DELINEATION OF THE MAIN CONDITIONS AFFECTING NITROGEN FORM IN SELECTED ANOXIC ALLUVIAL AQUIFERS IN SERBIA

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Abstract: Nitrogen complex chemistry is reflected in several oxidation states (from -3 to +5) in which nitrogen can persist depending on prevailing environmental biogeochemical conditions. The oxidation state of the elements in groundwater will preferably be determined by dissolved oxygen concentration and redox potential, followed by concentration and availability of present organic matter, nitrates, fero/manganese and sulfate ions, and dominant species of microorganisms. The aim of the paper is distinguishing which parameters are the main controllers of dominant reactions and final form of nitrogen present in anoxic alluvial aquifers, regardless of nitrogen origin. Research was conducted for selected drainage wells from Knićanin – Čenta (Danube and Tisa river), Kovin – Dubovac (Danube river) and selected wells from Belgrade groundwater source (Sava River). Parameters such as Eh, O_2, TOC, Fe^{2+}, H_2S concentrations and BART test results were all analyzed. The obtained results were used to define prevailing nitrogen form and dominant nitrogen transformation reactions, indicating different anoxic aquifers potential for nitrogen loss (N_2, N_2O) or conservation (NH^{4+}).The conclusion is that in anoxic alluvial groundwaters which were the subject of this research prevailing controlling factors are the concentration of total organic carbon (TOC), followed by presence of ferrous iron and sulfide, while C:N ratio would determine whether the nitrogen is subject of loss (N_2O, N_2) or conservation (NH^{4+}).

Keywords: nitrogen transformation, anoxic groundwater, BART test

INTRODUCTION

The amount of nitrogen present in soil, surface and groundwaters, is mainly affected by the lack of sewage systems, agricultural practice (fertilizer and manure application), increased cultivation of nitrogen fixing plants, and incorporation of residues. The main pathways of nitrogen enrichment of surface and groundwater are surface runoff, soil erosion and leaching. Coarse-textured soils and unconfined aquifers are usually at higher risk of NO_3^- leaching, although excessive rainfall or abundant watering can cause nitrates displacement beyond the root zone from any soil type. Nitrogen can be immobilized either by abiotic or biotic processes, where most of the immobilization to the soil organic matter is biotic (Bengtsson et al., 2003). Generally an accepted hypothesis is a positive relationship between the C:N ratio and rapid N immobilization and a negative relationship between the C:N ratio and N mineralization and nitrification. Bengtsson et al. (2003), suggest that soil C:N ratio may prove useful to predict site-to-site variations of the N transformations, while temporal variation of soil temperature and moisture will trigger dynamics of microbial biomass and activity that influence N transformations more than the spatial variation of the C:N ratio. The presence of sulfur as sulfate and thiosulfate has been shown to inhibit denitrification in soils, with the rate of denitrification negatively correlated to the sulfate (or thiosulfate) concentration (Kowalenko, 1979). In soil, sulfide has been shown to promote dissimilatory reduction of nitrate to ammonium (DNRA) rather than denitrification (Hiscock et al., 1991). Nitrate mobility in oxic environment is usually described almost as tracer's mobility, due to the stability in oxic environments and the inability of negatively charged ions to sorb to the sediment and soil particles. In anoxic, reducing conditions, nitrogen will be subject of reductive transformations: assimilation into biomass, respiratory denitrification, anaerobic ammonium oxidation (annamox), and dissimilatory nitrate reduction (DNR), usually associated with ammonium production (DNRA). Nitrate reduction processes as well as stoichiometry reactions in reductive groundwater are presented in Fig 1.

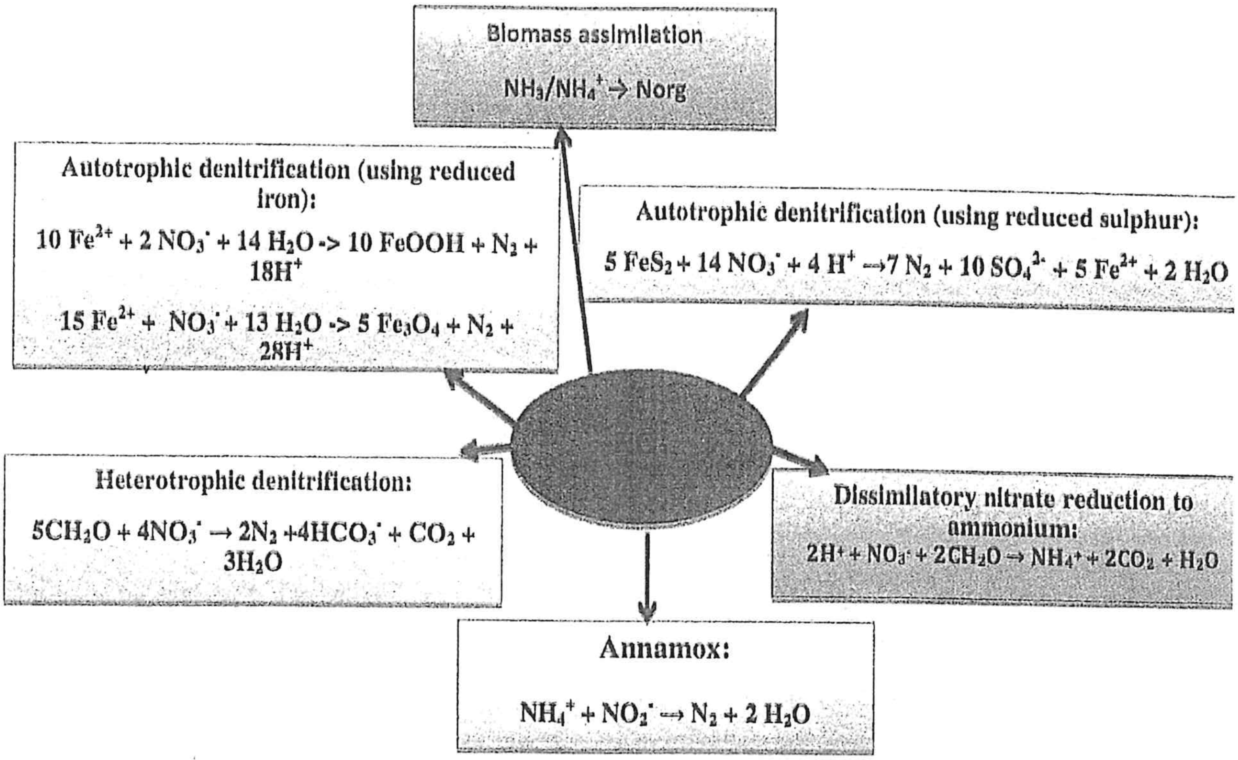

Figure 1: Illustration of possible nitrate transformation processes in reductive environment- red color denotes N conservation, while green color denotes nitrogen loss (N gasses production and loss to atmosphere)

MATERIALS AND METHOD

Drainage system Knićanin-Čenta

This drainage system is situated in the alluvial formation at the confluence of the Begej River into the Tisa River and Tisa River into the Danube. Medium-grain sands are dominant in the central and upper parts of the aquifer, while the lower part features sandy and fine-grain gravels (Majkić-Dursun et al., 2015). The base of the aquifer is made of clays while the top sediments are semi-permeable silty clays (Majkić-Dursun et al., 2015). For the definition of dominant nitrogen form, 4 facilities at the Tisa riverbank, and 3 facilities in Danube alluvial sediments were chosen. Lack of sewage network and intensive agricultural production are considered as the main sources of ammonium and nitrates in Knićanin-Čenta and Kovin-Dubovac alluvion.

Drainage system Kovin-Dubovac

Kovin-Dubovac is an alluvial plain of the left coast of Danube river between two settlements, Kovin and Dubovac, located in the southern part of the Banat depression. The deeper layers of the Danube's alluvial plain are composed of gravels and sandy gravels, with some cobbles even over 120 mm in diameter, with medium-grained sands, finely-grained silty sands, coal, alevrites and clay layers at their base. The upper part consists of semi-permeable sediments - silty sands, alevrites, silty clays and clay. In this paper, physicochemical and microbiological results are presented for 20 wells and piezometers situated at locations across the three drainage lines in Danube alluvial sediments.

Belgrade groundwater source

The Belgrade groundwater source is located along the Sava's river bank upstream from its confluence with the Danube. The Sava River alluvial formation was formed during several sedimentation cycles and sequences, and is comprised of: sandy gravels, sands of various grain sizes, and silty and clayey sediments (Majkić-Dursun et al., 2015). According to Dimkić and Pušić (2014) lower zone is consisting of coarse-grain sediments in which radial well laterals are installed. Occasionally clay, sandy clay and silt discontinuous beds and lenses may occur. The upper part consists of fine-grain less permeable sediments. In this paper, chemical and microbiological analyses from 12 piezometers and wells are presented. Nitrogen concentration is generally low for whole groundwater source which is in accordance with its use as a drinking water source.

Presented data are analyzed only for certain facilities, which were selected by simultaneous availability of chemical and microbiological data together. The data were collected with different frequency for the purposes of elaboration of different studies, under the Project No. TR37014 in the period 2010-2015 year.

In order to determine what are the relations existing among key factors affecting the final form of nitrogen in anoxic alluvial groundwater regardless of nitrogen origin, a comparative analysis of physicochemical and microbiological results for three alluvial aquifers with anoxic groundwater – Knićanin-Čenta, Kovin-Dubovac and Belgrade groundwater source, in R. Serbia, has been conducted. In-situ measurement of Dissolved Oxygen and Redox potential (Eh) were conducted using Multiparameter WTW 197i probes (Germany). Nitrate, nitrite and ammonium were analyzed using spectrophotometric methods, whereas volumetric method was used for sulfide. Detailed descriptions of the applied analytical methods are outlined in APHA (2005). TOC (total organic carbon) was quantified using Hiper TOC analyzer Thermo Scientific (United Kingdom). Performed BART tests (Hach Company, United States) have enabled detection of the following bacteria groups: Iron Related Bacteria (IRB-BART) with Winogradsky medium, Sulfate-reducing bacteria (SRB-BART) with Postgate C medium and Denitrifying bacteria (DN-BART) with nitrate-pepton medium. Defining present environmental groups of bacteria has enabled the estimation of current microbiological status of groundwater and potential transformation processes of nitrogen compounds. The correlation between the day, reaction type and the number of active cells in this study has been obtained by software QuicPop software 1.3 (Cullimor, 2010).

Location		Knicanin-Centa	Kovin	Belgrade water source
Sampling period (year)		2010-2012	2010-2015	2010-2015
Number of samples		22	125	61
Number of examined facilities		7	20	12
O_2 (mg/l)	min	<0.1	<0.1	<0.1
	max	1.6	2.35	2.17
	Avg.	0.2	0.15	0.3
Eh (mV)	min	23.5	20	48
	max	273	279	246.6
	Avg.	136.5	98.2	105.7
TOC (mgC/l)	min	1.2	1.1	1.1
	max	7.8	5.23	1.9
	Avg.	3.3	2.4	1.4
Fe^{2+} (mg/l)	min	0.18	<0.1	0.11
	max	3.7	5.41	4.13
	Avg.	1.1	1.82	1.4
H_2S (mg/l)	min	<0.02	<0.02	<0.02
	max	0.14	0.75	0.02
	Avg.	0.06	0.05	<0.02

Table 1. Average values of selected parameters for examined facilities affecting N-transformation

Information about the final compound of nitrogen resulting from transformation in water is important for two main reasons: 1) health and environmental hazard and 2) indication of aquifer potential for nitrogen conservation or loss. Acute or chronic ingestion of water with elevated nitrate concentration may exhibit various forms of harmful effects (Camargo and Alonso, 2006; Almasri and Kaluarachchi, 2004; Wolfe and Patz, 2002). Ingested nitrites and nitrates can induce methemoglobinemia in humans and have a potential role in developing cancers of the digestive tract through their contribution to the formation of nitrosamines (Wolfe and Patz, 2002). Some scientific evidences suggest that ingestion might result in mutagenicity, teratogenicity and birth defects, contribute to the risks of non-Hodgkin's lymphoma and bladder and ovarian cancers, etc. (Camargo and Alonso, 2006). Nitrate may indicate the presence of bacteria, viruses, and protozoa in groundwater if the source of nitrate is animal waste or effluent from septic systems (Almasri and Kaluarachchi, 2004).

RESULTS AND DISCUSSION

Rivett et al. (2008) suggested that after qualifying the environment (groundwater) based on redox value and oxygen concentration as reductive one, next influential factor in terms of nitrate transformation is the organic C concentration (in the paper TOC). In graphs (Fig.3, Fig.4, Fig. 5,) the parameters whose changes can be correlated to nitrogen transformation pathways are presented. In the case of high C content and sulfidic

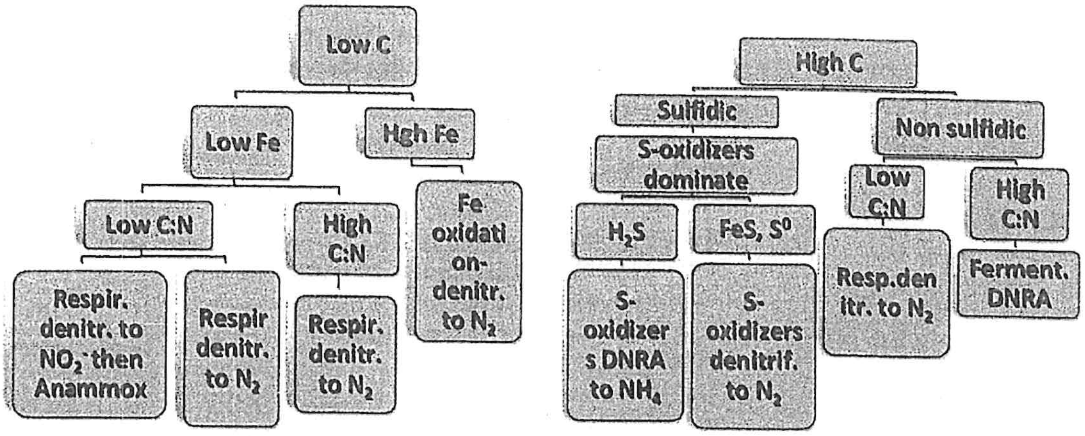

Figure 2: Illustration of the main pathway of nitrogen transformation and main conditions affecting dominant transformation. C:N ratios refer to the ratio of total organic carbon to nitrate (modified, Burgin and Hamilton, 2007)

waters (Knićanin-Čenta) correlation between parameters TOC, NH^{4+}, NO_3, Fe^{2+} and BART test results has been observed (Fig.3 and Table 2). In the case of variable high C / low C content, sporadicly sulfidic water (Kovin-Dubovac), correlation between parameters TOC, C:N, NH^{4+}, NO_3^-, Fe^{2+} and BART test results has been observed (Fig.4 and Table 2). For the third examined location (Belgrade groundwater source), carbon and nitrogen concentrations are low, with moderate activity of iron related and denitrificating microorganisms, indicating fulfillment of conditions for nitrogen loss (N_2, N_2O) by denitrification to N_2 (Fe oxidation) and respiratory denitrification to N_2O and anammox. This is the reason why correlation between TOC, NH^{4+}, NO_3^-, Fe^{2+} is presented (Fig.5).

Knićanin-Čenta

The average values for the selected physicochemical and microbiological parameters for seven sampling sites at Knićanin-Čenta facilities have been analyzed and compared with the mutual dependence of observed changes. BART tests showed that facultative anaerobes with anaerobes are dominant microorganisms in this area. Moderate to high activity of iron reduction is observed. Very high activity of sulfate reducers and heterotrophic bacteria which indicate anaerobic mineralization of organic matter dominate in the whole area. Parralel nitrate and iron reduction is probably the consequence of constant nitrate inflow, probably as a result of the absence of sewage network and intensive agriculture. Burgin and Hamilton, (2007) and Oshiki et al., (2013) stated sulfate reducers and iron related bacteria could be responsible for NH^{4+} concentration increase in anoxic groundwaters. Many authors observed that nitrate reduction by iron oxidation results in NO_2^- product, which transforms into N_2 or NH^{4+} and Fe^{3+} (Korom 1992; Rivett et al, 2008; Oshiki et al., 2013). Low TOC concentration (to 3 mg/l) is accompanied by low ferrous concentration (<0.68 mgFe^{2+}/l) (Fig.3), which according to Fig. 2 leads to nitrogen loss, whether by denitrification or denitrification coupled to anammox. The NO_3^- decrease and NH^{4+} increase are followed by simultaneous TOC increase (Fig 3.). Increase in TOC concentration above 3 mg C/l is accompanied by ferrous concentration increase, where dominant form of

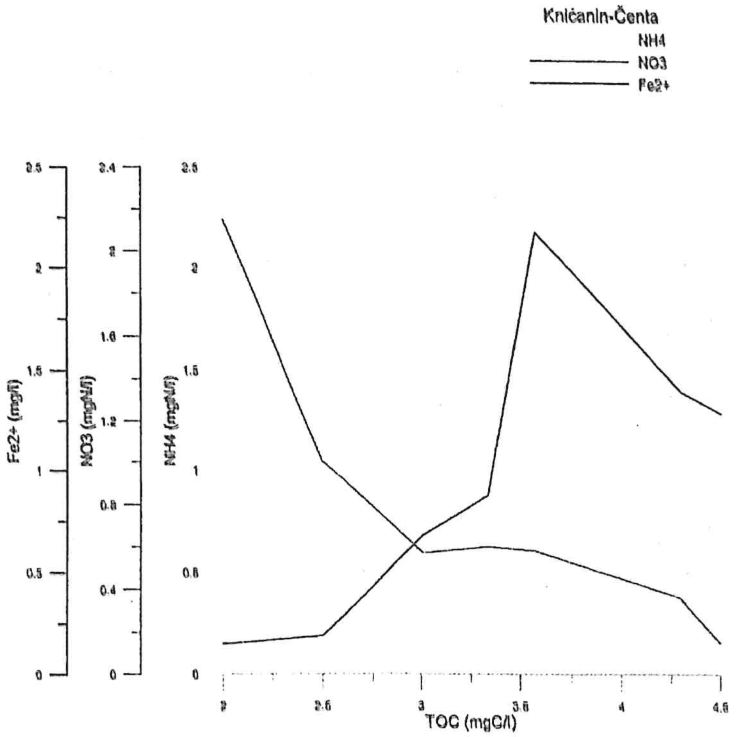

Figure 3: Comparative analysis of NH_4, NO_3 and Fe^{2+} concentration change with TOC increment in Knićanin-Čenta

nitrogen becomes NH^{4+}. NO_3 reduction to NH_4, might be attributed to secondary metabolism of sulfate reducers and DNRA process (avg. log SRB 3.41 p.a.c/ml) or even iron related bacteria (avg. log IRB 4.17 p.a.c/ml). If it is assumed that the first process might be nitrate reduction by iron oxidation (avg log IRB 4.17 p.a.c/ml), the resulting product is NO_2^-, which transforms into N_2 or NH^{4+} and Fe^{3+}. Fe^{3+} can be reduced back to Fe^{2+} in presence of organic matter. BART test results (Table 2.), and inverse dependency of NH^{4+} and NO_3 concentration might be related to TOC and Fe^{2+} increment and bacteria consortium observed in groundwater.

Location		Knicanin-Centa	Kovin	Belgrade water source
Sampling period (year)		2010-2012	2011-2012	2011-2012
Number of samples		21	27	24
Number of examined facilities		7	20	12
log p.a.c/ml IRB	min	0.30	0.90	2.14
	max	4.55	5.15	4.55
	Avg.	4.17	3.78	2.74
log p.a.c/ml SRB	min	0.90	0.90	1.0
	max	4.26	5.0	2.69
	Avg.	3.41	2.45	1.57
log p.a.c/ml DN	min	1.26	0.78	0.78
	max	4.24	4.24	4.24
	Avg.	2.92	2.38	2.20

Table 2. BART test results in log p.a.c/ml for selected facilities in Knićanin-Čenta, Kovin-Dubovac and Belgrade groundwater source

Kovin-Dubovac

For the Kovin-Dubovac area iron reduction, denitrification activity, and moderate to very high activity of sulfate reducers were observed. Examined area can be microbiologically characterized as reductive-anoxic with dominating reactions of Fe^{3+} reduction and dentrification, with observed sulfate-reduction which sporadically was very high. Based on high number of heterotrophic aerobic bacteria high concentration of organic matter is mineralized mostly under anaerobic conditions. Variably low C / high C concentrations indicate favorable conditions for both, N loss and conservation. Low C concentrations are accompanied by lower NH^{4+} concentrations. Low C concentration, according to Burgin and Hamilton (2007) might induce nitrogen loss. Sulfide concentrations are generally below detection limit (0,02 mg/l), with sporadic sulfide appearance. TOC concentration increase is followed by NH^{4+}, C:N ratio, and Fe^{2+} increase (Fig. 4). The assumption is that sporadic sulfide appearance and high C enables S-oxiders to perform their secondary metabolism of NO_3^- transformation to NH^{4+} - DNRA (max log p.a.c/ml SRB 5.0). High Fe^{2+} concentration is in accordance with intensive activity of iron reducing bacteria. When TOC is high and sulfides are absent, high C:N ratio indicates conditions for fermentative DNRA (Fig. 2.).

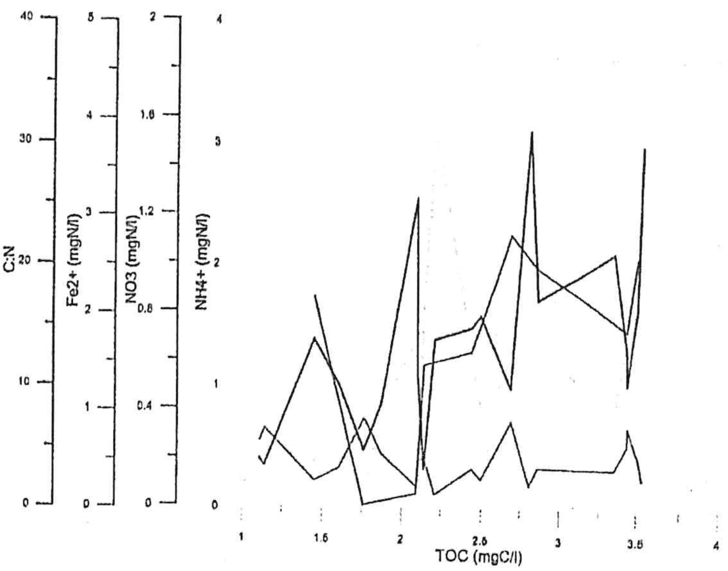

Figure 4: Comparative analysis of NH_4, NO_3, Fe^{2+} and C:N change with TOC increament in Kovin-Dubovac

Belgrade groundwater source

Generally nitrogen compounds concentrations are very low here, as well as TOC concentration. Mostly anoxic conditions, Fe^{2+} availability, low TOC concentration and mainly absence of nitrate indicate that any NO_3^- input will probably result in N loss (N_2O, N_2), rather than conservation (NH^{4+}). Out of all three sites, recorded bacteria activity in Belgrade groundwater source is the lowest (Table 2). Low Fe^{2+} concentration is accompanied by low C:N ratio, indicating conditions for respiratory denitrification to N_2O and then anammox. Low C and sporadically high Fe^{2+} will probably lead to Fe^{2+} oxidation and denitrification to N_2.

CONCLUSION

Complex transformation and transport processes of nitrogen compounds through unsaturated and saturated environment depend on mutual and oftentimes simultaneous combining of physicochemical-biological transformations, controlled by prevailing, usually variable, conditions of environment. Based on reviewed literature, and results of the research presented in this paper, the conclusion is that precisely defining of the leading process of any transformation is very hard, because of variable chemistry, availability of multiple electron donors and existing of different redox zones in close proximity. Also, the main limitation of BART assays is the fact that in the real environment bacteria commonly operate in communities containing up to one hundred different types all living in harmony and attempts to culture "pure" cultures essentially means concentrating on only those that are able to be cultured. The comparative analysis for three different alluvial groundwater sources has been presented trying to clarify which parameters might be the most important in determining the nitrate fate. The conclusion is that C increase can be correlated to the ammonium increase. This ammonium increase can be attributed to sporadic sulfide appearance (S-oxiders DNRA to NH^{4+}), or high C:N ratio (fermentative DNRA). N loss (evaporation of N_2, N_2O) is probably related to low C content, where Fe^{2+} concentration will determine whether the nitrate will undergo reduction by Fe-oxidation (high Fe^{2+} content), or it will be subject to respiratory denitrification or respiratory denitrification with anammox (low Fe^{2+} concentration).

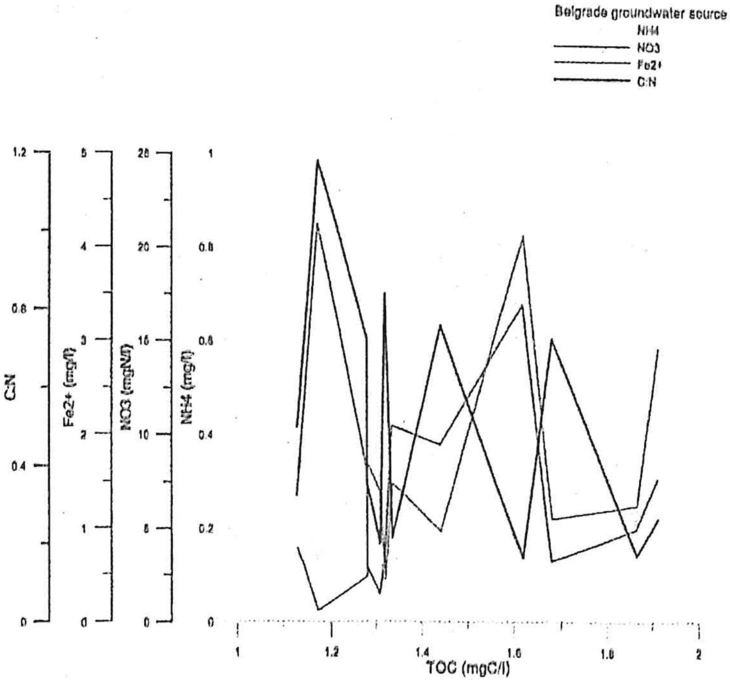

Figure 5: Comparative analysis of NH_4, NO_3, Fe^{2+} and C:N change with TOC increament in Belgrade groundwater source

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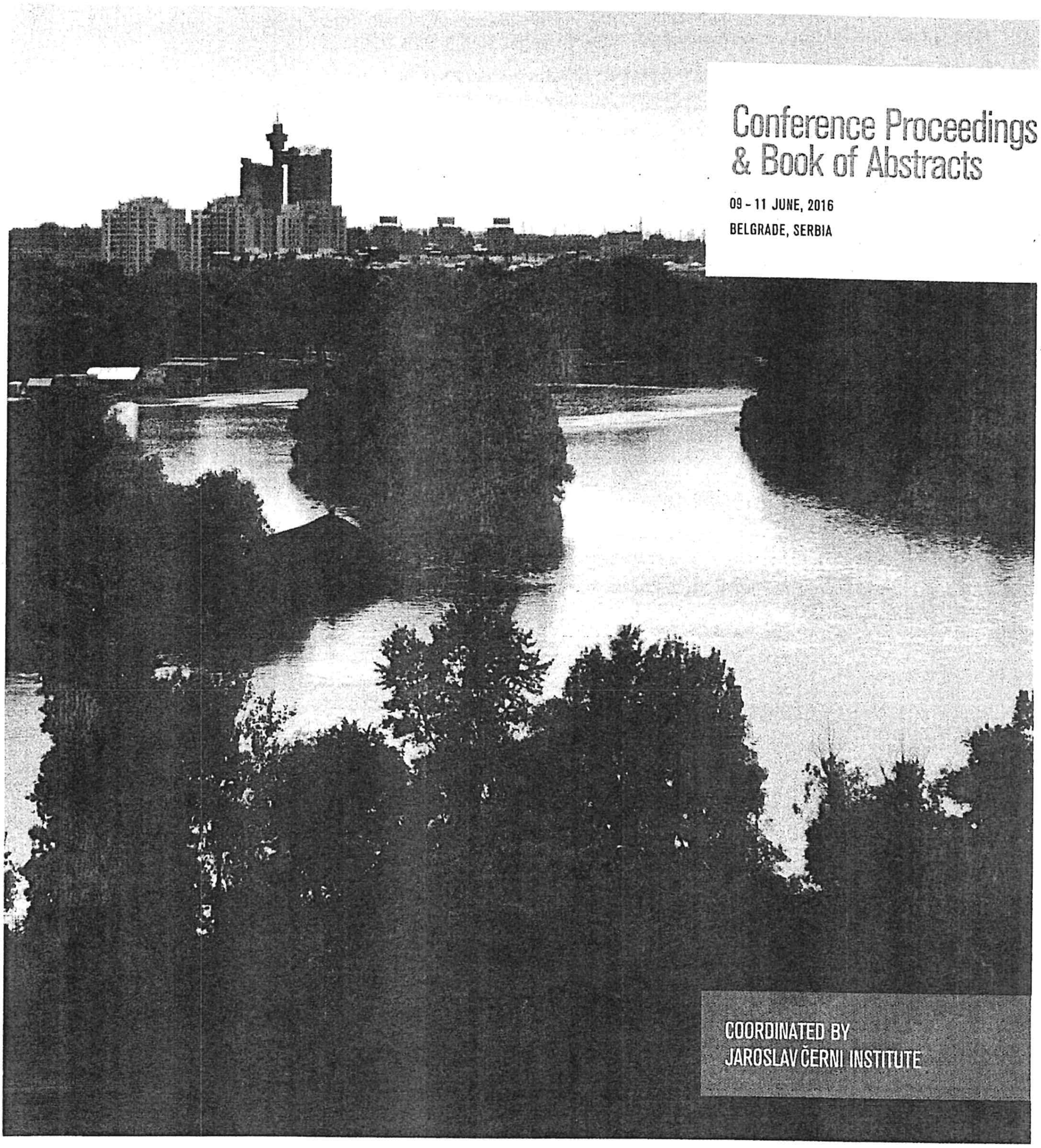

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INTRODUCTION

Water is the very essence of life on our planet. It constitutes 70% of our body. It is also one of the fundamental archetypes that define our existence and the state of our civilization. Sustainable management of natural resources, including surface water and groundwater, is a global necessity, which requires prudent management within watersheds and entire countries, for the benefit of each individual.

The relatively modest UN Millennium Development Goals related to progress in the areas of drinking water supply and sanitation until the year 2015 have not been achieved. We now have a new set of objectives ahead of us, as discussed at the 7th World Water Forum (Daegu, Korea, 2015), etc. The Sustainable Development Goals (SDGs), officially known as *Transforming our world: the 2030 Agenda for Sustainable Development*, were adopted at the UN Sustainable Development Summit September 25–27, 2015 in New York, USA. The goals incorporate in a balanced way all three dimensions of sustainable development (environment, economics, and society) and their interlinkages. In this context, a new goal related to water and sanitation has been proposed: *Ensure availability and sustainable management of water and sanitation for all.*

We are living in a time of strong pressures and contrasts, which threaten the continuation and development of the human society. Climate conditions, climate change, and social, economic and political pressures and disparities lead to crisis situations in social and economic spheres and in the area of sustainable use of water resources.

In ongoing circumstances, sustainable management is the promoted goal which the human society needs to attain. However, the success of achieving set objectives in the field of water is presently often relatively modest. Sustainable development of water management requires us to reach the needed level of scientific and technical knowledge, capacity, funding, and governance in general.

Groundwater is a crucial resource, as the basis of natural habitats, drinking water supply and irrigation. The goal is to attain sustainable groundwater management. The proposed topics of the Conference will virtually cover all of the scientific and technical knowledge in this field. The Conference is also expected to address very important issues and solutions relating to groundwater management.

The Conference will be the third IWA conference to be held in Belgrade (2007, 2011 and 2016), and will thus become traditional. It will also be held under the auspices of UNESCO - IHP, the Serbian Academy of Sciences and Arts, IAH, the Serbian Water Pollution Control Society, the Academy of Engineering Sciences of Serbia (AESS), IAWD, and ICPDR, and organized by the Jaroslav Černi Institute for the Development of Water Resources and WSDAC Center. We trust that this Conference, similar to the previous two, will be a significant contributor to improved groundwater management and the advancement of science in this field.

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