

## SEDIMENT MICROBIAL COMMUNITY PROFILE IN UPPER PART OF ISKAR RIVER

Yovana Todorova, Ivaylo Yotinov, Dayana Todorova, Yana Topalova

**Abstract:** Iskar River and its reservoir are the water sources in Bulgaria with the most important economical and social meaning because of their role for drinking water supply of Sofia. In this study, we focus on the sediment microbial community in upper part of Iskar River subcatchment as functional diversity related to main self-purification processes and as a risk factor for water quality. Microbial communities in freshwater sediments and their associated metabolic activity have fundamental role in ecology and metabolism of river ecosystems. The functional profile of sediment microbial communities was studied over a period of 8 years. The obtained results show that the microbial groups participating in organic carbon transformation and specific indicators (bacteria from g. *Pseudomonas* and coliforms) have higher quantitative parameters in sediments near to discharge of WWTP Samokov. The data for high ecological and chemical state of river sector confirm that the impact of WWTP discharge is in the frames of self-purification capacity of ecosystem and do not disturb significantly the water quality. In sediments of Palakaria River, the bacteria from nitrogen cycle are detected in permanently high number. These data are related to significant nitrogen loading identified in the river and present an adequate response of microbial community structure to this type of disturbance. The modeling of ecosystem response to risk situation of overloading with nutrients and organics confirms the high self-purification capacity of Iskar River. The importance of studied subcatchment for formation of water quality in Iskar Reservoir and literature data for sediment function as depot for coliforms in other rivers enforces the extension of existing monitoring program with regular assessment of this indicator in the sediment component of the ecosystem.

**Keywords:** microbial community profile, sediments, Upper Iskar, self-purification, transformation processes

### INTRODUCTION

Microbial communities in freshwater sediments and their associated metabolic activity have fundamental role in ecology and metabolism of river and stream ecosystems [3, 19]. The biomass and taxon richness in sediment are much higher than those of the corresponding water bodies [21]. Sediment receives deposition of bacteria and organic matter from the upper water layer and provides a matrix of complex nutrients and solid surfaces for microbial growth [20]. The sediment microbiota dominates in riverine biochemical cycling of nutrients, realizes the organic decomposition and leads to transformation of hazardous pollutants. The benthic microbiota associated with sediment biofilms and free organic matter particles is metabolically the most active and is the main biotic transformation factor in rivers [8]. Mapping and profiling of bacterial taxonomic and functional diversity in different lotic biomes, is related to the better understanding how this diversity is modulated by environmental and anthropogenic drivers and give the way for effective integration of bacteria into predictive ecological models [7]. The resistance and resilience of natural microbial community in sediments are important features in ecosystem response to all kinds of disturbance and microbial profile may provide a relatively stable indicator for ecosystem modifications, especially in long-term monitoring [9]. Allison and Martiny find that the

composition of most microbial groups is sensitive and not immediately resilient to disturbance, regardless of taxonomic width of the group or the type of disturbance. Their review demonstrates that changes in microbial community composition are often associated with changes in ecosystem process rates and may directly affect ecosystem processes [1]. The analysis of the kinetic parameters of the main transformation processes realized by microbiota is an important element in assessing the self-purification capacity of the river ecosystem and provides realistic information on the rate of elimination of the key types of pollutants.

The profiling of sediment microbial biodiversity should include another important element – a human health risk aspect of the problem. Freshwater sediments have recently attracted more attention as important depot of some potentially dangerous bacteria as coliforms and study of this interaction has a critical significance in hydrosystems used as strategic sources of drinking water [2]. The biodiversity and functional stability of autochthonous microbial communities are essential for the incorporation and survival of potentially dangerous allochthonous bacteria. The cumulative effect of the total indigenous microflora on *E. coli* survival for example is often negative as a result of predation, substrate competition and antagonism [5, 10, 16]. The diversity of the indigenous microbial communities is an important factor for regulation of

the population dynamics of invading *E. coli* [17, 18]. According to this view, the ecosystems with a higher level of biodiversity are more resistant to disturbances than these with a lower diversity [11].

In this study, we focus on the sediment microbial community in upper part of Iskar River subcatchment in both of discussed aspects: as functional diversity related to main transformation processes of pollutants and as a risk factor for water quality of drinking water source. The functional profiling is extended with data for ecosystem/biotic response at simulated risk loading with high organics and nutrients concentration. The modeling of kinetics of main transformation processes and its relationships with parameters of sediment microbial communities is important element in assessment of self-purification capacity of river and in prediction of rate of ecosystem metabolism.

## MATERIALS AND METHODS

### *Study area and site description:*

The study area is situated in upper subcatchment of Iskar River (892 km<sup>2</sup> with average latitude of 1314 m) before the Iskar Dam in north Rila Mountain, Bulgaria. Iskar River and its reservoir are the water sources in Bulgaria with the most

important economical and social meaning because of their role for drinking water supply of Sofia (capital of Bulgaria). The study sector of Iskar River is 25–35 m wide, depth ranges from 50 to 200 cm and the bottom substratum consists of pebbles, coarse and medium sands. It has a clearly determined seasonal character with summer and winter low flow (1–3 m<sup>3</sup>/s), a little increase in flow during the autumn (6–10 m<sup>3</sup>/s) and very expressive spring high water level (15–25 m<sup>3</sup>/s). The tributary Palakaria River is the longest in Iskar River upper subcatchment with the length of 44 km, an average stream flow of 1–2 m<sup>3</sup>/s and a basin area of 408 km<sup>2</sup>. For the last years it was in bad ecological status and had a possible risk for significant pollution with nutrients. There are several point sources identified with significant impact in Palakaria River – discharges from local wastewater treatment plant (WWTP), petroleum station and food industry. The studied sector of Iskar River was in unknown ecological status according to official monitoring information and in very good/good status according to scientific researches; only one significant impact was identified – discharge of WWTP Samokov. The land use in the upper Iskar subcatchment consists of agricultural lands (40–50%) and forests (30–50%) [6, 13, 15, 22].



Fig. 1. Study area and location of sampling sites

*Iskar – Iskar River, located on 903 m altitude, 42°21'963"N, 23°33'357"E, below the town of Samokov and after the influx of municipal wastewater treatment plant*

*Palakaria – Palakaria River, on 835 m altitude, 42°25'186"N, 23°31'445"E, before confluence with Iskar River*

Two sampling locations - one in main river channel of Iskar River and one in tributary Palakaria River were sampled during the low flow periods of 2008 and 2016. The sampling sites, target river component – sediments and investigated microbial groups have been selected by their key significance for ecosystem functioning and formation of water quality. The sampling sites are presented in Fig. 1.

### *Sediment microbial sampling and analysis:*

The samples were collected from sediments by manual dragging, transferred into sterile containers, stored at 4°C and processed within 24 h. Sediments were collected with unified particle size < 1 cm.

The functional profile of microbial community was studied from two points of view: (1) analysis of functional microbial groups with a key role for realization of self-purification processes in river ecosystem; (2) analyses of risk water quality factor – coliform bacteria.

The abundance of selected physiological bacterial groups was measured by use of count-plate or most probably number techniques. The sediments were preliminary treated with ultrasonic disintegrator UD – 20 automatic (3 times x 5 sec) for detachment of bacteria from sediment particles. The

following key microbial groups were analyzed by use of specific nutrient media according to BDS-EN-ISO standards (Table 1). The coliforms were detected additionally and confirmed by cytochrome oxidase test. The all data for microbial counts were presented as ln CFU/g dry weight.

Table 1. Microbial groups and methods for their determination

<i>Functional group</i>	<i>Nutrient media and cultivation</i>
Aerobic heterotrophic bacteria (AH)	Nutrient agar, 24-48 h at 35°C
Anaerobic heterotrophic bacteria (AnH)	Nutrient agar, 7-14 days under anaerobic conditions at 35°C
Oligotrophic bacteria (Oligo)	Diluted Nutrient agar (1:10), 48 h at 28°C
<i>Enterobacteriaceae</i> (Colif)	Endo agar, 24-48 h at 37°C
<i>Pseudomonas</i>	GSP agar, 48 h at 28°C
Nitrifying bacteria (Nitrif)	Sarachandra broth, 5 days at 28°C
Denitrifying bacteria (Denitr)	Giltay agar, 7-14 days in anaerobic conditions at 28°C
Ammonifying bacteria (Ammon)	Nutrient broth with Phenolrot, 3-5 days at 28°C

### **Calculation of kinetic parameters of main transformation processes in sediments**

The functional profiling of microbial communities in sediments was supported by data for biotic response to high organics and nutrient concentrations discharged in river ecosystem. This modeling simulates the ecosystem utilization of accidental discharge of untreated wastewater from municipal origin – one of the high risk impacts in the study area. The model risk concentrations were 10 times higher than the normal background and were measured at previous real critical event [13]. We used a modification of Nutrient Spiraling Concept for calculation of model kinetics of simulated risk situation in which the discharges of

WWTP were regarded as model of significant nutrient and organic loading [4]. The following parameters were calculated (Table 2).

For calculation of basic kinetics of nutrient and organic utilization, some chemical parameters of water were analyzed as normal background concentrations in watershed and at discharge of WWTP for simulation of risk event. The organic loading of water was measured as COD (Chemical Oxygen Demand – dichromatic EPA 410.4/ISO 6060 method). The ammonium and nitrates were determined by colorimetric methods according to the BDS-EN-ISO standards. The phosphorus was measured as phosphates (colorimetric method based on phosphomolybdic blue reaction).

Table 2. Model kinetic parameters and their definitions [4]

<i>parameter</i>	<i>calculation</i>	<i>definition</i>
net nutrient uptake length, $S_{net}$ , m	$C_0 = C_x e^{kx}$ $\ln(C_x/C_0) = kx$ $S_{net} = -1/k$	negative, inverse slope of the regression between the natural logarithm of the proportion of nutrient concentration in water and distance from the WWTP
mass transfer coefficient, $V_{f-net}$ , m/sec	$V_{f-net} = hu/S_{net} = Q/(S_{net} w)$	vertical velocity at which a nutrient molecule travels from the water column to the stream/river substrate
net nutrient uptake rate, $U_{net}$ , mg/ m <sup>2</sup> sec	$U_{net} = (C_b Q)/(S_{net} w) = V_{f-net} C_b$	uptake rate of nutrients for unit of stream/river substrate

\*where  $C_x$  is the nutrient concentration (mg/L) at distance  $x$  (m) from the WWTP,  $C_0$  is the nutrient concentration (mg/L) at the most-upstream site below the WWTP, and  $k$  is the nutrient change coefficient (1/m),  $h$  - average water depth (m),  $u$  - average water velocity (m/s),  $Q$  - average discharge (m<sup>3</sup>/s),  $w$  - average stream width (m),  $C_b$  - background concentration in watershed

## **RESULTS AND DISCUSSION**

### **Key microbial groups for realization of self-purification processes:**

Functional profile of sediment microbial communities in upper Iskar subcatchment is

analyzed in concern of biotic transformation cycles of organic carbon and nitrogen – pollutants with significant meaning according to risk analyses of anthropogenic impact [22]. The key microbial groups with an important role in organic matter

transformations - aerobic heterotrophic, anaerobic heterotrophic and oligotrophic bacteria are presented on Fig. 2. These three groups are included in effective utilization of varied concentrations of organics (a common situation in streams and mountain sectors of rivers) under conditions of quick alteration of aerobic/anaerobic microhabitats in sediments.

The long-term comparison for period of 8 years shows an increase of counts of these bacteria for two sampling sites. The increase is especially well presented for aerobic heterotrophic and anaerobic heterotrophic bacteria – these groups have the higher number with one order in 2016 compared to this in 2008. The oligotrophes retain their abundance on the similar level. This result indicates a frequent change in dominant microbial groups responsible for organics transformations in sediments and quick functional shift for utilization of different organics

concentration. The sediment functions of deposition and accumulation of organics and the variety of environmental conditions in microhabitats are the leading factors for this flexible adaptation of sediment heterotrophic bacteria. Their high number is indicator for good self-purification potential and ability for realization of anaerobic/aerobic transformation in the whole ecosystem. It can be assumed that the local sediment microflora is very well adapted to the permanent nature of the identified organic discharges and utilizes quickly and effectively the incoming organic matter.

The numbers of microbial groups responsible for nitrogen transformations in sediments are presented on Fig. 3. The bacteria participate in the first stages of non-specific nitrogen transformations /degradation of N-contained organics/ - ammonifying bacteria is the most abundant nitrogen-transforming group.

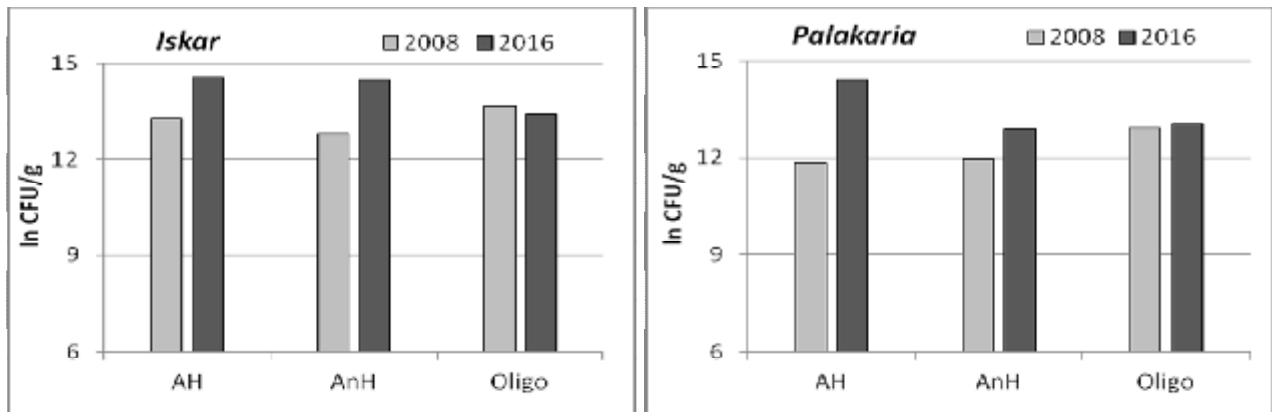


Fig. 2. Abundance of key microbial groups for realization of organic matter transformations in Iskar River (left) and Palakaria River (right) during the two studied periods

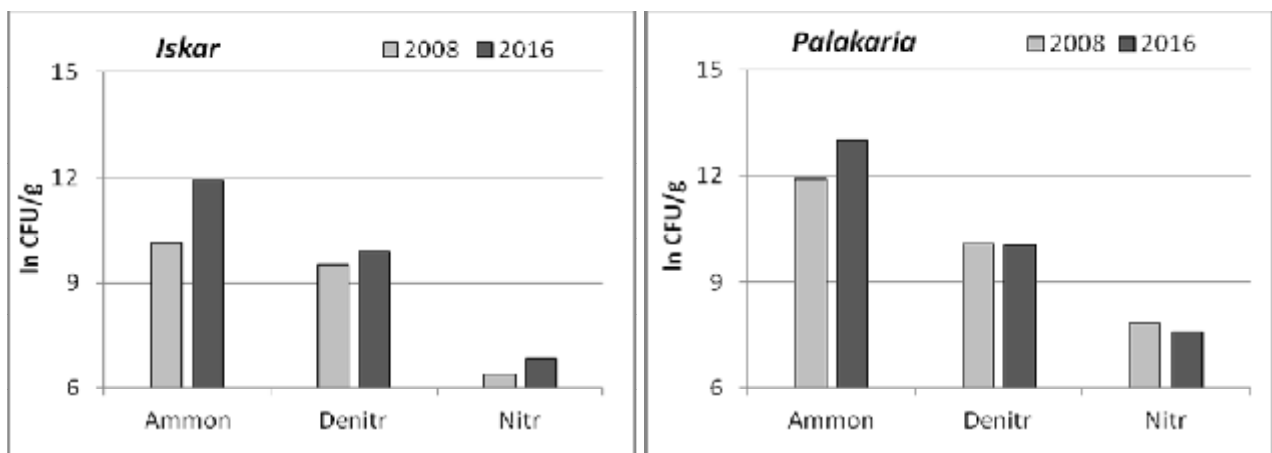


Fig. 3. Abundance of key microbial groups for realization of nitrogen transformations in Iskar River (left) and Palakaria River (right) during the two studied periods

Its quantitative parameters varied in the range of  $10^5$ - $10^6$  CFU/g. The more specific groups of denitrifying and nitrifying bacteria are less represented, as the nitrifiers are not exceeding 2000 CFU/g. These results determine the processes of nitrification and denitrification as critical for realization of nitrogen cycle still more the denitrification is the only way to eliminate completely from river ecosystem the surplus amount of nitrogen. In the past periods the local nutrient pollution was registered and the balance in nitrogen transformations was a natural highly effective mechanism for realization of successful self-purification [12].

The relationship between dynamics of sediment microbial community, its contribution for functioning of nutrient cycles and water quality in the subcatchment is supported by the comparison of the data in two sampling sites. In the both of studied years, the abundance of the three microbial groups is higher in sediments of Palakaria River. This result is in accordance with identified risk for significant pollution with nutrients and very high loading of nitrogen in Palakaria River (assess on more than 530 000 kg/year). For sector of Iskar River this annual loading is assess only on 89 000 kg/year [22]. The data present the strong interaction between sediment microflora and ecosystem response to high nutrient loading.

#### **Microbial groups with specific indicative role in sediments:**

The bacteria from genus *Pseudomonas* has been developed as an indicator for the biodegradation of xenobiotic organics, for flexible and adaptive metabolic potential of microflora [15]. In nature waters and drinking water sources, g. *Pseudomonas* is subject to assessment primarily because of the pathogenic representatives in both genera. The above discussed indicative abilities are still subject to further research, but it can be assumed that the high quantitative presence of the group is an indicator of the possibilities of natural microflora to realize the full set of metabolic potential to eliminate pollutants with a different hazardous nature, and to develop the self-purification full capacity on ecosystem level.

The dynamics of *Pseudomonas* during the two studied periods is shown on Fig. 4. In sediments of Iskar River next to the influx of WWTP, the pseudomonades have higher number than these in Palakaria River. The discharge of treated wastewaters is always considered as a critical factor for input of pollutants with different nature in river ecosystem (detergents, oil products, pharmaceutical and personal care products). In the whole upper subcatchment of Iskar River there are no deviations from the environmental quality standards for hazardous pollutants [6, 22] and the high abundance of *Pseudomonas* in this site is indicator primarily for a high biodegradation potential of sediment microflora and good self-purification capacity of the ecosystem.

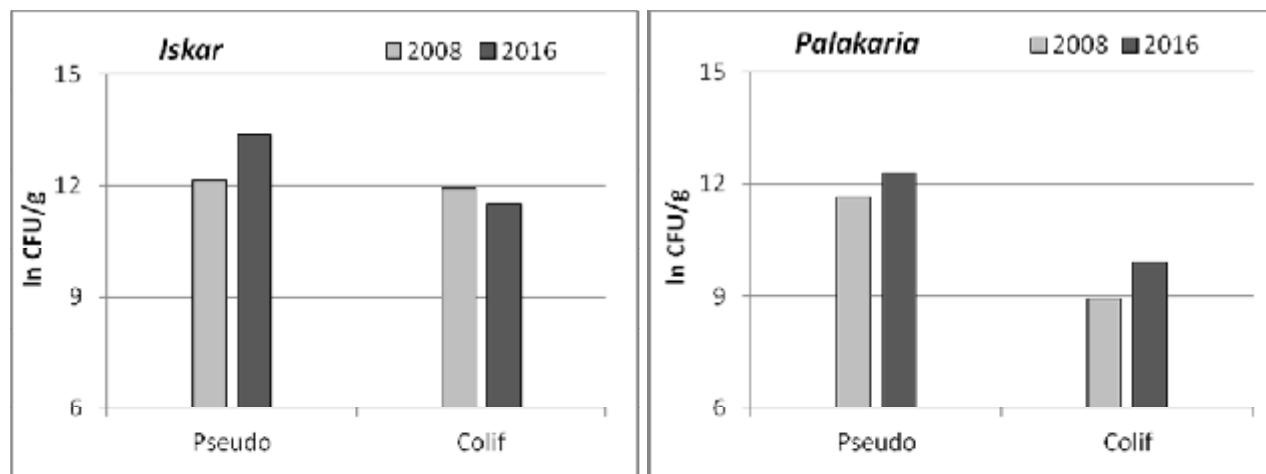


Fig. 4. Abundance of microbial groups with a specific indicator role

The indicator with a critical importance for the formation of microbiological parameters of water quality (coliforms) represents a considerable interest in studied subcatchment. In Iskar River sediments, the registered total counts of coliforms are lower in 2016 compared to these in 2008 but from the spatial

aspect the coliforms have significantly higher number than these in Palakaria River sediments for the both periods. These data are expected because the discharge of WWTP assumes to express presence of coliforms in river sector of impact. The freshwater sediments are important reservoir of

these bacteria ("natural depot") for surface waters and it again orientates to the recommendation the sediments to be incorporated into the system for monitoring of water quality in the upper subcatchment of Iskar River [12].

In sediments of Palakaria River, the coliforms are more abundant in 2016 and this result together with the similar data for counts of heterotrophic bacteria is indicator for increase of organic content in Palakaria River. This increase is favorable factor for functioning of sediment microflora and for development of ecosystem metabolism because in this tributary preliminary studies show that (1) the source of nitrogen and phosphorus is inorganic (agricultural fertilizers); (2) the organic content of tributary water is low and insufficient for bacterial growth; (3) the ratio C:N:P is unsuitable and first-rate factor for the bacterial abundance.

***Functional response of sediment communities to accidental loading with high organics and nutrient concentration***

The analyses of microbial communities in sediments of Upper Iskar subcatchment show the high functional diversity – base for intensive self-purification potential of ecosystem. For demonstration of this potential we model the ecosystem response at one simulated risk situation – overloading with nutrients and organics as a result of discharge of

untreated wastewater in river. The risk concentrations were 10 times higher than the normal background and were measured at previous real critical event [13]. The measured concentrations and kinetic parameters of their utilization are presented in Table 3.

The results show that the river ecosystem reacts adequately with intensive transformation processes target to utilization of organics and nutrients. The process lengths of net nutrient uptake are very short (<2 km) and the uptake rates are relatively high. The excessive nitrogen concentrations are eliminated within 1.5-2 km downstream and phosphates and organics - in the first few hundred meters. At normal concentrations the kinetic parameters are very different - for ammonium ions the inverse process of accumulation in the river system is realized and their concentration increases downstream. These data confirm the high functional activity of heterotrophic bacterial consortium - the capacity for organic uptake in ecosystem is high and well developed. Despite the good ecosystem potential for net uptake of nitrogen at risk situation, the parameter values at normal functioning of river ecosystem compared with the number of bacteria from nitrogen cycle confirm the key role of this nutrient in studied area and indirectly show the dominant source of nitrogen in watershed – permanent non-point pollution from agriculture.

Table 3. Kinetic parameters of nutrient and organic utilization at critical loading from WWTP

	$S_{net}$ (km)	$V_{f-net}$ ( $10^{-6}$ m/sec)	$U_{net}$ (mg/m <sup>2</sup> sec)
<i>nitrates</i>			
At risk concentration (10 mg/L)	1.477	158	0.209
At normal concentration (<1mg/L)	35.206	9.94	0.013
<i>ammonium</i>			
At risk concentration (4 mg/L)	1.885	1238	0.276
At normal concentration (<0.4 mg/L)	-16.143	-22	-0.005
<i>phosphates</i>			
At risk concentration (2 mg/L)	0.216	1082	0.316
At normal concentration (<0.2 mg/L)	1.950	180	0.052
<i>organics (as COD)</i>			
At risk concentration (60 mgO <sub>2</sub> /L)	0.564	974	0.302
At normal concentration (<5 mgO <sub>2</sub> /L)	2.032	102	0.118

**CONCLUSION**

The long-term profiling of functional biodiversity in sediment microbial communities from two sites in upper Iskar subcatchment shows several tendencies that can be summarized in following:

(1) The microbial groups participating in organic carbon transformation and specific indicators (bacteria from g. *Pseudomonas* and coliforms) have

higher quantitative parameters in sediments near to discharge of WWTP Samokov. This well presented abundance and diverse community structure are prerequisite for intensive functioning of ecosystem metabolism and effective utilization of this local impact. The data for high ecological and chemical status of river sector confirm that the impact of WWTP discharge is in the frames of self-purification capacity of ecosystem and do not disturb significantly the water quality. The modeling of

ecosystem response to risk situation of overloading with nutrients and organics confirms the high self-purification capacity of Iskar River.

(2) The well presented groups of anaerobic heterotrophs and bacteria from *Pseudomonas* in sediments of upper Iskar subcatchment is an indicator for existence of various metabolic pathways for utilization of different substrates.

(3) In sediments of Palakaria River, the bacteria from nitrogen cycle are detected in permanently high number. These data are related to significant nitrogen loading identified in the river and present an adequate response of microbial structure to this type of disturbance.

(4) The importance of studied subcatchment for formation of water quality in Iskar Reservoir and literature data for sediment function as depot for coliforms in other rivers enforces the extension of existing monitoring program with regular assessment of this indicator in the sediment component of the ecosystem.

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## МИКРОБНИ СЪОБЩЕСТВА В СЕДИМЕНТИ ОТ ГОРНОТО ПОРЕЧИЕ НА РЕКА ИСКЪР

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**Резюме:** Река Искър и едноименният язовир са водоизточниците в България с най-голямо икономическо и социално значение заради ролята им в питейното водоснабдяване на град София. В това изследване, фокусът е поставен върху седиментните микробни съобщества от горното поречие на река Искър като функционално разнообразие, пряко обвързано с реализирането на основните процеси на самоочистване и като рисков фактор за формиране качеството на водата. Микробните съобщества в седиментите и свързаната с тях интензивна метаболитна активност играят основна роля в екологията и метаболизма на речните екосистеми. Функционалният профил на седиментните микробни съобщества е изследван двукратно за период от 8 години. Получените резултати показват, че микробните групи, участващи в трансформацията на органиката и специфичните индикатори (бактерии от р. *Pseudomonas* и колиформи) имат по-високи количествени параметри в седиментите в непосредствена близост до заустването на ПСОВ Самоков. Данните за отличното екологично и химично състояние на речния сектор потвърждават, че въздействието на преработените отпадъчни води е в рамките на самопречиствателния капацитет на екосистемата и не оказва значително влияние на качеството на водите. От своя страна, в седиментите от река Палакария, бактериите от цикъла на азота са представени в доста по-висок брой. Тези данни са свързани със значителното натоваарване с азот, идентифицирано в притока, и представляват адекватен отговор на микробните съобщества към този тип замърсяване. Моделирането на екосистемния отговор при рискова ситуация на натоваарване с биогени и органика потвърждава високия капацитет за самопречистване на река Искър. Значението на изследвания водосбор за формиране на качеството на водата в язовир Искър и литературните данни за функцията на седиментите като депо за колиформи в други реки налагат разширяването на съществуващата програма за мониторинг с регулярна оценка на колиформните бактерии в седиментите на екосистемата.

**Ключови думи:** функционален профил на микробните съобщества, седименти, Горен Искър, самопречистване, трансформационни процеси

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