

Environmental impact assessment of discharge of treated wastewater effluent in Upper Iskar sub-catchment

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Abstract

The upper Iskar sub-catchment is one of Bulgaria’s most important economic and socially significant water sources because of its role in supplying Sofia with drinking water. Among the critical factors that carry potential high-risk levels for water quality in this hydrosystem are the discharge from the Samokov Wastewater Treatment Plant (WWTP), diffuse pollution from agriculture, and the percolation of untreated sewage from the small villages. In this study, we assessed the effect of treated wastewater effluent on water quality, and on the ecological state and microbial communities in the river sector of Samokov’s WWTP discharge area. The assessment was based on the complex use of chemical and microbiological indicators and biological quality elements. The concentrations of organics, nutrients and microcomponents were determined with results confirming the expected increase for parameters associated with the discharge of urban wastewater. The ecological state, according to macrozoobenthos indicators, was “good” throughout the river sector but local deterioration was registered in a proximal location downstream of the WWTP outfall. The analysis of stream water and bed sediment microbial communities by a fluorescent technique showed the high metabolic activity and intensive transformation processes in addition to high abundance registered with standard cultivation methods. The importance of the studied sub-catchment for the functioning of the urban water cycle, and for the quality of Sofia’s drinking water, underlines the need to extend an existing monitoring program with a more detailed assessment of the environmental impact of wastewater discharge.

Keywords

Ecological status, Iskar River, microbial community, pollution, self-purification, treated water discharge, WWTP

Introduction

The increasing release of different organic and inorganic pollutants, associated with rapid urbanization and industrialization, is one of the global environmental threats for the quality and ecological status of aquatic ecosystems. The prevention of pollution, and protection of the quality of natural and drinking water, is a primary concern for society in order to ensure healthy living conditions, as well as a high standard of public health (Fernandez-Luqueno et al. 2013; Giri 2021; Saravanan et al. 2021). Inland waters, especially rivers, are specific cross points in urban water cycles, considering their role as both freshwater sources and main recipients of wastewater effluents. The extensive use of wastewater treatment plants (WWTPs), implementation of different treatment facilities and advanced technologies for removal of pollutants in waste flows is currently an environmental standard in water management practice and significantly reduces the release of pollutants in the aquatic environment (Wang et al. 2022). Despite the innovations that are being applied to wastewater treatment processes, the effluent characteristics always remain worse than those in the receiving natural waters. That is why the continuous discharge from the WWTP is considered as a factor with a high-risk level for the functioning and ecological status of aquatic ecosystems (Drury et al. 2013). Effluents from WWTPs are significant sources of organics, nutrients, hazardous micropollutants and pathogenic microorganisms (Kiedrzyńska et al. 2014). The river ecosystems respond to this impact by a realization of different abiotic and biotic transformation processes known as self-purification. The specific transitional areas of WWTP discharges are important nodes in system functionality and keys for achieving effective utilization of pollutants. In the biotic part of self-purification, the contribution of microbial communities and their enzyme systems is fundamental for the fate of pollutants, carbon fluxes and nutrient cycles (Findlay and Sobczak 2000; Panigrahi et al. 2019). Microbial activity is a driving force in the processing of organic matter and the whole ecosystem metabolism in aquatic habitats (Zeglin 2015). In this context, the standard use of biological indicators, based on macroorganisms such as macrozoobenthos, macrophytes or fishes, can be successfully extended to include the assessment of microbial communities (Lau et al. 2015; Chen et al. 2021). The changes in microbial diversity, community structure and functionality have the potential to be sensitive indicators for the assessment of pollution impact and associated environmental risks from WWTP effluent discharge (Lear et al. 2012; Zhang et al. 2021; Sun et al. 2022). Another important factor must also be taken into account when evaluating the complex processes of pollution/self-purification in disposal areas of WWTP – the structural complexity of rivers. The sediment component with its stability and

heterogeneity has a dominant role in the functioning of these aquatic ecosystems as a trap for some pollutants but also as a habitat for metabolically active organisms, forming the specific sediment microbiome.

In Bulgaria, the upper Iskar sub-catchment (Danube River Basin) is one of Bulgaria's most important economic and socially significant water sources in Bulgaria because of its role in supplying the capital Sofia with drinking water and upholding the city's urban water cycle. The biggest reservoir in Bulgaria (Iskar Reservoir) is situated in this sub-catchment and provides more than 70% of Sofia's drinking water. According to official data, the waters of the Iskar River basin are in good/moderate ecological status to Sofia, after which the status is moderate except for the section after inflow of Vladayska River, where the status is categorized as bad (http://www.bd-dunav.org/uploads/content/files/upravlenie-na-vodite/ocenka-na-sustoianieto/povurhnostni-vodi/BDDR_analiz_SWB_2019-2020.pdf). The critical factors with potential high-risk levels for water quality in the upper Iskar aquatic ecosystems are the discharge from the WWTP in the town of Samokov, diffuse pollution from agriculture, and the penetration of untreated sewage from the small villages. Failing to make maximum use of the capacity of the treatment plants, exacerbated by an inflow of untreated water, as well as several diffuse sources, constitute some of the background explanations for pollution in the upper valley of Iskar (Ministry of Environment and Water 2021).

This study aims to assess the environmental impact of treated effluent discharge from the WWTP in Samokov municipality in the upper part of Iskar River. We use a complex approach with a combination of indicators (chemical, microbiological, biological quality elements) to assess local changes in water quality, ecological status and microbial communities. The paper is structured as follows: (1) Firstly, we discuss the effect of WWTP discharge on physicochemical parameters and nutrient concentrations on the water of the disposal area; (2) An analysis of the concentrations of selected hazardous and specific pollutants (microelements) in water samples is also presented; (3) The abundance and activity of microbial communities are assessed in water and sediments by standard cultivation and fluorescence techniques; (4) Finally, an assessment of ecological status in river sector by quality element "macrozoobenthos" is conducted and then discussed.

Materials and methods

Study area

The study area is located in the upper part of the Iskar River before the Iskar Dam in Northern Rila, Bulgaria. Iskar is the longest (wholly) Bulgarian river (368 km) with a river basin of 8 650 km². The study river sector is 10 km in length, and 25–35 m in width; its depth ranges from 50 to 200 cm and the bottom substratum consists of pebbles, coarse and medium sands. The seasonal character of flow is determined with summer and winter low flow (1–3 m³/s), a little increase in flow during the autumn

(6–10 m³/s) and very expressive spring high water level (15–25 m³/s). According to a map of the land use, agricultural land, pastures and forests dominate the area (Topalova et al. 2013; Todorova et al. 2017). In this river sector, one significant source of point pollution is registered – the discharge from WWTP Samokov (44 100 m³/day). The treatment plant treats the mixed wastewater from the town of Samokov (municipal, industrial and storm) and works on the denitrification/nitrification scheme.

Sampling and field analyses

The sampling design included sites upstream and downstream of the WWTP. We carried out two sampling campaigns in November 2020 and March 2021 when the average water flow is 5–8 m³/s. Paired water and sediment samples were collected from four sampling sites (Fig. 1).

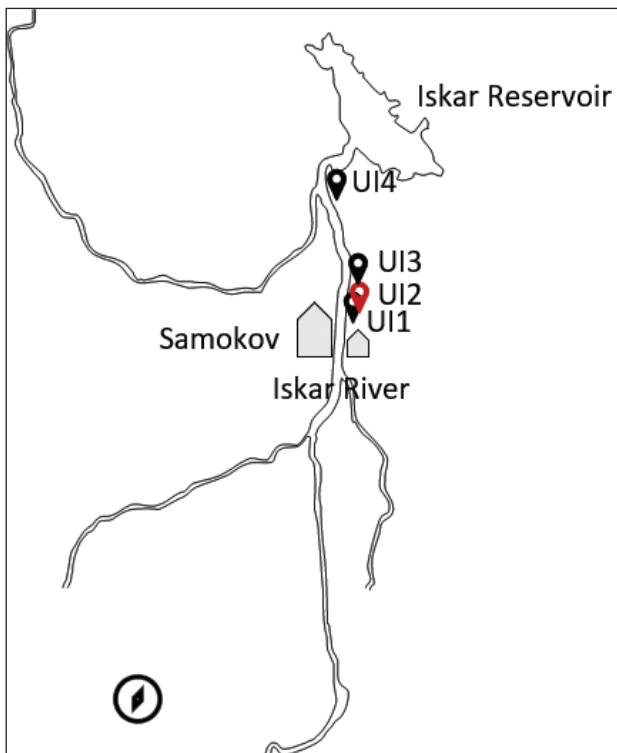


Figure 1. Location of the sampling sites in the study area of the Upper Iskar subcatchment

- Sampling site UI1 – Iskar above WWTP Samokov
- Sampling site UI2 – Discharge of WWTP Samokov
- Sampling site UI3 – Dragushinovo village, under WWTP Samokov
- Sampling site UI4 – Iskar River, near the Villa zone Mechkata (site from monitoring system for surface water bodies in Bulgaria)

We analyzed the physicochemical parameters (temperature, oxygen concentration, conductivity and pH) of the water *in situ* immediately after sampling with a portable oxygen and pH meters. The hand net was used for collecting the macroinvertebrates – up to 10 sub-samples were collected on one site according to multihabitat approach (EN ISO 10870:2012, EN ISO 16150: 2012). The assessment of the ecological status was conducted by metrics “biotic index” in ranges for river types R4 (semi-mountain rivers in 12 Ecoregion “Pontic Province”). The sediment samples for chemical, microbiological and fluorescent analyses were collected by manual dragging. The all water and sediment samples were transferred in sterile containers at 4 °C storage and processed within 24 h.

Chemical analyses

The determination of the organic loading in the water was performed by measuring the chemical oxygen demand (COD) by colorimetric dichromatic method. Nitrogen was measured as dissolved inorganic forms – ammonium and nitrate ions (colorimetric methods). The concentrations of phosphorus were defined as phosphates also by use of colorimetric method. Procedural details for measuring nutrients and organics were in line with standard methods recommended in EN ISO standards. The concentrations of selected microelements in the water samples were determined with inductively-coupled plasma mass spectrometry (ICP-MS, PerkinElmer SCIEX Elan DRC-e). The number of the decimal places is related to the precision of the measurements. The estimation of accuracy was conducted by the analysis of two water standard reference materials: SPS-SW2 (Reference Material for Measurement of Elements in Surface Waters, Spectrapure Standards, Norway) and NWTM-23.5 (Environmental matrix reference material, a trace element-fortified sample, Environment and Climate Change, Canada). The experimental results were in very good agreement with the certified values.

Data from chemical analyses of water and assessment of ecological status was compared with the requirements of Bulgarian legislation (Regulation No. H-4 of 14.09.2012 on characterization of surface water and Regulation No. 12 of 18.06.2002 on the quality of surface water intended for drinking and household purpose). Selecting microelements and ranking hazardous and specific pollutants was conducted on the basis of the EU-list of priority substances (Water Framework Directive 60/2000/EC (WFD) and Regulation on environmental quality standards for priority substances and some other pollutants, 2015) and the above-mentioned regulations.

Analyses of microbial communities

The total microbial count and number of coliforms was determined by the use of standard count-plate technique on Nutrient agar and Lactose TTC Agar with Tergitol 7 (Merck Millipore). The sediment samples were preliminarily treated with ultrasonic disintegrator VCX 750, Sonics & Materials Inc. (3 times × 10 sec). The data for microbial counts were normalized and presented as ln CFU/mL or ln CFU/g dry weight.

For analysis of changes in microbial activity of sediment communities, we used a modification of CTC/DAPI staining method with fluorescence imaging. CTC (5-cyano-2,3-ditolyl tetrazolium chloride) enters in cells and is reduced to CTC-formazan (fluorescent red signal). The content of reduced compound depends on the electron transport activity (activity of the dehydrogenase enzymes in viable cells) and is considered as an indicator of their metabolic activity. DAPI (4',6-diamidino-2-phenylindole) is fluorescent dye that specifically binds to nucleic acids but enters both in live and fixed cells. It is widely used for the enumeration of bacterial abundance. The combined staining with CTC and DAPI is applied to distinguish the active fraction in different microbial communities (Topalova et al. in press). The epifluorescent microscope Leica DM6 B was used to make fluorescence images, and then a digital image analysis (using the software *daime*) was applied to assess the total area, the area of fluorescent objects, and mean fluorescence intensity. From these parameters the percent of live, metabolically active cells in images was calculated (Daims et al. 2006; Topalova et al. in press).

Results

Effect of WWTP discharge on physicochemical parameters and nutrient concentrations in water

In the study area, the water temperature showed typical seasonal dynamics and varied from 4.2 °C at sampling site UI3 to 8.9 °C at sampling site UI2 in November (Table 1). The highest temperature in both seasons was reported in the water from the discharge area of WWTP. The values of pH varied between 7.63 and 8.80 – the highest value was measured at UI2. The content of dissolved oxygen is usually high for this part of the Iskar River because of its rapid flow rate. The lower concentrations in all sampling sites for this parameter were measured in March, when the water level and turbidity were higher as snow melted, and with the onset of the wet season. At the point of WWTP discharge, the oxygen concentration was significantly lower during both samplings and the conductivity was higher than the site located above. In March, the measured values for oxygen and pH in UI2 exceeded the recommended norms for high status but the fluctuations were assimilated downstream.

In Fig. 2 we present the impact of WWTP effluent on the dynamics of nutrients (nitrogen and phosphorus) in surface water for both sampling campaigns (Fig. 2A

Table 1. Physico-chemical parameters of the waters in the upper valley of the river Iskar.

Sites	Temperature °C		Oxygen, mg/L		Conductivity µS/cm		pH	
	Nov.	March	Nov.	March	Nov.	March	Nov.	March
UI1	4.7	5.0	12.14	7.71	114	295	8.21	7.63
UI2	8.9	8.8	9.39	6.04	291	341	8.00	8.80
UI3	4.2	5.3	12.80	8.25	90	209	8.19	7.68
UI4	4.9	6.2	12.33	8.73	177	422	7.75	7.73

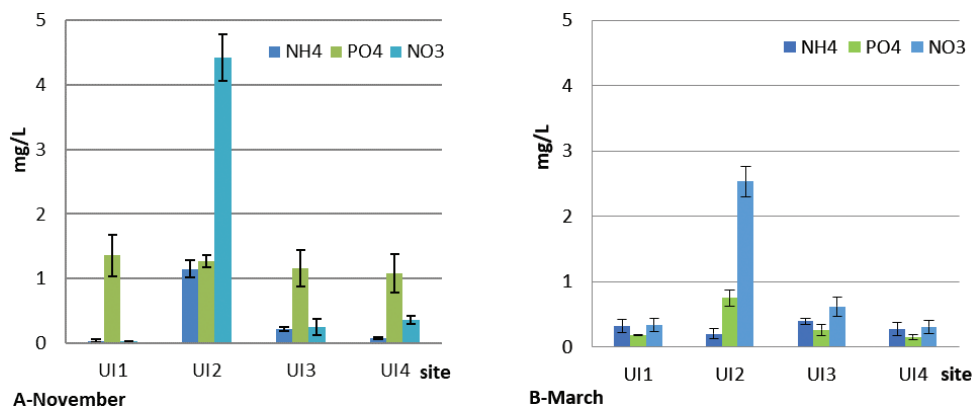


Figure 2. Dynamics of ammonium ions, phosphates and nitrates in surface water of study area in upper Iskar River (A – November, B – March).

– November and Fig. 2B – March). With regard to nitrogen, there was a significant impact on concentration of nitrates and ammonium ions from the discharge from the WWTP effluent. The highest values of nitrates were registered in water of sampling site UI2 during the both samplings. The concentration of nitrates increased almost 20-fold in comparison with the previous site in November and 8-fold in March. The situation was similar for ammonium during the autumn – the increase was more than 15-fold. Phosphate concentrations in surface waters were higher in November (between 1.087÷1.358 mg/L) compared to the values observed in March (0.188÷0.750 mg/L). During the autumn, the phosphate concentration retained similar very high levels in the whole river sector. In March, the discharge of WWTP effluent affected the phosphate concentration in the main river channel and the measured value at UI2 site was 0.750 mg/L. The nitrates were in the range acceptable for surface waters, although the WWTP effluent affected their concentration, but the ammonium ions and phosphates exceeded the values admissible according to the stipulations of Bulgarian legislation.

The values for chemical oxygen demand (COD) during the two samplings at three of the four sampling sites were below 10 mgO₂/L. The highest concentration was measured in site UI2 in March – 11.98 mgO₂/L.

Analyses of selected microelements (metals and metalloids) in water

The concentrations of the selected microelements in the water samples are presented in Table 2. The data showed an increased level of risk, associated with seasonal dynamics of Hg, being a priority pollutant (Regulation on environmental quality standards for priority substances and some other pollutants, 2015) and Cu – the measured values in November were higher than those measured in March for all sampling sites. The highest mercury concentration was determined in the water of site UI3 (after discharge of WWTP) – 1.42 µg/L; for copper – 44 µg/L was measured at site UI2. The concentrations of Cu increased more than 10–30 times in the autumn. The concentrations of the

Table 2. Concentrations of selected microelements in the water of the study area (the values exceeding the maximum admissible concentrations are marked in gray).

		Pb	Cd	Hg	Ni	Mn	Cr	As	Cu	Fe	Zn
		µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L
UI1	Nov.	0.150	0.031	0.12	1.04	2.20	1.830	1.15	21.60	0.120	0.062
UI1	March	0.113	0.028	<LOD	0.480	1.10	0.166	0.163	1.56	0.098	0.004
UI2	Nov.	0.032	0.073	0.13	0.90	0.18	0.820	0.85	44.00	0.051	0.045
UI2	March	0.011	0.352	0.27	2.470	0.43	0.526	0.408	1.48	0.006	0.019
UI3	Nov.	0.084	0.030	1.42	0.30	1.13	1.100	0.59	21.30	0.112	0.058
UI3	March	0.022	0.035	<LOD	0.548	0.37	<LOD	0.245	1.29	0.027	0.003
UI4	Nov.	0.008	0.071	0.24	0.46	0.90	1.039	0.50	24.20	0.094	0.076
UI4	March	0.167	0.072	<LOD	1.640	1.46	0.498	0.310	1.81	0.184	0.003

rest of the microelements were below the admissible values in both seasons. As a result of increased anthropogenic activity in the post-summer period, significant seasonal changes are worth noting in all sampling sites for Zn (till 25 times in UI4), Cr (till 11 times in UI1) and As (till 7 times in UI1). To a certain extent, the same tendency is also established for Fe with the exception of sampling place UI4. The contents of the toxic elements Cd, Ni, Pb, being also priority pollutants, and Mn showed relatively close values in the studied sampling sites and significant stability between the seasons.

Analyses of water and sediment microbial communities

The data from determination of total microbial count and coliforms in water and sediments are presented in the figures below (Fig. 3, 4).

In water samples from the studied part of the river, we enumerated stable values for total culturable microflora – in the range of $10^3 \div 10^4$ CFU/mL. The coliforms were constantly presented in water samples with abundance of $10 \div 10^2$ CFU/mL. According to site location, the numbers of two indicator groups showed a slight increase in the sampling site of WWTP discharge during the each season studied. In the sediment samples, the total microflora was more abundant and variable. The numbers fluctuated between $10^5 \div 10^7$ CFU/g. In the sediments of discharge area of WWTP, the increase in numbers of two indicator groups was significant, especially for coliforms ($3\ 000 \div 11\ 000$ CFU/g).

The mean fluorescence intensity and percent live cells were calculated from the total area and the area of fluorescent objects on images from CTC/DAPI analysis (Table 3). The fluorescence intensity as an indicator of viability and activity of cells was assessed on CTC staining images. It can be seen that the parameter was up to 2.3 times higher in UI3 when compared to the other two sites. The metabolic activity of the bacteria in that site remains high also in March of the following year (with up to 35%). The share of live cells was calculated as ratio of bacteria with metabolic activity (CTC) to total bacteria (DAPI). At sampling site UI3, a high share of live cells (2.20%) was also detected in November, but their share decreased by 10.5 times in March. The data for UI2 revealed clear differences in the share of live cells in sediment samples compared to

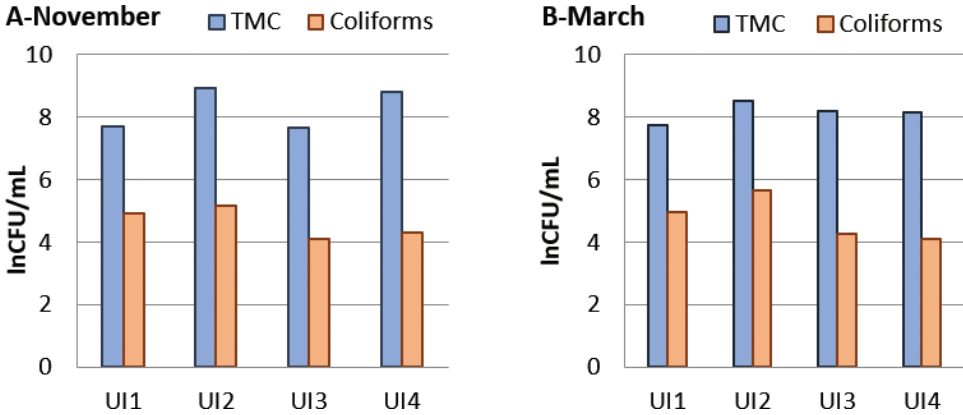


Figure 3. Total microbial count and coliforms in surface water **A** November **B** March.

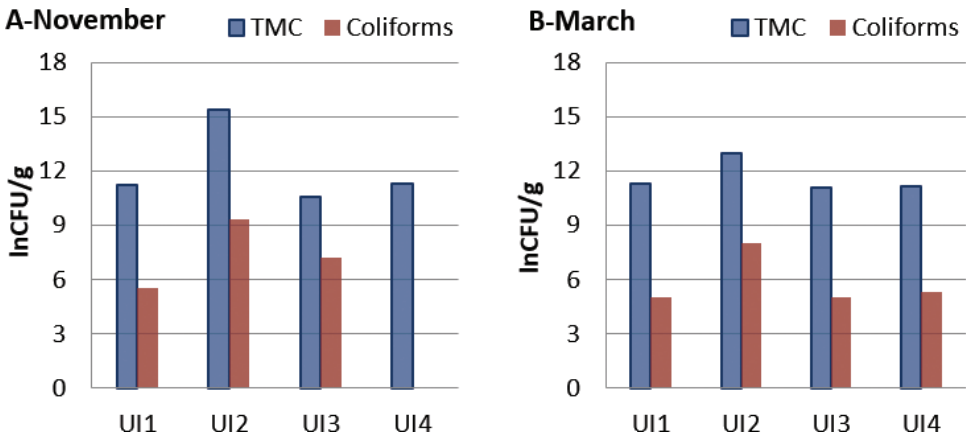


Figure 4. Total microbial count and coliforms in sediments **A** November **B** March.

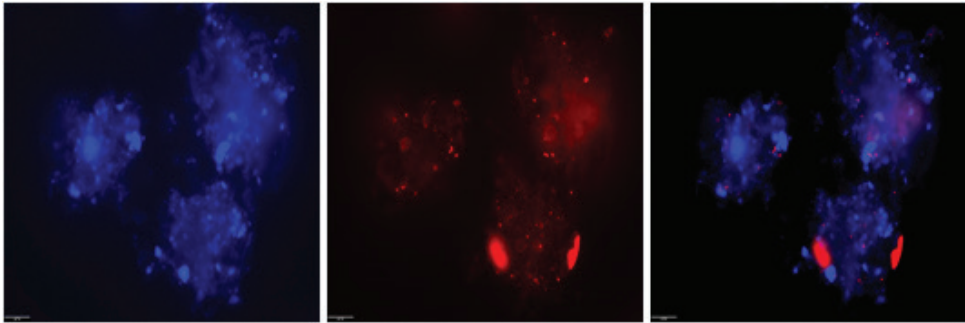
UI1 – the live cells were about 3% in both seasons. The fluorescent images of sediment communities in sampling site UI2 were presented in Fig. 5.

Assessment of ecological status

The ecological status of the studied river sector was assessed as “good” according to WFD and Regulation N-4/2012 using macroinvertebrate bioindicators. The metrics Biotic index had a score of 4 in both sites for macrozoobenthic analyses (UI1, UI3). Despite similar ecological status, in sampling site UI1 species tolerant to deterioration in environmental conditions were found – *Hydropsyche* sp. (Trichoptera), Chironomidae (Diptera), *Erpobdella octoculata/monostriata* (Hirudinea). After discharge of WWTP Samokov, in sampling site UI3, the total taxa number was higher and *Baetis* sp. (Ephemeroptera) was also found.

Table 3. Mean intensity and percent live cells in sediment samples.

Sites	mean fluorescence intensity, CTC		percent live cells, CTC/DAPI	
	November	March	November	March
UI1	80.25	99.67	0.40	0.83
UI2	79.67	96.67	3.07	2.81
UI3	182.33	130.00	2.20	0.21

**Figure 5.** Fluorescent images of sediment communities in sampling site UI2 (left – DAPI staining, middle – CTC staining, right – CTC/DAPI staining).

Discussion

Many previous studies have discussed the response of aquatic ecosystems to WWTP effluents by assessing various indicators, but in most cases, the analyses were performed on specific communities or groups of indicators (Todorova and Topalova 2009; Drury et al. 2013; Zeglin 2015; Todorova et al. 2017; Kenderov and Trichkova 2020; Wang et al. 2022). In our study, we applied a comprehensive approach to assess the impact of WWTP effluent on water quality characteristics, the changes to the number and functioning of aquatic and sediment microbial communities, as well as an overall assessment of the ecological status. The synthesis of obtained results demonstrates the expected negative impact of WWTP discharge on water quality – the waters in the area of discharge had higher values of temperature, conductivity, nitrates, ammonium ions, phosphates and lower concentration of dissolved oxygen. This impact of WWTP effluent is partially assimilated along the river, but the analyses performed in other sampling sites reveal the salient problems affecting the whole study area – high concentrations with seasonal fluctuations of ammonium ions, phosphates and some hazardous/specific pollutants (Hg, Cu). At subcatchment scale, considering the location and strategic role of Iskar Reservoir, it is necessary to pay special attention to these results and implement better control measures for these parameters. The risks, associated with the potential eutrophication of stagnant water bodies and toxic effects of Hg and Cu, are serious threats for water quality and ecological status.

The data about nitrates, ammonium ions and phosphates also are of interest when we refer them to the measured low organic content in the waters of the studied area. The discharge of the WWTP does not lead to additional organic loading and in the

aqueous phase there is an imbalance in the ratio C:N:P, which further complicates the utilisation of nitrogen and phosphorus by heterotrophs. However, if we look at the river ecosystem in its heterogeneity, namely the sediment component, the high abundance of microorganisms in the sediment microbiome is impressive. The sediments are stable, active habitat where the predominant part of the transformation processes is probably quickly realized. The more diverse redox and oxygen regimes, the different ecological niches, and the longer retention of organic matter suggest that the sediments are the habitat where the full variety of self-purification processes unfolds. This is confirmed by the high metabolic activity and the share of live cells in sediments – these indicators have the higher values in the discharge area of WWTP and at the sampling site located downstream. The registered high fluorescence intensity at the site under the treatment plant shows that the activity of sediment microbiome remains high downstream, despite the fact that the number and share of live cells decreased. The assessment of the ecological status confirms the role of the sediments for the retention of the organics and the fast realization of the self-purification processes after the discharge. At the same time, the fact that the sediment habitat also serves as a potential refuge for opportunistic and pathogenic microorganisms must be taken into account. The high abundance of coliforms shows the potential role of sediments as a “natural depot” and bring to the fore the recommendation for including the sediment component into the system of water quality monitoring in the upper subcatchment of Iskar River.

Compared to our earlier studies in this part of river subcatchment (Todorova and Topalova 2009; Todorova et al. 2017; Kenderov and Trichkova 2020), the current ecological status and water quality have deteriorated. The discharge from WWTP is combined with negative effects of intensive agriculture and livestock breeding in area – the cumulative environmental impact is significant and can have consequences in the future, although self-purification is currently working effectively enough.

Conclusion

Along with the positive aspects of the increased number of WWTPs worldwide, the associated environmental risks of their operation must be taken into account, especially in terms of the functioning of urban water cycles. The importance of the studied sub-catchment of Upper Iskar for the quality of drinking water of Sofia enforces the extension of an existing monitoring program with a more detailed assessment of the environmental impact of wastewater discharge.

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