

2-ci Xəzər Beynəlxalq Su Texnologiyaları konfransının materialları

Azərbaycan, Bakı 11 aprel 2014-cü il

2nd Caspian International Aqua Technologies Conference Materials

Azerbaijan, Baku 11 April 2014



Bakı - 2014

THE REPUBLIC OF AZERBAIJAN
“AZERSU” OPEN JOINT STOCK COMPANY

**2nd CASPIAN INTERNATIONAL
AQUA TECHNOLOGIES**

**Conference
MATERIALS**

(Azerbaijan, Baku, 11 April, 2014)

Baku - 2014

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Chairman of "Azersu" OJSC

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2nd Caspian International Aqua Technologies. Conference materials (*Azerbaijan, Baku, 11 April, 2014*).
– Baku: Mutarjim, 2014. – 640 p.

ISBN: 978-9952-28-164-4

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M.A.Musayeva. The nature of snow cover distribution and the role of the drinking water supply in the north-eastern slope of the Greater Caucasus in Azerbaijan	333
I.M.Mammadzade. Water management associations - Way to ensure participation in the water resources management.....	339
N.T.Safarova, L.J.Abdullayeva, T.R.Guliyev. Methods of preventing contamination of drinking water	347
Z.S.Allahverdiyev, S.A.Rzayeva. The distribution of the average salinity on the surface waters of the Caspian Sea and the use of seawater with the purpose to meet the demand for fresh water in the future	350
Pasuqale Scandizzo, Diego Rodriguez, Rovshan Abbasov. Willingness to pay and integrated water demand: a case study for the greater Baku area, in Azerbaijan.....	356
M.A.Teymurov. Important areas of water resources and water supply of mountainous Shirvan economic region.....	362
E.S.Badalov. Water supply problems of Absheron economic-geographical region.....	368
D.A.Repin. Increasing productive capacity, improvement of water supply of oil refineries at the stage of reorganization conditions	373
S.M.Isgandarov, I.S.Aliyeva. Water reservoirs siltation in arid climatic condition.....	377
I.H.Garayev. Some water supply problems in Salyan and Neftchala districts.....	382
Y.A.Garibov, N.S.Ismayilova. X-ray diaphragmatic analysis of rivers in north-eastern flank of the Caucasus and its impact on irrigation landscape formation.....	390
Z.A.Tarikhazar, M.T.Huseynzade, Z.A.Jabraylova, G.A.Eldarova. Scientific recommendations on organization of ecological monitoring for water supply and sanitation facilities	394
A.H.Hummatov, Sh.Kh.Osmanov. Mathematical and economic assessment of water supply management in agriculture	401
S.K.Nagiyev. Historical development stages of water supply	409
I.N.Shirinov, E.M.Musayeva. Use of drainage water in water supply of Shirvan plain	413
E.M. Barishnikov. Providing reliability, durability and ecological safety of water supply using ductile iron pipes.....	419

IV. Green Planet. Waste Water Treatment and Reuse

I.Ivancev-Tumbas, A.Leovac, A.Tubic, J.Agbaba, J.Molnar. Significance of natural organic matter in drinking water treatment	432
Asadov M. Y., Osmanov T. A. Clean water problem, adaptation of irrigation and water management sector to global climate changes	438
E.A.Tamas, I.Matrai. Importance and methodology of simultaneous data collection on morphology, hydrology and water quality	444
R.A.Ismayilov, R.UAbdulazimov. Assessment of lake formation processes and geo-ecological condition of lakes in Absheron Peninsula	454
Kh.A.Asadov, N.S.Bababeyli, M.M.Bakirova. Hydro chemical regime of the rivers of the Nakhchivan AR	461
K.F.Ibrahimov, A.Q. Seyidov. Original water plant found in Old city.....	466
V.A.Mammadov, M.S.Alosmanov. Formation of lakes, transformation and assessment of ecological condition in Absheron Peninsula	470
Dusan Kostic, Ana Blagojevic, Gordana Subakov Simic, Zorana Naunovic, Marko Ivetic. Late Autumn Bloom of Potentially Toxic Cyanobacteria Planktothrix rubescens in the Reservoir Vrutci (Serbia).....	479

LATE AUTUMN BLOOM OF POTENTIALLY TOXIC CYANOBACTERIA *PLANKTOTHRIX RUBESCENS* IN THE RESERVOIR VRUTCI (SERBIA)

Dusan Kostic¹, Ana Blagojevic¹, Gordana Subakov Simic¹,
Zorana Naunovic¹, Marko Ivetic^{1*}

¹University of Belgrade -UNESCO Chair in Water for Ecologically Sustainable Development,
*markoi@hikom.grf.bg.ac.rs

Abstract: A multipurpose reservoir Vrutci, with traditionally very good quality of water, after more than thirty year of operation, in the second half of December 2013, was a scene of intensive bloom of potentially toxic cyanobacteria *Planktothrix rubescens*, causing disruption of regular water supply to more than 60000 people from the Uzice Municipality, for almost two months. Everything happened in a short period of time, and consumers and relevant institutions were taken by surprise. However, careful examination of activities in the watershed feeding the reservoir had revealed scores of mismanagement, like illegal urbanization, excessive hydropower utilization, untreated waste water, ilegal fishing etc. Most of them were noticed by the Public Water Supply Utility of Uzice and reported to higher instances, but without timely (re)action. The paper gives the overview of the event, measures undertaken to overcome the problem and the lessons learned. Interestingly enough, even during the peak of cyanobacteria bloom, the other water quality indicators remained within acceptable limits for oligotrophic/mesotrophic lakes, indicating that the monitoring program has to be improved to meet emerging situation.

Keywords: mutipurpose water reservoir, cyanobacterial bloom, field measurements, risk management

1 Introduction

The reservoir Vrutci, constructed 1984., serves as a main source of water for Uzice water supply system, which provides potable water for the Uzice Municipality, suburbs, industry and surrounding settlements. On December 14, 2013, it was reported that a strange red spill of an approximate area equal to 0.5 ha, was noticed in the upstream part of the reservoir. On the same day, Municipal Public Health Office and Center for Emergency Situations sent their experts to the spot. It was suspected that some substance was intentionally spilled into the reservoir. On December 19, in two out of ten samples, MPHIO indicated the presence of algae at the intake of raw water, and samples were sent to the microbiological laboratory of Public Water Supply Utility (PWSU) Krusevac, neighbouring city, for further analyses. On the next day, for the first time, but still unofficially, the presence of potentially toxic cyanobacteria *Planktothrix rubescens* (DeCandolle ex Gomont) Anagnostidis et Komarek was confirmed. Additional samplings were done by the Republic Public Health Institute of Serbia "Batut" and the PWSU of Krusevac, on December 23, and December 25, respectively.

On December 26, cyanobacteria were detected in the water distribution network. Ministry of Public Health of Serbia and Sanitary Inspection announced the ban on using water for drinking and food preparation. Alternative ways of potable water supply were organized. At the same time, samples of water were sent abroad to several referent laboratories for toxicologic analyses.

Two days later, on December 28, upon the invitation of PWSU Uzice, the team from the UNESCO Chair in Water for Ecologically Sustainable Development, University of Belgrade started the field measurements. Despite the obvious purple-red water caused by cyanobacteria bloom, the results of measurement did not indicate significant anomalies in the water quality measured indicators. Only chlorophyll *a*, an indicator of primary production of phytoplankton was detected at greater depths (over 30 meters in the reservoir Vrutci), indicating that the *Planktothrix rubescens* also settled relatively dark layers of the water column, making it impossible to avoid by changing the elevations of the selective water intake.

Planktothrix rubescens is potentially toxic cyanobacteria, which was, untill recently, classified in *Oscillatoria* genus. Pigment phycoerythrin is responsible for its red color. In the

process of photosynthesis, they can use the whole spectrum (from 400 till 700nm) of the photosynthetically active radiation, which enables its active presence even in deep water (Micheletti et al., 1998). Contrary to the majority of cyanobacteria, *Planktothrix rubescens* can use phosphorus of organic and inorganic origin. This gives a great adaptability to the cyanobacteria, and hard time to remove from the reservoir. It is characteristic for cold water lakes and reservoirs at higher altitudes, like Alpine lakes (Geneva Lake, Lake Garda in Italy, Lake Bled in Slovenia, Lac du Bourget in France etc.). In Serbia it was detected in water reservoirs on Uvac River (Blaženčić et al., 1990; Svirčev et al., 2007), and very recently there was a cyanobacteria bloom in the Vrutci Reservoir. Experiences from similar cases indicate that they do not exist in lakes where concentrations of total phosphorus are below 10 microgram per liter (Jacquet et al., 2005).

The results of toxicological analyses performed abroad were negative for the presence of cyanotoxins in drinking water. In the meantime, the alternative water source "Sušička vrela" was activated and connected to the raw water pipeline. After several days of intensive flushing of raw water pipeline and secondary water supply network, and several consecutive proofs that biofilm deposits and cyanobacteria that found their way through sand filters in the water treatment plant, were removed and inactivated, the ban on use of water was finally lifted.

2 Methodology

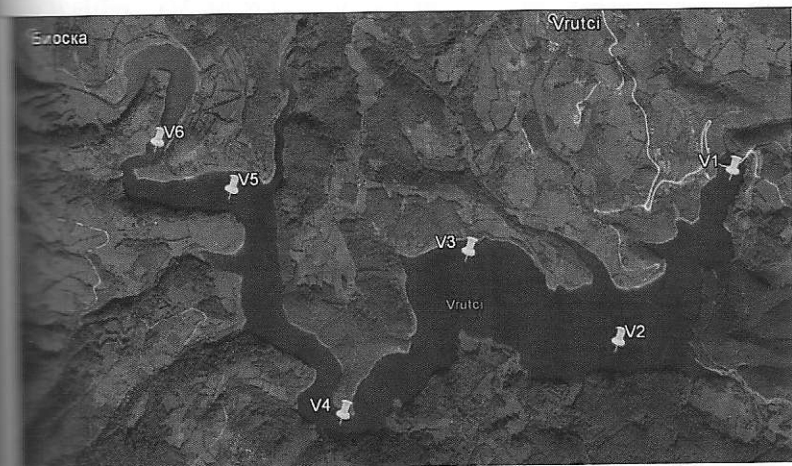
2.1 Water Reservoir Vrutci and Djetinja River

Multipurpose reservoir Vrutci (43° 50' 34" N, 19° 41' 36" E) was formed in 1984 by damming river Djetinja with an arch concrete dam with the height of 77 meters. Bulkhead place is located 12 kilometers upstream from Uzice, at the entrance of Djetinja gorge. Medium perennial flow of Djetinja at that section is 1.87 m³/s. The Djetinja watershed area (160 km²), is rarely populated without significant economic activity. Up to the top of dam overflow (627 m asl), the reservoir has a maximum capacity of 54 million m³, and the volume of 40.2 million m³, at a normal operation level, 621.3 m asl. Reservoir Vrutci is the main source of the Uzice PWSU, and also has roles in flood control, sediment control and enriching the low flows in the Western Morava basin (KRO Bioktoš, 1984). Since the year 2010, a mini hydropower plant (MHP) was connected to the raw water pipeline, and the environmentally required minimum flow was diverted through that connection. Like most other reservoirs in Serbia, Vrutci is a popular summer swimming and picnic area, and also is a part of the fishing area. In its nominal morphometric characteristics, and the surface of water table of 1.92 km², the mean depth of 20.8 m, Vrutci is expected to be an oligotrophic lake with trophic index below 40 (Grašić, 2013). Djetinja River is a merger of several tributaries, some of which bring organic pollution, like untreated wastewater from villages Kremna and Bioska, small solid waste landfills etc.

2.2 Field measurements of water quality

Field measurements conducted by the team of UNESCO Chair in Water, on December 28, was the first detailed survey in the thirty years long history of the Vrutci reservoir. Prior to that, monitoring by the relevant state agency was not systematic, and with long periods without any data. In the present measurement campaign, water quality indicators like temperature, dissolved oxygen concentration, dissolved oxygen saturation level, chlorophyll *a*, pH, specific conductivity and total mineralization, were measured by a multi-parameter probe at 156 points. Measuring points are arranged in six verticals (V1, V2, V3, V4, V5, and V6), evenly distributed over the longitudinal axis of the reservoir, from the dam to the confluence of tributaries (Figure 1a). The multi-parameter probe, type 6600 V2 -2 (Figure 1b) belongs to the latest generation of producers YSI Inc. (Yellow Spring Instrument, <http://www.ysi.com>), which has optical sensors for measuring the concentrations of dissolved oxygen and chlorophyll *a*. The quality of water is measured out of the boat, and the probe was lowered from the surface to the bottom in depth increments of approximately 1m. A very similar methodology and the same measuring equipment has been used within the research

project TR 37009 - Measurement and modeling of physical, chemical, biological and morpho-dynamic parameters in rivers and water reservoirs (financially supported by the Ministry of Education, Science and Technological Development of Republic of Serbia). Faculty of Civil Engineering in Belgrade on a monthly basis, examines the quality of water in the reservoirs Celije and Gruza (Nenadic et al., 2013).



a) Locations of water columns where measurements were taken in 156 points on December 28, 2013. b) Probe YSI 6600 V2-2

Figure 1. Vrutci water reservoir with the indicated (yellow placeholders) positions of measurements (a), where basic indicators of water quality were measured by a multi-parameter probe YSI 6600 V2-2 (b).

3 Results, Observations and Discussion

3.1 Results of Measurements

The measurement results are presented graphically in diagrams (Figure 2 and Figure 3). Data on vertical distribution of water temperature indicate nearly completely mixed vertical water column (Figure 2a). In the profile V1, which is closest to the dam, the water temperature is approximately constant up to a depth of 32 meters and is slightly less than 6.0 °C. Because of the great depth of water at the profile and because of the relatively high ambient temperature, convective mixing is slow. Also, the layer of the water column near the bottom is slightly warmer with temperatures about 6.5 °C. For this phenomenon we do not have a reliable explanation. Measurements conducted by PWSU Krusevac and by the Institute "Batut", obtained similar values, and confirmed the validity of measurement. Increased temperature in the vicinity of the sediment may indicate the extensive biological degradation that releases heat. The upstream profile, V6, is the most affected by tributaries. The small mass of water in this part of the reservoir and the small heat capacity, cause the fastest response to the external forcing. In profile V6, water temperature at the surface was slightly above the freezing point, and in this part of reservoir the ice cover was formed.

Water electro-conductivity, the ability of an aqueous solution to conduct electricity, is directly dependent on the water temperature and the number, mobility and valence of ions dissociated in water. The specific conductivity is the value of conductivity normalized at the water temperature of 20°C. All measured values of specific electric conductivity in the reservoir are in the range of 350 to 430 $\mu\text{S}/\text{cm}$ (Figure 2b). These values are much smaller than the value defined as the natural level (1000 $\mu\text{S}/\text{cm}$), the limit for the first class of ecological status. Completely pure water (distilled or demineralized) has a specific conductivity of 0.05 $\mu\text{S}/\text{cm}$.

The TDS (Total Dissolved Solids) or total mineralization is a measure of the presence of cations of magnesium, calcium, sodium, potassium, and anions of carbonate, hydrogen carbonate, chloride, sulfate, nitrate and nitrite. Although the most reliable method to

determine TDS is gravimetric, it is possible to relate it with the electro conductivity. Due to the almost complete mixing of the lake water in that period, the standard deviations of all the measured parameters are small. Total dissolved solids range in the narrow band of 220 to 260 mg/L (Figure 2d), which is smaller than the concentration of 1000 mg/L, or natural level, the boundary between the first and second classes of ecological status. Increased concentration of dissolved solids were observed just above the sediments at the location of V1, where the temperature is increased and concentration of oxygen is low, which indicate the intense process of degradation of the organic substances.

At the time of measurement the Vrutci Reservoir was relatively rich in dissolved oxygen. In over 80 % of measured points in the concentration ranged between 8 and 10 mg/L (Figure 3a). For given water temperature and the corresponding height of the water column, this concentration range corresponds to the degree of saturation between 60 and 80 % (Figure 3b). The presence of significant amounts of oxygen up to the depth of 20 meters is probably due to convective mixing of the water column and the current cyanobacterial bloom (cyanobacteria produce oxygen through photosynthesis), but it cannot be completely confirmed, as there are no data measurements of water quality in summer, when the reservoir was thermally stratified. For depths greater than 20 meters, oxygen decreases slightly, while for depths greater than 32 meters, there is a rapid drop. In the profile of V1, at the dam, near the bottom, nearly anoxic conditions are detected as a result of bacterial decomposition of organically precipitated matter. It is very important that water is evacuated from the reservoir bottom outlet because it contains the largest amount of phosphorus, whose presence is a prerequisite for development of cyanobacteria and other algae. Analyses of PWSU Kruševac (Grašić, 2013) indicate that the total phosphorus concentration in the bottom layer is 3 to 4 times greater than the average value for the whole of the reservoir. Significant amounts of dissolved oxygen and the absence of cyanotoxin in the water are the main reasons why there was no dead aquatic organisms spotted.

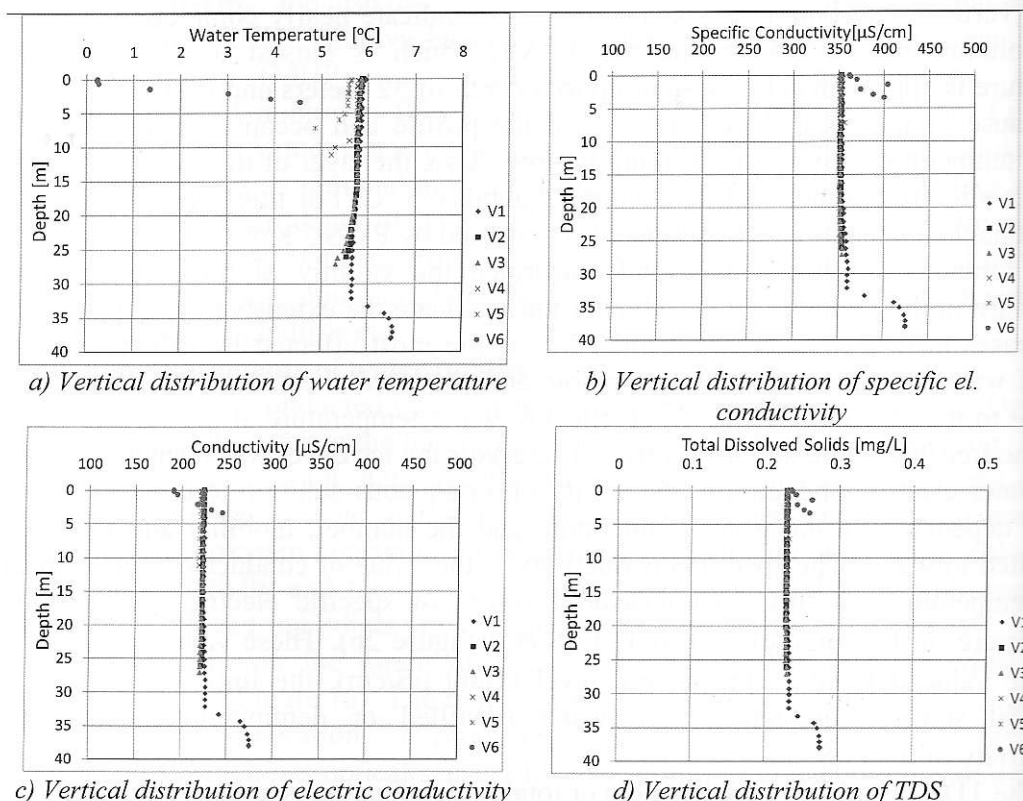


Figure 2. Vertical distribution of: a) water temperature; b) specific e. conductivity; c) electric conductivity and d) Total Dissolved Solids

The vast majority of measured concentrations of chlorophyll *a* are in the range between 5 and 8 $\mu\text{g/L}$, which are relatively small concentrations, but being detected at greater depths, up to 30 meters (Figure 3c), means that the cyanobacteria occupied the entire volume of the lake, and that it is impossible to avoid them by changing the water intake elevation. Small concentrations of chlorophyll and dissolved oxygen saturation level that does not exceed 100 %, testifies the presence of cyanobacteria which are not significant producer of oxygen, but still able to synthesize organic matter under conditions of low temperature and low light conditions. For example, during the summer blooming of certain species of algae or cyanobacteria, oxygen concentration corresponding to the saturation of 150 % and more, could be detected.

The most of the measured pH values in the Vrutci reservoir were in a relatively narrow range between 8.2 and 8.5, which indicate an alkaline environment. Elevated pH values are the result of photosynthetic activity of cyanobacteria and geological composition of the basin of Djetinja River. In the profile V6, the highest value of pH, equal to 8.62 was measured. This indicates a relatively high alkalinity of tributaries (hard water), which was confirmed by chemical analysis of PWSU Krusevac.

During thermal stratification of the water column, *Planktothrix rubescens* takes position of metalimnion, a narrow layer of the water column around the thermocline, making it difficult or impossible to be visually detected. Therefore, in summer it is necessary to sample at a number of depths by VanDorn bottle, or by other container. However, these samplings have to be preceded by measurements with multiparameter probes that have sensors for temperature, dissolved oxygen, and for chlorophyll *a*, in order to determine the depth from which the bottles will be sampled. Regarding the Vrutci reservoir, it would be very good to upgrade the probe with the phycoerythrin sensors to detect the presence of *Planktothrix rubescens*, and other cyanobacteria that possess this pigment.

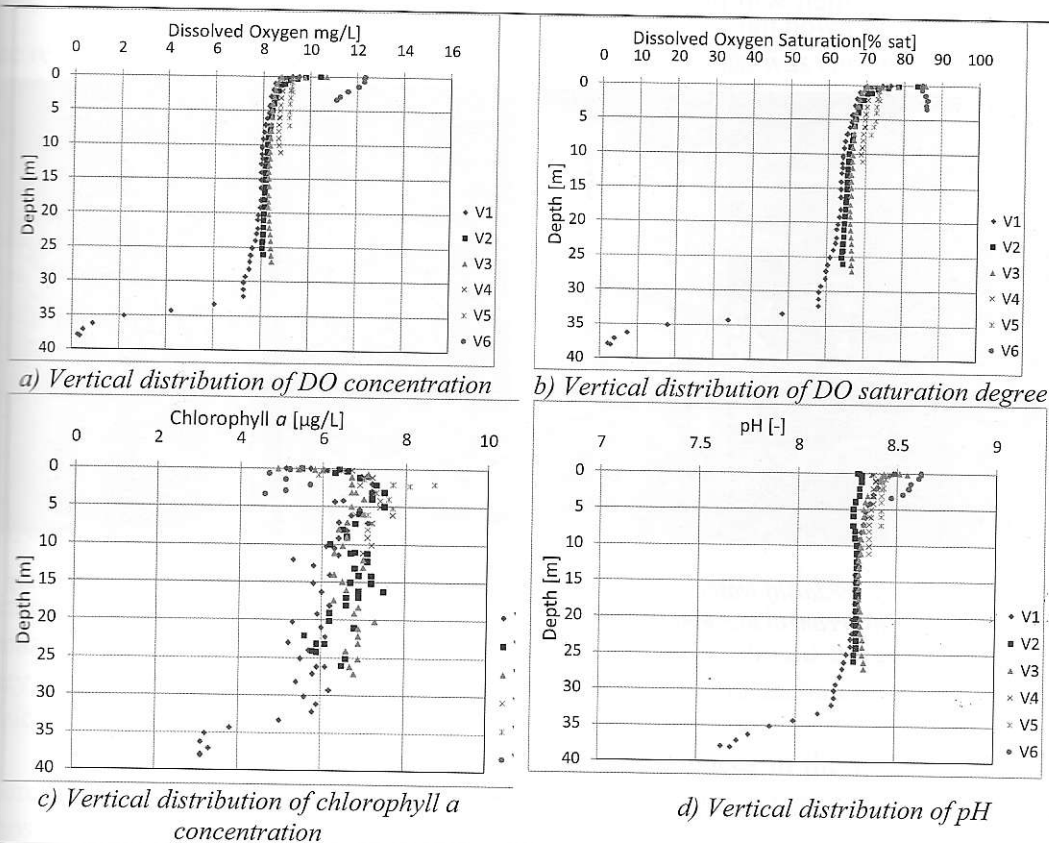


Figure 3. Vertical distribution of: a) dissolved oxygen (DO) concentration; b) DO saturation degree; c) concentration of chlorophyll *a* i d) pH value.

3.2 Visual observations

On the December 28, the day of measurements, the reservoir was covered with a thick fog, and visibility was reduced. The next visit to the reservoir and tributary was organized on January 2, on a clear day. Elevation of the water level was at 614 m above sea level, which is 7 m lower than the design water level. The low water levels provided an insight into the lower littoral zone. Large amounts of sediment from torrential tributaries into the reservoir indicate an urgent need for the construction of torrential erosion control structures in the watershed. In Jokic meadows the dog was spotted while drinking water from the reservoir, and near the dam, an allegedly vital otter (*Lutra lutra*), dived and appeared out of the water, in a place where there was a thick layer formed from dead cyanobacteria (Figure 4).

The upstream part of the reservoir is now without water, which provides insight into the bottom covered with sediment deposits (Figure 5), brought by river Đetinja from the watershed. Organic forms of nitrogen and phosphorus compounds are oxidized in air and transformed into inorganic forms, which are then available to all primary producers, including the cyanobacteria. During the period of high water, tributaries wash these layers, bringing them directly into the reservoir. It is therefore necessary to maintain the elevation of the water level as close to the normal elevation as possible, to allow establishing of populations of submersed macrophytes (aquatic plants) by biotechnical measures, or naturally. Macrophytes will assimilate nitrogen and phosphorus compounds more efficiently, provide shelter for fish, and help the restoration of a disturbed ecological balance. In the case of proliferation of macrophytes, their biomass is simply regulated by underwater harvesting, a practice that gives excellent results on the Sava Lake in Belgrade that despite the huge number of visitors in summer, manage to retain first-class ecological status. The most important objective of reservoir management is to avoid unnecessary changes of the elevation of the water table below the design level, which will provide storage for flood waves of 50 years return period, without overflowing.



Figure 4. A dog drinking (reddish) water from reservoir Vrutci (left), and an otter (*Lutra lutra*) swimming upstream of the dam in a zone of thick layer of cyanobacteria.



Figure 5. The most upstream part of the reservoir with sediment deposits revealed when the water level is lowered, either as an activity in flood management strategy, or due to hydropower utilization. This part of reservoir could serve as a habitat for water plants which can have an active role in intercepting incoming pollution and nutrients.

4 Concluding Remarks

It has to be noted that on the basis of present investigation, in spite of intensive *Planktothrix rubescens* bloom, which made water colored in red, there were no significant anomalies in measured water quality indicators. The only exception is that chlorophyll *a*, as the indicator of primary photosynthetic phytoplankton production, was detected in layers more than 30 meters below free surface, which was a proof that *Planktothrix rubescens* has occupied the whole water column, and that selective water intake cannot help in avoiding the risk of cyanobacteria entering the system.

The most important general conclusion relates to the unknown history of water quality in the reservoir Vrutci. The current monitoring, which in the past was carried by the State MetOffice and has recently taken over by the Agency for environmental protection, was sporadic, with lots of missing data, so the timing of *Planktothrix rubescens* bloom and possible causes for blooming, one can only speculate.

The absolute priority in the reservoir management was given to flood control and electricity generation at MHP, with no regard for the possible consequences on water quality. The water level in the reservoir was significantly lowered down in late autumn, in order to provide space for the storage of the spring floods. In addition to this too early emptying, instead of using the bottom outlet, "clarified" and rich-in-oxygen water, from the raw water pipeline and MHP was disposed. In this way, the water with high turbidity, with low dissolved oxygen content and high phosphorus content, was retained in the reservoir, which was not beneficial to the reservoir ecosystem. According to local sources, fish stocks management was arbitrary and non-transparent. Almost all relevant republican institutions showed indifference and inertia to assist local government.

On the other hand, the reaction of the neighboring cities, their Public Utilities and health centers in terms of providing water tanks in taking care of seriously ill patients etc., was amazing. The support provided by Waterworks Krusevac should be emphasized. In the past they were facing similar challenges, and their expertise and technical support to Waterworks Uzice are highly appreciated.

Based on the literature review and similar experiences in the world, it is likely that the aforementioned cyanobacteria will remain in the Vrutci reservoir until the level of total phosphorus is reduced below the threshold of 10 mg/L, when the cyanobacteria, according to data from the literature, is being eradicated. Roughly estimated, it will take at least 10 years. Therefore, it is imperative for PWSU Uzice, to improve the water treatment process to be able

to handle cyanobacteria breaches, and to increase the capacity and number of alternative sources of water supply.

Acknowledgements. The paper presents results of the research grant TR37009: *Measurement and modeling of physical, chemical, biological and morpho-dynamic parameters in rivers and water reservoirs -MORE*, funded by the Ministry of Education, Science and Technology of Serbia, coordinated by the University of Belgrade, Faculty of Civil Engineering. We wish to thank Public Water Supply Utility of Uzice on financial, technical and logistic support during field measurements.

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