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WATER QUALITY OF SPRINGS ALONGSIDE SREBARNA LAKE: PRELIMINARY CHEMICAL ANALYSIS

JAKOŚĆ WODY ŹRÓDLANEJ WZDŁUŻ JEZIORA SREBARNA: WSTĘPNA ANALIZA CHEMICZNA

Abstract: The Srebarna Lake Biosphere Reserve is a protected natural site of national and international significance. It characterizes with extremely high biodiversity, being one of the last wetlands along the Lower Danube preserved in relatively natural state; the lake area is NATURA 2000 site. This study was designed to further research on the impact of intensive agriculture on the lake's ecological status. First task was to find an evidence for link between intensive agriculture and the loading of the lake with nutrients. The second was a study on the PAH's penetration into the lake ecosystem. An overview was made on drinking water data for 2 underground (drinking water springs). Dyakova spring's water displayed good ecological state after measured parameters PO_4^{3-}, NO_3^{2-}, NO_2^{-} and pH. Todorankina spring did not fell within the boundaries of good and very good conditions. Increased nitrate levels in water samples leads us to conclude that agriculture in this part of the surrounding area affect groundwater quality in the biosphere reserve. Results from GC-MS analysis proved, that there is no groundwater contamination, responsible for the existing (although low) concentrations of PAH found in the lake.

Keywords: eutrophication, drinking water springs, chemical analysis, gas chromatography-mass spectrometry (GC-MS), PAH, biogens, intensive agriculture

Beyond human advanced technologies, there are places on Earth, functioning as natural wastewater treatment plants. These places are aquatic ecosystems, composed of variety of flowing and stagnant water and associated groundwater.

The process of water self-purification, occurring within the marshes makes these wetlands unique. Nevertheless, in the past they were often perceived as unnecessary, even human harming landscapes and people unaware of their significance.

Understanding the importance of wetlands to humanity undergoes significant development during the last century. In the mid-century a priority has been given to drainage of the marshes as a source of disease (malaria, etc.) and conquers territories for agriculture. Gradually this consumption treatment gave way to contemporary view that

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wetlands are extremely valuable for preserving biodiversity and ensuring the human growing demand for fresh water.

The Ramsar Convention declares that, “wetlands” are: wetlands, bogs, or open water, natural or artificial, permanent or temporary, static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters. Members of the Convention are 111 countries in the world and each one offers wetlands within its territory to be included in the List of Wetlands of International Importance. Bulgaria is the eighth country to join the Convention in 1975, initially with two wetlands - Srebarna Lake Biosphere Reserve and Arkutino marsh.

The role of wetlands in the hydrological cycle has been already well documented [1] and moreover, groundwater is an integral part of the wetlands in the hydrological studies [2-6].

In conservation history of the Srebarna Lake, four main periods can be distinguished [7]. In the “pristine” period the lake have had a natural connection with Danube River. In 1954 a dike was built and the connectivity with the river had been interrupted. The lack of adequate contact with the river led to shallowing and accumulation of mud and organics. In 1994, the link was restored by new channel. Ecological situation has been estimated as a disaster in the early nineties of the last century [8]. Nowadays significant improvement of the environmental parameters and eutrophication level of the lake is registered in comparison with previous periods [9].

According to FAO Reports [10], eutrophication is the process of change from one trophic state to a higher trophic state by the addition of nutrient. Srebarna, as an eutrophic lake tend to be shallow and suffer from high rates of nutrient loadings from point and non-point sources. Agriculture is known as a major factor in eutrophication of surface waters. Any groundwater artificial development has some impact on the water balance components and often on water quality. Two main penetration paths of nutrients in the ecosystem are predicated on the west coast where the developing of intensive agriculture is notable: with groundwater and surface inflow.

PAHs (Polynucleararomatic hydrocarbons) are a class of hydrophobic organic compounds, which brings together more than 100 representatives drawn from two or more fused aromatic rings [12]. Representatives of PAHs ranging from naphthalene ($C_{10}H_8$, two rings) to the crown ($C_{24}H_{12}$, seven rings). PAHs are generated by natural and anthropogenic sources, which may be aquatic, terrestrial and atmospheric. Anthropogenic sources of PAHs are combustion and pyrolysis of fossil fuels and wood, and mainly of petroleum products which account for more than half received surfactant in water ecosystems worldwide [10-12]. Sources are also municipal and industrial wastewater, creosote, oil spills, urban and agricultural effluent, asfalt production, waste incineration. The fate of PAHs in the aquatic environment is determined mainly by the size, which affects their most important physical and chemical characteristics. With increasing molecular weight and increased hydrophobicity of the surfactant and reduces the fraction is in solution and thus removed by evaporation. The half-life in water strongly depends on the nature of the system [13].

According to [14] in the early 90s the samples from Lake Srebarna displayed background character, with decreasing concentrations in the more recent top samples and a relative enrichment in the core taken near the village Srebarna. Situation with PAHs in this samples is identical mainly phenanthrene, fluoranthene and pyrene (low %) were found, indicating that their likely origin is pyrosynthetic sources. However, it is important to follow their accumulation over time.

That is what determined the aim of our study, which shows a examining the hypothesis of a link between the nutrients and PAH's loading of the lake and the intensive agriculture developed in the area.

Sampling points

This study was designed to refer further research on the impact of intensive agriculture on the lake ecological status. First main task was to find if there is an evidence for link between intensive agriculture and the loading of the lake with nutrients through the groundwater. The second was to give suggestion on the PAH's penetration path into the lake ecosystem. An overview was made on drinking water chemical analysis data. A survey was built on 2 single underground objects (drinking water springs), recharging the wetland.

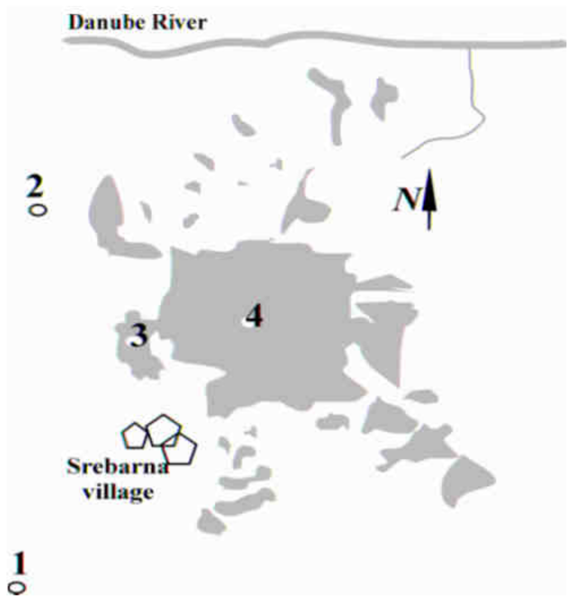

Fig. 1. Schematic map of Srebarna Lake with 4 sampling points: 1 - Dyakova spring; 2 - Todorankina spring; 3 - Lateral pool; 4 - Central Open Water Body

Material and methods

Sampling was made on November 21, 2010 at 4 sampling points: 2 springs and 2 points in the lake.

Biological drift

Biological drift was collected with plankton net (88 μm diameter of the eye) for 22 hours. Water quantity flowed within the net was estimated, based on the measured water volume, ongoing for 1 min. Five individuals from Amphipoda class were observed by naked eye. For this biological samples were submitted to the expert from IBER - BAS Dr. L. Kenderov for species level determination.

Hydrochemical analyses

Determination of NO_3^-; NO_2^- and PO_4^-

Values of pH, concentration of nitrates(V), nitrates(III) and phosphates(V) in the samples were measured *in situ* using portable photometer (WTW PhotoFlex) following the specified methodology. In the lake hydrochemical parameters were measured *in situ* using Multi1970i on 3 depths (0, 1 m and 3 m), corresponding to the surface, midwater and bottom. Dataset for concentrations of nitrates(III), nitrates(V) and phosphates(V) were compared with the reference values of the parameters for good and very good ecological state according to the Regulation. №7/ (1986) (bulgarian ecological legislation).

Non-metric MDS analysis [15, 16] on Euclidean distances of normalized data was made (s = 0.01) with PRIMER v6.

Determination of Polycyclic Aromatic Hydrocarbons (PAHs)

The PAH in water samples were determinate by Gas Chromatography/Mass Spectrometry (TERMO Science) system with an ZB-5MS (Zebron) capillary column (20 m length, 0.18 mm I.D., 0.25 μm film thickness). Ultra-pure helium (constant flow) served as carrier gas, and injector temperature was at 325ºC. The present work analyses the yield and precision of extraction of PAHs by *solid phase extraction* (C18 SPE discs), the method used for aqueous samples. Samples with changed and not changed pH of drinking water, were tested to assess the influence of pH on PAH recovery. The purpose of changing the pH of the water to check its influence in the extraction of PAH's from the middle and consequently varying their concentration.

The used reagents and materials for pretreatmeant of water samples are:

- Agilent SampliQ C18 6 cm^3, 500 mg (PN 5982-1365)
- The PAH standards were: mixtures of 16 PAHs (PAH Mixture, Agilent; concentration 2.5 μg/dm^3. For spiking the PAH standards were diluted with a polar solvent (CH_2Cl_2)
- 50 cm^3 sample reservoir (P/N 5982 - 9305); coupling fitting; vacuum manifold (12-port)

Pretreatment of water samples:

A 4 · 500 cm^3 samples of water filtered through glass-fibre Whatman GF/C filter (pore size 9.0 nm). Before extraction the samples from series 1 were acidified with hydrochloric acid (solution of 2M HCl) to pH = 3÷4. The samples from series 2 are pH = 8 without the change pH. After that the samples were spiked with 0.02 cm^3 of PAH standard 1 and then left overnight at room temperature. Mixed it. In all 500 cm^3 water samples add 50 cm^3 isopropyl alcohol and mixed it very well. Therefore, we analysed the samples changing the pH and without changing.

Solid Phase Extraction of Polycyclic Aromatic Hydrocarbons (PAHs) in water make in 4 steps: **Activation of cartridge**: Add 5 cm^3 methylene chloride (CH_2Cl_2) to the cartridge. Apply vacuum and discard the eluant. Repeat with 5 cm^3 methanol (MeOH) then 5 cm^3 water. Do not allow the sorbent to go dry at any point during this step; **Load:** Attach a sample reservoir to the top of the cartridge. Add to the reservoir water sample. Apply the vacuum and discard the eluant. The flow rate should be no greater than 10 cm^3/min. You must to be carefully the sorbent do not be drying; **Rinse:** Add 3 cm^3 of acetonitril/water (1 : 1 ratio) to the cartridge. Apply vacuum and discard the eluant. Leave the vacuum on for 30 seconds and after that repeat the procedure for washed of samples; **Elution:** Place a collection tube beneath the cartridge. Add 3 cm^3 CH_2Cl_2 to the cartridge. Apply vacuum and collect the eluant concentrate to (50; 200) mm^3 under a stream of dry nitrogen. Do not heat the samples during concentration - this will result in the substantial loss of many of the smaller ring size PAHs. Add methylene chloride to bring the final volume to 1000 mm^3. Inject 1 mm^3 into the GC/MS.

Results and discussion

According the reference data, at the early 20th Century the Srebarna Lake was mesotrophic to eutrophic water body [17]. Then isolation from the Danube in 1948 accelerated the process of eutrophication [18]. Another indirect evidence for changes in trophic status is the disappearance of the Ostracoda community presented there in the recent past in the *central open water body* (COWB) [19] as an indicator of typical lake conditions. Recently the Srebarna Lake is classified in hypertrophic state with ecological attributes anoxic hypolinmia, possible macrophyte problems.

All the analyzed parameters showed typical values for the season (Table 1).

Table 1

In situ measured parameters on 3 sampling points into Srebarna Lake: COWB and LP- Pristan

LP-Pristan	**pH**	**O_2 [mg/dm^3]**	**O_2 [%]**	**T [^{o}C]**	**Red/Ox**	**Cond. [µS/cm^3]**
0m	8.52	7.25	63.72	12.8	-77	557
1m	8.5	7	66.4	12.7	-76	559
3m	7.6	0.14	1.8	12.7	-34	568
COWB						
0m	8.52	6.37	59.4	12.7	-78	560
1m	8.52	6.25	58.8	12.7	-78	560
3m	8.02	0.25	4	12.6	-49	538

Table 2

In situ measured concentrations of nitrates(III), nitrates(V) and phosphates(V) on 2 points into Srebarna Lake and 2 sampled springs

	NO_2 [mg/dm^3]	**NO_3 [mg/dm^3]**	**PO_4-P [mg/dm^3]**
Todoranka	0.13	1.00	0.45
Dyakova cheshma	0.08	3.50	0.40
COWB	0.15	0.20	0.11
LP	0.16	0.00	0.19
Very good to Good ES	0.06	3.00	0.20
Good to Moderate ES	0.12	6.00	0.80

Temperature was relatively equal in the whole water column. The values of nutrient concentration suggested intensive degradation processes in the bottom layer. Nutrients concentrations in the lake increase with the inflow from both the Danube and the watershed, and decrease as a result of the consumption by primary consumers and of the processes of nutrient recycling. In the lake we did not found any dissolved nitrates but significant loading with nitrates was found in the water from Todoranka spring (Table 2).

Fig. 2. MDS analysis (stress 0.01) on Euclidean distances of normalized data for concentrations of nitrates(III), nitrates(V) and phosphates(V) and the referent values for *ecological state* (ES), recommended (Refferent) from WFD 2000/60/EC

Three groups of points with different ecological state (ES) appear on the MDS analysis plot (Fig. 2). Euclidean distance of 0.71 is recorded between Dyakova spring and recommended values for nitrates(III), nitrates(V) and phosphates(V) concentrations for very good to good ES. We classified water quality of the given spring as near to that reccomended in European ecological legislation. Both the Todoranka's spring and lake's water quality are fall into second group (Euclidean distance between them 1.0). High values of Euclidean distance are observed between the EU water in the lake and fountains (3.86). The third group consists only values of concentrations for the nutrients, pointing moderate ES. Neither lake water quality, nor spring water quality could be classified as moderate ES.

The samples of filtered water from 4 sampling points (2 from springs and 2 from the lake) were analyzed for 16 individual PAH compounds. The results (Fig. 3) are arranged by methodology of pretreatment. In the middle of figure is shown a chromatogram of using standard solution with concentration neat to our MLD = 2.5 ng/cm^3. Detection signal for all PAHs compounds at our real samples were generally low or undetectable. Usually, if the detection signal is small and does not allow quantifying the concentration, indicate that it is below the detection limit. While PAHs do not show extremely high acute toxicity to aquatic organisms, the lower molecular mass compounds tend to exhibit higher lethal toxicity than the larger PAH [18, 19]. It was reported that the *lethal concentration* (LC) of PAHs in the range up to and over 104 ng/dm^3 for most organisms. It is clear from Figure 3 that the concentrations of total PAHs in each sample of the 4 sites are not detectable in two different pH values, therefore, the toxicity of PAHs are not expected to occur in organisms. Overall, a very low total PAH concentrations (~0.03 μg/cm^3, in recovery times ~85÷102% for PAHs) was observed in samples. The pH pretreatmenat of samples with aim to facilitate the process of PAHs elution not influenced them concentration. High-molecular mass PAHs, such as benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene and dibenzo[a,h]anthracene, were not detected in the water samples, this fact may be attributed to their lower water solubility. Other PAHs moleculs were obsoroved but in levels under of our MLD concentration.

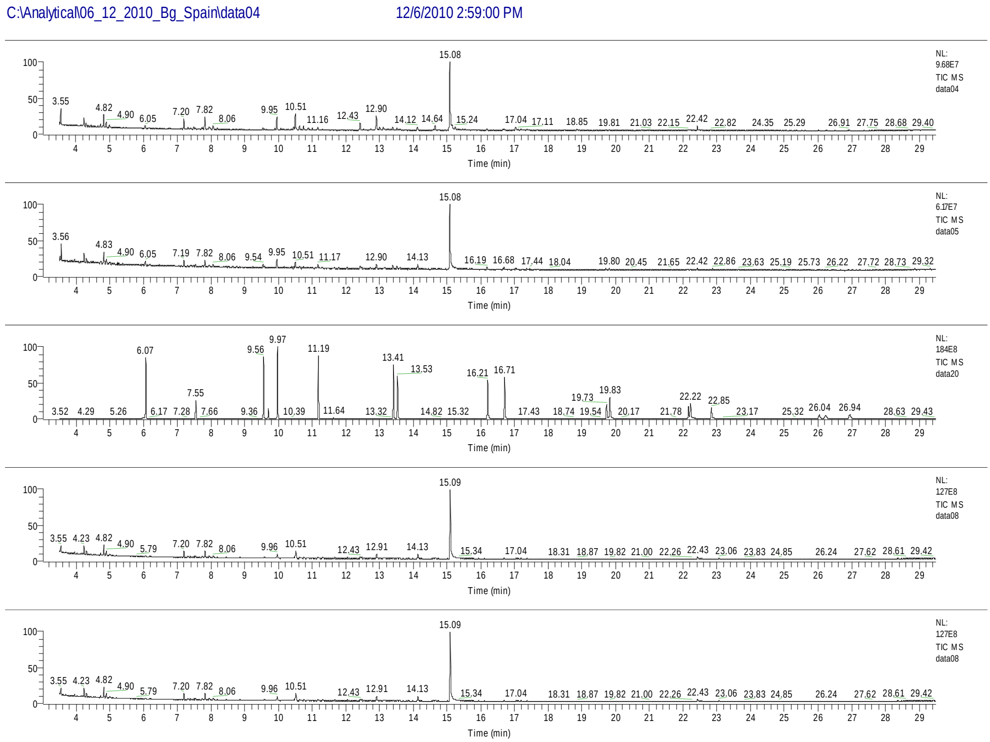

Fig. 3. GC-MS analysis of samples: Standard solution of PAHs (concentration, 2.5 ng/cm^3) - middle position; SPE extract of a non-spiked organic soil (down) and SPE extract of a spiked organic soil (up)

Conclusions

Qualitative and quantitative analysis of samples shows that groundwater in the watershed of the lake is relatively clean and the contents of the PAH are below acceptable standards. PAHs are ubiquitous and persistent contaminants, with the potential to harm both aquatic resources and human consumers of shellfish, and further study is needed on their environmental distribution and behaviour.

Groundwater feeding is notable source of nutrients for the lake ecosystem. A comprehensive study of environmental and biotic parameters of underground water would give valuable results in terms of groundwater quality in the area of the Srebarna Biosphere Reserve and its impact to the whole ecosystem. Continuous monitoring of groundwater in the area is suggested notably as useful addition to the regular monitoring of the lake ecosystem. Data collected could be used for qualitative modeling of the impact of intensive agriculture on the lake for giving to all the stakeholders an understandable explanation of the complex processes in the ecosystem.

Acknowledgments

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JAKOŚĆ WODY ŹRÓDLANEJ WZDŁUŻ JEZIORA SREBARNA: WSTĘPNA ANALIZA CHEMICZNA

Abstrakt: Rezerwat Biosfery Jezioro Srebarna jest chronionym obiektem przyrodniczym o znaczeniu krajowym i międzynarodowym. Charakteryzuje się bardzo dużą różnorodnością biologiczną, jako jeden z ostatnich terenów podmokłych wzdłuż dolnego Dunaju zachowany w stanie względnie naturalnym; region jeziora jest objęty programem Natura 2000. Przeprowadzone badania dotyczą wpływu intensywnej gospodarki rolniczej na stan ekologiczny jeziora. Pierwszym zadaniem było znalezienie dowodów na związek pomiędzy intensywną gospodarką rolniczą i wzbogacaniem jeziora w substancje odżywcze. Drugim celem było zbadanie translokacji WWA w ekosystemie jeziora. Badaniom poddano dwa źródła wody pitnej. Woda ze źródła Dyakova charakteryzowała się dobrym stanem ekologicznym, co stwierdzono na podstawie wartości stężeń PO_4^{3-}, NO_3^{2-}, NO_2^{-} oraz wartości pH. Woda ze źródła Todorankina nie mieści się w granicach jakości dobrej i bardzo dobrej. Zwiększone stężenie azotanów w próbkach wody prowadzi do wniosku, że rolnictwo wpływa na jakość wód gruntowych w tej części rezerwatu biosfery. Wyniki analizy GC-MS pokazały, że wody podziemne nie są odpowiedzialne za istniejące niewielkie zanieczyszczenie jeziora przez WWA.

Słowa kluczowe: eutrofizacja, źródła wody pitnej, analizy chemiczne, chromatografia gazowa-spektrometria mas (GC-MS), WWA, biogeny, intensywna gospodarka rolna