Tracer Test and Monitoring of Behavior and Transport of Selected Pharmaceuticals

S. Kovačević*, N. Živančev*, M. Perović**, V. Bežanović*, P. Vojt**, A. Petković**, M. Dimkić**

*University of Novi Sad, Faculty of Technical Sciences, Department of Environmental Engineering and Occupational Safety and Health, Trg Dositeja Obradovića 6, 21000 Novi Sad
(E-mail: *srdjankovacevic@uns.ac.rs; nevenasenk@uns.ac.rs; vesko1989@gmail.com)*
**Jaroslav Černi Institute for the Development of Water Resources, Jaroslava Černog 80, 11000 Pinosava-Beograd, Republic of Serbia (E-mail: *marijaokuka@yahoo.com; predrag.vojt@jcerni.co.rs; andjelka.petkovic@jcerni.co.rs; milan.dimkic@jcerni.co.rs)*

Abstract
Pharmaceuticals are a group of emerging substances which are present in the environment and appear to be relatively constant at low concentrations. With the development of modern analytical methods more pharmaceuticals can be detected in the water sphere of the environment. Study of the behavior and transport of pharmaceuticals in groundwater is significant for understanding the processes of natural attenuation and potential use of filtration through the aquifer to evaluate the most effective way to remove micropollutants that occur under anthropogenic influence. Due to insufficient quantities of data on the characteristics of the subsurface structure and mechanism and behavior of the studied pharmaceuticals during groundwater transport, tracer experiments can be a very effective method for the characterization and examination of pharmaceutical behavior in relation to transportation of non-reactive tracer. The implementation of tracer experiment, provide data on effective parameters and data about breakthrough curves of pharmaceuticals in relation to the breakthrough curve of non-reactive tracer, on which basis parameters of transport can be determined and used for further modeling and forecasting. Removal of pharmaceuticals occurs mainly due to biological degradation of micropollutants, while on the other hand sorption retards the transport. Quantifying the effects of biodegradation and sorption during a field tracer experiment was insufficiently studied, and therefore the implementation of tracer experiment represents very useful method to obtaining better and more precise data. This paper represents the results of field research on the location of the drainage system Kovin Dubovac, during which tracer tests experiment was conducted and behavior of selected pharmaceuticals was monitored (Trimethoprim, Carbamazepine, Diclofenac and metamizole metabolites 4-AAA and 4-FAA). Aim of this paperwork is to show and analyze the results of tracer experiment during which the tracer NaCl was discharged, and to correlate the obtained data on the characteristics of the subsurface, as well as the results of the breakthrough curve of selected pharmaceuticals so the effects of sorption and biodegradation can be quantified.

Keywords
Tracer test; transport; pharmaceuticals; field experiment

INTRODUCTION

In Intergranular aquifers simulation and prediction of groundwater flow and transport of substances in this case pharmaceuticals require a detailed knowledge of the nature and spatial distribution of aquifers. In most cases the above data may not be easy to collect and require detailed research and implementation of experiments. Pharmaceuticals belong to the group of the emerging contaminants in the environment and tracer test and monitoring of their behaviour was not conducted in Republic of Serbia. Dimkić conducted tracer tests and monitored behaviour for heavy metals and phenols near Žičko Polje like it is described in the literature (Dimkić, 2008). Basic need for conducting tracer test was the lack of experimental data on the behaviour of pharmaceuticals in groundwater.

Tracer test represent very powerful tool and method for characterization of subsurface. There is a large variety of application possibilities: tracer test methods can be applied for classical subsurface investigation purposes yielding effective transport parameters (transport velocity, porosity, and dispersivity), which may describe nonreactive as well as reactive (contaminant) transport processes within an aquifer like it is case for this study. Information on subsurface structure (preferential flow paths, and structural anisotropy) can be obtained as well (Käss, 1998). Tracer tests provide a database (tracer breakthrough curves and their statistical moments respective derived transport parameters) too, which can be applied for testing of forward transport predictions obtained from deterministic or stochastic model approaches, for the reduction of prediction uncertainty within stochastic modeling frameworks, or for the development and application of inverse stochastic flow and transport modeling methods (Ptak, 2004).

This tracer test was conducted under forced gradient conditions, induced by groundwater pumping because of the experimental duration and other limiting factors. In this test convergent flow field approach was used and groundwater was pumped out of an extraction well, the tracer was injected continuously, over a limited period in the injection piezometer, and breakthrough curves are measured at the extraction location based on example (Ptak and Schmid, 1996). As a tracer in this case NaCl was used because of many practical reasons: nontoxic, available and affordable, good solubility for injection, low detection limits, low natural background concentrations, negligible effect on transport properties (density, viscosity, pH, etc.), stable or well-characterized low and very slow degradation, thermal or radioactive decay acceptable and no sorption processes on tracer substance. During this experiment concentration of selected pharmaceuticals (Trimethoprim, Carbamazepine, Diclofenac and metamizole metabolites 4-AAA and 4-FAA) on extraction well was monitored and after that sampling program and analysis was established so the breakthrough curve could be defined and obtained.

MATERIALS AND METHODS

Experimental setup

During tracer test, nonreactive tracer NaCl and reactive pharmaceuticals were injected in injection piezometer Bp-2/P6, approximately 8,5 meters from monitoring well Bp-2 and 20 meters depth. In monitoring well peristaltic pump was set up with a constant flow. Also monitoring of groundwater levels was conducted with CERA divers. Flow was monitored with Thompson overflow, volumetric method and ultrasonic flow meter Nivus PCM Pro.

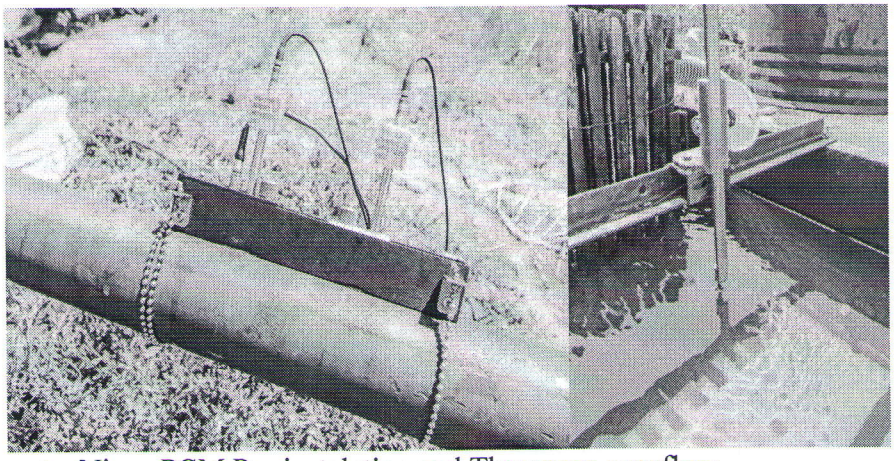


Figure 1. Flometer Nivus PCM Pro instalation and Thompson overflow

Tracer NaCl was mixed and dissolved in 1000 liters of water extracted from monitoring well Bp-2 and pharmaceuticals obtained from Sigma Aldrich were dissolved in 1 liter of methanol and after that mixed with 100 liters of water and injected in same piezometer as tracer NaCl. In monitoring well peristaltic pump was set up with a constant flow of 6 Ls^{-1}. During the experiment only specific conductivity was monitored as a parameter through which accompanied the concentration of tracer NaCl and for reactive compounds samples were collected periodically and frozen for further investigation.

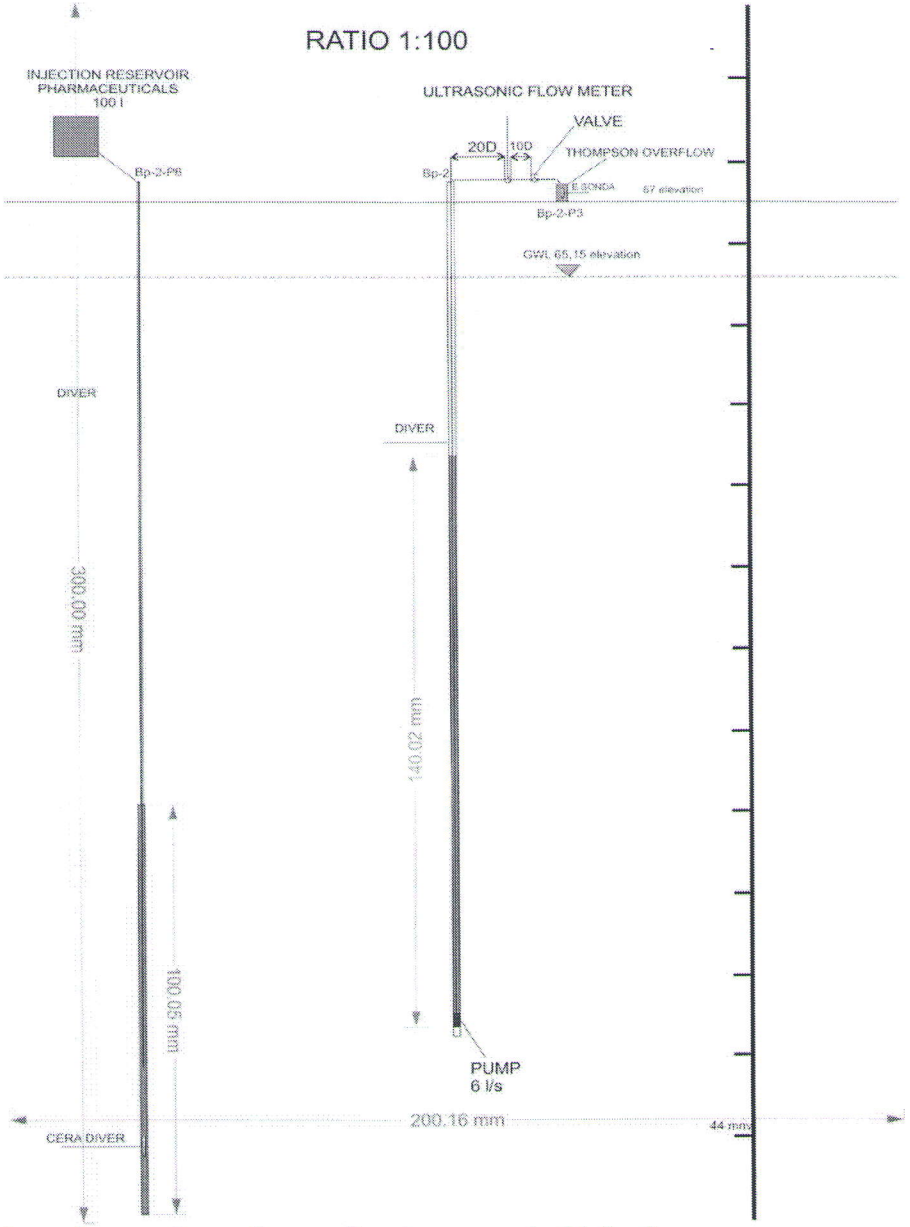


Figure 2. Schematic experimental setup for pharmaceutical injection

Experimental Methods

New boreholes

Drilling of new monitoring well - piezometer was performed by classical method with the use of flasks. During drilling, water was collected from each three meters, and then composite sample was extracted from pelit fractions for further examination.

Each core was packed into crates and transferred to the Jaroslav Černi Institute for the Development of Water Resources for further investigation and mapping and taking samples for detailed analysis. The purpose of the drilling was to form injection piezometer near drainage well Bp-2 on Kovin Dubovac drainage system where experiment took place. Figure 3 shows the gran size of sample from water bearing layer and core of the sample. Determination of particle size distribution curve was conducted using the method of dry sieving -Standards SRPS. U.B1. 018. Particle size distribution is determined by sieving materials using sets of sieves according to JUS-u L.J9.010 with the following openings in mm: 0,063; 0,090; 0,125; 0,250; 0,500; 0,710; 1,0; 2,0; 4,0; 8,0; 11,2; 16,0; 22,4; 31,5; 63,0; 125,0. Based on the data obtained by sieving analysis calculated filtration characteristics investigative through use of empirical equations, where $d_{ef}=d_{10}$

$$K = d_{ef}^2 \quad (1)$$

According to obtained particle size distribution curve filtration coefficient was calculated and K value is 7 x10^{-4} ms^{-1}.

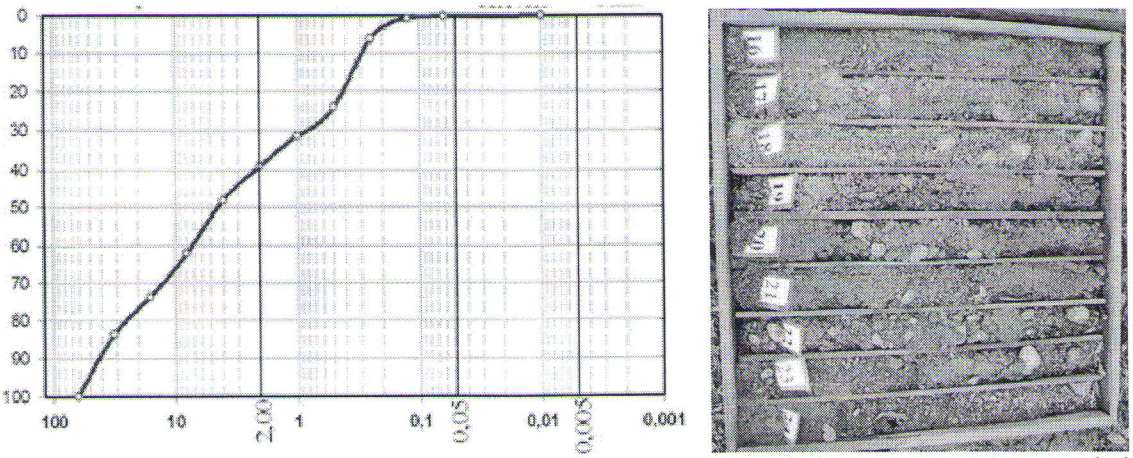


Figure 3. Sample core and particle size distribution curve for sample between 16-24 meters below surface

Sampling

All groundwater samples for pharmaceutical analysis were collected in 1-L plastic bottles from outflow of well and kept refrigerated without preservatives until they were processed, usually few days after sampling. Once prepared collected samples were kept at 4° C until arrival to the laboratory and processed within 48 h.

Sample Analysis

"In situ" monitoring was carried out with the probe immersion at the outflow of the well and electric conductivity was monitored continuously during the experiment with "HQ40d" multi parameter probe. Before experiment started baseline quality was monitored in order to determine the initial state. Based on the results average electric conductivity before experiment started was 649 μScm^{-1}. Previously developed multi residual method for analysis of most commonly used pharmaceuticals and two metamizole metabolites was employed (Grujić et al., 2009). Water samples were prepared with method of extraction on solid phase which was developed on Faculty of Technology and Metallurgy (TMF) in the Laboratory for the mass spectrometry at the department for the Analytical Chemistry and Metallurgy (TMF) in Belgrade. This method comprises purification of the sample, as well as traces of the extraction and before concentrating traces of pharmaceuticals. Calibration was performed using the standard addition method.

Surveyor LC system (Thermo Fisher Scientific, Waltham, MA, USA) was used for the separation of the analytes on reverse-phase Zorbax Eclipse® XDB–C18 column, 75mm×4.6mm ID and 3.5μm particle size (Agilent Technologies, Santa Clara, CA, USA). A pre-column, 12.5mm×4.6mm ID and 5μm particle size (Agilent Technologies), was also used. Mass spectra were obtained using the LCQ Advantage quadrupole ion trap mass spectrometer with electrospray ionization technique (Thermo Fisher Scientific). All compounds were analyzed in the positive ionization mode. For the LC–MS^2 analysis of pharmaceuticals and pesticides in the positive ionization mode, the mobile phase was composed of methanol (A), deionized water (B) and 10% acetic acid (C), and gradient was changing as follows: 0.0min, A 14.5%, B 85%, C 0.5%; 35.0 min, A 99.5%, B 0.0%, C 0.05%; 46.0 min, A 99.5%, B 0.0%, C 0.5%. The initial conditions were re-established and held for 10 min. The flow rate of the mobile phase was 0.6mlmin^{-1}. The injection volume was 10μl. The optimal source working parameters for monitoring all ions were as follows: source voltage (4.5 kV), sheath gas (25 au, i.e. 25 arbitrary units, from a scale of arbitrary units in the 0–100 range defined by the LCQ Advantage system) and capillary temperature (290 °C).

Table 1. Physico-chemical characteristics of the investigated compounds

Compounds	log K_{ow} (25°C)	Water Solubility (mg L^{-1}) (25°C)	pKa	K_{oc} [b] (mL g^{-1})	Sources
Trimethoprim	0.91	2334	7.1	905	a, b
4-FAA	0.5	11300	–	63.6	a
4-AAA	–0.13	1590	–	240.7	a
Carbamazepine	2.45	17.7	2.4	3871	a. b
Diclofenac	4.51	4.5	4.1	833.3	a, b

[a]The e–Chemical Compound Database, http://www.chemspider.com;
[b]The e–Hazardous Substances Data Bank, http://www.toxnet.nlm.nih.gov;

Table 2 shows limits of detection and quantfication. The values shown in the table below were obtained experimentally by recording standard low concentrations.

Table 2. Limit of qantification (LOQ) for analysed pharmaceuticals and pesticides

Analytes	LOQ (ngL^{-1})
Trimethoprim	5
Carbamazepine	5
Diclofenac	15
4-formylamino antipirine(4–FAA)	15
N-acetyl-4-aminoantipyrine (4–AAA)	5

REASULTS AND DISCUSSION

After successful dissolution of NaCl in the 1000 liter tank for injection, injection of tracer NaCl was started at 15:07 and it lasted 52 minutes. Average specific electric conductivity in the injection reservoir was 74.3 mS / cm. At the beginning of experiment 50 kg of NaCl was dissolved in the reservoir. The measured value shows that the amount of pure NaCl, which is poured into the reservoir, was 35.7 kg, calculated on the basis of dependence diagrams between electrical conductivity and concentrations of NaCl.

Next picture shows the breakthrough curve for the tracer NaCl. Maximum concentration of tracer NaCL was established after 201 minutes from beginning of experiment which is consistent with the data obtained by analysing the filtration properties of the aquifer and average filtration coefficient of $7x10^{-4}$ ms^{-1}.

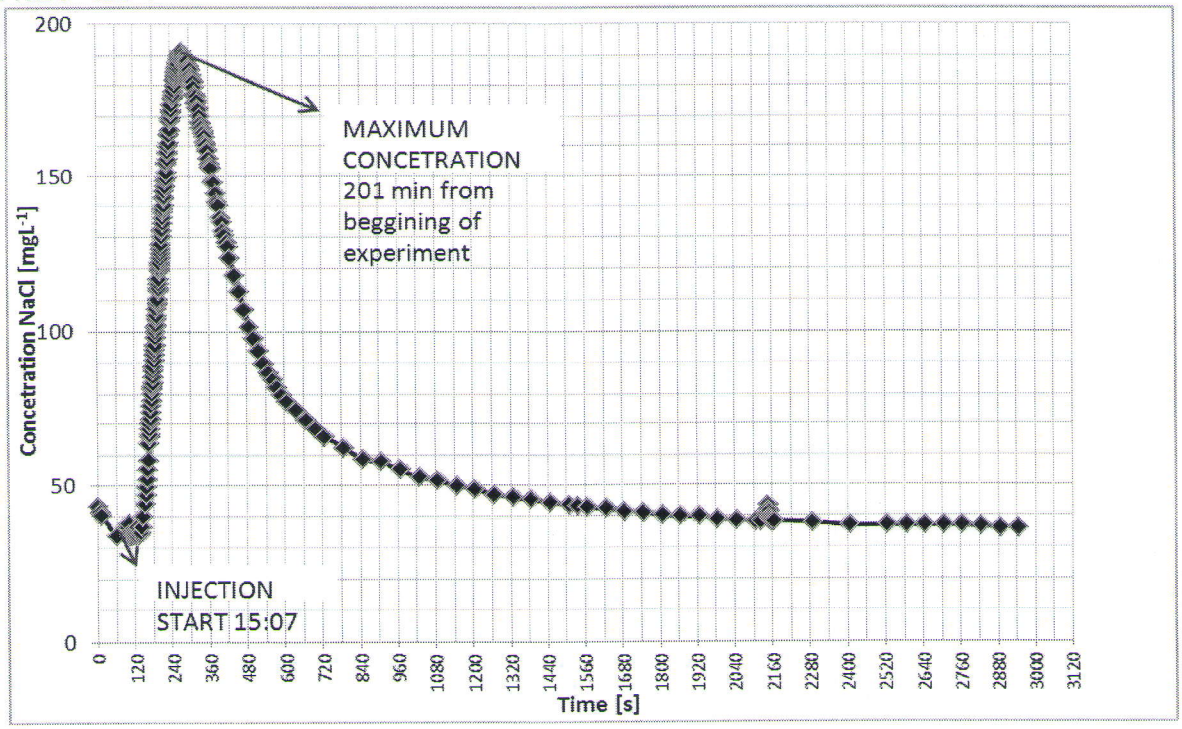


Figure 4. Tracer NaCl breakthrough curve

After successful injection of tracer NaCl, injection of pharmaceutical started. Initial concentrations in injection reservoir of 100 litres were: Trimethoprim 2.5 mgL^{-1}, Carbamazepine 1 mgL^{-1}, Diclofenac 1 mgL^{-1}, metamizole metabolites 4-formylamino antipirine (4–FAA) 0.1 mgL^{-1} and N-acetyl-4-aminoantipyrine (4–AAA) with concetration of 1 mgL^{-1}. After injection which lasted continuously about 36 minutes, sampling started based on the established program. Next diagram shows the representative breakthrough curve for Diclofenac. Based on the results obtained from the experiment we can conclude that for example Diclofenac reached maximum concentration after 315 minutes or that the delay of Diclofenac with respect to tracer NaCl was approximately 1.56 times. Based on the following equation we can calculate Kd for all pharmaceuticals.

$$Rd = \frac{v_{NaCl}}{v_{pharmaceutical}} = 1 + \frac{\rho_b}{n} Kd \qquad (2)$$

where *Rd* is Retardation coefficient; v_{NaCl} is the velocity of tracer NaCl; $v_{pharmaceutical}$ is velocity of pharmaceutical; ρ_b is bulk density ; *n* is porosity; *Kd* is the partition coefficient.
The next table shows data calculated from the experimental results for Kd.

Table 3. Partition coefficient for analysed pharmaceuticals

Analytes	Kd [cm^3g^{-1}]
Trimethoprim	0.36-1.63
Carbamazepine	0.15
Diclofenac	0.1
4-formylamino antipirine(4–FAA)	0.4
N-acetyl-4-aminoantipyrine (4–AAA)	0.15

According to results of tracer experiment velocities for tracer NaCl and pharmaceuticals were calculated, other data (n-porosity and ρ_b – bulk density was calculated from grain size curve and theoretically.

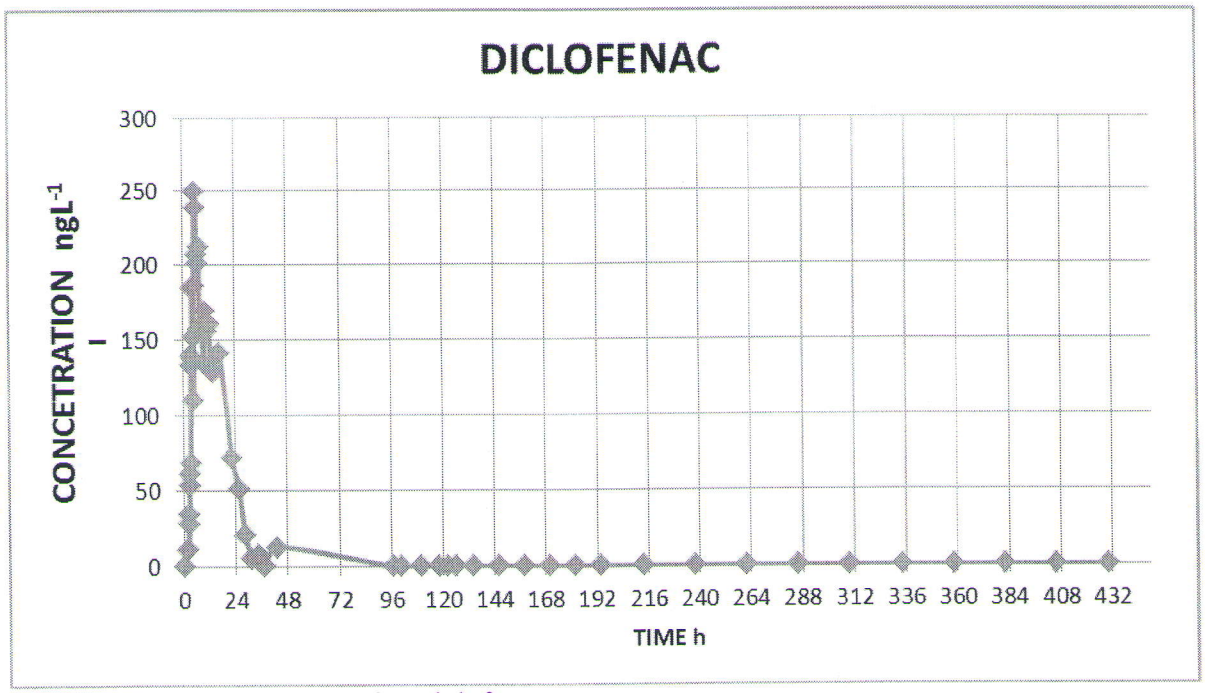


Figure 5. Breakthrough curve for Diclofenac

On the other hand the degradation is calculated based on the amount of matter that was lost after the implementation of the experiment, and based on the budget balance of selected pharmaceuticals during the experiment. Results for budget balance were calculated using next equation

$$m = (T_{n+1}+T_n)/2) \cdot (C_{n+1}-C_n) \cdot Q \cdot 60s \quad (3)$$

where m - is mass of the pharmaceutical, T_{n+1} and T_n is time of sample collection and C_{n+1} and C_n is concentration after time T_n and T_{n+1}, Q – is average flow at the outlet of well.

After the calculation of the remaining mass of pharmaceuticals after experiment, degradation time can be calculated. Next table shows the results of degradation time for selected pharmaceuticals.

Table 4. Degradation constant for analysed pharmaceuticals

Analytes	Half life [day]
Trimethoprim	11.6
Carbamazepine	27.7
Diclofenac	31.3
4-formylamino antipirine(4–FAA)	75
N-acetyl-4-aminoantipyrine (4–AAA)	20

CONCLUSIONS

Based on the results of tracer experiment sorption capacity can be easily calculated of selected pharmaceuticals under experimental conditions. It is very important that sorption of selected pharmaceutical with relatively small calculated partition coefficients compared to the literature data rep-

resent a very important factor that influences the behavior of pharmaceuticals in groundwater during filtration.

On the other hand many literature data gave the results for laboratory experimental conditions and the mentioned results cannot be applied in practice when calculating real transport parameters Groundwater represents a huge bioreactor in which numerous processes taking place in different conditions so even with a very small extension of transport time it seriously affects the removal of these compounds in groundwater and in this way it is possible to make a concept to protect existing and future drinking water sources and health of people.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, under the Project No. TR 37014 and project III 46009.

REFERENCES

Dimkić, M., Brauch, H.J., Kavanaugh, M., Groundwater management in large river basins. IWA Publishing, Alliance House, 12 Caxton Street, London SW1H 0QS, UK.

Grujić, S., Vasiljević, T., Laušević, M., (2009) Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography–ion trap–tandem mass spectrometry. *Journal of Chromatography A*, 1216, 4989–5000.

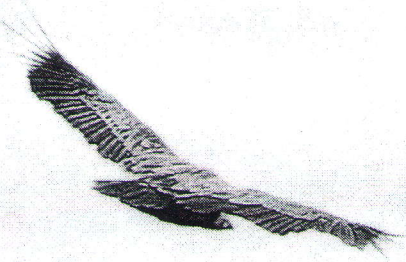
Käss, W., (1998) Tracing Techniques in Geohydrology, A.A. Balkema, Rotterdam.

Ptak, T., Schmid, G., (1996) Dual-tracer transport experiments in a physically and chemically heterogeneous porous aquifer: Effective transport parameters and spatial variability. *Journal of Hydrology,* 183(1-2), 117–138.

Ptak, T., Piepenbrink, M., Martac, E., (2004) Tracer tests for the investigation of heterogeneous porous media and stochastic modelling of flow and transport—a review of some recent developments. *Journal of Hydrology* 294 122–163.

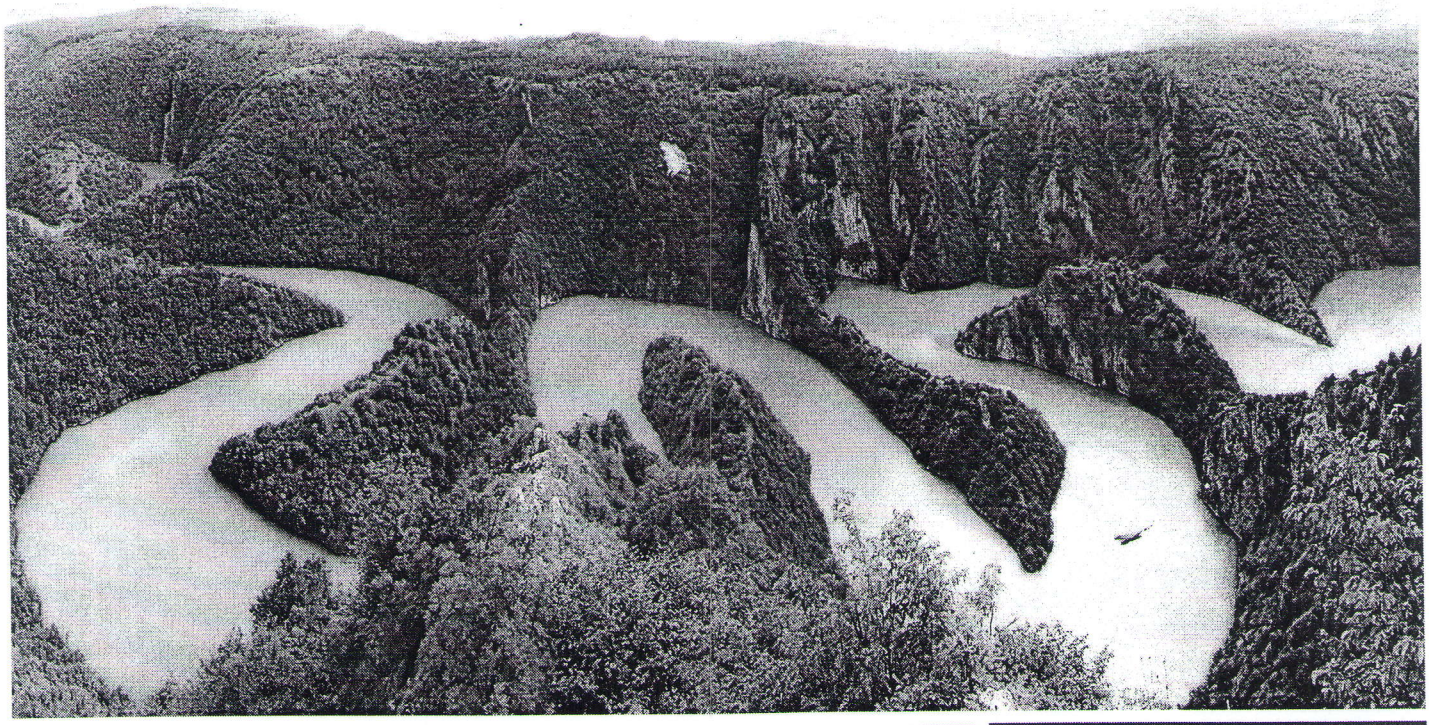
7th Eastern European Young Water Professionals Conference

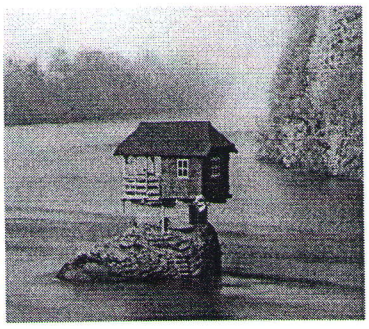
the international water association

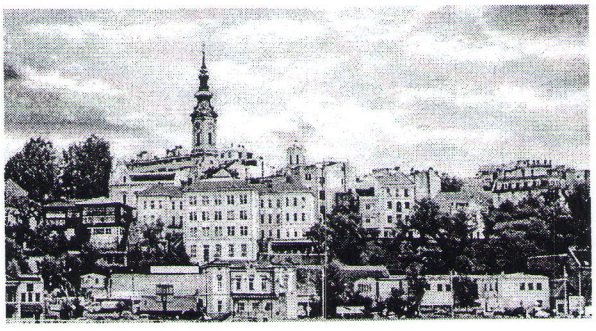


Conference Proceedings

17-19 September 2015
Belgrade, Serbia
www.eu-ywp.pwr.wroc.pl







Organised by:

UNESCO-IHE Institute for Water Education

Faculty of Civil Engineering University of Belgrade

Jaroslav Černi Institute for the Development of Water Resources

University of Novi Sad

Wroclaw University of Technology

IWA YOUNG WATER PROFESSIONALS
the international water association

Proceedings of the IWA 7th Eastern European Young Water Professionals Conference Belgrade, 17-19th September, 2015 - 654p.

Organising Committee

Chairman: Maryna Feierabend
Mob.:+32 492 27 19 34
E-mail: iwa.ywp.2015@gmail.com

Committee members:

Zeljka Rudic (Serbia)
Olga Tiron (Romania)
Anja Randelovic (Serbia)
Jürgen Heinrichmeier (Germany)
Arlinda Ibrahimllari (Albania)
Dr. Jakub Drewnowski (Poland)
Dr. Olha Novytska (Ukraine)
Dr. Malgorzata Szlachta (Poland)
Ina Bulskaya (Belarus)
Dr. Brankica Majkić-Dursun (Serbia)
Dr. Vesna Tripkovic (Serbia)
Dr. Jelena Molnar Jazić (Serbia)
Dr. Ivana Mihajlovic (Serbia)
Maja Djogo (Serbia)
Milena Stosic (Serbia)
Dr. Branislava Lekic (Serbia)
Dr. Florin Iliescu (Romania)
Dr. Edip Avsar (Turkey)

Programme Committee

Chairman: Assoc. Prof. Dr. Nemanja Trifunovic
Tel.: +31 152 15 18 58
E-mail: n.trifunovic@unesco-ihe.org

Committee members:

Dr. Stojanovic Z. ("Jaroslav Cerni", Serbia)
Prof. Dr.Vaseashta A. (Institute for Advanced Sciences Convergence & Clean Water Institute, USA)
Prof. Pupyrev E. (MosvodokanalNIIProekt, Russia)
Mr. Foerster G. (WTE, Germany)
Dr. Wójtowicz P. (Wroclaw University, Poland)
Dr. Vakuliuk P. (National University of Kyiv-Mohyla Academy, Ukraine)
Prof. Dr. Gogina E. (MGSU, Russia)
Dr. Bumbac C. (ECOIND, Romania)
Dr. Maletic S. (University of Novi Sad, Serbia)
Prof. Dr. Agbaba J. (University of Novi Sad, Serbia)
Dr. Roncevic S. (University of Novi Sad, Serbia)
Prof. Dr. Vojinovic Miloradov M. (University of Novi Sad, Serbia)
Prof. Dr. Radnovic D. (University of Novi Sad, Serbia)
Prof. Dr. Simeunovic J. (University of Novi Sad, Serbia)

Organising Committee

Chairman: Dr. Maryna Feierabend

Mob.:+32 492 27 19 34
E-mail: iwa.ywp.2015@gmail.com

Committee members:

Zeljka Rudic (Serbia)
Olga Tiron (Romania)
Anja Randelovic (Serbia)
Jürgen Heinrichmeier (Germany)
Arlinda Ibrahimllari (Albania)
Dr. Jakub Drewnowski (Poland)
Dr. Olha Novytska (Ukraine)
Dr. Malgorzata Szlachta (Poland)
Ina Bulskaya (Belarus)
Dr. Brankica Majkić-Dursun (Serbia)
Dr. Vesna Tripkovic (Serbia)
Dr. Jelena Molnar Jazić (Serbia)
Dr. Ivana Mihajlovic (Serbia)
Maja Djogo (Serbia)
Milena Stosic (Serbia)
Dr. Branislava Lekic (Serbia)
Dr. Florin Iliescu (Romania)
Dr. Edip Avsar (Turkey)

Programme Committee

Chairman: Assoc. Prof. Dr. Nemanja Trifunovic

Tel.: +31 152 15 18 58
E-mail: n.trifunovic@unesco-ihe.org

Committee members:

Dr. Stojanovic Z. ("Jaroslav Cerni", Serbia)
Prof. Dr. Vaseashta A. (Institute for Advanced Sciences Convergence & Clean Water Institute, USA)
Prof. Pupyrev E. (MosvodokanalNIIProekt, Russia)
Mr. Foerster G. (WTE, Germany)
Dr. Wójtowicz P. (Wroclaw University, Poland)
Dr. Vakuliuk P. (National University of Kyiv-Mohyla Academy, Ukraine)
Prof. Dr. Gogina E. (MGSU, Russia)
Dr. Bumbac C. (ECOIND, Romania)
Dr. Maletic S. (University of Novi Sad, Serbia)
Prof. Dr. Agbaba J. (University of Novi Sad, Serbia)
Dr. Roncevic S. (University of Novi Sad, Serbia)
Prof. Dr. Vojinovic Miloradov M. (University of Novi Sad, Serbia)
Prof. Dr. Radnovic D. (University of Novi Sad, Serbia)
Prof. Dr. Simeunovic J. (University of Novi Sad, Serbia)

Sponsors

Gold Sponsor - company WTE (Germany)

wte
EVN Group

Sponsor: MosvodokanalNIIProject

YWPs (six previous IWA YWP Eastern Europe Conferences)

YOUNG WATER PROFESSIONALS

IWA: Alliance House • 12 Caxton Street • London SW1H 0QS • United Kingdom
Tel: +44 (0) 20 7654 5500 • Fax: +44 (0) 20 7654 5555 E-mail: water@iwahq.org • Website: www.iwahq.org •
Registered in England No.3597005 • Registered Charity (England) No.1076690

Kostić D., Blagojevic A., Subakov Simic G., Predojevic D., Naunovic Z., Jacimovic N., Grasic S.
"Blooming" Reservoir Response to a High Inflow Event - Case Study: the Vrutci Reservoir (Western Serbia) **97**

Kovačević S., Živančev N., Perović M., Bežanović V., Vojt P., Petković A., Dimkić M.
Tracer Test and Monitoring of Behavior and Transport of Selected Pharmaceuticals **105**

Lavrnić S., Mancini M.L.
Water Scarcity and Wastewater Reuse Standards in South Europe: Focus on Agriculture **113**

Lazić G., Grubač S., Bugarski D., Lupulović D., Lazić S., Knežević P., Petrović T.
Detection of Viruses in Water Environments in Serbia **122**

Majkić-Dursun B., Tončić J., Petković A., J. Čolić
Redox Conditions and Groundwater Quality Issues in Selected Alluvial Aquifers in Serbia **130**

Milišić H.
Transport Processes in Rivers Modelling Using Field Experimental Data **138**

Murenji S., Maletić S., Agbaba J., Rončević S., Tubić A., Molnar Jazić J., Kragulj-Isakovski M., Dalmacija B.
Anaerobic Degradation of Hydrocarbons from Aquatic Sediment with Sulphate as an Electron Acceptor **146**

Nikić J., Agbaba J., Watson M., Maletić S., Molnar Jazić J., Dalmacija B.
Adsorption Mechanism of As(V) and As(III) on Fe–Mn Binary Oxides in Synthetic and Real Water Matrices **153**

Olenici A., Momeu L., Tornes E., Baciu C.
Morphological Abnormalities of Benthic Diatom Communities from Mining-Impacted Abrud River (Rosia Montana, Romania) **162**

Petrović Pantić T., Veljković Ž., Samolov K.
Creating the Basic Hydrogeological Maps (BHGM) with Purpose of Managing the Groundwater Resources in Serbia **173**

Poguberović S.P., Krčmar D.M., Dalmacija B.D., Maletić S.P., Tomašević-Pilipović D.D., Kerkez Dj.V., Rončević S.D.
Removal of Ni(II) and Cu(II) from Aqueous Solutions using "Green" Zero-Valent Iron Nanoparticles Produced by Oak and Mulberry Leaf Extracts **179**

Randelovic A., Prodanovic V., Jacimovic N., McCarthy D., Deletic A.
Assessing Uncertainty of a Water Quality Model for a Stormwater Biofiltration Treatment System **188**