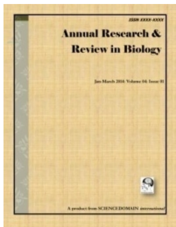

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Importance of Main Stream/Tributary Interface for Nutrient Dynamics and Microbial Communities in Upper River Subcatchment

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Authors' contributions

Author Y. Todorova performed the laboratory and statistical analysis, wrote the protocols, and wrote the first draft of the manuscript. Author Y. Topalova designed the study, managed the experimental design and all analytical procedures. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: A significance of tributary confluence for dynamics of nutrients and microbial communities in main river channel was studied in the Iskar River upper subcatchment (Bulgaria).
Methodology: The fluctuations of physicochemical parameters, nutrients, abundance of total bacteria, oligotrophes and coliforms in main stream and tributary were estimated seasonally by standard methods in small spatial scale (<2 km).
Results: The $P\text{-}PO_4$ concentrations in river and tributary waters were higher in cold seasons (between 0.14÷0.53 mg/L) compared to values observed in the other seasons. The quantity of nitrate nitrogen in tributary waters showed a statistically significant difference in comparison with the concentration in site upstream from the inflow but not in the site downstream from the inflow. Similar differences were observed in the nitrite dynamics even though this nitrogen form was less abundant. Ammonium nitrogen concentrations were generally in the range 0.03-0.6 mg/L in main stream waters and 0.15-1 mg/L in tributary waters. There were not statistically significant differences among sites but the seasonal profiles indicated the difference in ammonium concentrations during the autumn.
Conclusion: The results showed that tributary effect is more significant during the autumn

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and winter. The water quality of study area was disturbed in these seasons and the content of total suspended solids, phosphorus, ammonium and bacterial indicators for fecal contamination might be increased to a critical significance in sites with higher anthropogenic impact.

Keywords: Main stream; tributary inflow; nutrient concentrations; bacterial abundance; water quality.

1. INTRODUCTION

Catchments consist of upland ecosystems, where substantial material transformation and transport occurs, and aquatic ecosystems (rivers and their tributaries), which receive water and materials from uplands or urban areas and further transform and transport them [1]. The degree of loading and transformation of materials (suspended solids, nutrients, toxic substances, bacteria, etc.) determines water quality and illustrates how lotic water quality responds to human impacts.

Although the importance of channel network structure for material dynamics has gradually been recognized [2], when most of the earlier conceptual and field studies were conducted, watershed processes were assumed to be linear, and thus network structures such as tributary pattern, density, and junction effects were disregarded. The river continuum concept [3] evokes not a network (branching shape), but a linear concept from upper to lower stream reaches. Similarly, the nutrient spiraling concept [4] presents a more complex, but still linear, abstraction of solute dynamics in stream ecosystems within channels and hyporheic zones [5]. Fisher et al., 1998 [1] noted that a paradigm shift from linear to network (branched shape) systems is necessary to understand the processes and linkages of physical and biological dynamics in stream ecosystems. The tributary junctions are very important as network nodes for regulating material flows in watersheds, and they have unique hydrologic, geomorphic, and biological attributes. Heterogeneity of water, sediment, and organism communities is higher at tributary junctions. These sites are specific ecotone zones and the interface main stream/tributary has a crucial meaning for control of river metabolism, pollution and self-purification processes and water source management [6,7,8,9]. The longitudinal linkages, lateral exchange and nutrient fluxes between uplands and lotic ecosystems, the vertical interactions in hydrological system illustrate a complexity of tributary influence on the main stream. The temporal variability in hydrological and climatic conditions must also be considered when assessing nutrient and microbial loading within this dynamic interface.

Much of the work done to date has focussed on understanding flow and mixing regimes at tributary and main stream confluences and the relationships between sediment transport, morphology and stratigraphy. Until relatively recently, less attention had been paid to the biological attributes of confluences [8]. At a slightly larger scale than the confluence itself, the confluence zone is considered to be an important site for storage of sediment and organic materials in the form of fans and terraces while, at the larger scale of reaches, main stream adjustments to the influx of water, sediment and organic materials are know to influence abiotic and biotic processes [8,10]. Just as tributary flows can contribute significantly to sediment and debris loads, they also affect water quality in the receiving channel. The most conspicuous effect is increased turbidity due to suspended solids [10]. Tributary flows can also cause variations in temperature, nutrients and contaminants, and create or disrupt gradients in the water chemistry in the main channel [9,11]. Confluences are often associated with increased productivity due to the supply of nutrients, drift and detritus from the tributary [12].

The objectives of this paper are to (1) determine the tributary effect in small spatial scale to the nutrient concentrations and microbial communities in main stream; (2) discuss some of ways to affect water quality in rivers; (3) compare microbiological and chemical parameters of quality in main stream and tributary seasonally.

2. MATERIALS AND METHODS

2.1 Study Area

The study area includes a 2 km sector of Iskar River and the confluence of its tributary – Palakariya River. This sector is situated in upper subcatchment of Iskar River before the Iskar Dam in north Rila Mountain, Bulgaria. The study reach 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. According to the digital map of land use, the catchment area consist of 3% urban area, 8% barren, 10% meadow, 10% agricultural land, 25% pastures, 44% forest and scrub [13].

Iskar is the longest Bulgarian river (368 km) with 8 650 km² basin area. The 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 studied upper part of the river has a clearly determined seasonal character with summer and winter low flow (1–3 m^3/s), a little increase in flow during the autumn (6–10 m^3/s) and very expressive spring high water level (15–25 m^3/s). The tributary Palakariya River has a length of 40 km, an average stream flow of 1–2 m^3/s and a basin area of 402 km² (4.7% of total watershed of Iskar River). This tributary is the longest in Iskar River upper subcatchment and has a possible risk for significant pollution with organics and nutrients.

2.2 Experimental Design

The selected river sector has a critical significance for integrated management of whole subcatchment because of its proximity to the Iskar Reservoir and the infusion flow of Palakariya River – the most polluted tributary in this area. Nutrients and microbial abundance were studied in two sites of Iskar River (1 km upstream and 1 km downstream from the tributary confluence) and one of Plakariya River (tributary) (Fig. 1). The confluence of tributary has been considered as an impact event for main stream; the sampling site upstream from this impact was a control site; the sampling site downstream from the tributary junction was the impact site. The timing of sampling was the same for the three sites: once a month for nutrient concentrations analyses and twice a season for microbial parameters during one year.

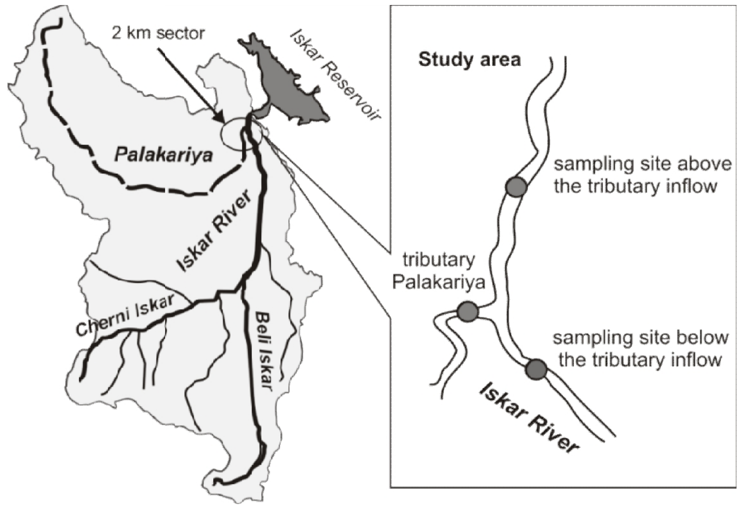

Fig. 1. Location of sampling sites in the Iskar River upper part subcatchment

2.3 Sampling and Analytical Procedures

Water samples were collected from the main stream and tributary, transferred into bottles or sterile containers, stored (at 4°C) and processed within 4 hours. The physicochemical parameters (temperature, oxygen concentration, and pH) of water have been analyzed *in situ* immediately after sampling with portable Oxy-meter Handylab Ox1/set and pH-meters Handylab pH11/set (Schott Instruments, Germany).

After transfer of samples to the laboratory, the waters were analyzed in triplicate for determination of total dry residue and total suspended solids (total SS) by drying to constant mass (gravimetric method). Total dry residue (also referred to as total solids) is the term used for material left in a container after evaporation and drying of a water sample. Total dry residue includes both total suspended solids, the portion of total solids retained by a filter and total dissolved solids, the portion that passes through a filter [14]. The estimation of organic loading of waters was done as COD measurement (Chemical Oxygen Demand – dichromatic EPA 410.4/ISO 6060 method) [14]. The nitrogen was measured as dissolved inorganic forms – ammonium, nitrite and nitrate nitrogen (colorimetric methods according to the ISO and EPA standards). The relation of nutrients concentrations and stream flow data was analyzed through the parameter Total Calculated Nitrogen (TCN) – sum of all nitrogen forms. The phosphorus was determined as $P\text{-}PO_4$ (colorimetric method based on phosphomolybdic blue reaction).

The density of studied physiological bacterial groups was measured by use of count-plate technique. Plates were incubated at 28-30°C for 24-32 hours. The following microbial groups were analyzed by use of specific nutrient media, Scharlau Chemie, S.A.: (1) TMC - total microbial count, determined on Nutrient Agar; (2) Oligo - count of oligotrophic bacteria as indicator of low organic concentration in stream ecosystems; cultivation was carried out on 1:10 diluted Nutrient Agar; (3) Coliforms - density of coliform bacteria on Endo media used as indicator of health risk caused by fecal contamination.

All data are presented at the mean ± SD for the each of season. The normality and homogeneity of variance were tested and the analysis of variance (ANOVA) was chosen to test for differences between the different sites or seasons and to assess the tributary effect. The statistical tests were performed on raw data for physicochemical parameters and nutrient concentrations. In all the statistical methods, differences were considered significant at $P = 0.05$. Analyses were performed with SigmaStat 3.10, Systat Software, Inc.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Parameters

The fluctuation of the physicochemical parameters of the waters in the Iskar River and Palakariya River is given in Table 1. Water temperature showed typical seasonal variations and ranged from 3.5°C during the winter to 21°C during the summer in site downstream from the tributary. The tributary Palakariya showed high water temperatures during the spring - 17.9±2.98°C, while the lower temperature was detected in the main stream. The variation of the pH values along the Iskar River and its tributary was not significant – the pH mean values varied between 7.18 and 7.99. It is obvious that the pH was relatively stable among and within the sampling sites during the year.

Dissolved oxygen concentration is typically high for this part of Iskar River subcatchment, both for main stream and the tributary. During the low flow cold period of winter, the water of river and tributariy had an oxygen concentration over 11.2 mg/L. In the summer, the mean values were in the range of 7.8÷8.7 mg/L. In spring, corresponding to the higher temperature in the waters of tributary, the oxygen concentration of Palakariya River was lower than this in Iskar River before confluence - 8.7±1.04 mg/L.

The total amount of dry residue for the waters in upper Iskar subcatchment fluctuated between 60÷230 mg/L (Table 1). The values were higher during the summer (180÷230 mg/L) and in Palakariya River (170÷230 mg/L). The total suspended solids varied between 7÷33 mg/L. The water of tributary differed significantly from the water in main stream by these parameters ($P < 0.05$, One Way ANOVA). The effect of Palakariya River on the concentration of total suspended solids and dry residue was crucial and the confluence of this tributary reflected negatively on the water quality in the main stream. The tributary confluence may have a negative effect on water quality of near situated Iskar Reservoir and be a prerequisite for future problems with quality of drinking waters.

Table 1. Physicochemical parameters of water in the main stream (above and below the tributary inflow) and in tributary waters at four seasons

Sampling sites	Above	Tributary	Below
Autumn			
T air, °C	9.8±4.5	11.8±5.1	11.2±4.0
T water, °C	8±3.32	7.5±5.47	8.8±4.12
oxygen, mg/L	10.30±1.81	10.70±2.53	11.90±2.13
pH	7.18±0.26	7.75±0.21	7.39±0.08
total DR, mg/L	60±12	190±27	100±19
total SS, mg/L	10±1.50	20±1.32	15±2.03
stream rate, m/s	0.86±0.11	1±0.21	1.83±0.31
Winter			
T air, °C	4±0.9	5.6±2.1	3.5±1.8
T water, °C	5.1±1.35	5±0.96	4.8±1.86
oxygen, mg/L	11.50±0.76	11.40±1.96	11.20±2.14
pH	7.99±0.31	7.56±0.15	7.61±0.24
total DR, mg/L	60±11	190±9	200±31
total SS, mg/L	10±2.69	30±3.02	15±3.62
stream rate, m/s	0.50±0.09	1.25±0.25	0.90±0.11
Spring			
T air, °C	14.3±5.2	14.8±6.3	14±7.1
T water, °C	11.1±5.69	17.9±2.98	12.8±4.14
oxygen, mg/L	10.40±2.13	8.70±1.04	9.60±2.59
pH	7.10±0.56	7.81±0.12	7.33±0.41
total DR, mg/L	60±28	170±38	70±19
total SS, mg/L	7±0.85	20±3.14	17±4.19
stream rate, m/s	1.11±0.65	1.25±0.26	1.40±0.31
Summer			
T air, °C	17±8.3	18±7.6	21±6.3
T water, °C	14.5±5.11	14.6±4.23	14.6±6
oxygen, mg/L	7.80±1.96	8.70±1.78	8.40±2.54
pH	7.32±0.24	7.62±0.18	7.78±0.13
total DR, mg/L	180±22	230±41	150±26
total SS, mg/L	15±1.51	33±5.67	10±2.48
stream rate, m/s	0.80±0.15	0.80±0.27	1.00±0.3

Mean values ± Standard error of means of six samples
T – temperature of water; DR – dry residue; SS – suspended solids

3.2 Tributary Influence on the Nutrient Concentrations

3.2.1 Phosphorus

Consumption of phosphorus does not appear to affect human health but high phosphorus levels in the water can have dramatic effects on aquatic life. Concentrations of over 0.03 mg/L can provoke excessive plant growth, increase demand of oxygen and reduce fish stock [15]. Most concentrations, measured in this study and presented in Fig. 2 are over 0.05 mg/L $P\text{-}PO_4$ and exceed the limits for A1 category (see Council Directive 75/440/EEC). The $P\text{-}PO_4$ concentrations in river and tributary waters were higher in cold seasons (between 0.14÷0.53 mg/L) compared to values observed in the other seasons (0.04÷0.17 mg/L). The largest amounts of $P\text{-}PO_4$ were noted in site downstream from the tributary inflow – 0.53±0.7 mg/L and in tributary – 0.50±0.9 mg/L during the autumn. The tributary and the river after its confluence in winter were also discharged with high phosphorus concentrations – over 0.2 mg/L.

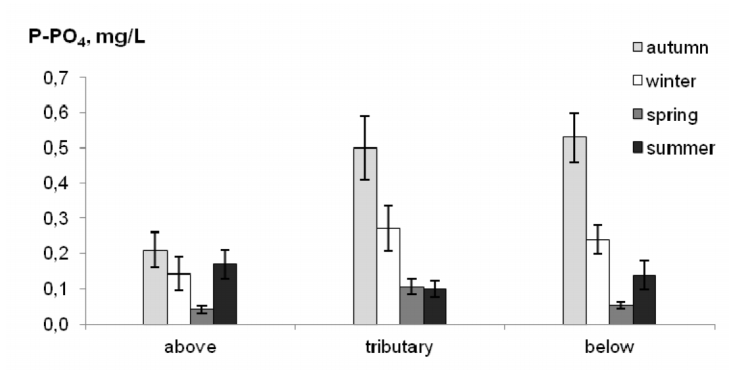

Fig. 2. Influence of tributary on the phosphorus concentrations (PO_4-P) of Iskar River.
Mean values ± Standard error of means of six samples

3.2.2 Nitrogen

In the aquatic environment, nitrogen can be found in various forms: molecular nitrogen, organic nitrogen, ammonium nitrogen, nitrite and nitrate. Domestic and industrial sewers along with animal excrement and fertilizers are the main sources of nitrogen. Nitrite is usually associated with active biological processes influenced by organic pollution. Nitrate is more associated with the use of organic and inorganic fertilizers [15].

The major form of nitrogen in upper Iskar subcatchment was nitrate: means of 0.609±0.406, 1.297±0.434 and 0.855±0.563 mg/L were measured in the site upstream from the tributary inflow, tributary and site downstream from the inflow, respectively. Seasonally, the nitrate concentrations fluctuated between 0.2 and 1.7 mg/L in the main stream and the periods with low values were observed – spring and summer (Fig. 3). In tributary, the N-NO_3 concentrations were permanently high and the temporal variation was less: between 1 and 1.8 mg/L. The quantity of nitrate nitrogen in tributary water showed a statistically significant difference in comparison with the concentration in site before the confluence (P= 0.005) but not in the site after the confluence (P= 0.055, One Way ANOVA; Holm-Sidak method for multiple comparison).

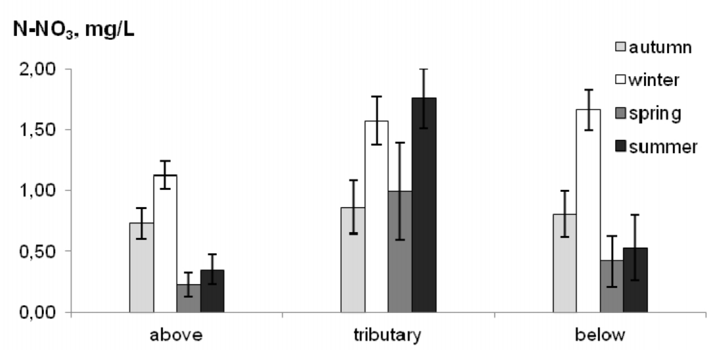

Fig. 3. N-NO_3 concentrations in waters of Iskar River and its tributary Palakariya River.
Mean values ± Standard error of means of six samples

Differences were observed in the nitrite dynamics of the main stream and tributary even though this nitrogen form was less abundant (Fig. 4). Concentrations of $N\text{-}NO_2$ in the upper Iskar subcatchment were between 0.002-0.01; 0.01-0.03; 0.008-0.01 mg/L for sampling sites upstream from the tributary inflow, tributary and downstream from the inflow. The maximal value was measured in Palakariya River during the summer – 0.033 mg/L $N\text{-}NO_2$ and the minimal in site upstream the tributary inflow during the spring – 0.0024 mg/L $N\text{-}NO_2$. One-way ANOVA results showed that the nitrite concentration in tributary differed significantly from the quantities in main stream (P= 0.008; Holm-Sidak method for multiple comparison). The spatial distribution pattern of $N\text{-}NO_2$ is similar to that of nitrates (Fig. 3 and 4).

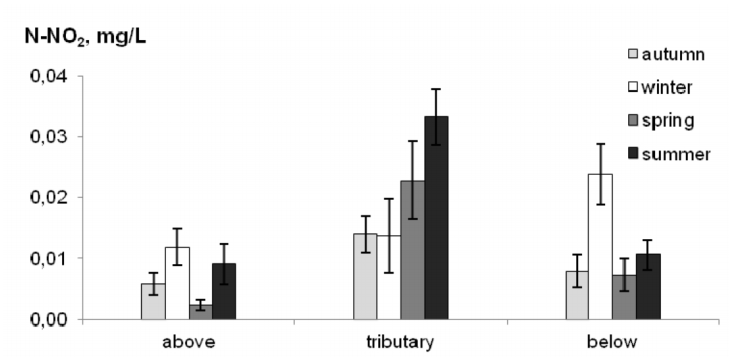

Fig. 4. $N\text{-}NO_2$ concentrations in waters of Iskar River and its tributary Palakariya River.
Mean values ± Standard error of means of six samples

Ammonium nitrogen concentrations were generally in the range 0.03-0.6 mg/L at Iskar River and 0.15-1 mg/L at Palakariya River (Fig. 5). Maximum concentrations were observed at tributary Palakariya in autumn – 0.964 mg/L $N\text{-}NH_4^+$. There were not statistically significant differences among sites but the seasonal profiles indicated the difference in ammonium concentrations in subcatchment during the autumn in comparison with the other seasons (P= 0.0007).

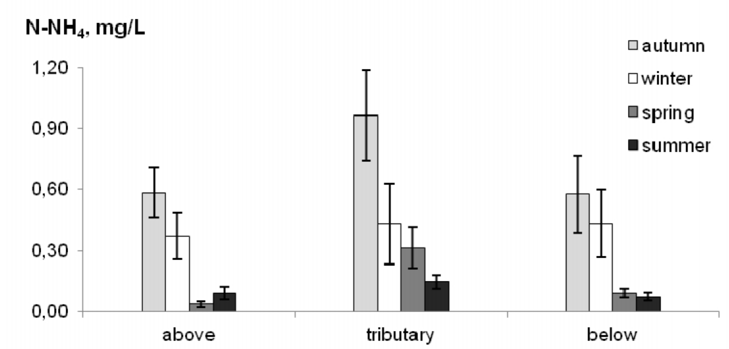

Fig. 5. $N\text{-}NH_4$ concentrations in waters of Iskar River and its tributary Palakariya River.
Mean values ± Standard error of means of six samples

The requirements for nitrogen content in surface waters according to the European legislation determine stronger limits for the ammonium concentration than the limits for nitrates. The measured ammonium nitrogen concentrations were under 1 mg/L but over advisable values for A1 category (0.05 mg/L NH_4^+, see Council Directive 75/440/EEC). The most critical point was the confluence of Palakariya during the autumn and winter, especially as this area was situated before Iskar Dam. Palakariya River represented a significant source of nitrogen for Iskar River.

On Fig. 6 was shown the relation of nutrient concentrations (P-PO4 and Total Calculated Nitrogen) and stream flow data of Iskar River. The hydrological data [16,17] had normal for the moderate continental climate zone seasonal flow variations – with summer and winter low flow (1–3 m^3/s), a little increase in flow during the autumn (6–10 m^3/s) and very expressive spring high water level (15–25 m^3/s).

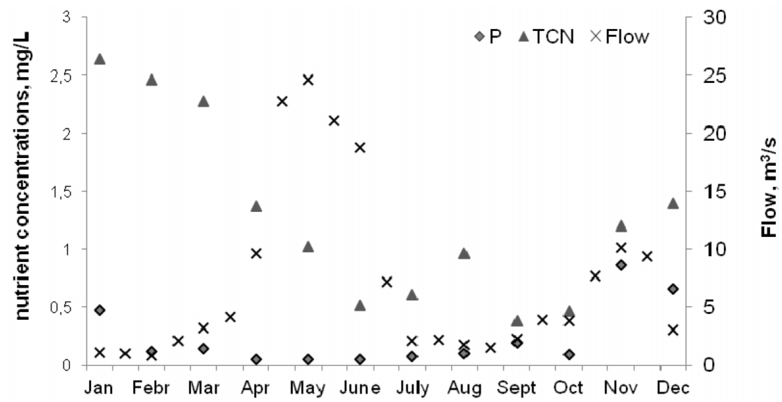

Fig. 6. Relation of nutrient concentration (phosphorus and Total Calculated Nitrogen) and monthly run off of studied river sector.

The observed seasonal variations in nutrients concentrations are typical for upper stream subcatchment. During the spring active vegetative season, there is an increase in periphyton and riparial vegetation growth and increased consumption of nutrients ($N\text{-}NO_3$, $N\text{-}NO_2$, $N\text{-}NH_4$, $P\text{-}PO_4$), while in the autumn and winter, because of low vegetation growth, the nutrient contents remain high in the water. The cumulative effect of fall of the leaves, first flash events and intensive fertilizers use in the subcatchment further increased the nutrients concentrations in autumn.

High level of nutrients in Palakariya River is most probably due to the extend use of pesticides and fertilizers at near farming areas Agricultural runoff has been well documented as a non-point source pollutant in streams, especially of nutrients [18].

3.2.3 Chemical Oxygen Demand (COD)

Headwater streams transport organic matter including dissolved organic carbon, particulates and woody debris into larger channels [5,19], all of which directly affect water quality at the confluence. Furthermore, confluences are often sites of storage of benthic organic matter, particularly where large boulders and woody debris trap fine material, although much of this material is eventually transported downstream [10].

The impact of tributary on COD in water of Iskar River was not determined clearly (Fig. 7). The values of COD varied in interval of 1-90 mgO_2/L. A sharp decline was observed during the spring in Palakariya River – its organic loading detected by COD was insignificant. This season is characterized by large fluctuations in COD level and high values in main stream.

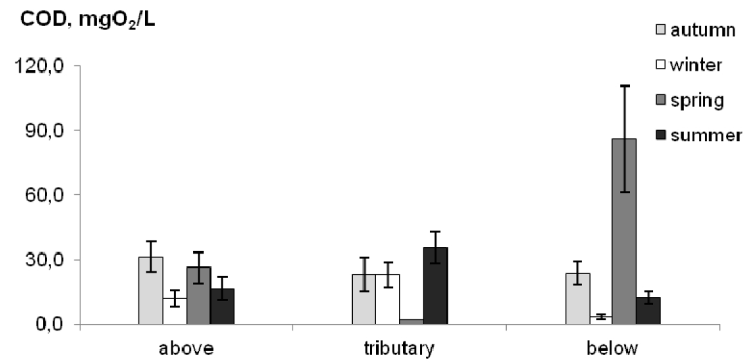

Fig. 7. COD (mgO_2/L) in waters of Iskar River and its tributary Palakariya River.
Mean values ± Standard error of means of six samples

3.3 Microbiological Aspects of MS/Tr Interface

In this study the highest bacterial abundance was registered during the autumn high flow and winter low flow – the microbial counts were greater by an order than the other seasons (Fig. 8). The oligotrophic bacteria were the predominant group in upper Iskar subcatchment – 1500÷47000 CFU/cm^3. The obtained results confirm previous studies on microbial communities in the Iskar River [20,21]. The assessment of influence of Palakariya River on the microbial abundance in Iskar River presented unexpected results. Measurements of phosphorus and nitrogen concentrations showed the role of this tributary for the formation of water quality in the main stream. But the microbial parameters did not confirm that, especially in spring. Simultaneous analysis of obtained results for nutrients and bacterial concentrations gave an explanation. At high spring level the concentrations of $N\text{-}NH_4$, $N\text{-}NO_3$ and $P\text{-}PO_4$ in the water of Palakariya River were very high, but the COD content was low. These data showed that: (1) the source of nitrogen and phosphorus was inorganic (agricultural fertilizers); (2) the organic content of tributary water was low and insufficient for bacterial growth; (3) the ratio C:N:P was seasonally variable and first-rate factor for the bacterial abundance.

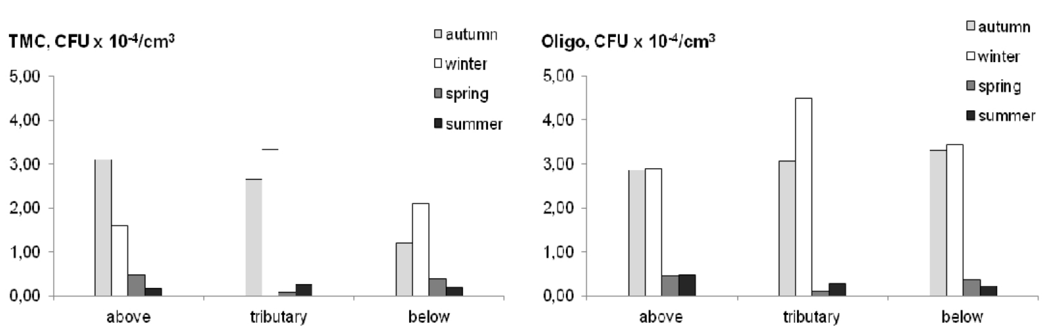

Fig. 8. Effect of tributary confluence on microbial parameters Total Microbial Count (TMC) and count of Oligotrophic bacteria (Oligo) in Iskar River.

The counts of coliform bacteria in upper Iskar subcatchment corresponded to A2 category (Council Directive 75/440/EEC). The most critical season was the autumn high flow – the level of 5000 CFU/100 cm^3 was reached in sampling sites of main stream (Fig. 9). Values in Palakariya River remained the same throughout the study period – 1000÷1350 CFU/100 cm^3.

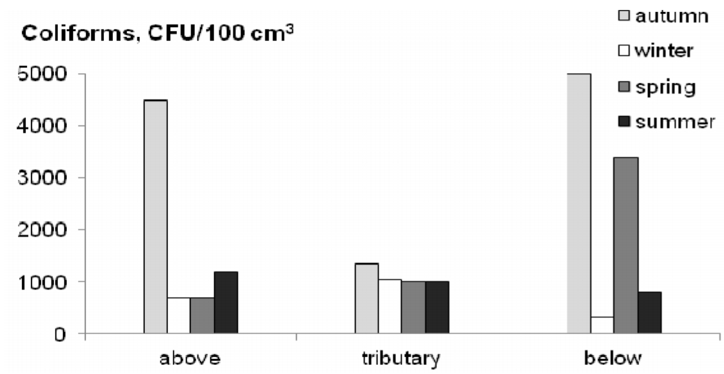

Fig. 9. Effect of tributary confluence on coliform bacteria in Iskar River.

4. CONCLUSION

The assessment of tributary effect on water quality in upper Iskar subcatchment shows the complex nature of main stream/tributary interface. The influence is not unidirectional but the seasonal study of nutrient concentration showed that tributary effect is more significant during the autumn and winter. In this season, the influence of others factors (near situated villages; agriculture and tourist activity) is stronger and accumulation of impacts may be result in a temporal deterioration of water quality in Iskar River. The water quality of study area was disturbed by the tributary confluence and during the low water level periods the content of total suspended solids, phosphorus, ammonium and bacterial indicators for fecal contamination might be increased to a critical significance in sites with higher anthropogenic impact.

Excessive nutrient runoff, mainly nitrogen and phosphorous, into main stream can cause a potential risk of eutrophication in Iskar Reservoir and problems with drinking water supply. High nutrient levels allow expansive plant and algal growth, and deterioration of water quality. Non point source nutrients loads in upper Iskar subcatchment may be reduced through the implementation of specific management strategy and practices. An appropriate management strategy should contain the following elements:

(1) extended water quality monitoring program for the main stream and tributary with more intensive sampling, sites and parameters;
(2) control and reduction of fertilization through implementation of good agricultural practices for assessment of approximate nutrient budget of soils;
(3) limiting of sewage inputs by construction of local wastewater treatment plants;
(4) conservation and restoring of riparian ecotones.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fisher SG, Grimm NB, Marti E, Holmes RM, Jones JBJr. Material spiraling in stream corridors: A Telescoping Ecosystem Model. Ecosystems.1998;1:19-34.
2. Benda L, Dunne T. Stochastic forcing of sediment routing and storage in channel networks. Water Resources Research. 1997;33:2865-2880.
3. Vanotte RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences. 1980;37:130-137.
4. Newbold JD, Elwood JW, O'Neill RV, Van Winkle W. Measuring nutrient spiraling in streams. Canadian Journal of Fisheries and Aquatic Sciences. 1981;38:860-863.
5. Gomi T, Sidle RC, Richardson JS. Understanding processes and downstream linkages of headwater systems. BioSciense. 2002;52:905-916.
6. Benda L, Poff NL, Miller D, Dunne T, Reeves GH, Pess GH, Polluck M. The network dynamics hypothesis: How channel networks structure riverine habitats. Bioscience. 2004;54:413–427.
7. Benda L, Andras K, Miller D, Bigelow P. Confluence effects in rivers: Interactions of basin scale, network geometry and disturbance regimes. Water Resources Research 2004;40:WO5402,doi:10.1029/2003WR002583.
8. Rice SP, Rhoads BL, Roy AG. Introduction: river confluences, tributaries and the fluvial network. In: Rice SP, Roy AG, Rhoads BL, editors. River Confluences, Tributaries and the Fluvial Network. John Wiley and Sons; 2008.
9. Kiffney PM, Greene CM, Hall JE, Davies JR. Tributary streams create spatial discontinuities in habitat, biological productivity, and diversity in mainstream rivers. Canadian Journal of Fisheries and Aquatic Sciences. 2006;63:2518–2530.
10. Dye A. Contribution of unregulated tributaries to the ecological functioning of the main channel of rivers. Snowy River Recovery: Snowy Flow Response Monitoring and Modelling. NSW Office of Water, Sydney; 2010.
11. Gooseff MN, Bencala KE, Wondzell SM. Solute transport along stream and river networks. In: Rice SP, Roy AG, Rhoads BL, editors. River Confluences, Tributaries and the Fluvial Network. John Wiley and Sons; 2008.
12. Rice SP, Kiffney P, Greene C, Pess GR. The ecological importance of tributaries and confluences. In: Rice SP, Roy AG, Rhoads BL, editors. River Confluences, Tributaries and the Fluvial Network. John Wiley and Sons; 2008.
13. Ribarova I, Ninov P, Cooper D. Modelling nutrient pollution during a first flood event using HSPF software: Iskar River case study, Bulgaria. Ecological Modelling. 2008;211(1-2):241-246.
14. APHA-AWWA-WEF. Standards methods for the examination of water and wastewater. Washington, DC. 1998.
15. Maillard P, Santos NP. A spatial-statistical approach for modeling the effect of non-point source pollution on different water quality parameters in the Velhas river watershed. Brazil Journal of Environmental Management. 2008;86(1):158-170.

16. Ribarova I, Topalova J, Ninov Pl, Kukurin Kr, Kalinkov P, Dimova G. Assessment of the flush event significance for a river with typical seasonal run off. Proceeding of IWA Watershed and River Basin Management Conference, Canada. 2005.
17. Ninov Pl, Ribarova I, Nikolaidis N, Tzoraki O, Kalinkov P, Kukurin K. Testing of the HSPF model for simulation of the nutrients loads in the Upper Iskar basin. Proceeding of IWA Watershed and River Basin Management Conference, Canada; 2005.
18. Sharpley AN, Daniel T, Gibson G, Bundy L, Cabrera M, Sims T, Stevens R, Lemunyon J, Kleinman P, Parry R. Best management practices to minimize agricultural phosphorus impacts on water quality. U.S. Department of Agriculture, Agricultural Research Service, ARS–163; 2006.
19. Bigelow PE, Benda LE, Miller DJ, Burnett KM. On debris flows, river networks and the spatial structure of channel morphology. Forest Science. 2008;53(2):220-238.
20. Todorova Y, Topalova Y. Functional profile of microbial community in critical points of upper Iskar subcatchment /Bulgaria/. Natura Montenegrina. 2008;7(2):345-356.
21. Todorova Y, Topalova Y. Microbial response to accidental organic pollution in selected part of Iskar River. Biotechnology & Biotechnological Equipment. 2009;23(SE):434-437.

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