SUSTAINABLE DEVELOPMENT OF THE WATER ECOSYSTEMS

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MICROBIAL STATUS OF DOSPAT DAM LAKE, BULGARIA

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Abstract

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The aim of this study was to determine the total count of main microbial indicators for the water quality of Dospat Dam Lake. Samples were taken from six stations situated in the aquatory of the reservoir and one station at Dospatska River. The seasonal dynamics of the total viable count (TVC 20°C), total coliforms (TC), *E. coli*, fecal streptococci (FS) and *C. perfringens* were studied for the period April 2011 – March 2012. The values for the TVC 20°C were within range of 1.10^3cfu.100 mL^{-1} to 39.10^3 cfu.100 mL^{-1}, without statistically significant differences between stations (ANOVA, $P > 0.05$). The average values for TC varied from 10 cfu.100 mL^{-1} to 100 cfu.100 mL^{-1} and in August it rose to 1000 cfu.100 mL^{-1} with higher numbers near the net cage farms (ANOVA, $P < 0.005$). Presence of *E. coli*, FS and *C. perfringens* during the spring months was not established. Slight increase of the sanitary-state indicator was observed in August with values of 26 cfu.100 mL^{-1}, 20 cfu.100 mL^{-1} and 10cfu.100 mL^{-1} for *E. coli*, FS and *C. perfringens* respectively, with similar trends in November. Coliforms were characterized by low species diversity with domination of *Serratia marcescens, Pantoea aglomerans, Hafnia alvei* and *Enterobacter cloacae* at stations tree, four and five. The highest number of species was observed at station six (near Sarnitza village).

Key words: Dospat Dam Lake, TVC 20°C, TC, *Escherichia coli*, FS, *Clostridium perfringens*

Introduction

Inland fresh water reservoirs represent an alternative venue for culture fisheries (Usha et al., 2006). Bulgaria is among the countries with a growing interest in the field of net cage fish farming and construction of new farms in the inland water bodies. The number of newly constructed net cage farms has been steadily increasing over the period 2007–2012, reaching a total of 38 active farms in 2012. Intensification in the sector has a direct impact on water quality.

Deployment of a large number of net cages drastically increases sedimentation, which in combination with the retention of water masses, caused by resistance from the nets, create conditions for the deterioration of water quality (Beveridge, 1984) and changes in the composition of the microflora under the net cage farms. The qualitative and quantitative changes are a result of the inoculation of the water column and sediments with a significant number of microorganisms from the digestive tract of fish (Pond et al., 2006).The composition of microbial communities under the net cage farms is formed by the natural microflora and the bacterial species entering the sediment through excreted feces and uneaten food. The environmental effects of aquaculture range from highly detrimental to highly beneficial, depending on the perspective of the observer and the type of aquaculture activity (Bert, 2007), as bacteria are known to efficiently decompose organic matter and regenerate minerals in aquatic ecosystems, and their abundance represents an index of heterotrophic activity (Overbeck and Chrost, 1990; Zmyslowska et al., 2000; Zmyslowska and Golas, 2003).

In recent decades there has been increased urbanization and development of coastal areas, which increases the risk of further contamination with household waste waters. This requires that greater attention is paid to the water quality, including assessment of microbiological pollution and sanitary status of waters used for aquaculture.

The aim of this study was to determine the total count of main microbial indicators for the water quality of Dospat Dam Lake for the evaluation of the anthropogenic impact on the water body.

Materials and Methods

The research was carried out in the aquatory of Dosapt Dam for the period April 2011 – March 2012. Water samples were collected according to standard procedures for water sampling (EN ISO 25667-2) from six sampling stations located in the aquatory of the reservoir. Each station includes three sampling horizons, respectively at the epi-, meta- and hypolimnion water layer when there is a clear stratification of there servoir. In November and March, during fall and spring turn over, samples were taken at 50 cm below the surface, at 1 m above the bottom and at the middle water layers. The exact location of the stations was determined by GPS receiver Garmin 76CSx (Figure 1).

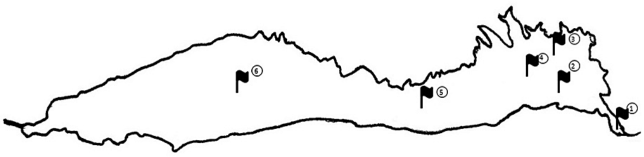

Fig. 1. Location of the sampling stations in the aquatory of Dospat Dam

The total count of the microbial indicators was determined after membrane filtration through filters (Membrane Solutions) with a poresize of 0.45 µm. For *Eschericha coli* and total coliform count (TC) filters were transferred for cultivationon SLS Coliform Agar (Hi Media, India) for 24 h at 37°C. The medium allows simultaneous counting of the number TC and *Escherichia coli* in the sample. The irnumber was established after direct counting of specifically colored colonies. The number of the fecal streptococci was determined by standardized test method ISO 7899-2, 2000. *Clostridium prefringens* count was established after incubation of the membrane filter on tryptose-sulphite agar with cycloserine under anaerobic conditions at 44°C ± 1°C for 24 h.

Eighty strains be longing to the total coliform group were isolated from the water column at each sampling station in August 2011. Identification was carried out by biochemical reactions, based on the rapid identification system Enterotube II (ENCISE). Confirmatory test were conducted with MERLIN MICRONAUT system (Merck).

Statistica V. 10 was used for statistical evaluation of the results.

Results

Seasonal dynamics of Total Viable Count (TVC 20°C)

The waters of Dospat Dam Lake were characterized by relatively low number heterotrophic bacteria (TVC 20°C) for the period of the research. The only exception was the month of August, when a sharp increase in the values of the parameter was recorded (Figure 2).

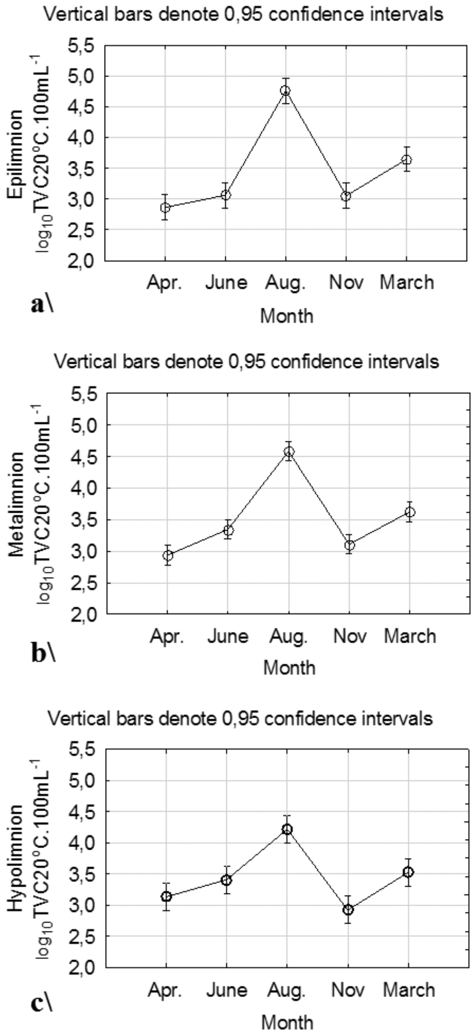

Fig. 2. Monthly average values and 95% Conf. Intervals of the Total Viable Count in the waters of Dospat Dam for the period April 2011 – March 2012 (epilimnion (a), metalimnion (b) and hypolimnion (c))

The seasonal dynamics were relatively less pronounced. The lowest count of 1.10^3 cfu.100 mL^{-1} was recorded in April 2011. With the rise of water temperature in June, an increase of TVC 20℃ up to 2.10^3 cfu.100 mL^{-1} was observed, with no significant differences in the mean values for the different sampling horizons (ANOVA, $P > 0.05$). The highest mean count - 39.10^3 cfu.100 mL^{-1} was recorded in August, when was also recorded the highest temperature in the Dam. During this period was established a vertical differentiation of the indicator, with highest values of 6.10^4 cfu.100 mL^{-1} in the surface layer, while in the hypolimnion the total count of microorganisms decreased to $1,8.10^4$ cfu.100 mL^{-1} (ANOVA, $P < 0.005$). During the autumn period (November) was recorded a new drop in the count, reaching values close to those for April.

During the period of spring turnover in March, the index TVC 20°C reached mean values of $3.5.10^3$cfu.100 mL^{-1} in the entire water column. The slight increase in comparison to the autumn period and the late spring months is most likely due to the vertical movement of water masses and the transfer of organic matter from the bottom layers vertically in the water column. This process was also established for other Dam lakes in the country (Traykov et al., 2003; Iliev et al., 2012).

For the entire period TVC 20° in the waters of Dospatska River, at the station situated near the village of Sarnitza was several times higher compared to the values in the Dam. The values were in the range 9.10^3 to 12.10^3 cfu.100 mL^{-1}, again with the exception of August, when was recorded an increase in the count to 7.10^5cfu.100 mL^{-1} (ANOVA, $P < 0.0005$).

Spatial variation of Total viable count TVC 20°C

The data for the horizontal distribution of the total count of microorganisms are shown in Figure 3.

During the spring period were not reported statistically significant differences in mean values of the indicator between stations in the aquatory of the Dam. The highest count was reported in the bottom water layer, where it did not exceed $1.5.10^3$cfu.100 mL^{-1} in April 2011. Only at station III, the number of heterotrophic microorganisms reached $3.3.10^3$ cfu.100 mL^{-1}. For June the recorded values in hypolimnion were within $2.6.10^3 \pm 500$ cfu.100 mL^{-1}.

In August microbial titer rose sharply. The highest values were recorded in the epilimnion layer 76.10^3 cfu.100 mL^{-1} and 90.10^3 cfu.100 mL^{-1} respectively at stations III and IV, with no statistically significant differences in comparison to other stations (ANOVA, $P > 0.01$). In the free of net cages aquatory, were recorded 58.10^3 cfu.100 mL^{-1} and 54.10^3 cfu.100 mL^{-1} in the surface water layer respectively at stations V and VI. In the zone of the thermocline and the hypolimnion the count was in the range $33.5.10^3 \pm 4.10^3$ cfu.100 mL^{-1}. Higher values 70.10^3 cfu.100 mL^{-1} were recorded again only at station III (ANOVA, $P > 0.5$).

In general, the surface water layer contains a higher number of microorganisms in summer compared to spring and autumn. Towards the bottom the number of microorganisms in the summer decreased, possibly because of the decrease in oxygen concentration in the bottom layers.

For the whole period the values for TVC 20°C did not exceed 50.10^3 cfu.100 mL^{-1}. Based on the assessment on the load of the reservoir with easily assimilated organic matter, the waters of the lake belong to 1-st category (waters with very low organic load), only in August at station III were recorded deviations from these values, which implies a higher load (Albinger, 1992).

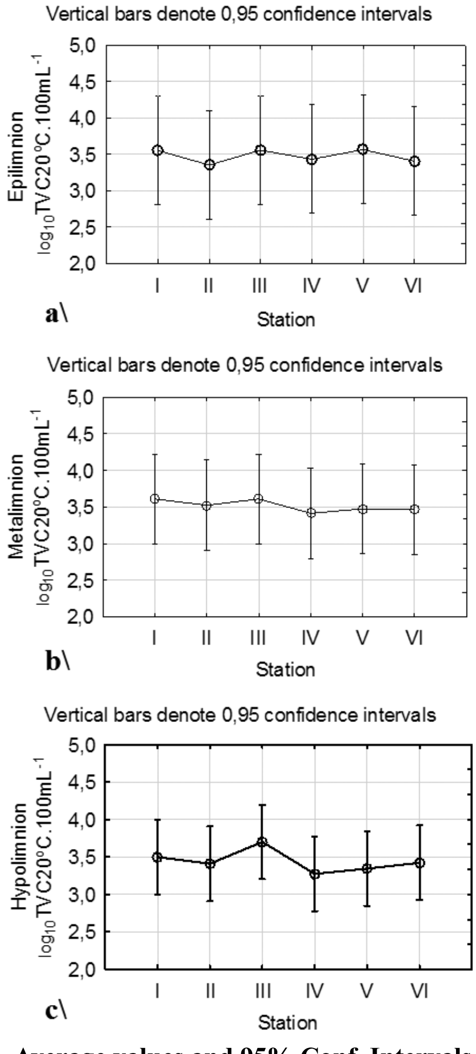

Fig. 3. Average values and 95% Conf. Intervals of the total viable count (TVC 20°C for the period April 2011 – March 2012 in epilimnion (a), metalimnion (b) и hypolimnion (c) of Dospat Dam at the different sampling stations

Spatial and temporal variation of the Total coliform count, Escherichia coli, fecal streptococci (FS) and Clostridium perfringens

The results from the research on the values of the index total count of coliforms in the aquatory of Dospat Dam Lake are shown in Figure 4.

Total coliform count demonstrated a clearly defined seasonal dynamics with values varying from >10 cfu.100 mL^{-1} in April to 1.10^{3} cfu.100 mL^{-1} in August. With the exception of the summer period the mean values of this indicator did not exceed 150 cfu.100 mL^{-1} in the aquatory of the Dospat Dam (ANOVA, $P < 0.005$).

Similar correlation was observed during the analysis of the waters from the station at Dospatska River, where for the entire period were recorded values higher or close (June) compared to those reported in the Dam. Due to the low water flow of the river during most of the year its waters do not significantly affect the microbiological status of the study area in the reservoir.

In April 2011 were not recorded statistically significant differences in respect to the mean values of the indicator TC (total count of coliforms) between the different station, and in the entire water column did not exceed 10 cfu.100 mL^{-1} (Figure 5). During that period in 100 ml of the examined water samples was not detected the presence of the studied indicator species

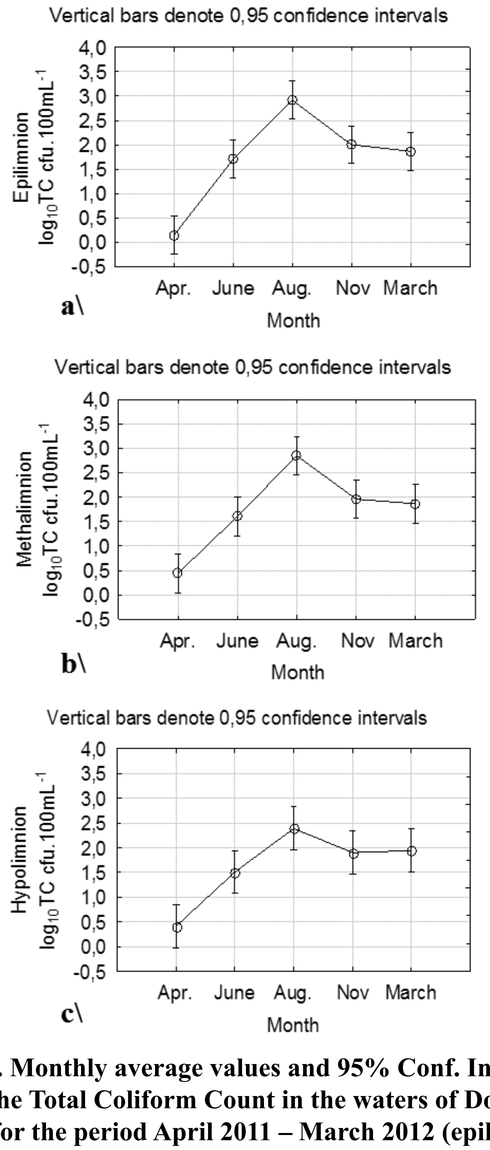

Fig. 4. Monthly average values and 95% Conf. Intervals of the Total Coliform Count in the waters of Dospat Dam for the period April 2011 – March 2012 (epilimnion (a), metalimnion (b) and hypolimnion (c))

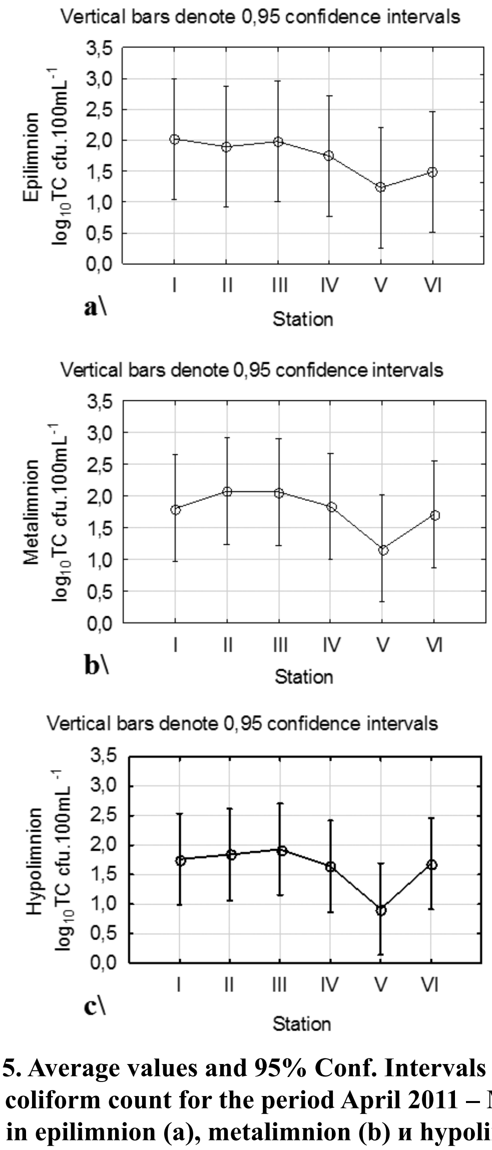

Fig. 5. Average values and 95% Conf. Intervals of the total coliform count for the period April 2011 – March 2012 in epilimnion (a), metalimnion (b) и hypolimnion (c) of Dospat Dam at the different sampling stations

Table 1

Average values of the monitored microbial parameters (cfu.100 mL^{-1}) in the aquatory of reservoir Dospat and river Dospatska for the period April 2011 – March 2012

Station	Parameter	Sampling date				
		April 2011	June 2011	August 2011	November 2011	March 2012
I	*E. coli*	< 1	2 ± 1	7 ± 9	25 ± 4	134 ± 60
	Faecal streptococci	< 1	< 1	8 ± 12	14 ± 4	>300
	C. perfringens	< 1	< 1	1 ± 1	2 ± 1	< 1
II	*E. coli*	< 1	4 ± 3	1 ± 1	11 ± 6	30 ± 29
	Faecal streptococci	< 1	< 1	< 1	5 ± 1	13 ± 10
	C. perfringens	< 1	< 1	2 ± 1	1	< 1
III	*E. coli*	< 1	3 ± 2	10 ± 13	18 ± 6	41 ± 31
	Faecal streptococci	< 1	< 1	9 ± 10	2 ± 1	13 ± 11
	C. perfringens	< 1	< 1	7 ± 5	5 ± 4	< 1
IV	*E. coli*	< 1	< 1	2 ± 3	< 1	3 ± 1
	Faecal streptococci	< 1	< 1	3 ± 2	< 1	3 ± 1
	C. perfringens	< 1	< 1	2 ± 1	< 1	< 1
V	*E. coli*	< 1	< 1	< 1	< 1	1
	Faecal streptococci	< 1	< 1	< 1	< 1	< 1
	C. perfringens	< 1	< 1	< 1	1	< 1
VI	*E. coli*	< 1	< 1	6 ± 7	< 1	4 ± 1
	Faecal streptococci	< 1	< 1	2 ± 1	2 ± 1	10 ± 12
	C. perfringens	< 1	< 1	1 ± 1	< 1	< 1
River Dospatska	*E. coli*	< 1	26	70	283	108
	Faecal streptococci	< 1	41	65	68	149
	C. perfringens	< 1	< 1	70	< 1	19

Escherichia coli, fecal streptococci and *Clostridium perfringens* (Table 1).

TC remained under 10 cfu.100 mL^{-1} in the area free of net cages at station V and VI. At stations II and III, situated in immediate proximity to the net cage facilities was recorded an increase of the TC in the entire water column compared to station V (ANOVA, $P < 0.05$) *Escherichia coli* was present only near the farms with 6 ± 2 cfu.100 mL^{-1}.

During the summer period was recorded a significant increase in the values of the studied indicators (ANOVA, $P < 0.05$). The values were highest in the epi- and metalimnion water layer. A second increase in the count along the longitudinal axis of the water body was reported in the area of the thermocline and bottom water layers at station VI (Figure 5).

The count of *Escherichia coli* and FS during that period reached respectively 26 cfu.100 mL^{-1} and 20 cfu.100 mL^{-1} in the surface layer at station III and 18 cfu.100 mL^{-1} and 23 cfu.100 mL^{-1} at the dam wall. At the bottom anoxic layer were recorded values of 10 ± 3 cfu.100 mL^{-1} for *Clostridium perfringens*.

Higher values for the studied indicators were recorded in the waters of Dospatska River as well, where the high count of coliforms was accompanied by an increase in the count of *E. coli* to 70 ± 6 cfu.100 mL^{-1} and of FS to 65 ± 12 cfu.100 mL^{-1}. The total increase in the count is most likely due to a fecal contamination of river waters, but the low count of *E. coli* and FS did not allow determination of the origin of the contamination.

Spatial differences in coliforms species composition in the aquatory of the reservoir

Species composition of the total coliform group was determined in order to evaluate the potential health risks. The identification of the isolated strains demonstrated a presence of 10 species of the family *Enterobacteriaceae*, belonging to the genera *Escherichia*, *Klebsiella, Enterobacter, Citrobacter, Serratia, Hafnia, Pantoea* and *Yersinia*. The first four of the more used as an indicator of fecal contamination (WHO 1998). Station VI was characterized with highest species diversity, where 80% of the total number of coliforms was formed from representatives of fecal coliforms (FC) *E. coli* (24%), genus *Citrobacter* (12%), and *Enterobacter cloacae* (44%). At stations IV and V species composition is formed by representatives of the natural microflora of the reservoir from genus *Serratia* (73%), *Hafnia alvei* (17%) and genus *Enterobacter* (10%). At the other stations again was reported an increase in species diversity, possibly due to the filtration of wastewater from the town of Dospat near the Dam wall (Figure 6).

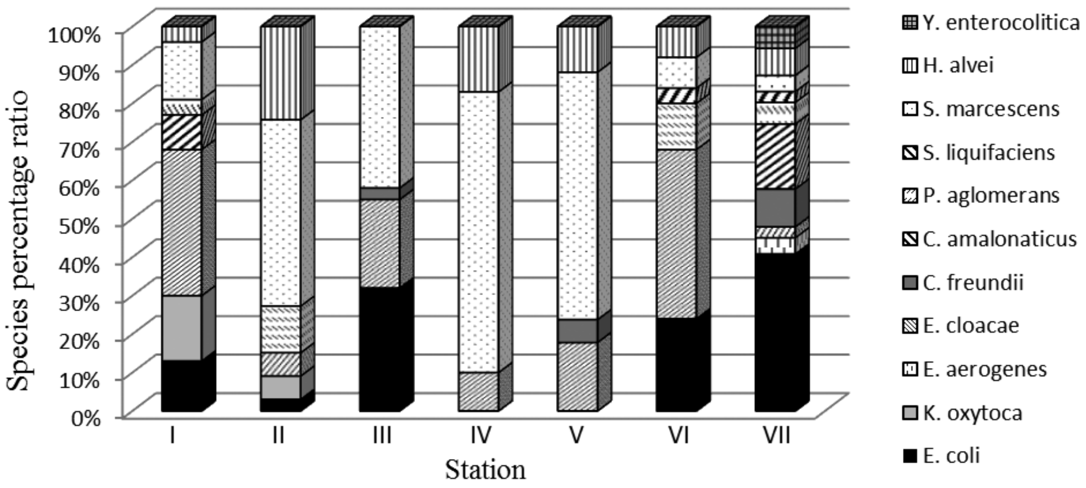

Fig. 6. Percentage ratio of the identified genera from family Enterobacteriaceae in the waters of Dospat Dam and River Dospatska in August 2011

Discussion

The research results are to a great extent consistent with those obtained by Naumova and Zhivkov in their study on the influence of intensive trout rearing in net cages on the physicochemical and hydrobiological quality of the water in Dospat Dam (1988). The established low average annual count of heterotrophic microorganisms in the waters of the reservoir compared to the values of the indicator in the Kardzhali Dam lake for the same study period (Todorov et al., 2012) is largely predisposed by the specific characteristics of the reservoir, such as high altitude (1200 m) and a relatively high retention time (RT) of over two years determining the high self-purification capacity of the Dam. The decrease of the count in depth in August is largely due to a sharp decrease of the temperature in the thermocline and hypolimnion and development of oxygen deficiencies as a result of the thermal stratification of the reservoir in this period (Hadjinikolova and Iliev, 2011). The obtained data for the decrease in the count of TVC 20°C from station VI towards station IV is in accordance with the tendencies established by Straskraba (1998) for the decrease of trophic levels along the longitudinal axis in water bodies with high RT from the tail section towards the Dam wall. This is related to a decrease of the organic matter and microbiological titer (Zmislowska and Golas, 2003; Nahurska and Deptula, 2004). According to Shiranee et al. (1993) and Markosova and Jezek (1994) the amount of incoming organic matter in the water layer is a major factor for the determination of the TVC. For that reason the established secondary increase in the total count of microorganisms at station III in the reservoir, is most likely a result of the higher load in that area, due to the input of organic matter in form of fecal mass and uneaten food from the fish farm.

The mean values of TC in spring and in the autumn period are close to those reported for other Dam lakes in the country (Iliev et al., 2012). The low values during the summer period in comparison to those recorded in Kardzhali Dam by Iliev et al. (2012) are explained with the low monthly average water temperatures, which constrain the development of this group of microorganisms (Baudisova et al., 1997).

The analysis on the species composition of coliforms, showed significant differences in water quality between the stations situated before the net cage farm (stations IV and V) and the remaining stations. The majority of genera of coliforms found at the stations in the area free from net cage farms are species that grow in natural habitats rather than in the digestive system of humans and animals. The species diversity includes mainly representatives form genus *Serratia* and genus *Hafnia*,which are widespread in surface waters (Villalobos et al., 1997), as well as genus *Enterobacter*, participating in the formation of bacterial communities in the environment (Hinton and Wotson, 1995). Their presence is a result of natural ecological processes and is not due to contamination with household waste water. The higher count of TC in the area of the net cage farm is again probably due to local load with organic matter. The absence of *E. coli* and FS from the samples from station V, despite their presence at station VI, is due to the dilution of the contaminants, the natural sedimentation and the absence of local sources of pollution. Similar results were obtained from Niewolak (1999) in a study of Lake Wigri in Poland.

Conclusion

The analysis of theTVC 20°C and TC showed a pronounced seasonal dynamic in Dospat Dam. The waters of River Dospatska were characterized with higher levels of microbial load in comparison with the open aquatory of the water body for the whole period of the study. The higher level of occurrence of the monitored microbial parameters, recorded at station III evidence that net cage farm acts as a secondary source of organic contamination in the area. The species composition of the coliforms confirms the significant differences in water quality between stations along the shore line and those situated in the open water area of the reservoir established earlier. The total count of coliforms at station IV and V is formed mainly by species which are part of the natural microflora of the water bodies such as *S. marcescens*, *H. alvei* and genus *Enterobacter*. Never the less the similarity of the results from researches conducted within 25 years in the aquatory of the reservoir (Naumova and Zhivkov, 1988) testifies for a stable ecological state of Dospat Dam Lake.

Acknowledgements

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