. In the tributaries higher levels were detected. The most contaminated tributary river was the Arges. The highest levels for most chemicals in the Danube were detected in the area around Budapest.

The chemicals detected in the Danube at the highest median concentrations were: benzotriazole (213 ng/l), tolyltriazole (81 ng/l), caffeine (80 ng/l), nonylphenoxy acetic acid NPE1C (49 ng/l), carbamazepine (37 ng/l), sulfamethoxazole (17 ng/l), perfluorooctanoate PFOA (14 ng/l) and desethylatrazine (11 ng/l).

The highest perfluorooctanoate (PFOA) level was detected in the River Inn (Germany); a concentration of 60 ng/l was measured. This river is the major PFOA source for the Danube. In the Danube downstream of this influent, PFOA levels up to 46 ng/l were found. PFOA was diluted along the river course, reaching levels around 14 ng/l at the end of the river. It has to be mentioned that the emission of PFOA to the Inn was stopped in 2008 as PFOA has been replaced in the relevant technological process.

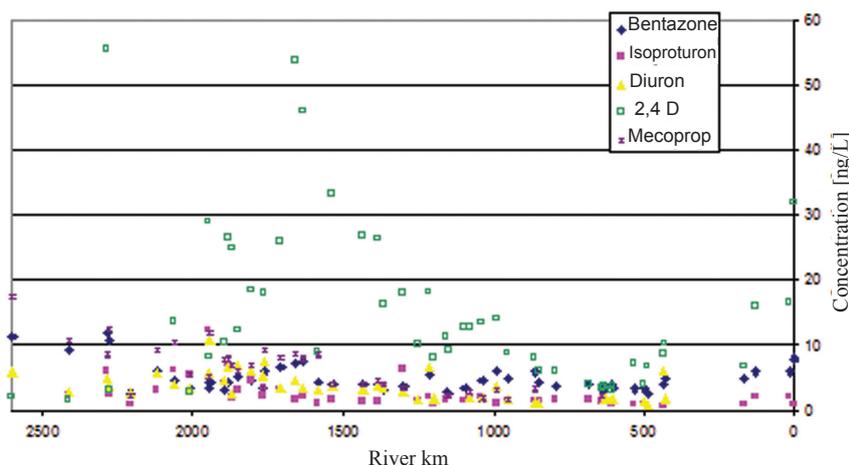


The highest pharmaceutical levels were found for carbamazepine and sulfamethoxazole, which can be explained by their persistency. For carbamazepine in the Upper Danube, slightly higher levels were detected than in the lower reach (around 50-60 ng/l around Budapest and ~30 ng/l in samples downstream of JDS50, which corresponds to the Tisa and Sava tributaries). For sulfamethoxazole levels ~20 ng/l were detected along the whole river. These anthropogenic substances are “discharged” along the whole river.

Concentration levels observed for bezafibrate, ibuprofen (5-10 ng/l), and diclofenac (<5 ng/l) were lower. Further downstream in Romania, diclofenac and bezafibrate were not detected anymore, which could be explained by the biodegradability of these compounds. Ibuprofen was found after the Velika Morava tributary (JDS56) in concentration levels between 9-27 ng/l, and at the Danube Delta ~5 ng/l.

The highest concentrations of 2,4-D, one of the most widely used herbicides in the world, were found in the area around Budapest (~50 ng/l). In the Austrian part of the Danube concentrations were measured at ~20 ng/l and in the downstream part ~10 ng/l were found. Bentazone levels were ~5-10 ng/l in the whole Danube. Simazine, atrazine, isoproturon and diuron levels were low (<10 ng/l) in the whole Danube. Terbutylazine

levels were slightly higher, between 10-20 ng/l around Budapest, and between 5-10 ng/l in the downstream region of the Danube. The concentrations for the metabolites desethylatrazine and desethylterbutylazine were in the range of 5-20 ng/l with maximum levels around Budapest.



In the Danube River the concentration levels of estradiol and ethinylestradiol were in all cases below the LOD (5 ng/l). Estrone could be detected in some samples in the area of Budapest and downstream of the Velika Morava confluence at concentrations around 1-2 ng/l. Moreover, 2 ng/l of estrone were detected in the Rus-senski Lom, and by far the highest measurement was taken in the Arges tributary (71 ng/l).

Benzotriazole (130-300 ng/l) and tolyltriazole (62-130 ng/l) were only analysed in selected sites and were found at all of them. They are persistent chemicals used e.g. as corrosion inhibitors in dishwashers.

Conclusions

According to the Blueprint to Safeguard Europe's Water Resources and the Commission assessment of the Member States River Basin Management Plans the assessment of chemical status indicates that a large proportion (about 40%) of the EU water bodies has an unknown status due to insufficient monitoring in many Member States. This unfavorable situation also holds for the Danube and is even more critical in case of the emerging substances for which no legislative monitoring requirements exist. To address this issue the third Joint Danube Survey in 2013 will monitor not only EU WFD priority and candidate substances but will also focus on river basin specific pollutants already defined in the Danube countries as well as on the emerging substances identified to be of relevance at the EU and/or Danube basin level based on previous results, national lists and national results or results from Europe-wide surveys. In addition to that the target analysis of emerging substances in terms of the effect-based monitoring of a wide range of chemical substances and of passive sampling will be applied during JDS3 to identify Danube river basin specific substances.

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PSEUDOPERSISTENCY, LOW DOSES EFFECT AND MASS LOAD FOR THE EMERGING SUBSTANCES

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Abstract

In recent years, emerging substances, EmS, have received much attention as pollutants of wastewater, surface water, water bodies or drinking water resources due to possible negative effects on humans or on the ecosystem even at very low concentrations. The considerable progress has been made regarding the knowledge of the specific physico-chemical characteristics, low doses effects, pseudo-persistence, the exposure of water bodies, aquatic bio system, and human to the EmS, up-to-date analytical determinations, process of prioritization, PEC, PNEC and other phenomena. This paper summarizes the main and specific physico-chemical properties of EmS with focus onto pseudo-persistence, low doses effects and mass load through river basin to the Black sea.

Keywords: emerging substances, pseudopersistency, low doses effect and mass load

Introduction

The presence of EmS/pharmaceuticals in surface, wastewaters and potentially in drinking water, is documented in the literature (Verlicchi et al., 2012). Emerging pollutants are substances of concern due to their potential genotoxic effects, disruption of aquatic ecology, promotion of antibiotic resistance, low doses effects, pseudo-persistency, modern technologies for wastewater treatments, and possibly increased human health risks.

EmS belong to the old chemical classes which are newly recognized as well as to the new chemical classes, with the new types of use, new effects, not well known mechanism of action, sources and way of release into environment/water bodies and the pathway of exposure. For the most EmS, there is currently little information about their potential toxicological significance in ecosystems, particularly from long - term low - level environmental exposure. The fate and the transport of EmS in natural aquatic media are practically unknown, especially in the context of water/soil/sediment distribution and partitioning processes. Wastewater treatment processes achieve variable and often incomplete removal of EmS resulting in discharge of emerging chemicals into environmental and receiving waters.

Physicochemical characteristics of EmS

The dominant and specific physicochemical characteristics of EmS are: low doses occurrence and effects, pseudopersistency or persistency, low or nondegradability, hydro or lipophilicity, bioconcentration /bioaccumulation/biomagnification in aquatic organisms, interreaction with proteins, toxicity with acute, but rather chronic effect. EmS could be endocrine/modulating molecules, with suspected teratogenic and carcinogenic consequence in low and sublow doses, volatile, non/semivolatile compounds, water soluble, polar or non polar molecules. Emerging pollutants can be neutral, acidic, basic, in ionic or zwitter ionic status.

Their main physical and chemical properties are characterized by following constants of: protonation (Log pKa) which lie in the range of 9.6 – 2.5, octanol–water partition coefficient (Log Kow) in the range 0.03 – 6.8 and solubility (Sw) from $1 \cdot 10^6$ mg/l to 0.02 mg/l, and constants of sludge–water distribution coefficient as Log Kd, reaction rate constant k_{biol} and molecular charge at pH 7.

The sorption removal of EmS are relatively low. Chemicals with $K_d > 500$ L/kg ($\text{Log } K_d > 2.7$) potentially tend to adsorb onto sludge and particles. According to the literature references, if $\text{Log } K_d$ values for some EmS are lower than 2.7 it could indicate relatively small tendency to adsorb (Verlicchi et al., 2012). The value of EmS/pharmaceuticals molecular charge at pH 7 provides information about its potential to create electrostatic interactions with the negatively charged soil/sediment surface. Sorption of EmS compounds is in general pH dependent.

Attempts to correlate biodegradation removal of EmS to its molecular structure demonstrate that a large set of emerging organic chemicals including type of esters, nitril and aromatic alcohols may increase biodegradability while aromatic amines, iodide, nitro- and azo- groups increase the persistency of the compound. The long and highly branched side chains (i.e. ranitidine) could provide EmS compounds more persistent as well as complicated aromatic ring structure (i.e. diazepam) and halogen group.

Mobility of EmS as the aqueous transport is the most important environmental process and environmental behaviour of emerging chemicals. In water chemicals are carried downwards, by percolating rain and irrigation waters, and upwards by capillary action in poorly drained and arid situations and laterally on slopes and in groundwater aquifers.

It is significant to have information on the following values of the constants for EmS: pKa, log Kow, Sw, log Kd, k_{biol} . According to the available literature data, following EmS with corresponding values of the constants are presented: Aspirine – 3.5, 1.13, 5295, nd, nd; Carbamezapine – 13.9, 2.45, 17.66, 0.1, <0.008; Diclofenac – 4.15, 4.51, 4.52, 1.2, <0.002; Ibuprofen – 4.51, 3.90, 41, 0.9, 1.33; Naproxen – 4.2, 3.18, 144.9, 1.1, 0.08; Ciprofloxacin – 6.38, 0.4, $1.2 \cdot 10^4$, 4.3, nd; Doxycycline – 3.5/ 7.7/9.5, -0.02, 312.9, nd, nd; Peniciline V – 2.79, 1.87, 101.1, nd, nd; Sulfamethoxazole – 5.7, 0.89, 3942, 2.7, 0.3 (Verlicchi et al., 2012).

Persistency and Pseudo-persistency

Persistency is one of the most important criteria in the environmental assessment of chemicals. Resistance to chemical transformation/degradation is typically used to evaluate the persistence of emerging chemicals. Persistent describes the retention of chemicals beyond the usual period and survival in the environment without the change in function or structure. Persistency in abiotic matrices (soil, water, sediment), is determined by the rates of the chemical removal by physical, biological and chemical processes. Chemical processes of removal include degradation processes like hydrolysis, direct/indirect photolysis (photolytic degradation), and oxidation/reduction reactions. Microbial degradation of chemicals is crucial and the most important process in biotic matrices and are depending on the nature of the EmS, persistence of EmS in the environment, temperature, moisture, pH of aquatic media and others. Emerging chemicals-contaminants/pollutants which are continually released to aquatic environment are attributed as pseudo – persistent even if their half-lives, $t_{1/2}$, are short.

Pseudo-persistency is the consequence of the constant supply of emerging contaminants/pollutants, which are continually replenished in environment, especially in aquatic media, receiving water or open aquatic bodies. The phenomena of pseudo persistency belong to the second order persistency and thermodynamic kinetic equilibrium processes could be presented by mathematical relation in which the rate of input of EmS (v_i) is considerably higher than the rate of EmS output (v_{ou}): $v_i \gg v_{ou}$. That means that the rate of input (release, emission, generation) of EmS into receiving water is much higher than the rate of output, namely, the kinetic rate of chemical and biological degradation processes, (transformation and mineralization processes) of EmS are slow at given environmental conditions, such as oxic and non-oxic, high or low temperature, acidic or alkaline properties of the aquatic media and others (Vojinović Miloradov et al., 2013).

When calculating the degradation rate of EmS and the kinetic parameters, first order process of degradation could be assumed because the EmS are present in a very low concentration versus the other reactants as well as water. The degradation processes follows pseudo-first-order kinetic behaviour $C_t = C_0 e^{-kt}$, and the value of the rate constant can be calculated from the plot of $\ln C_t/C_0$ vs. time. The straight line behaviour is confirmation of the first order kinetic, actually pseudo-first-order, because of the „high concentration of the water as the reactant“.

Long life of “overall persistence” is based on the chemical fate modeling of EmS. In general persistent and bioaccumulative pollutants have the following average half-lives in days: in water 182 days, sediment 365 days and soil 182 days.

The most EmS are by nature, biologically active and hydrophilic, and the human body can take them up easily. Their removal in WWTPs is variable and depends on the properties of the emerging substance and process conditions, e.g. sludge retention time, hydraulic retention time (HRT), temperature and other parameters and types of treatment. Although the concentration levels of EmS are very low, they are detectable in influent and effluent by sophisticated analytical equipment (HPLC tandem MS, and others).

Low doses effect and non monotonic dose response

Although present at low concentrations in the aquatic environment, emerging chemicals can have adverse effects on aquatic organisms. These effects are chronic and depend on exposure and bioavailability of biota, as well as the lipophilicity/hydrophilicity, water solubility, polarity, degradability and other physico-chemical properties of EmS.

At the beginning of 21st century, a “low dose” effect emerged as a new paradigm that hormonally active environmental emerging agents could cause a variety of adverse effects, mainly reproductive and developmental. One of the main physicochemical characteristics of EmS in surface and waste water is very low and sub low concentrations in the range of nano- and pico- molar level. Natural hormones act at extremely low serum concentrations. There are two major characteristics of endocrine disrupting/modulating chemicals (EDCs)/EMCs low dose exposures and non monotonic dose response curves (NMDRCs). EMCs can act in nanomolar to micromolar range, which is the same concentration level in which they were detected in surface and wastewaters, and potentially in drinking water. Low doses include a dose below the lowest dose at which a biological change for a specific chemical could be measured. Any dose below the lowest observed effect level or lowest observed adverse effect level (LOAEL) is considered a low dose.

NMDRCs present a challenge to traditional approaches in toxicology, which assume that the dose response curve is monotonic. There is now substantial evidence that the low doses of EDCs/EMCs have adverse effects on human health as well as on wildlife, including no mammalian species. The traditional thinking in toxicology studies are not adequate to assess adverse effects from these hormonally active EmS because they do not recognize the effects occurring at “low doses.” “Low dose” effects are occurring at levels comparable to those to which humans are being exposed. “Low dose” effects of EMCs could be defined as specific phenomena that occur in the range of human exposures or effects observed at doses below concentration level that are used for traditional toxicological studies. Non-monotonic dose response curves, defined as a nonlinear relationship between dose and effect is the slope of the curve changes sign somewhere within the range of doses examined. Figure 1 presents the response within monotonic and non monotonic events with emphases that low dose effects can not be predicted from high dose testing.

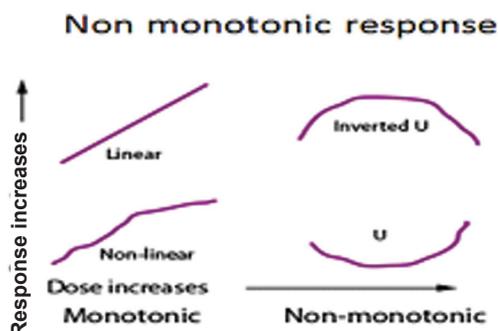


Figure 1. Monotonic and non monotonic

Molecules of EmS in aquatic solutions in very low molar concentrations exist in the state of mono molecules and are free in the sense of activity, rate and effects. Free mono molecules have open shape with protruding and openly exposed active centers (active functional group) which provide mono-molecules of EMCs to have high reactivity and high mobility. Such bioactive mono-molecules of EmS, as individual chemical species, can easily “attack” the different susceptible hormone molecules in living organisms and causing adverse effect. Low concentrations of EmS in water solution are effective concentrations of EMCs with the factor of activity concentration $f=1$. These adverse and hazardous effects of EmS are primarily a result of a low concentration levels (nano-, pico-, or even lower). The number of active molecules in picomolar and nanomolar concentrations is in the ranges from $6.023 \cdot 10^{11}$ to $6.023 \cdot 10^{14}$. These concentrations of EmS are equal and correspond to the biological concentration of hormone molecules.

Fate and persistence of EmS in the environment

Several processes can affect the fate and transport of organic compounds in the environment including: sorption, biotic transformation as well as non-biotic transformation/degradation. Awareness of the chemical properties and structures of compounds (QSAR) can allow preliminary estimation of their fate and persistence in the environment.

The persistence and transport of antibiotics in the environment have been determined for sulfonamides and fluoroquinolones which are the most persistent, followed by macrolides; tetracyclines can persist for relatively long periods if sunlight is not present, but tend to be less mobile; and aminoglycosides and β -lactam antibiotics show the least persistence (Milić et al., 2012). Tetracyclines and fluoroquinolones readily adsorb to soils, sediments, and/or sewage sludge, macrolides moderately, sulfonamides moderately to weakly, and aminoglycosides and β -lactams have relatively weak adsorption. The likelihood of detection depends on both the compound's initial effluent concentration and its environmental persistence. It is important to realize that it is not essential for EmS to be persistent in the environment in order to be detected.

Three antibiotics: sulfamethoxazole, trimethoprim, and ofloxacin, were present in both the influent and effluent samples. Removal efficiencies ranged between 20% and 77%. Sulfamethoxazole, (a sulfonamide) demonstrated poor removal (20%), where as, trimethoprim and ofloxacin (a fluoroquinolone) experienced greater removal (69% and 77%, respectively). Sulfamethoxazole appeared to be resistant to transformation and persisted in the river over a distance of approximately six kilometres with no change in concentration (Stamm et al., 2008).

Mass discharge load/emissions from Danube to the Black Sea

Information and the comprehension of mass discharge load of EmS and priority substances from the Danube to the Black Sea are very challenging and give useful information about the transport, accumulation and sedimentation to the Black Sea. Knowing the concentrations of persistent chemicals in the river water and the river flow (m^3/s) can lead to a simple calculation of the mass load of the EmS emitted to the Black Sea (kg/day or tons/year). This calculation and assessment of mass load of EmS deposited in the Black Sea are with relatively high level of uncertainty due to the limited and short period of monitoring data.

Mass discharge loads/emissions of persistent chemicals from the Danube River to the Black Sea is calculated with the flow rate of $6420 \text{ m}^3/\text{s}$ (Loos et al., 2010) and also with the flow rate of $3000 \text{ m}^3/\text{s}$ at the 1255 km of Danube in Novi Sad focused on alkyl phenolic compounds, caffeine and BPA and pharmaceuticals (Grujić Letić et al., 2012; Kovačević et al., 2013; April Progress report for NATO Project ESP.EAP.SFP 984087, 2013).

Table 1. Mass discharge loads of emerging persistent chemicals from the Danube river to the Black Sea with flow rate of 6420 m³/s* and for average river flow of 3000 m³/s at the Novi Sad

Chemical	Concentration downstream Arges before Danube delta [ng/L]	Mass load [t/year]	Mass load for flow rate of 3000 m ³ /s [t/year]
Benzotriazole*	167	33.8	15.8
Caffeine*	152	30.8	14.4
Tolyltriazole*	84	17.0	7.9
Carbamazepine*	25	5.1	2.4
Sulfamethoxazole*	23	4.7	2.2
PFOA*	12	2.4	1.1
PFOS*	6	1.2	0.6
4-AAA	520	105.3	49.2
4-FAA	213	43.1	20.2
Trimethoprim	223	45.1	21.1
Azithromycin	56	11.3	5.3
Metoprolol	35	7.1	3.3
Pentachlorobenzene	0.010	0.002	0.001
(4-(1,1',3,3'-tetramethylbutyl)-phenol	0.02	0.004	0.002
Nonylphenols	0.13	0.026	0.012
DiButylPhthalate	1.16	0.235	0.11
Di(2-ethylhexyl)phthalate (DEHP)	0.77	0.156	0.073
Caffeine	84	17.0	7.95
BPA	221.6	44.9	20.96

Summary and conclusion

Emerging substances, EmS, are pollutants with growing concern of their occurrence in environment, toxic effect at very low concentrations, distribution, transport and fate in aquatic environment (wastewater, surface water, water bodies in general or drinking water resources) with possible negative effects on humans and bioindicator aquatic ecosystem. The research progress has been made regarding the information, data monitoring and knowledge of the specific physico-chemical characteristic as the low doses effects, pseudo-persistence, the exposure of water bodies, aquatic bio system, and human to the EmS, up to date analytical determinations, process of prioritization, PEC, PNEC and other phenomena. This paper also presented general and specific physico-chemical properties of EmS with focus onto pseudo-persistence, low doses effects and mass load through river basin to the Black Sea.

Acknowledgement:

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FATE OF EMERGING POLLUTANTS IN ALLUVIAL GROUNDWATER OF BIG RIVERS IN SERBIA

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Abstract

Groundwater abstraction is main source for public water supply in Republic of Serbia. Abstraction of alluvial groundwater is over 50% at the national level; while in AP Vojvodina 70% of the amount of groundwater withdrawn for drinking water supply comes from the alluvial groundwater source (Dimkić et al., 2007.)

The processes which take place in an aquifer are numerous and diverse. In addition to convective transport of solutes, groups of processes include: dispersion processes, exchange processes between the solid and the liquid phase, and degradation processes (resulting in the “disappearance” of solutes from the liquid and solid phases of the aquifer (Dimkić et al., 2007.). Bank filtration efficiency in organic matter removal can be traced based on the all parameters e.g., total organic carbon and dissolved organic carbon.

The results demonstrates that bank filtration through water-bearing medium reduces total organic carbon concentrations (TOC), and micro pollutants concentrations mainly pharmaceuticals and their metabolites. Total organic carbon concentration in Sava River is 2,5 mg l⁻¹ on average, while in the Belgrade groundwater wells this concentration is approximately 1,5 mg l⁻¹ (Dimkić, Brauch & Kavanaugh, 2008).

The contamination of water resources with thousands of micro pollutants is one of the key environmental problems humanity is facing, even though compounds tend to be present at low concentrations, it is a growing concern and relatively poorly understood compared to other freshwater pollution sources (Schwarzenbach et al., 2006, Massmann et al., 2008) and the need for further research exists.

The ‘emerging organic contaminants’ (EOCs) are of particular interest. This term do not refer only to newly developed compounds but also to compounds newly discovered in the environment due to improvement of the analytical methodologies. These recently discovered contaminants are present in environment, as a result of their expansion but mainly due to the fact that they were not considered as contaminants before. Among emerging pollutants, a particular attention focuses on pharmaceuticals since they may exert their activity at the low ng l⁻¹ range.

Numerous and long-term studies have demonstrated that the River Bank Filtration is very effective way to reduce the pharmaceutical load in raw water (Jekel i Grünheid, 2006; Schmidt i Brauch, 2006).

Since several pharmaceutical residues are not completely removed during conventional wastewater treatment. (Zhang et al., 2012, Sipma et al., 2010), or as it is case in the Republic of Serbia, approximately 15 % of wastewater is treated (RSO Serbia 2012), significant number of pharmaceutical reach the surface water.

The aim of the study was to examine the occurrence and behavior of selected pharmaceuticals and their residues during passage through the riverbed and aquifer sediments and to correlate those results with the model for the transport of pharmaceuticals in alluvial groundwater by application of the Groundwater Vistas software package. As a result the kinetics of biological degradation of analyzed pharmaceuticals are determined based on the hydrogeological conditions and susceptibility for sorption which were calibrated in the model.

The results of the analyses

During the research period (2009 -2012) the total number of 19 pharmaceuticals has been included in analyses (Trimethoprim Sulfamethoxazole, Azithromycin, Doxycycline, Erythromycin, Bromazepam, Carbamazepine, Lorazepam, Diazepam, Diclofenac, metamizole metabolites - 4-formilaminoantipyrine (4-FAA) i N-acetyl-4-aminoantipyrine (4-AAA), and cardiovascular drugs: Enalapril, Atorvastatin, Amlodipin, Cilazapril, Metoprolol, Bisoprolol, Simvastatin. With the purposes for the further research and transport micro model setup, the analyses results of the presence in surface water and groundwater for the following pharmaceuticals are used: Trimethoprim, Carbamazepine, 4-formilaminoantipyrine (4-FAA) i N-acetyl-4-aminoantipyrine (4-AAA).

Table 1. Summary of results for detection of selected pharmaceuticals

Surface water samples				
Substance	Carbamazepine	Trimethoprim	4-FAA	4-AAA
Total analysis/Rivers	49	49	49	49
Positive detection	23	7	26	29
% Positive detection	47%	14%	53%	59%
Concentration range (ngl ⁻¹)	6-68	4-223	9-213	75-520
Groundwater samples				
Total analysis/Profiles	73	73	73	73
Positive detection	7	0	14	12
% Positive detection	9%	0%	19%	16%
Concentration range (ngl ⁻¹)	5-23	0	31-150	25-128

Within the research period (2009 -2012) 122 samples for surface water and groundwater have been analysed, 49 locations of the selected monitoring points are within the major rivers basins: Danube River – Novi Sad, Smederevo, Kovin and Veliko Gradište, Tisza River – Titel, Velika Morava River - Ljubičevski Most.

During the observation period from 2010 to 2012 the total number of 73 groundwater samples from the significant groundwater sources in the Republic of Serbia are included in analyses: Belgrade groundwater source , Drainage system Kovin – Dubovac, Drainage system Knićanin – Čenta, groundwater source Trnovče, groundwater source Ključ, groundwater source Medijana – Niš .

Localities for modelling samples

Collection sites for sampling and input data for the development of the hydrogeological and hydrodynamic micro model and transport modelling are within the area of Danube River Basin in Serbia, specifically location close to the municipality of Kovin, at the 1098 km of Danube. Sampling localities for groundwater include drainage system close to the Danube river bank at the well Bp-12 and two associated piezometers namely, Bp12/P1 and Bp-12/P2 within the drainage system Kovin-Dubovac.

The sediments of the uppermost porous aquifer abstracting bank filtrate are older Quaternary and upper part of the deposits at the end of the late Pleistocene and Holocene deposit in gravel-sand, and the lower alevrites clay sediments in the upper part of the alluvial plain. Thickness of gravel-sand deposits of younger Quaternary in this region of 1.5 - 20 m. Quaternary sediments lays uncomfortably over the Pontic upper layers. The shelves of the gravel-sand deposits are alevrites sands, and puddle clay and peat. Shelf layer thickness is on average about 7.5 m. Drainage well (Bp-12) with accompanying piezometers (Bp-12/P1 and Bp-12/P2) was screened in the groundwater at 3-4 -meters below terrain elevation, at a distance of approximately 350 m from the Danube shoreline (shoreline moves slightly over the year).

Sampling and analysis

In drainage wells measurements were made with probe immersion in water to the level of filter design and in piezometers the probe was dived to the filter structure after throwing at least three volumes of water from the piezometers using peristaltic pumps.

The selected pharmaceuticals were analyzed by solid phase extraction (SPE) followed by liquid chromatography–tandem mass spectrometry analysis (LC–MS²) according to a method described in the literature (Grujić et al., 2009). Analyses for the selected pharmaceuticals and sorption isotherms development are accomplished in cooperation with the Faculty of Technology and Metallurgy (TMF) in Belgrade in the Laboratory for the mass spectrometry at the department for the Analytical Chemistry

All relevant analyses regarding to the groundwater quality and quantity are completed in the Laboratory for the physicochemical and biological water resources analyses at the Institute for the development of water resources “Jaroslav Černi” in Belgrade.

Based on the results of analysis for the presence of selected pharmaceuticals in surface and ground waters, average pharmaceutical concentration of in the Danube River and accompanying drainage wells and piezometers were calculated.

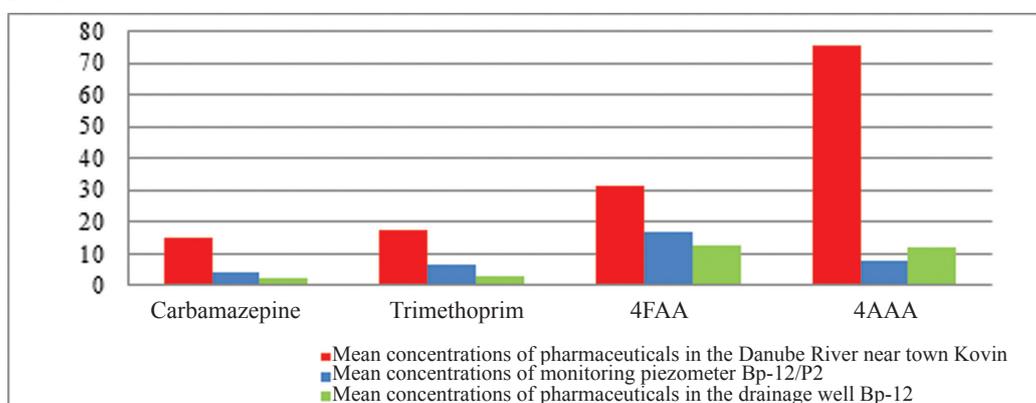


Figure 1. The results of determination of the average concentration for selected pharmaceuticals in the river Danube, the drainage well Bp-12 and observation piezometer Bp-12/P2

According to analyses results there is a strong evidence that significant decrease in pharmaceutical concentrations in surface water results from the flow through the river bank and bank filtrate all a way to the drainage wells and associated piezometers.

The goal of the mathematical modeling is to define the existing effects and processes that happen during the bank filtration, given the established natural hydrogeological conditions and observed and averaged EmS concentrations, and influence of before mentioned effect on pharmaceutical concentration changes in surface water in comparison with groundwater. Mathematical calculations and model development for the micro locations at the inception phase are realized in the Laboratory for the Numerical Analyses at the Institute for the development of water resources “Jaroslav Černi” in Belgrade. The essential input data for the hydrogeological and hydrodynamic parameters and bench marks establishment are based on the field works for the following projects: “Methodology for the assessment, design and maintenance of the alluvial groundwater sources with respect to the aerobic condition degree” TR37104 and “Wells ageing and infrastructure maintenance” supported by the Ministry for agriculture, forest and water management.

Processes involving the migration of a substance through a porous medium are generally described by the following equations:

Continuity equation for the flow of water in an aquifer

$$\nabla \cdot (T \nabla \Pi) = \varepsilon \frac{\partial \Pi}{\partial t} - q \quad (3)$$

Where: Π is the piezometric level of the water-bearing layer, [m]; T is the coefficient of transmissivity, [$\text{m}^2 \text{s}^{-1}$]; ε is the specific yield of the aquifer, [-]; q is the water flow to the water-bearing layer per unit area, [m s^{-1}]; ∇ is Hamilton's¹ operator; and t is time, [s].

¹Depending on the context, Hamilton or nabla operator may denote the gradient $\nabla f = \left[\frac{\partial f}{\partial x}, \frac{\partial f}{\partial y}, \frac{\partial f}{\partial z} \right]$ and the divergence $\nabla \cdot \vec{v} = \frac{\partial v_x}{\partial x} + \frac{\partial v_y}{\partial y} + \frac{\partial v_z}{\partial z}$

Continuity equation for the migration of a substance in an aquifer:

$$\nabla \cdot (D' \nabla C) - \nabla (C \vec{v}) - nW_1 C - W_2 (1-n) S = n \frac{\partial C}{\partial t} + (1-n) \frac{\partial S}{\partial t} \quad (4)$$

Where: C - the concentration of the substance in water, [kg m^{-3}]; \vec{v} is Darcy velocity; S is the concentration of the substance on soil particles, [kg kg^{-1}]; n is the rock porosity, [-]; D is the coefficient of hydraulic dispersion, [$\text{m}^2 \text{s}^{-1}$]; W_1 is the rate of degradation of the substance in the liquid phase, [s^{-1}]; W_2 is the rate of degradation of the substance in the solid phase, [s^{-1}]; and t is time, [s].

As a result of the transport model calculation the relevant current image and isolines of alluvial groundwater flow to the drainage well Bp-12 were obtained, as well as the approximate kinetics of degradation for carbamazepine, figure 2. Groundwater flow is 89% from River Danube, while the result of the calculation indicates that the path of groundwater from the Danube River to the drainage well-Bp 12 is within the framework of 30 to 40 months.

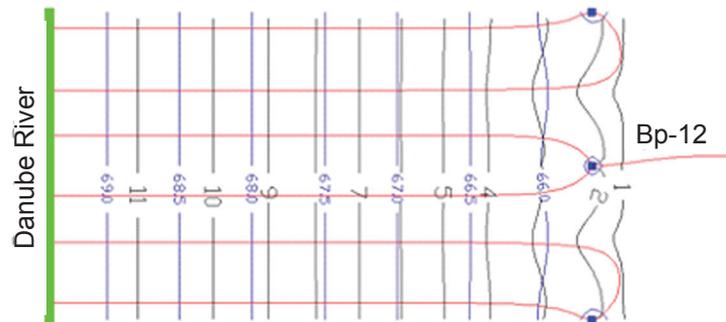


Figure 2. The groundwater flow from Danube River to drainage well Bp-12, with relevant current image isolines and average concentration of carbamazepine

The results for linear sorption coefficient value in transport model calculation are significantly smaller than the sorption coefficient based on experiments. This difference is result of the composition of the aquifer and different particles size in water-bearing layer along the model boundaries that affects the sorption kinetics.

Degree of carbamazepine biodegradation is very low since breakthrough curve has shown that even after 40 years we could expect carbamazepine breakthrough concentration of 2 ng l^{-1} , and same concentration is also measured in the monitored well. Thus, it can be indicated that after 40 years carbamazepine was poorly degraded and it could be found in drainage well Bp-12 after few decades.

Bp-12

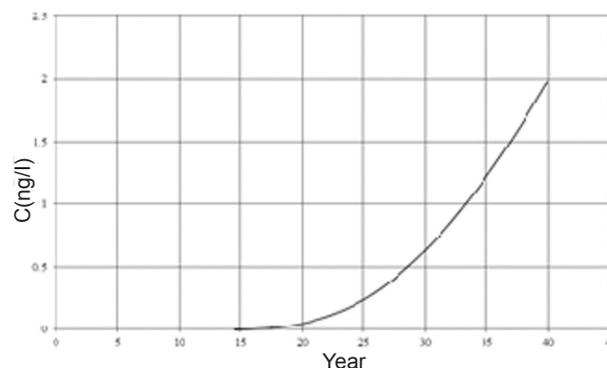


Figure 3. Carbamazepine breakthrough curve

Acknowledgements:

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CLAY AND FERROUS IRON STRATIFICATIONS IN A TROPICAL SAVANNAH RICE IRRIGATED VALLEY BOTTOM SOIL

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Abstract

High ferrous ion Fe^{2+} concentration, inserted into dense clay strata, constitutes an important threat to the rice production in several tropical Savannah valley irrigation schemes, and a lot of efforts are currently mobilized to oppose iron toxicity. In this study, we have investigated the presence of clay and ferrous iron stratifications within a typical valley bottom soil profile called Tiefora in Burkina Faso. Taking into account the multiple slopes of the valley, two randomized soil samplings were implemented at various depths. Samples were collected as deep as 500 cm, but especially at 30 cm, 50 cm and 100 cm depths. The clay percentage was determined by grain size analysis. Ferrous iron concentrations were determined by the reflectometric method. The stratifications of clay and ferrous ion Fe^{2+} were checked using statistical hypothesis testing (ANOVA and Kruskal-Wallis). Clay percentage, 28.92%, was found twice higher within the first 100 cm top soil than in the layers underneath. Furthermore, ferrous iron was mainly located in the top 30 cm of this 100 cm top clay, with a mean concentration of 994.1 mg/l. This is much higher than found at depth 50 cm (47.5 mg/l). This striking stratification suggests several means of alleviating iron toxicity. Among these means, we propose the simple removal of the contaminated 30 cm top soil followed by maintaining permanent wet conditions in the irrigated lands.

Keywords: ANOVA, iron toxicity, Kruskal-Wallis, Minitab, Savannah, soil sampling

Introduction

In order to provide means for alleviating iron toxicity, various researchers brought contributions to the characterization of valley bottom redoximorphic soils (Ogban and Babalola 2009, Barron and Torrent 1986, Kessler and Oosterbaan 1974). However, previous research does not provide detailed information about the soil stratification and ferrous iron distribution within the soil (Jackson and Sherman 1953). This potential stratification can help building a strategy against iron toxicity. In the current research, we have hypothesized that both clay content and ferrous iron Fe^{2+} concentrations are stratified in the soil, at least under certain conditions. To check these assumptions, a randomized block sampling followed by a statistical analysis was implemented in the case of the typical tropical Savannah valley bottom of Tiefora.

Materials and methods

Site location

The site is geographically located at 4°33'13.19" longitude West and 10°37'33.56" latitude North, some 800 m downstream an earthen dam.

Soil sampling and statistical analyses

The investigations focused on finding the relationships between one explanatory variable – the depth in the soil – and two response variables: the percentage of clay in the soil and the ferrous iron Fe^{2+} concentrations.

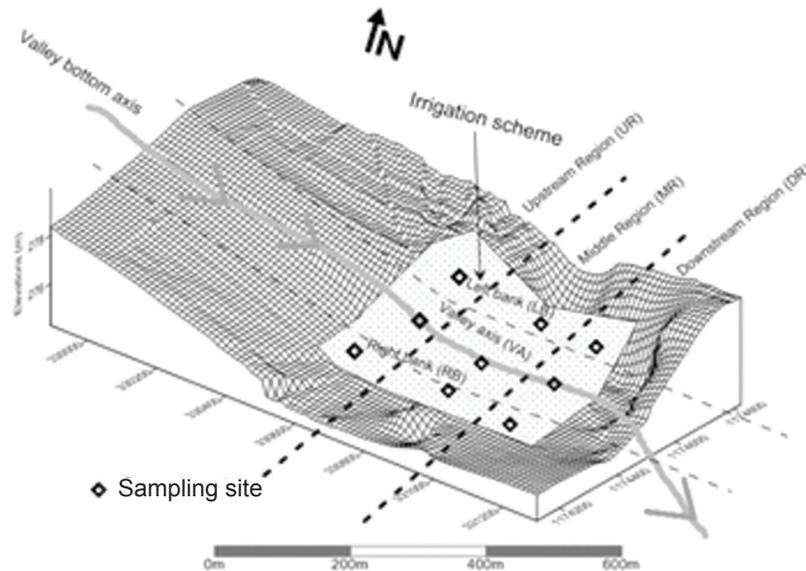


Figure 1: Valley bottom randomized block sampling sites

One can see the different sloping conditions from upstream to downstream and from the left to the right bank. This led to dividing the valley bottom into nine sampling sites. For each site, several soil extracts were taken at various depths.

The numbers on XY axes are the UTM coordinates

The V-shape of the Tiefora valley bottom suggested making a randomized block sampling (Spiegel et al., 2001, Boslaugh and Watters 2008). Two series of soil extraction operations were conducted. The ferrous iron concentrations were measured using a reflectometer with Fe^{2+} reflectoquant strips (Persson 1997). For both groups of soil extracts, the clay proportions were obtained by grain size and sedimentometry analyses according to the ASTM norm (American Society for Testing and Materials).

Statistical analyses

The three conditions of applicability of ANOVA – the normality of the underlying populations, the equity of variances and the equity of the number of samples – were checked before implementation (Boslaugh and Watters 2008). These conditions were met with the 44 extracts of soils. However, the important difference in variances did not permit the application of ANOVA to the 25 other soil extracts. Consequently, a non-parametric method – Kruskal -Wallis test (Breslow 1970) – was applied.

Results and discussion

Clay stratification occurrence

One-way ANOVA test can be applied with the 44 soil extracts despite the differences in the number of observations (Table 1) according to the depths. Our null hypothesis is that there is no stratification in the soil. The results show that clay content in the soil reduces steadily but definitely with the depth (Figure 2). Because of the small p-value (1.5%), we reject the null hypothesis that all depths are equivalent in terms of clay content and that there is no stratification. The soil is stratified.

Table 1: Clay percentage of the soil extracts taken in the valley bottom

Depths (cm)	Number of observations	Mean %Clay	StDv	Variiances	Min	Max
00-100	25	28.92	12.27	150.65	3.60	56.40
100-200	9	19.60	11.37	129.17	1.10	36.60
200-300	5	18.08	10.40	108.26	3.10	31.00
300-400	4	15.85	11.57	133.84	4.10	26.90
400-500	6	13.85	06.96	48.46	6.00	23.10

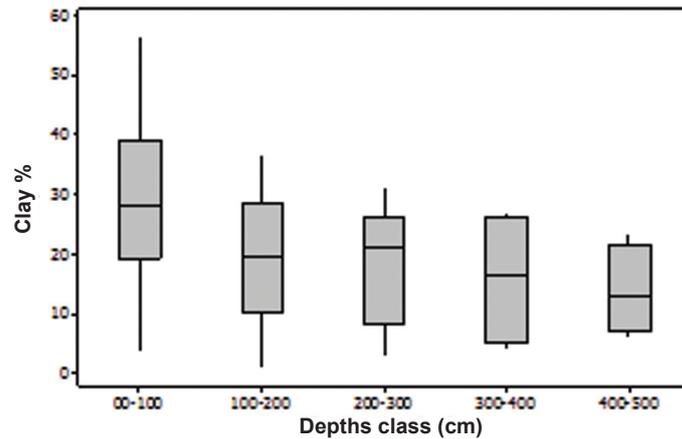


Figure 2: Clay percentage dispersion and decrease with the soil depth

Ferrous iron concentration is stratified in the rootzone

One of the most certain way to test the hypothesis of ferrous iron stratification in the 100 cm top soil was to apply the non-parametric Kruskal-Wallis Test (Breslow 1970), which is based on the comparison of the medians of the 3 underlying populations of the 25 soils extracts. On could not apply the one-way ANOVA because of the great difference within the variiances (Figure 3). In fact, both the Bartlett’s and the Levene’s tests of equality of variiances (Carroll and Schneider 1985) performed at 5% of significance level led to the rejection of the equalities of the variiances (p-value = 0%). Therefore Kruskal-Wallis Test was performed at 5% of significance level. The null hypothesis is then that the 3 soil depths were equal in terms of ferrous iron concentrations (no stratification). The test led to the rejection of this null hypothesis in favor of the stratification with a p-value = 0.00.

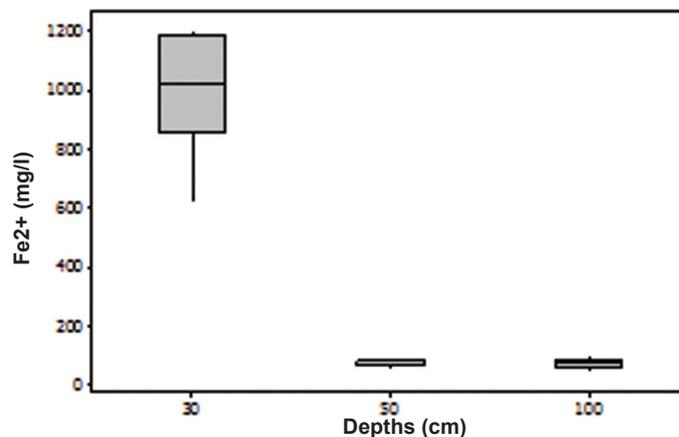


Figure 3: Ferrous iron concentration dispersion and decrease with the soil depth.

It is also noteworthy to observe that within this non-stratified clayey 30 cm-top soil, even though the variation is important (variance = 46363.3 mg²/l²), the ferrous iron concentration is very high everywhere in the valley (average = 994.1 mg/l). There is no single location where the phenomenon is not present. This is also in favor of the explanation that water evaporated leaving behind the ferrous iron.

Conclusions

The stratification of clay and ferrous iron concentration can help to develop a strategy to alleviate iron toxicity in tropical savannah rice irrigated soils. In our case study, the results show that clay content is significantly much higher in the 100 cm top soil, with a mean of 28.92% (stdev of 12.27%). It drops to 13.85% at 400 cm of depth. We have found that ferrous iron was mainly concentrated within the top 30 cm of the clayey topsoil, reaching 994.1 mg/l (stdv of 215.1). The iron concentration drops much more quickly than the clay since already at 50 cm depth, the value is only 47.5 mg/l (stdev of 10.93). Moreover, this high iron concentration is observed within the top 30 cm of soil all over the valley bottom, supporting the idea that it is was left behind after evaporation. These extreme stratification and concentration in the top soil provide several ways to fight against iron toxicity. The most immediate way is to remove the top 30 cm of soil and replace it. A second solution, which can be associated with the previous one, is to maintain the soil saturated all over the year. Finally, when the clay permeability is not too high, subsurface drainage can be a mean for removing the excess of ferrous iron.

Acknowledgements

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ENVIRONMENTAL OCCURRENCE AND POTENTIAL ADVERSE EFFECTS OF THE RESIDUES OF CYTOSTATIC DRUGS TO AQUATIC ORGANISMS

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Introduction

Pharmaceuticals have brought enormous benefits to humanity in terms of healthier and longer lives. It is now not possible to imagine life without pharmaceuticals and huge quantities of pharmacologically active substances are consumed annually. Thus it is not surprising that active pharmaceutical ingredients are emerging as a new group of environmental contaminants the potential adverse environmental effects of which are yet to be fully investigated.

Pharmaceuticals are excreted through faeces and urine as a mixture of metabolites and the unchanged parent compound. Predominantly, they enter the aquatic environment via hospital and wastewater treatment plant (WWTPs) effluents, landfill leakages and to a minor extent in the discharge from the pharmaceutical industry. Due to their ubiquitous presence in the environment arising from continual input into the aquatic compartment, they are considered as “pseudo” persistent pollutants (Hernando et al., 2006). To date, the majority of environmental studies regarding occurrence and impact on environmental health of the pharmaceutical residues focused on those drugs that are used in relatively high quantities such as those used for regulation of blood pressure, lipid lowering drugs, and nonsteroidal anti-inflammatory drugs. The main reason is that predicted environmental concentrations (PEC) of these pharmaceuticals exceed 10 ngL^{-1} in surface waters, which is the limit at which the European Medical Agency (EMA) requires environmental risk assessments (ERA) before the pharmaceuticals are launched on to the market (EMEA, 2006).

Cytostatic (also known as antineoplastic) drugs are a group of highly active chemotherapy agents that either prevent or disrupt tumor cell division via interference with the genetic material and cell signalling. However, they may be not selective enough to target only tumor cells, but would act on different types of dividing cells and tissues of exposed organisms. Due to their genotoxicity, exposure to cytostatic even at very low doses can induce mutations with delayed effects resulting in cancers and other chronic diseases, reproductive effects, heritable disease, and as shown by more recent studies, also neurodegenerative effects. Because cytostatics interfere with the function of DNA and genetic material, which is common in all organisms, they have the potential to harm a broad variety of species including microorganisms, plants and animals. At environmental concentrations and through continuous exposure such adverse effects that impact reproduction and population fitness, may in principle, also be expected to occur in aquatic organisms. In the REACH legislation the chemicals with carcinogenic, mutagenic and/or reproductive toxicity, the so called CMRs, are considered as priority pollutants regardless of production quantities.

Despite the fact that cytostatics belong to highly hazardous chemicals their potential adverse effects when released to environment have so far not been addressed. One of the reasons is that their occurrence in the environment is, compared to other types of pharmaceuticals, relatively low. Most studies focus on wastewaters from hospitals and municipal wastewater treatment plants where cytostatic pharmaceuticals were in most cases detected in the ng/L range while in natural waters they were generally undetectable (Heath and Kosjek, 2012). However, the reason for the latter is the current lack of analytical methods with adequate sensitivity, and that no regulatory monitoring is required to follow the presence of the residues of these drugs in the environment. Consequently also the ecotoxicological data for these compounds are very scarce.

In addition, when data are available they are mostly based on the results of the acute ecotoxicity testing, which are not relevant for the prediction of the long-term and delayed effects upon chronic exposure to low doses.

Occurrence of cytostatic drugs in aquatic environment

To estimate the occurrence of the residues of cytostatic drugs in aquatic environment we analysed consumption amounts of cytostatic drugs in Slovenia and calculated their predicted environmental concentrations (PEC) (Figure 1). The data showed the highest consumption of antimetabolites, followed by protein kinase inhibitors, alkylating agents, monoclonal antibodies, platinum based alkylating agents, topoisomerase and mitotic inhibitors, and cytotoxic antibiotics. The investigations of consumption trends between the years 2009 and 2011 showed clear increase in the overall consumption of these drugs with the highest increase in the consumption of protein kinase inhibitors and monoclonal antibodies. Similar pattern of consumption of antineoplastic drugs has been recently reported by Besse et al (2012) for France in 2004 and 2008.

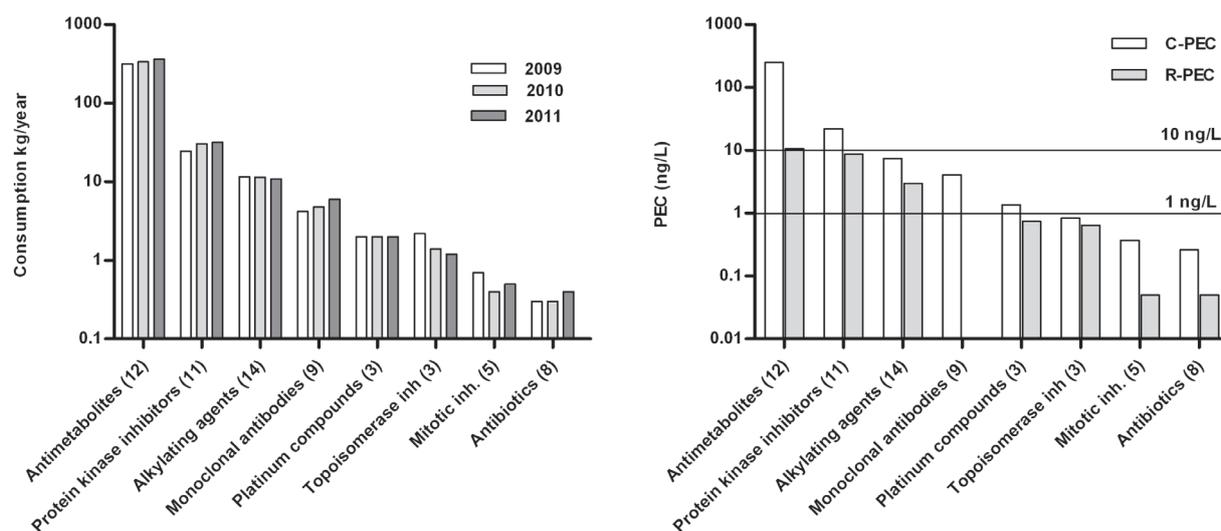


Figure 1: Consumption of cytostatics in Slovenia in the years 2009, 2010 and 2011 (left panel) and predicted environmental concentrations (conservative - C-PEC and refined - R-PEC) calculated based on the consumption in 2011 (right panel).

The antimetabolite capecitabine (a prodrug of 5-fluorouracil) exceeded conservative PEC (C-PEC) value of 10 ng/L, which is the value at which for pharmaceuticals ecotoxicological data for the environmental risk assessment should be obtained (EMEA, 2006). However, due to the mechanism of action of cytostatic drugs, which may theoretically upon long term exposure exert adverse effects to non target organisms at very low concentrations we considered C-PEC ≥ 1 ng/L as the value relevant for identifying the cytostatic drugs of priority for the ecotoxicity studies. Three alkylating agents (dacarbazine, temozolomide and ifosfamide), seven protein kinase inhibitors (imatinib, sorafenib, lapatinib, nilotinib, erlotinib, gefitinib, pazopanib), three antimetabolites (5-fluorouracil, gemcitabin, methotrexate), and three monoclonal antibodies (bevacizumab, rituximab, trastuzumab) exceeded PEC value of 1 ng/L. The refined PEC (R-PEC) values, which take into account also the metabolism of these compounds, indicate that none of the single cytostatic exceeded 10 ng/L. R-PEC values ≥ 1 ng/L were calculated for the antimetabolites capecitabin and methotrexat, alkylating agent dacarbazin and protein kinase inhibitors lapatinib, sorafenib, nilotinib, imatinib.

Based on the obtained PEC values and taking into account different mechanism of action four cytostatics were selected to be studied for their potential adverse effect to aquatic organisms: an antimetabolite 5-fluorouracil (5-FU), a protein kinase inhibitor imatinib mesylate (IM), a topoisomerase inhibitor etoposide (ET) and a platinum based alkylating agent cisplatin (cDDP).

Eco(genotoxicity of selected cytostatic drugs

The ecotoxic and genotoxic properties of the four drugs were determined in experimental models covering aquatic organisms from different trophic levels: algae, cyanobacteria and higher plants as producers, crustacean and mussel as primary consumers, and vertebrate (*Danio rerio* – zebrafish) as secondary consumers. As expected the acute toxicity of the four compounds in crustacean (*D. magna* and *C. dubia*) and zebrafish was relatively low; toxic effects were observed at concentrations in the range of mg/L – g/L that are several orders higher from those expected in the environment. Higher sensitivity was observed in the reproduction assay with algae (*P. subcapitata*) and cyanobacteria (*S. leopoliensis*) in which toxic effects of 5-FU, cDDP and IM were observed at µg/L concentrations. In the reproduction assay with crustacea (*D. magna* and *C. dubia*) toxic effects of the four cytostatics were observed at several order lower concentrations than in the acute toxicity assay. cDDP, after chronic exposure, caused toxic effects at concentrations below µg/L.

The genotoxicity of the selected cytostatics to the aquatic organisms was studied with the comet assay that detects DNA strand breaks and with the micronucleus assays that detects chromosomal aberrations. In *D. magna* 5-FU, cDDP, ET and IM induced clear dose dependent increase in DNA damage at the µg/L concentration range. The most potent was cDDP, followed by IM, ET and 5-FU. Increase in DNA damage was observed also in mussel *U. tumidus*, though at higher concentrations. The most potent was 5-FU followed by ET, while cDDP did not induce DNA damage. In higher plants (*Allium* and *Tradescantia* assays) all four cytostatics induced micronuclei formation, however at relatively high concentrations. The exception was cDDP, which in *Allium* test induced micronuclei formation at 15 µg/L. In the two generation study with zebrafish that were exposed to 0.01, 1 and 100 µg/L 5-FU an increase in DNA damage was observed in the liver, gonads and blood cells, but not in gills. In blood erythrocytes a significant increase in micronuclei formation was detected at all doses of 5-FU.

Conclusions:

These studies clearly showed that data on the acute toxicity of cytostatics to aquatic organisms are irrelevant for prediction of adverse effects during the chronic exposure to low doses, which is typical in the aquatic environment. The chronic exposure assays associated with the detection of genotoxic effects showed differences in the susceptibility of different aquatic organisms to the toxic and genotoxic effects of the tested cytostatic drugs as well as differences in toxic and genotoxic potential of different cytostatics. These results indicate that data from long term in vivo tests focusing on specific effects (i.e. genotoxic, reprotoxic...) are needed to determine the significance of the presence of these drugs in the environment. The results also showed that of the four cytostatics included in this study 5-FU and cDDP exerted toxic and genotoxic effects at concentrations that are close to predicted environmental concentrations and those found in hospital wastewaters. This means that certain cytostatic may pose threat to aquatic organisms indicating the need for further investigations of their ecotoxicological properties as well as investigations of the real occurrence and fate of cytostatic drugs in the environment.

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OCCURENCE AND ECOTOXOLOGICAL EFFECTS OF RESIDUAL PHARMACEUTICALS ON AQUATIC ENVIRONMENT

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Introduction

According to the monitoring studies, the pharmaceutical compounds (PHCs) are found in the most water sources, especially in wastewaters and surface waters. Although, they are found in very low concentrations (from ng/L to µg/L) these substances may represent a real danger to aquatic ecosystems. In this work we tried to find the answer to the question: „Have the pharmaceuticals toxic effects on aquatic organisms?”

Over 80 active PHCs have been detected in rivers and lakes in Austria, Brazil, Canada, Norway, Ukraine, USA. In Romania, the study of the PHCs contamination of the aquatic environment started recently. There are some investigations in surface waters (Somes River). Fifteen compounds including nervous stimulants, anti-rheumatic, anti-epileptic, analgesic, disinfectant and anticoagulant drugs were detected in concentrations ranging from the 30 ng /L to 10 µg / L, using GC/MS technique (Moldovan, 2006).

Since the increasing of drugs environmental discharges represent a serious threat for human and animal health, the worldwide organizations such European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA), have developed and implemented various environmental risk assessment guidelines (Fent et al., 2006). In 2006, EMA have published the Guideline of Environmental Risk Assessment of medicinal products for human use that impose the assessment of acute and chronic effect of new pharmaceutical chemicals (EMA, 2006; Straub, 2001).

Due of the lack of European and international environmental legislation concerning the admissible limits of these compounds in wastewaters and surface waters, the municipal WWTPs and medicinal producers are not constrained to discharge wastewater effluents without PHCs contamination.

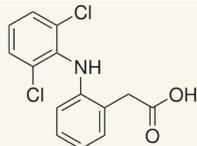
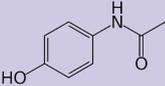
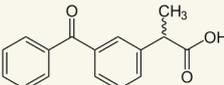
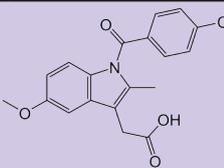
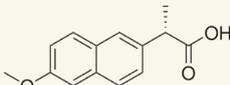
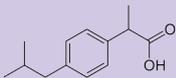
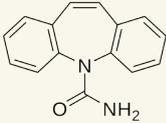
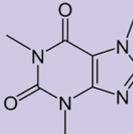
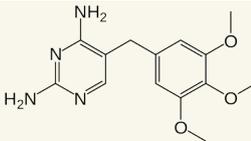
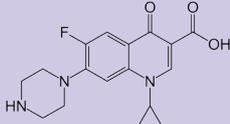
The aim of the present work was to assess the effects of 10 PHCs (analgesics, antiepileptic drug, nervous stimulant and antibiotics) on aquatic organisms and preliminary risk assessment in Romanian surface waters according to actual international norms and base on our toxicity and monitoring data and from literature.

Materials and methods

Chemicals

Six anti-inflammatory drugs (ibuprofen, diclofenac, acetaminophen, naproxen, indomethacin, and ketoprofen), a nervous stimulant (caffeine), an antiepileptic drug (carbamazepine) and two antibiotics (trimethoprim and ciprofloxacin), were studied, in terms of acute toxicity on aquatic organisms throughout the food chain (fish, crustaceans, algae and bacteria). All the PHCs analytical standards were purchased from Sigma-Aldrich (Seelze, Germany). The PHCs selected for this study are characterized in Table 1.

Table 1. CAS number, chemical structure, water solubility, octanol / water partition coefficient (logKOW), molecular mass (MM) and medical application of selected PHCs

Compound	CAS	Chemical structure	Application
Diclofenac	15307-79-6		Analgesic Anti-inflammatory
Acetaminophen	103-90-2		Analgesic Antipyretic
Ketoprofen	22071-15-4		Analgesic Antipyretic
Indomethacin	53-86-1		Analgesic Anti-inflammatory
Naproxen	22204-53-1		Anti-inflammatory
Ibuprofen	15687-27-1		Anti-inflammatory
Carbamazepine	298-46-4		Antiepileptic
Caffeine	58-08-2		Nervous stimulant
Trimethoprim	738-70-5		Bacteriostatic antibiotic
Ciprofloxacin	85721-33-1		Bacteriostatic antibiotic

Sampling

In order to assess the aquatic risks of the studied PHCs in the field, some representative samples were chosen:

- wastewater samples (influent and effluent) collected monthly (from January until September 2011) from 3 municipal WWTPs;
- surface water samples collected from Ghimbasel Stream (Brasov area), Mures River (Targu Mures town area) and Arges River (Pitesti area) upstream and downstream of WWTPs.
- surface water samples collected from Danube River (Romanian sector) and Danube Delta (Sf. Gheorghe Branch), in March 2011 and January 2013, in different sites (Bazias, Giurgiu, Tulcea, Mahmudia, Uzlina, Murighiol, Sf. Gheorghe and Black Sea Confluence).

Detection methods

LC-UV analysis. The chromatographic conditions were modified and adapted from an analytical method previously developed and described elsewhere (Santos, 2005). Liquid-chromatographic separation of selected PHCs was investigated by using an Agilent 1100 (Agilent Technologies, USA) system equipped with a degasser, quaternary pump, autosampler, column thermostat and multiple wavelength detectors (MWD).

HPLC-MS/MS analysis. An analytical procedure was developed based on EPA Method 1694 with some modification (EPA 1694). LC-MS/MS measurements were carried out with an HPLC system Agilent 1290 Infinity coupled to an Agilent 6410 triple quadrupole MS equipped with an electrospray ionization (ESI) source (Agilent Technologies, Waldbronn, Germany) in multiple reaction monitoring mode (MRM).

Ecotoxicology methods

Toxicity assessment was carried out according to OECD and ISO methodologies using conventional and alternative methods (microbiotests). The effects of tested PHCs were assessed on aquatic organisms as following: fish (*Cyprinus carpio*), planktonic crustacean (*Daphnia magna*), green microalgae (*Selenastrum capricornutum*), marine luminescent bacteria (*Vibrio fischeri*), and other bacteria (gram negative and positive bacteria). In Table 2 are presented the biological tests battery, the monitored endpoints and the ranges of tested concentrations.

Table 2. Battery of aquatic toxicity tests used in PHCs effect assessment

Bioassay / microbiotest	Species	Type of test	Endpoints	Test period	Range of tested concentrations
OECD 203	<i>Cyprinus carpio</i>	acute	Mortality and behavior	96h, 21-22°C	1-500 mg/L
OECD 202 Daphtoxkit F magna ^a	<i>Daphnia magna</i>	acute	Mortality and behavior	24-48h, 20°C	0.01-100 mg/L
OECD 201 Algaltoxkit F ^a	<i>Selenastrum capricornutum</i>	acute/ chronic	Growth inhibition	72h, 21-25°C	0.01-100 mg/L
DIN EN ISO 11348-3 BioFix Lumi, Multi-Shot kit ^b	<i>Vibrio fischeri</i>	acute	Luminescence inhibition	15min, 20°C	0.01-100 mg/L
MARA test (Microbial Array for toxicity Risk Assessment) ^c	<i>Microbacterium sp.</i> , <i>Brevundimonas diminuta</i> , <i>Citrobacter freundii</i> , <i>Comamonas testosteroni</i> , <i>Enterococcus casseliflavus</i> , <i>Delftia acidovorans</i> , <i>Kurthia gibsonii</i> , <i>Staphylococcus warnerii</i> , <i>Pseudomonas aurantiaca</i> , <i>Serratia rubidaea</i> , <i>Pichia anomalia</i>	acute	Microbial growth inhibition	18 h, 30°C	0.4- 200 mg/L

^a MicroBioTests Inc., Belgium; ^b Macherey–Nagel GmbH & Co. KG, Germany; ^c NCIMB Ltd., Scotland.

Aquatic risk assessment methodology

The aquatic risk assessment of the studied PHCs was conducted according to Environmental Risk Assessment EMEA Guideline (2006). For hazard characterization of the studied drugs, predicted effect concentrations (PEC) and predicted no effect concentrations (PNEC) were estimated. The PNECs were calculated using the lowest acute toxicity values (LC₅₀/EC₅₀) and the PECs were estimated based on monitoring data (pharmaceuticals concentrations obtained in different Romanian surface waters and also in effluents of three WWTPs). The PEC/PNEC ratio is widely accepted as endpoint in aquatic risk assessment models.

Results and discussions

Environmental PHCs concentrations

All PHCs were found in the influents and effluents from the three WWTPs studied with mean concentrations varying from 0.13 µg/L (naproxen) to 21.48 µg/L (ibuprofen). The concentrations average of PHCs in surface water were between 0.22 µg/L and 11.05 µg/L, and the most abundant compounds were ibuprofen and caffeine. Generally, in Mures River, Arges River, Danube River and Danube Delta (Sf. Gheorghe Branch), PHCs concentrations were detected less than 1 µg/L, excepting ibuprofen (2.16 µg/L) and caffeine (1.78 µg/L).

Aquatic toxicity of PHCs

Results indicated moderate and low acute toxicity values, in the most cases. $LC_{50(96h)}$ ranged from 43.65 to >100 mg/L (for fish *Cyprinus carpio*), $EC_{50(48h)}$ ranged from 12.02 to >100 mg/L (for planktonic crustacean *Daphnia magna*) and $IC_{50(15 min)}$ / MTC ranged from 6.02 to 77.62 mg/L (bacteria, *Vibrio fischeri* and other). Antibiotics showed a acute toxicity to bacteria < 5 mg/L. As it is known that antibiotic are harmless for microbial flora (Hamre, 2006; Halling-Sørensen et al., 2000), the studied antibiotics (ciprofloxacin and trimethoprim) revealed a high toxicity on bacteria using MARA test, the most sensitive bacteria being the *Microbacterium sp.*

Aquatic risk assessment characterization

For each PHCs risk coefficients were calculated taking into consideration four risk scenarios. All the PHCs were found in the investigated surface waters in concentrations over 0.01 µg/L and the risk started with the Phase II Tier A – toxicity assessment using aquatic organisms. In order to estimate the PNEC values, the lowest EC50 values were selected and an extrapolation factor of 1000 was applied.

The estimated generic consumption of PHCs in Romania highlighted an insignificant or low environmental risk for all PHCs (most of PEC/PNEC ratios were <0.1 and in the range 0.01 – 1).

PEC / PNEC ratios revealed that the presence of these compounds in the aquatic environment leads to lower aquatic risk, but it should take into account the bioaccumulative proprieties with synergic and antagonist effects, developing resistant bacterial strains.

We consider that there are still many gaps in the research on the effects of pharmaceuticals on environmental health, due to the abundance of compounds, the low number of advanced detection methods and high analysis costs and lack of an integrated ecotoxicity assessment methodology.

Acknowledgement

The authors would especially like to thank to National Research Program (CORE) - PN no 13N/2009 for the financing support of the research experiments.

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NEW 3D MODEL FOR ANALYSIS OF POLLUTANT TRANSPORT IN GROUNDWATER

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Abstract

Bank-filtration type groundwater sources are often encountered in practice. One of their advantages is that they are located near consumers: large cities are often located on rivers, which implies lower costs of water transportation. Maybe even more important advantage is that such sources provide satisfactory and stable water quality. Of course, this depends not only on the river water quality (which is the main source of bank-filtration source groundwater), but also on the hydrological composition and groundwater filtration conditions. In other words, the quality of groundwater (well water quality, assuming that wells are used to extract water) is a result of the aquifer filtration potential.

We single out only a few processes which are schematically represented in a mathematical model of groundwater mass transport. These are: dispersion, sorption and degradation.

Owing to dispersion, dissolved matter moves faster than the main flow, causing the concentration to move in front of the filtration flow. On the other hand, sorption significantly slows down the mass transport, allowing for intervention in a case of an accident. In stationary conditions and in the absence of degradation, concentration with sorption is the same as without sorption. Any type of degradation reduces the concentration and, combined with sorption, is an important factor of aquifer filtration processes.

This implies that hydrogeological conditions of aquifer filtration potential have a decisive role in filtrating dissolved substances and ensuring basic water quality.

Belgrade water source is an example. This bank-filtration groundwater source is probably one of the largest in the world by the number of radial wells (99), whose manholes are located immediately by the river, with most of drains under the river. Most of this water source is within the urban area. Sava river, which has an international character more than twenty years already, carries a variety of substances that can be considered pollutants. In spite of this, and in spite of the strain that the city imposes on the source, the well water quality is, surprisingly, not jeopardized.

Belgrade water source aquifer is composed of alluvial river sediments formed by multiple sedimentation cycles, following the simplified bottom-up scheme: gravel – sand - clay. The whole aquifer pack is dominated by sand sediments, coarser and with more gravel fraction at the bottom. One of the source characteristics that was not noticed or researched before is the existence of one or more weakly permeable inter-layers in the largest part of the source. These inter-layers are composed mainly of dust and clay.

These inter-layers have considerably higher sorption capacity in comparison to sand sediments. Even though they are relatively thin (usually thinner than a meter), they retard the transport by one or two orders of magnitude and, combined with degradation, they present a strong barrier to pollution penetration into the well.

Another specific characteristic of Belgrade Groundwater Source allowing these processes is the vertical water movement. Radial well horizontal drains are mostly orthogonal to the river bank at the depth 22-28 m. They are 50-60 m long and mostly under the river bed. This allows for one or more weakly permeable inter-layers between the drains and the river bed (see Fig. 1).

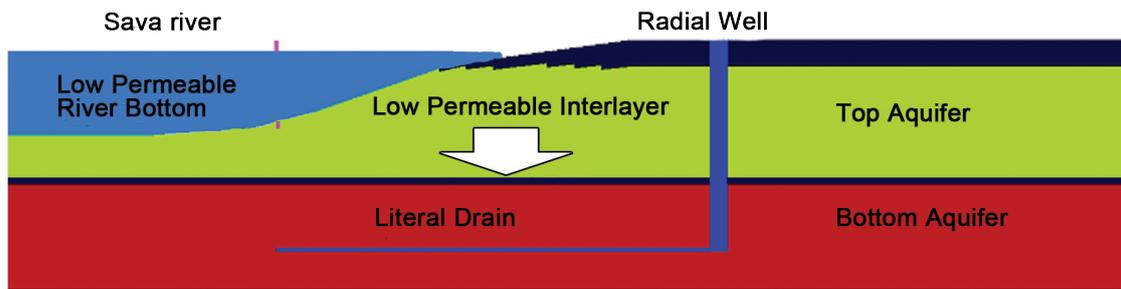


Figure 1: Vertical profile of a single drain model, with aquifers and a weakly permeable interlayer.

Although the groundwater flow is usually imagined to be horizontal, the flow from the river towards the drains is predominantly three-dimensional (See Fig. 2 and 3).

Owing to the position of the drains under the river bed, river water filtration is predominantly vertical, forming a three-dimensional flow field in the drain zone. Due to the river water level oscillations and a variable well operation regime, the part of the river bed close to the bank is sometimes saturated and sometimes not (see Fig. 3).



Figure 2: Streamlines from the river to the radial well in a profile normal to the drain

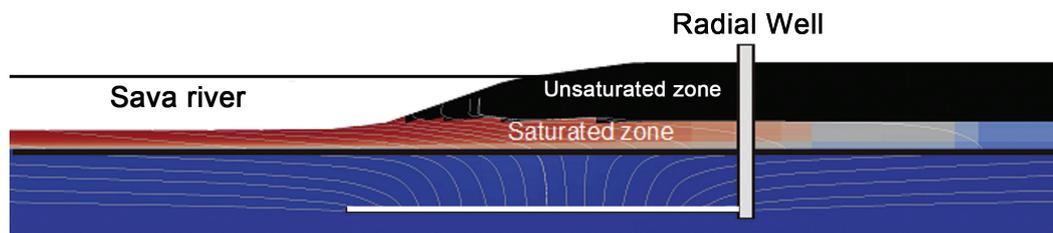


Figure 3: Streamlines and an unsaturated zone under the river bed

Therefore the pollutant transport did not become so prominent in comparison with some other groundwater sources where horizontal groundwater movement is dominant. The flow spreads horizontally and filtrates vertically through a large surface of the low-permeable inter-layer. This explains why the solved matters do not appear in the wells, even though they have been registered in the river water.

Micropollutants have come under the spotlight recently, owing in the first place to the development of their detection equipment. They are regularly monitored in the surface water and in the groundwater. Their kinetics of establishing equilibrium is significantly faster, and concentrations are small, so one can apply the linear (Henry) sorption model in computations.

Special software has been developed to solve this type of problem. Specific requirements have been imposed in respect to the user-friendly definition of river bed geometry, limited weakly permeable inter-layers (lenses), horizontal well drains and appropriate boundary conditions.

In order to model the transport through a variably saturated groundwater environment, one needs to solve two partial differential equations. One is Richards equation, describing a partially saturated water flow

$$\frac{\partial \theta}{\partial t} = \nabla \cdot (k_r(\psi) K \nabla h),$$

where θ is the water content i.e. effective porosity \mathcal{E} times the saturation, h is the hydraulic head (piezometric level), k_r is the relative permeability computed from the pressure head $\psi=h-z$ together with the saturation using Van Genuchten – Mualem formulas, z is the elevation relative to an arbitrary reference point, K is the hydraulic conductivity, and t is the time.

Another one is the mass transport equation:

$$\frac{\partial(\theta C)}{\partial t} + b(1-\varepsilon)\frac{\partial C}{\partial t} + \nabla \cdot (D \nabla C) = -\theta \frac{h}{t_{1/2}} C - b(1-\varepsilon) \frac{h}{t_{1/2}} C,$$

where C is the concentration, b is the ratio of concentrations in the solid and in the liquid (i.e. bC is the concentration in the solid),

$$D = (\theta D_d + \alpha_T \|\mathbf{u}\|)I + (\alpha_L - \alpha_T) \mathbf{u} \mathbf{u}^T / \|\mathbf{u}\|$$

is the diffusion-dispersion tensor, D_d is the diffusivity, α_L and α_T are the longitudinal and transversal dispersivity respectively, $\mathbf{u} = -k_r(\psi) K \nabla h$ is Darcy velocity, $\|\mathbf{u}\|$ is the intensity of this velocity, I is the unit matrix, and $t_{1/2}$ and $t_{1/2}$ are the half-lives in water and in solid respectively.

These equations are solved using a non-linear finite volume method described in [1] and [2] in W.O.D.A. solver (Well Outline and Design Aid) on models created in graphical software Lizza [3]. These programs allow for precise modeling of complicated discontinuous geological layers geometry, river beds, as well as radial and ordinary wells, using unstructured prismatic grids.

In order to demonstrate that the high quality of Belgrade water is due to the sorption and degradation in weakly permeable inter-layers, we have constructed a simplified model of a well with a single horizontal drain 50 m long which extends under the river bed.

Fig. 1 shows a vertical profile along the well drain. Four layers have been defined with conductivities (top-down): $K=5 \cdot 10^{-5}$ m/s, $K=5 \cdot 10^{-4}$ m/s, $K=5 \cdot 10^{-7}$ m/s and $K=8 \cdot 10^{-4}$ m/s. Porosity is 0.2 everywhere. Transfer coefficient K/d is $1.8 \cdot 10^{-8}$ 1/s in the colmated river bed and $K/d=6 \cdot 10^{-8}$ 1/s at the drain entrance. River water level is 71.7 m and well water level is 53 m. Model width is 300 m because this is the typical distance between wells. The computed stationary well flux is 11.8 l/s.

Concentration of 14 ng/l has been imposed in the river. The computed stationary concentration in the well is 0.11 ng/l. We have chosen $b=500$ in the inter-layer (the third layer from above) and $b=0.1$ in other layers. Dispersivity is $\alpha_L=0.2$ and $\alpha_T=0.02$. Half-life is two years in water or solid. The concentration obtained in the well agrees with the typical values measured in practice. Fig. 4 shows the sudden drop in the concentration when passing through the inter-layer.

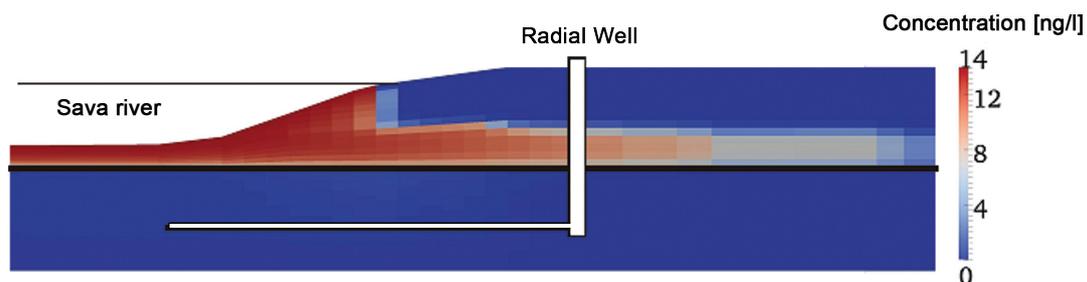


Fig. 4: Vertical stationary concentration distribution

The developed software facilitated the simulation and monitoring of basic mass transport processes in the relatively complex geometry of radial wells near the river bed. It is also applicable in well capacity dimensioning in the projected operational regime as well as in the case of an accident. Using simulation in various conditions it is possible to determine elements for well capacity dimensioning, in respect to the required well water quality.

Acknowledgment

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THE SELECTION OF PRIORITY SUBSTANCES IN NOVI SAD MUNICIPALITY, SERBIA

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The modern approach based on Quantitative Structure-Activity Relationships (QSAR) was applied for prioritization of emerging compounds, detected in surface and groundwater and related aquifers used for supply of drinking water in Novi Sad municipality. The water samples were collected from Danube River near Novi Sad during 2011-2012 years, and both screening and target monitoring were carried out.

Keywords: water pollutants, gas chromatography-mass spectrometry, Danube River, target analysis, screening analysis

Introduction

Accordingly to the World Health Organization guidelines for Drinking water Quality, “access of safe drinking-water is essential to health, a basic human right and a component of effective policy for health protection” (WHO, 2011). Moreover, the legislation of the European Union provides the strict requirements for concentration parameters of emerging contaminants. Thus the situation with quality of surface water becomes very crucial in the case of cities with developed industrial zones, especially when some military campaigns took place. For instance, Novi Sad as Serbia’s second largest city with a population of more than 350,000 has faced a specific problem - deriving of drinking water either from the Danube River or from several groundwater reservoirs. In the Danube River, the drinking water abstraction point is located only several hundred meters downstream of the municipality’s wastewater discharge point. Additionally, several groundwater abstraction points are used; however, all of them are located under densely populated urban areas, and one of them is located in the proximity of the oil refinery with area covers about 250 ha. UNEP (UNEP and UNCHS, 1999) declared Novi Sad an Environmental Hotspot in its report on the Kosovo Conflict, its main concern was the risk that groundwater polluted with petrochemicals from the oil refinery could enter drinking water wells, given the location of the wells near the refinery area. Apart from the refinery, there are other sources of pollution, thermoelectric and heat generating plant, and developed agricultural activity in this area.

Furthermore, it should be noticed that destruction of Novi Sad’s oil refinery during NATO bombing attack caused a spill of over 70,000 tons of crude oil, of which more than 5,500 tons were discharged in the Danube River and almost 2,000 tons spread out over the refinery area. As a result of this, the Danube sediment was heavily polluted with PAHs and other oil derivatives (B. Dalmacija et. al., 2000). Ten years after the destruction of the three bridges and Oil refinery in Novi Sad, providing safe drinking water and protecting the environment is still a major challenge.

However, no detailed and systematic monitoring programme for the screening of river pollutants and emerging substances in Novi Sad municipality was performed within the last 5 years, and in common control of selected target substances was a subject of several domestic research projects. For example, The *Eko bilten* Journal (Eco-Bulletin), Secretariat for Environmental Protection has reported higher residue values of chlorine, manganese, total iron, chloroform, ratio of dichlorobromomethane and chloroform as the most common pollutants in the drinking water in Novi Sad. Quantitative GC-MS analysis of the groundwater samples taken at a location along the refinery fence in the direction of the water supply source was carried out in March 2000, to check the presence of phenols, hydrocarbons, aromatic and polycyclic aromatic hydrocarbons. A detailed investigation

yielded the assessment of the time span of the pollution propagation in the direction of the water supply source and the direction of Danube-Tisa-Danube Canal of about 40 months (B. Dalmacija et al., 2000). Recently published results of monitoring studies performed in 2007 showed, among others, the presence of benzotriazole derivatives (R. Loos et al., 2010).

This work is focused on the identification of organic pollutants in surface water and related aquifers used for the abstraction of drinking water and their subsequent monitoring. The results will be used for determination of list of specific Danube basin pollutants for Novi Sad municipality.

Experimental part

Selection of sampling points/Sampling

In order to create relevant monitoring network of the surface Danube water in the vicinity of Novi Sad, selection of sampling points and defining of monitoring dynamic in this study have been defined upon proposal, knowledge and recommendations of the experts in close co-operation with Public Utility Company Waterworks and Sewage Novi Sad. The selected sampling sites were located in municipal wastewater collectors (GC1', GC2', RP', RO'), and 100 meters downstream of the each discharge in the Danube's riverbed (GC1'', GC2'', RP'', RO'', RIB). A sampling site RIB is located upstream of the city of Novi Sad, before all discharge points has been selected in order to assess the ecotoxicological status of the river Danube prior to any local urban impact.

The sampling on all selected localities has been conducted under equal hydrometeorological conditions. Samples were collected in plastic and glass bottles from eleven sampling sites, and stored at 4°C until analysis. Sampling for the preliminary analysis was made on June, October and December 2011 and the same months in 2012.

Gas chromatography analysis

Stir bar sorptive extraction (SBSE) and liquid-liquid extraction (LLE) have been used as sample treatment methods. A stir bar (1 cm long) coated with 0.5 mm layer of polydimethylsiloxane was used for sorption of organic compounds, while solution was stirred for 24 hours at 900 rpm. In LLE, water samples were extracted with two 50 ml portions of dichloromethane for 20 minutes using automatic shaker device. After extraction, both extracts were combined, dried and placed into heart-shape flask for evaporation to final volume 1 ml. Large volume injection was used for introduction of 30 µl extract to GC system. The GC analysis was performed using Agilent 7890 gas chromatograph coupled to Agilent 5973 mass spectrometric detector. The system was equipped with PTV injector system. Capillary GC analysis was performed on a 30 m x 250 mm x 0.25 mm DB-FFAP column. Helium was used as carrier gas. Oven temperature program started at 50 °C and this temperature was held for 2 min, then it increased with a gradient 12 °C /min to 140 °C, then 14°C /min to 310 °C (3 min). Identification of compounds was performed using Wiley7n and NIST08 mass spectrum libraries.

Results and Discussion

Based on the conducted three screening and two target analyses, around 300 compounds have been selected for ecotoxicity assessment by using QSAR approach. Selected compounds represent the most frequent groups that were identified, such as linear and branched alkanes, carboxylic acids, alcohols, pesticides, hormones and others. QSAR approach is based on prediction of critical effect concentrations (PNEC - predicted no effect concentration) based on physico-chemical properties, known toxicity parameters and inherent molecular descriptors using regression analysis and discriminant analysis (von der Ohe et al., 2011). PNEC values were obtained in three different ways: through EU directives (Directive 2011/876/EC, Directive 2008/105/EC and Water Framework Directive 2000/60/EC), ecotoxicity databases such as AQUIRE and ETOX, and finally QSAR approach.

In order to identify the extent of exceedance of ecotoxicity thresholds, PNEC values were compared with Maximum concentration (MC) of detected compounds obtained from target and screening analyses. The concentrations of tentatively identified compounds from GC-MS screening were estimated through comparison of the signal of an unknown compound to the signal generated by the known concentration of an internal standard (J. Slobodnik et al., 2012).

Consequently, the exceedance of environmental thresholds was used to generate the list of substances considered as a specific priority substances for Danube basin in Novi Sad municipality. The indicator for ranking of compounds shows the exceedance of the compound's concentration respective to the corresponding PNEC value. Tab. 1, 2 shows the list of the compounds observed in target and screening analyses of the samples.

Tab. 1. List of compounds – Target analysis

CAS	Compound name	Lowest PNEC ng/l	Max concentration	Exceedance of lowest PNEC
76-44-8	Heptachlor	0,0002	420	2100000,00
1024-57-3	Heptachlor epoxide	0,0002	50	250000,00
56-55-3	Benzo(a)anthracene	1,8	210	116,67
206-44-0	Fluoranthene	6,3	510	80,95
50-29-3	DDT-4,4'	10	500	50,00
115-29-7	Endosulfan-alpha	5	230	46,00
5598-15-2	Chlorpyrifos	1	40	40,00
60-57-1	Dieldrin	10	270	27,00
72-54-8	DDD-4,4'	25	620	24,80
129-00-0	Pyrene	20	490	24,50
85-01-8	Phenanthrene	30	360	12,00
35694-08-7	PCB-194	0,2	1,2	6,00
58-89-9	Hexachlorocyclohexane-gamma	5,5	30	5,45
1806-26-4	Octylphenol	100	540	5,40
118-74-1	Hexachlorobenzene	10	50	5,00
72-55-9	DDE-4,4'	25	110	4,40
67-66-3	Trichloromethane	2500	9720	3,89
84852-15-3	Nonylphenol	300	1150	3,83
104-40-5	4-nonylphenol	300	1140	3,80
84-74-2	1,2-benzenedicarboxylic acid, dibutyl ester	600	2150	3,58
120-12-7	Anthracene	100	280	2,80
117-81-7	Di(2-ethylhexyl)phthalate (DEHP)	1300	2630	2,02
86-73-7	Fluorene	100	140	1,40
218-01-9	Chrysene	100	130	1,30
140-66-9	4-(1,1,3,3-tetramethylbutyl)-phenol	100	110	1,10
108-88-3	Toluene	4300	4410	1,03
1582-09-8	Trifluralin	30	30	1,00

Tab. 2. List of compounds – screening analysis

CAS	Compound name	Lowest PNEC ng/l	Max concen- tration	Exceedance of lowest PNEC
473-03-0	Ambrein	0,0000024	100,59	42802953,66
630-01-3	Hexacosane	0,00022	781,56	3627715,43
629-99-2	Pentacosane	0,00054	956,51	1779488,14
646-31-1	Tetracosane	0,0013	662,10	494513,44
593-49-7	Heptacosane	0,000086	38,16	442598,74
629-97-0	Docosane	0,0083	590,54	71455,38
6418-47-9	Heneicosane, 3-methyl-	0,012	364,63	30312,91
25117-37-7	Heneicosane, 5-methyl-	0,012	208,55	17378,90
629-94-7	Heneicosane	0,020	230,91	11280,05
83-47-6	Stigmast-5-en-3-ol	0,059	402,05	6849,28
629-92-5	Nonadecane	0,125	732,75	5869,04
593-45-3	Octadecane	0,307	1227,21	3997,94
27400-77-7	Nonadecene	0,388	1222,93	3154,82
1560-84-5	Eicosane, 2-methyl-	0,122	338,25	2781,64
6418-45-7	Nonadecane, 3-methyl-	0,297	437,10	1473,89
57-11-4	Octadecanoic acid	1,318	1094,29	830,20
57-88-5	Cholesterol	0,536	424,50	791,97
142-91-6	Isopropyl palmitate	2,520	1859,61	738,00
333-41-5	Diazinone	0,200	146,06	730,29
83-46-5	beta-Sitosterol	0,059	40,14	683,83
629-78-7	Heptadecane	1,072	725,81	677,00
54833-23-7	Eicosane, 10-methyl-	0,128	54,55	425,94
629-59-4	Tetradecane	2,913	1096,47	376,42
123-95-5	Octadecanoic acid, butyl ester	0,210	74,63	355,37
57-10-3	Hexadecanoic acid	9,622	3273,90	340,24
629-62-9	Pentadecane	1,550	425,89	274,77
6765-39-5	Heptadecene	1,977	420,11	212,52
112-80-1	9-octadecenoic acid	17,636	3317,34	188,10
112-88-9	Octadecene	0,867	131,15	151,27
60-33-3	9,12-octadecadienoic acid	24,692	3713,77	150,40
544-76-3	Hexadecane	8,100	1160,33	143,25
601-53-6	Cholestan-3-one	4,243	436,49	102,88
80-97-7	Cholestanol	12,260	1215,18	99,12
6418-44-6	Heptadecane, 3-methyl-	1,578	155,83	98,74
111-06-8	Hexadecanoic acid, butyl ester	0,738	66,82	90,54
538-23-8	Glycerol tricaprilate	1,323	111,51	84,27
19466-47-8	Stigmastanol	3,156	251,97	79,84
58-08-2	Caffeine	100,000	4600,14	46,00
646-13-9	Octadecanoic acid, 2-methylpropyl ester	0,356	15,63	43,88
3055-98-9	Octaethylene glycol monododecyl ether	32,227	1130,50	35,08
544-63-8	Tetradecanoic acid	69,405	2057,13	29,64
1120-36-1	1-tetradecene	22,873	494,59	21,62

The highest exceedance of PNEC, for compounds detected in screening analyses, was obtained for is a fragrant substance Ambrein with very low value of PNEC which was detected only in one wastewater sample. It was not found in Danube surface water, due to its low concentration at the discharge and the dilution factor. Stigmast-5-en-3-ol is one of several phytosterols with the basic cholestane structure. This compound has poor water solubility; however, it wasn't detected in Danube surface water due to biological degradation processes and dilution factor. Diazinone is nonsystemic organophosphate insecticide, currently used in agriculture. It has been used more often due to the ban of DDT, and it represents the reason for its occurrence in wastewater samples. Diazinone is relatively stable under standard ambient temperature and pressure, but instead of sulphur and phosphorous can be degraded. As a consequence it is not detected in Danube water samples, even though its exceedance of PNEC is very high.

Benzo(a)anthracene and Fluoranthene, belonging to the group of PAHs, have similar values of MC/PNEC ratio, and were only detected in wastewater samples. According to their low solubility in water and high potential to sorb to sediments their concentration levels in Danube surface water were very low. Other compounds such as: Cholestan-3-one, Stigmastanol, Dodecane, and others, which were detected only in wastewater samples collected at discharges, have much smaller MC/PNEC ratios. Due to the dilution factor they were not measured in Danube surface water samples. Compounds with high exceedance detected only in Danube surface water samples, such as Heptachlor epoxide, Octadecene and Chlorpyrifos occurred in water due to several factors: upstream Danubian countries, soil runoff, atmospheric deposition, chemical reactions in aquatic environment.

Conclusions

Accordingly to the developed prioritization list of substances detected in the Danube River near Novi Sad, the most hazardous compounds that are considered for permanent monitoring by EWS are classified as linear and branched alkanes. The received result is not surprising due to the vicinity of Oil refinery. Other important groups of compounds include hormones, pesticides (Heptachlor epoxide, DDT and DDD), industrial chemicals, and PAHs, originated from different sources, e.g. municipal discharges and agricultural activity.

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FRESHWATER MUSSELS AS BIOINDICATORS IN THE ECOGENOTOXICOLOGICAL STUDIES

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Introduction

The simple detection of pollutants fails to provide the information on the relationship between contaminant exposure and biological effects in aquatic organisms and therefore usage of biomarkers becomes essential for assessing the condition of aquatic ecosystems (Nigro et al., 2006; Jha, 2008). Presence of pollutants in aquatic ecosystems can be detected by a range of physiological, histological and molecular responses, including abnormal morphology, alterations of antioxidative status and DNA integrity (Pavlica et al., 2001; Bolognesi et al., 2004; Kolarević et al., 2012).

The integrity of cellular DNA is continuously attacked by various agents in the environment resulting in DNA lesions such as strand breaks, modified bases, DNA–DNA cross-links and DNA–protein cross-links. Unrepaired DNA lesions may block replication and transcription, potentially leading to cell death, or may give miscoding information, generating mutations (Simić et al., 1998; Vuković-Gačić et al., 2006). As a result, a number of biological consequences can be initiated at the cellular, organ, and whole animal and finally community and population levels. Studying the origin of genotoxic pollution, as well as the effects of pollution on individuals and populations are the main objectives of ecogenotoxicology.

Freshwater mussels as bioindicators

Mussels are commonly employed in the ecogenotoxicological studies. They have several characteristics, such as wide distribution, filter feeding, a sessile life form and an ability to accumulate pollutants, which makes them favourable organisms for estimating the environmental pollution level and the bioavailability of various types of pollutants (Roméo et al., 2003; Andral et al., 2004; Amiard et al., 2006). In response to environmental stress they show a range of physiological, histological and molecular responses, including abnormal morphology, alterations of antioxidative status, induction of DNA strand breaks, etc. (Pavlica et al., 2001; Binelli et al., 2007; Bolognesi et al., 2004; Coffinet et al., 2008).

In ecotoxicological studies, different approaches are used for assessing the conditions of ecosystems. Passive biomonitoring employs collection of the specimens from selected locations, while the active biomonitoring entails the use of bioindicator organisms obtained from unstressed populations and their subsequent exposure at polluted sites (De Kock and Kramer, 1994; Smolders et al., 2003). Active biomonitoring is increasingly used for quantifying the impact of pollutants on aquatic ecosystems because of the numerous advantages over the passive, such as avoiding the biological variability in the responses related to different age and the reproductive status of the organisms *in situ*. In addition, it can overcome the hydrological, hydrochemical and other abiotic and biotic factors that can influence species distribution, contaminant bioaccumulation and biomarker responses (Cossu et al., 2000; Arbuckle and Downing, 2002; Andral et al., 2004; Viarengo et al., 2007).

One of the major issues in ecogenotoxicological studies is providing data from the animals at unpolluted sites which can be used as control values of DNA damage for *in situ* assessment of genotoxicity. Active biomonitoring also requires the specimens from unpolluted sites to be used for translocation. However, finding an unpolluted site is not always possible. The acclimation of mussels in controlled laboratory conditions could provide an adequate solution for obtaining the control values i.e. the baseline DNA damage, as described in different mussel species (Fedato et al., 2010).

Studying the effect of exposure to certain compound or mixture of compounds in controlled laboratory conditions is employed in *ex situ* assessment of genotoxicity. The compounds that are mostly used are pollutants which are suspected to be threat to environment.

Residues of human pharmaceuticals are raising concerns about their potential effects in non-target species (Fick et al., 2010). Great attention is dedicated to anticancer drugs due to their cytotoxicity, genotoxicity, mutagenicity and teratogenicity (Kosjek and Heath, 2011).

Detection of genotoxic effect with Comet assay (SCGE)

The comet assay, also known as single cell gel electrophoresis (SCGE), is a sensitive and rapid technique for detection of DNA damage in individual cells based on the migration of denatured DNA during electrophoresis, in which damaged nuclei form comet-like shapes. Comet assay has been accepted as one of the major tools for assessing pollution related genotoxicity in aquatic organisms (Dixon et al., 2002). It has been used in many ecogenotoxicological studies on freshwater mussels (Pavlica et al., 2001; Klobučar et al., 2003; Guidi et al., 2010, Kolarević et al., 2013, Vuković-Gačić et al., 2013) and show correlation with other genotoxicity tests such as chromosomal aberration, sister chromatid exchanges and micronucleus assay (Dhawan and Bajpayee, 2009). The modified alkaline version of the comet assay, described by Sing et al. (1988), enables detection of both single and double DNA strand breaks, as well as alkali labile sites. In our studies the comets are scored and analyzed using Comet IV Computer Software (Perceptive Instruments, UK). Tail intensity - TI (the percent of DNA fluorescence in the comet tail) and Olive tail moment - OTM (calculated as a product of the TI and the distance between the means of the head and tail distributions, are most often used as a measure of DNA damage.

In mussels, detection of DNA damage is usually performed on haemocytes and gill cells. Gills have a high efficiency in genotoxicity monitoring due to their large surface and constant exposure to environment. Haemocytes have a role in processes such as the transport and digestion of nutrients, and elimination of toxic substances and small particles, which makes them constantly exposed to water-borne pollutants (Soares-da-Silva et al., 2002; Dhawan and Bajpayee, 2009). Haemolymph can be easily collected from the adductor muscle and, most importantly, collecting does not require sacrificing animals.

Assessment of genotoxicity - *in situ* assessment and active biomonitoring

The result of the *in situ* assessment performed on freshwater mussel *Sinanodonta woodiana* indicated presence of genotoxic pollution in the Velika Morava River (Kolarević et al., 2013). Employment of active biomonitoring with freshwater mussels *Unio pictorum* and *Unio tumidus* indicated significant genotoxic potential of untreated wastewaters in the urban area of the Belgrade city (Vuković-Gačić et al., 2013). Moreover, the results of these studies indicated correlation in the level of DNA damage in mussels with concentrations of dissolved heavy metals in water. Also, we have shown the ability of DNA damage recovery in mussel species from the Unionidae family.

Assessment of genotoxicity - *ex situ* assessment

Impact of acute exposure to the most frequently used cytostatics (5-FU, cisplatin, etoposide and vincristine) was studied on haemocytes of freshwater mussels *Unio pictorum* and *Unio tumidus*. The results of exposure show that majority of tested cytostatics can induce DNA damage in haemocytes of freshwater mussels *U. pictorum* and *U. tumidus*, even when present in concentrations which are far below administered in clinical treatment.

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ANTIBIOTICS IN ENVIRONMENT - FRIENDS OR FOE?

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Abstract

The presence of the emerging substances, especially antibiotics, has become the subject of growing concern, because of their extensive use in human and veterinary medicine. Most of them could not be completely removed using conventional WWTPs and therefore they are frequently detected in the aquatic environment in ppb, ppt or lower concentrations. Although the growing resistance of the vast groups of bacteria is registered, there is still no scientific evidence about the negative consequences of the sub-low levels of antibiotics for the humans and the environment. The information about the antibiotics has mainly been focused on the USA and Western Europe, but in the Western Balkan region, especially in Serbia, the reports about their occurrence are still rare and limited on only few compounds and sampling points. According to these findings, it is necessary to continuously monitor large river basins and to pay the special attention to the arising problems of the serious overuse of antibiotics and possible risks to both health and environment.

Keywords: pharmaceuticals; antibiotics; emerging substances; aquatic environment

Introduction

Pharmaceuticals are one of the emerging substances of concern that could potentially cause adverse environmental effects. Among different classes of pharmaceuticals, special attention has been paid to antibiotics due to the possible formation of the resistant bacterial strains, which can pose a serious threat to the human health and the environment.

Antibiotics are chemical substances that are capable either to suppress the bacterial growth (bacteriostatic) or to kill them (bactericidal). They are extensively used for the prevention and treatment of diseases in human and veterinary medicine and their consumption increases every year. Penicillins, followed by tetracyclines, macrolides, fluoroquinolones and sulfonamides are most commonly prescribed in human practice as well as in veterinary medicine for the treatment and control of infectious diseases such as mastitis, enteritis, peritonitis and pneumonia (Milić et al., 2012). Also, they are used in concentrated animal feeding as growth promoters, for prophylaxis and in aquaculture as well. More than 8000 tons of antibiotics are added in the feed and water for promoting animal growth in China, but still without the regulations (Ben et al., 2009). Although the growth promoters have been banned from the European agriculture by Regulation No. 1831/2003, the use of antibiotics as growth promoters is widespread in the USA (Kemper 2008).

According to all, the most important global sources of antibiotics in the environment are wastewaters. After the administration, antibiotics are only partially metabolized and discharged into wastewater. Most of them could not be completely removed using conventional wastewater treatment plants (WWTPs). As a consequence, a large percentage of antibiotics is released into the aquatic environment and due to their inherent bioactivity could have a negative impact on the environment and public health.

Considering all of that this paper gives an overview of the most frequently detected antibiotics in the aquatic environment and demonstrates the need for further investigation of their occurrence and necessary assessment of the potential risks to both health and environment.

Sources, Pathways and Fate

The consumption of antibiotics increases every year and the estimated annual world consumption is between 100,000 and 200,000 tons (Senta et al., 2013). Only in Europe 10,000 tons of antibiotics were consumed in 1998 and half of them was used in veterinary practice (Kemper 2008).

After the administration antibiotics are mainly excreted as glucuronide and sulfate conjugates that can easily hydrolyze in the environment. However, large amounts of them are eliminated without metabolism and in an unchanged form discharged into the water system. Most of them could not be completely removed using WWTPs. The removal of trimethoprim by wastewater treatment plants is less than 10% of while fluoroquinolones can be 10-80%. The disappearance of the parent compound does not necessarily mean that the treatment was successful because of the possible presence of its metabolites or complexes (Petrović et al., 2011).

Despite their positive effects, serious overuse of antibiotics has become a new environmental problem. More than 70% of bacteria are insensitive to at least one antibiotic. According to the continuous input of these substances into in the aquatic environment further development of pseudo-persistence could be expected (Vojinović Miloradov et al., 2013).

The most prescribed antibiotics, penicillins and cephalosporins are not recognized as serious threat to the environment due to the poor stability of β -lactam-ring which can easily be hydrolyzed, either chemically or microbiologically. However, sulfonamides, fluoroquinolones and macrolides are more stable and widely detected in aquatic environment and present important environmental contaminants.

Besides the worrying impact on public health caused by spread of bacteria resistant to high doses of antibiotics, scientific evidences about systematic uptake and input of low antibiotics doses and their influence on humans and environment are still very limited. Kümmerer (2003) documented that the impact of various antibiotics remaining active against bacteria living in wastewater but effects of sub-inhibitory concentrations against non-marine aquatic bacteria were mainly unknown. The resistant and multi-resistant bacteria were detected in the wastewater and sewage treatment plants and the resistance to erythromycin, chloramphenicol, cefurixime and cephalothin were noticed in some *Vibrio* species in the wastewater effluents in South Africa (Igibinosa et al., 2011).

Antibiotics in Aquatic Environment

During the recent years the investigations of the occurrence of antibiotics in the aquatic environment have been carried out and more than 30 antibiotics have been found in urban and agricultural wastewaters, meat industry wastewaters and surface water and even in ground and drinking waters.

Table 1. Concentration level of antibiotics in water from selected literature

Class	Substance	Concentration ($\mu\text{g l}^{-1}$) Maximum value	Source
Macrolides	Lincomycin	21.10	Surface water
	Clarithromycin	0.26	Surface water
	Erythromycin	1.70	Surface water
Sulfonamides	Azithromycin	1.14	Municipal wastewater
	Roxithromycin	0.56	Surface water
	Sulfadiazine	4.13	Surface water
	Sulfamethazine	0.24	Ground water
	Sulfamethoxazole	0.48	Surface water
	Sulfamethoxazole	0.41	Ground water
	Sulfamethoxazole	11.60	Municipal wastewater
Trimethoprim		0.20	Surface water
Trimethoprim		2.55	Municipal wastewater
Fluoroquinolones	Ciprofloxacin	2.61	Municipal wastewater
	Ciprofloxacin	0.03	Surface water
	Norfloxacin	2.94	Municipal wastewater
	Norfloxacin	0.12	Surface water
Chloramphenicol		0.06	Surface water
Tetracyclines	Tetracycline	0.40	Ground water
	Oxytetracycline	32.00	Overland flow water
	Oxytetracycline	4.49	Surface water

The continuous presence of low concentrations of antibiotics (ppt, ppb or lower) in aquatic environment has become of great concern among the scientists and public, but not among the policy makers and stakeholders. The concentration limits of antibiotics in the environment are not regulated by either the USA or the EU. Also, the defined standardized techniques and methods for the antibiotic detection in water matrix have not been established yet in the EU. The maximum detected concentration of antibiotics in the aquatic environment in Germany, Switzerland, Western Balkan Region and the USA is presented in Table 1 (Kolpin et al., 2002; Boxall et al., 2005; Kemper 2008; Terzić et al., 2008).

Conclusions

The presence of the emerging substances, especially antibiotics, has become the subject of worrying concern, because of their extensive use in human and veterinary medicine. Evaluation of the occurrence of these contaminants is especially important due to the growing resistance of the vast groups of bacteria.

The appropriate use of antibiotics in human and veterinary medicine, more efficient treatment of municipal and industrial wastewaters and the reduction of their emission into the environment in accordance with proper legislation and management of this field are essential.

The future investigations should be focused on the quantitative structure-activity relationships (QSARs) analysis, in order to evaluate the ecotoxicological risk of the low doses of antibiotics and their impact on human health and the environment.

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MEAT PROCESSING INDUSTRY AS SOURCE OF EMERGING AND PRIORITY SUBSTANCES IN WATER RESOURCES OF VOJVODINA - GEOSPATIAL ANALYSIS AND MODELLING

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Introduction

The industry sector emerges as one of the significant contributors to the most pressing environmental problems in Republic of Serbia, especially to pollution of natural water bodies [1]. Meat processing industry represents an important industrial segment in past decade, which contributes about 10% of industrial sector in Vojvodina [2]. Meat industry is a branch that consumes and discharges a significant quantity of water. Due to the different techniques of discharge and discharge possibilities in studied area there is a notable need for broadening of methods used for evaluation of obtained analytical data. Waste water discharged from meat processing industry is a complex mixture with high organic and significant inorganic loads that can contribute to environmental disbalance in recipient water body – either surface or underground water. It will be shown the possible impacts of meat processing wastewater on recipient water bodies, due to the different processes of discharged wastewater into environment. The research is structured of two parts – analytical and geospatial. Within the analytical part of research the detection and identification of priority and emerging substances have been conducted . on Agilent GCMS system. During the analytical part of research preparation method and analysis optimization was conducted for meat processing wastewater with emphasis on priority pollutants and emerging substances. Within the second part of the research, the implementation of geoinformation system for monitoring of water pollution loads due to the meat processing industry effluent discharging was examined. Geospatial identification of pollutant sources with different types of discharge techniques and possibilities, was conducted due to the possibilities of cumulative pollution effects in surface water or infiltration into the underground water. Accordingly, 2D model of aquifer distribution and depth at the territory of Vojvodina was created in Quantum GIS 1.8.0 for the purpose of geospatial analysis of possible effluent discharge impact onto the groundwater bodies.

Sampling campaign and optimization of analysis for meat processing wastewater samples

Sampling campaign - Water samples were collected in 2 separated sampling champagnes –in October 2012 and November of 2012. The importance of spatial positioning of sampling sites is in order to explore the possibilities of overlapping and cumulating effects of expected and detected pollutants, and their direct or indirect effects on the River Danube as well as on groundwater bodies which is the prevalent source of drinking water for the population of Vojvodina. **Optimization of analysis for meat processing wastewater samples** - Sample preparation, as the important factor of environmental research process, was submitted to optimization during this research. Liquid-liquid extraction is one of the most widely utilized sample preparation techniques for qualitative and quantitative analysis. [3]



Figure 1. a) LLE with pre-concentration, b) evaporation in rotary evaporator and a) filtration process

It was concluded that LLE is an optimal preparation technique for meat processing wastewater samples after 3 unsuccessful preparation techniques for this type of samples: filtration, evaporation in rotary evaporator, filtration with active carbon. [6] Analysis of prepared samples was conducted on GC-MS system. Agilent 7890N GC, in SCAN mode was used for analysis of prepared extracts, coupled with Agilent 5975 MS Detector (*Carrier gas*: helium, 1 ml/min; *Capillary Column*: DB-FFAP 30m x 250mm I.D., 0.25mm; *Oven program*: 40°C, 10min holding time; rate 2°C /min to 230°C). Different oven programs were conducted to prolong the separation of compounds.

Identified compounds of interest – priority pollutants and emerging substances

Total of 259 different compounds have been detected in all 6 samples [1].

Table 2. Identified substances during analytical research in meat processing wastewater samples [2]

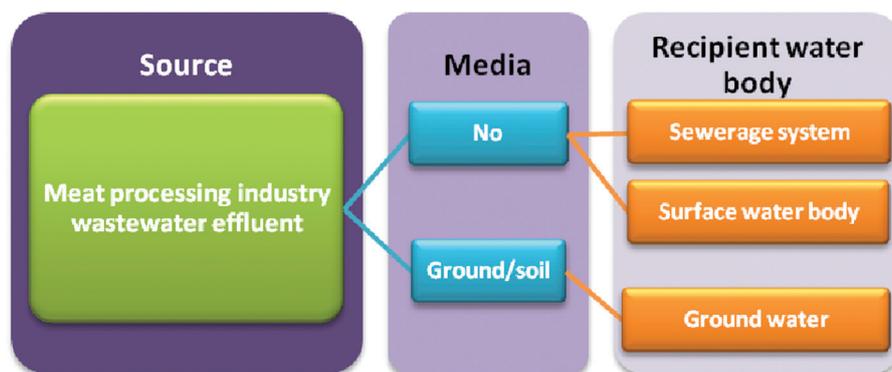
Compound	Samples
NORMAN's list of emerging substances	
1,4-dimethyl-benzene; (p-Xylene)	MI1,MI2, MI3, MI4
7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-; (Isocineole)	MI6
Cyclohexene, 1-methyl-4-(1-methylethenyl)- (CAS);dl-Limonene	MI1, MI2, MI3, MI4, MI5, MI6
2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl-; (1,8-Cineole)	MI1,MI4, MI5, MI6
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-; (Carvone)	MI2, MI6
Phenol, 2-methoxy-4-methyl-; (p-Cresol)	MI2, MI3, MI5, MI6,
Phenol, 4-methyl-; (p-Cresol)	MI1, MI2, MI3, MI4, MI5, MI6
2,4,7,9-Tetramethyl-5-decyn-4,7-diol;(Surfynol 104A)	MI6
Hazardous priority substances	
2-Octenal, (E)- (CAS);	MI2, MI5, MI6
2,4-Decadienal, (E,E)-	MI2, MI4, MI5, MI6
Phenol, 2-methoxy-; (o-Guaiacol)	MI3, MI4, MI5, MI6
Hexanedioic acid, dioctyl ester; (Dioctyladipate)	MI6
2,6-Dimethylphenyl isocyanate	MI2
Phenol, 2,6-dimethoxy-4-(2-propenyl)-	MI5
Phthalic acid, allyl ethyl ester; (Ethylallylphthalate)	MI2
9-Octadecenoic acid (Z)-, methyl ester; (Emery)	MI5
Phenol, 2-methoxy-4-(1-propenyl)-, (E)-; ((E)-Isoeugenol)	MI5
1-Docosanol; (Behenic alcohol)	MI2
1-Octen-3-o	MI2, MI3, MI4, MI5

Compounds identified during analytical study are on NORMAN's list of emerging substances and on EPA's list of priority pollutants are shown in Table 2.

Discharge techniques and possibilities of monitored meat processing industry entities

Industrial wastewaters that represent taken samples have different treatment in the end of use. Due to obtained facts from the field, it is concluded that there are 3 different processes of wastewater disposal:

1. Full treatment and disposal of wastewater according to regulations and legislations;
2. Primary treatment or no treatment and disposal into the sewerage;
3. Disposal of wastewater onto the soil or local dump for communal waste.



Graph 1. Meat processing industry wastewater disposal techniques and possibilities

In this sense it is necessary to broaden the scope of research from co-relations between communal waste water and surface water, to ground water as well, as it has a possibility of impact due to presented discharged possibilities and techniques.

Table 3. Locations and types of treatment for meat processing industry wastewater samples obtained

Meat processing industry (MI) WW samples	Location of MI	Treatment/recipient
MI1	Pecinci	after the treatment process/sewerage
MI2	Novi Sad 1	after the treatment process/sewerage
MI3	Kulpin	no treatment/local dump/soil
MI4	Pecinci	before the treatment/sewerage
MI5	Novi Sad 2	primary treatment/sewerage
MI6	Novi Sad 3	primary treatment/sewerage

Geospatial context

Potential organic load analysis of water resources in the physical environment of Novi Sad is the initial analysis phase of the meat industry waste streams impact onto the recipients. The analysis of the meat industry facilities in terms of technological capacity for adequate treatment and discharge of waste water into the environment is also an inevitable stage of the above monitoring. Analytical and technical-technological approach to the phenomenon of environmental pollution and the environment itself should not be extracted from the spatial context. The spatial distribution of potential pollutants in relation to the distribution of natural resources, in a great extent, determines the preservation of the environment. For the purpose of the comprehensive examination of meat industry facilities impact on water resources, geospatial display of identified pollutants with associated data on the characteristics of each facility as well as data on detected substances of concern in wastewater was created. Based on the paper *M. Sremacki et al, Geospatial support for the analytical study of water pollution loads due to wastewater from the meet industry facilities in Novi Sad* [2], the scope of analysis was impact of organic load from meat industry wastewater effluent into a communal wastewater system and surface water resources. With the aim of expanding the research it is necessary to consider the possibility of indirect effects of targeted sources of pollutants on soil and groundwater. In that sense, for the analysis of drinking water resources exposure to emerging and priority substances emanating from the meat industry process, it is necessary

to collect information on the distribution and depth of the aquifer in the area of interest. Accordingly, 2D model of aquifer distribution and depth at the territory of the Vojvodina have been created in Quantum GIS 1.8.0 for the purpose of geospatial analysis of possible effluent discharge impact onto the groundwater bodies.

Methods for 2D aquifer model creation

“Map of the complex “B” aquifer with isobaths of the lower limits with elevations in relation to the level of the Adriatic Sea” is used as an input data for the creation of the desired model. “Map of the complex” “B” is a part of the Volume 6 of the project named “Hydrological base maps of AP Vojvodina” that have been realised within Scientific and Education Institute for Water Management, at the Faculty of Agriculture in Novi Sad, in March 1983. (Figure 3) [3].

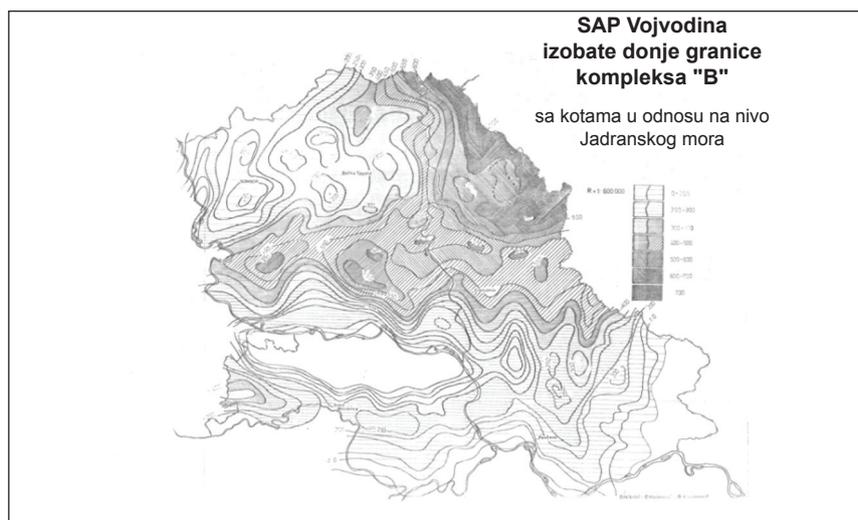


Figure 2. Map of the complex “B” aquifer with isobaths of the lower limits with elevations in relation to the level of the Adriatic Sea

Aquifer at the territory of Vojvodina is divided into two zones: the complex “A” is the upper zone artesian aquifer at a depth of 150-300 m and complex “B” is lower aquifer zone that extends to greater depths [3]. Map of the complex “B” is data in analogue form. For the purposes of processing of data displayed on the map by using geo-information technology, the map was scanned and converted to digital form. Then, digitalized map (raster) was imported into QGIS software application for the purpose of georeferencing. Appropriate coordinates of corresponding spatial reference system were assigned to each pixel of the raster, which is the basis for creating a model of the real system. Produced digital map was used as a base map for creating the vector display of aquifer distribution with associated data of the lower boundary of aquifers in Vojvodina. Vectorization was performed by using line geometry in the spatial reference system MGI / Balkans zone 7, which is a national reference system of the Republic of Serbia. After the vectorization, a visual representation of the obtained vector distribution was created by use of plugin for the interpolation, which is integrated into QGIS 1.8.0 application. For the purpose of interpolation Inverse Distance Weighting (IDW) method was used. Numerical model of continuous spatial variations [4] which are visually presented by using geo-information technologies, allows us to further analyse characteristics and behaviour of the presented physical system.

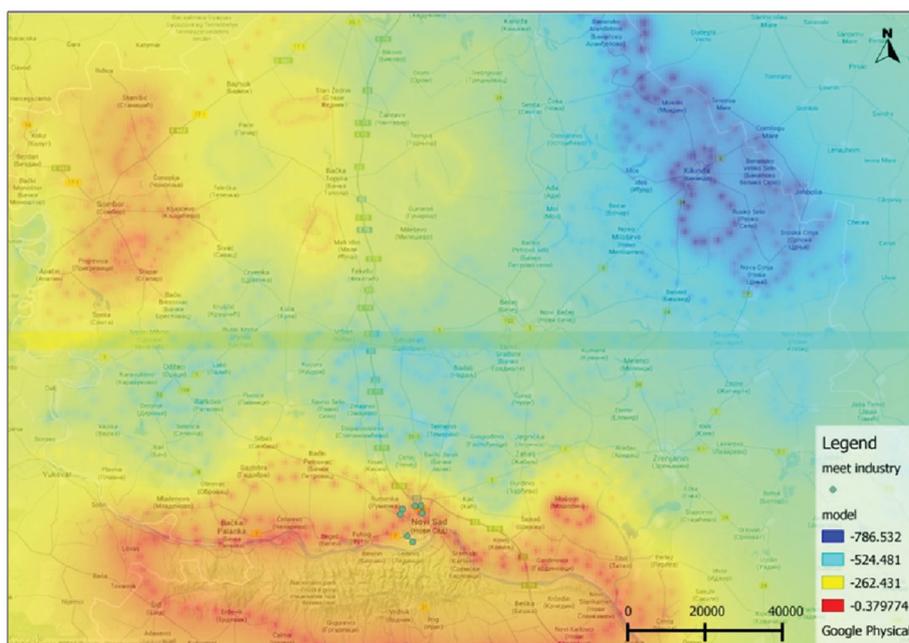


Figure 3. 2D model of aquifer distribution and depth at the territory of Autonomous Province of Vojvodina

Conclusion and discussion

First conclusion derived from this research is optimum preparation technique for this type of ‘dirty’ sample, the meat processing industry effluent sample, is an LLE - liquid-liquid extraction technique. This technique has shown the best possible results of extraction of organics from sample:

- high efficiency of process,
- relatively low time consumption,
- efficient separation in GC system and
- high peak quality in obtained chromatograms.

Obtained results of analytical experiments have confirmed that hazardous priority pollutants have been successfully removed during treatment that is suggested by legislation, which is not the case for emerging pollutants. This can be observed from samples taken from location Pećinci, before and after treatment. This conclusion is one of the most pressing which shows the need to broaden the scope of research onto the ground water. The question is if the soil can be natural filter for removal of emerging substances, which cannot be removed from wastewater with any standardized commercial wastewater treatment technique. For this diverse scope of pollutants it is concluded that in the sense of overall and quality monitoring it is necessary to broaden the research methods (analytical coupled with *geospatial modeling*) as well as mediums selected in previous phase of study (communal wastewater, meat industry wastewater, surface and *underground raw water*). Result of overlapping created models of distribution and lower limits of the aquifers in Vojvodina and the engineered vector layer that displays studied locations with an associated database of analytically identified emergency and priority substances [2], is an information on the potential impacts of the studied pollutants in water resources, surface and ground water. Consideration the distance between aquifer and possible sources of pollution, leads to notion about the potential of soil sorption and reactions of identified organic substances. In the context of modeling geospatial, data on average and maximum groundwater levels, will contribute to obtaining the information about the volume of groundwater aquifers, which opens new possibilities for the analysis of the impact of effluents on water resources. Furthermore, the mapping of the drinking water source that covers the territory of Vojvodina would allow the analysis of topological relations between sources of pollution and significant locations in terms of groundwater protection. The required number of sampling and field analyses is often not possible, to achieve representable data for monitoring in the purpose of scientific research. Therefore, the model is an abstraction of the real system that is invaluable in the study of the phenomenon of environmental pollution in terms of identification, quantification and monitoring of pollution dispersion in environmental media.

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EMERGING POLLUTANTS IN BELGRADE WASTEWATER

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Pharmaceuticals are emerging contaminants with damaging effects to the environment and human health. In the recent years, pharmaceuticals have been frequently detected in aquatic ecosystems [1–3]. This is mainly caused by their high level of production and everyday consumption. Since significant amounts of pharmaceuticals present in the aquatic environment originate from the urban wastewater, the control of such compounds has become an important topic of water monitoring and analysis [4].

In this work seven pharmaceuticals were monitored in the municipal wastewater discharges in Belgrade in Jul and July 2011. Method developed for analysis of pharmaceutical residues in surface and ground water was applied to the wastewater samples after validation [1]. The analysis of the selected analytes was performed using procedure based on solid-phase extraction as sample preparation technique combined with liquid chromatography coupled with tandem mass spectrometry (LC–MS²) using positive electrospray ionization. The investigated compounds include: sulfamethoxazole, trimethoprim (antibiotics); diazepam, bromazepam, lorazepam, carbamazepine (sedatives) and diclofenac (analgoantipyretic).

Sample preparation consisted of solid-phase extraction using Oasis HLB cartridges, 100 mL of the wastewater sample (pH = 6), and methanol-dichloromethane (1:1) as extraction solvent. As for the LC–MS² method, the chromatographic separation of compounds was performed on a reverse-phase Zorbax Eclipse® XDB–C18 column, 75 mm × 4.6 mm ID and 3.5 µm particle size. The mobile phase consisting of water, methanol and 10% acetic acid. The mass spectrometric measurements were obtained using the quadrupole ion trap mass spectrometer. Electrospray ionization was used to perform the mass spectrometric analysis operating in the positive ionization mode. Quantification of the selected pharmaceuticals was performed using matrix-matched standards.

Recoveries of the method validated for wastewater samples were in the range 80–118%. Repeatability of the method, expressed as the relative standard deviation, was in general lower than 20%. Matrix-matched calibration curves displayed good linearity. Low limits of detection (0.2–5 ng L⁻¹) and quantification (0.4–16 ng L⁻¹) were achieved for all investigated analytes.

Validated method was successfully applied in the analysis of samples from Belgrade urban wastewater system. Wastewater samples were collected at seven different sampling sites of the municipal wastewater discharges in Belgrade in June and July 2011. Sampling of wastewater was carried out by the automatic sampler for every hour over 24 hours. Mixing specific volumes of each of the collected samples was obtained composite sample was analyzed. Sample collection sites include 80% of all municipal wastewater discharges in Belgrade. Sampling was carried out on the following locations: Sajem (1) Ušće (2) Lasta (3), Istovar (4), Ada Huja 1 (5), Ada Huja 2 (6) and Višnjica (7) (Figure 1.)



Figure 1. Sample collection sites

The results are presented in Table 1. Out of seven monitored compounds, five were found to be present in the analyzed samples. Pharmaceuticals, belonging to the classes of antibiotics and sedatives, were the most frequently detected contaminants. Bromazepam and carbamazepine were not detected in samples while the most commonly detected analytes were lorazepam and diclofenac. The frequency of pharmaceutical detection were highest at sampling sites Sajam and Ada Huja 1. The lowest concentration was measured for diclofenac (58 ng L^{-1}) and highest for sulfamethoxazole (1184 ng L^{-1}).

Table 1. Pharmaceutical residues detected in wastewater samples (ng L^{-1})

Pharmaceuticals	Sajam		Ada Huja 1		Ada Huja 2		Ušće		Lasta		Istovar	Višnjica
	June	July	June	July	June	July	June	July	June	July	June	
Trimethoprim	122		482									
Sulfamethoxazole			1184									
Lorazepam	171		77		105		583	105	84		167	
Diazepam	163											
Diclofenac	442		782		479		58		579	471		310

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EMERGING POLLUTANTS IN RIVER SEDIMENTS

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Human and animal sterols can be used as indicators of fecal contamination of the environment, as well as for distinguishing between sources of contamination based on their ratios [1]. Elevated concentrations of plant sterols and hormones can cause a number of adverse effects on the human body [2-4]. Traces of these new pollutants can be detected in surface and ground water. Due to the accumulation, these contaminants can also be detected in river sediments [3]. The aim of this work was development and optimization of analytical method for extraction of river sediment and determination of human, animal and plant sterols, and hormones. In the method development, extraction solvent, mass of sediment and time of ultrasonic extraction were optimized. For a quantitative analysis of the obtained extracts, chromatographic separation of twenty sterols and hormones was optimized using liquid chromatography, with the ion trap as a mass detector (LC-MS/MS, Liquid Chromatography-Tandem Mass Spectrometry) and atmospheric pressure chemical ionization.

For the extraction of analytes from the sediment, six solvents of different polarity were tested: methanol, acetone, ethyl acetate, acetonitrile, dichloromethane and hexane. Mass of the sediment sample was also optimized, with the aim of achieving higher preconcentration factor and extracting lower amounts of impurities. In order to shorten the sample preparation, different times of ultrasonic extraction were tested.

In the finally developed and optimized sample preparation procedure, the sediment was extracted in an ultrasonic bath for 10 min. Sample was then centrifuged and the supernatant was separated. Extraction procedure was repeated two times and the resulting supernatants were mixed. The extract was evaporated and quantitatively transferred to the purification column packed with silica gel and anhydrous Na₂SO₄. The elution of column was carried out with a methanol-ethyl acetate mixture (1:1). The obtained purified extract was evaporated to dryness and reconstituted in methanol, filtered and analyzed.

In the developed LC-MS/MS method, the mobile phase consisted of methanol and deionized water. It was found that for the optimal chromatographic separation of twenty selected sterols and hormones it was necessary to develop two methods, because it was not possible to separate a significant number of analytes, especially hormones. It was shown that 17 α - and 17 β -estradiol, then estrone and estriol, and also epicoprostanol, α - and β -cholestanol have identical MSⁿ fragmentation reactions, but they can be chromatographically separated using the appropriate mobile-phase gradient. Based on the results of MSⁿ analysis, characteristic fragmentation reactions were selected for quantification and confirmation of each analyte in the developed LC-MS/MS methods.

The recoveries of the optimized methods were high (81–120%), with a relative standard deviation less than 22% (Figure 1).

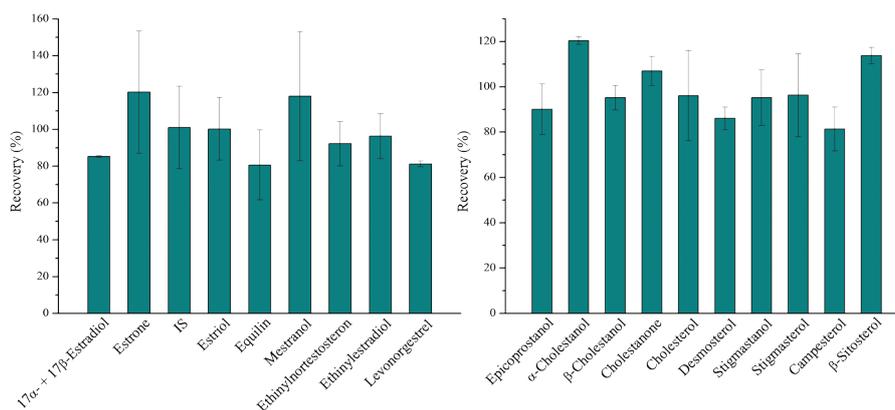


Figure 1. The recoveries of the optimized extraction method

The sample preparation method was successfully applied in the analysis of the sediment from the Morava River in which traces of human, animal and plant sterols were detected. It was determined that human and animal sterols (epicoprostanol, α -cholestanol, β -cholestanol, cholesterol) in the range 0.76 – $2.94 \mu\text{g g}^{-1}$, as well as plant sterols (stigmastanol, stigmasterol, campesterol, β -sitosterol) in the range 0.52 – $2.21 \mu\text{g g}^{-1}$, were present in the river sediment (Figure 2).

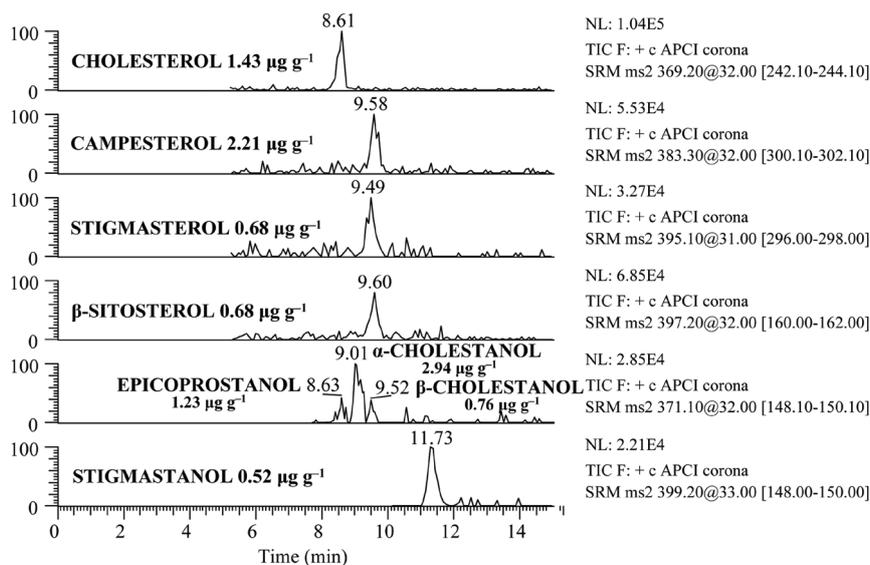


Figure 2. Chromatogram of the sediment sample from the Morava River with amounts of detected sterols

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