Impact Assessment of the Wastewater Treatment Plants' Discharges on Maritsa River

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Abstract: *Data analysis of wastewater samples at the outlets of wastewater treatment plants (WWTPs) of Pazardzhik, Plovdiv and Svilengrad, which discharge into the Maritsa River is presented. Total monthly loads for 2017 at the outlets are calculated using the monthly averages for the concentrations of chemical oxygen demand, biochemical oxygen demand, total phosphorus and total nitrogen (TN) and the monthly averages for the flow rates. The contributions of the WWTPs to the total river loads emphasize that the impact of WWTPs of Pazardzhik and Plovdiv is significantly greater than WWTP of Svilengrad. Additionally, river water samples were collected before and after the discharge points of the WWTPs in August 2018 and analyzed for water quality parameters listed in Directive 75/440/EEC. Comparison between the river concentrations before the outlet of WWPT – Pazardzhik and after the last sampling point (the outlet of WWTP – Svilengrad) indicates an increase for all the studied parameters, except for Al and Cu. Based on the results obtained for TN, the category of the surface water is significantly deteriorated after discharge of the WWTP – Plovdiv. Wastewater effect on the river surface water is also estimated by using a battery of ecotoxicological tests. The results are presented and compared by the classical approach using categorization based on water quality indicators.*

Keywords: *Surface water, Maritsa, Physicochemical parameters, Trace elements, Ecotoxicity, Wastewater treatment plant.*

Introduction

Regardless of how much water there is on planet Earth (about 2×10^9 km^3), nearly 3% of it can be used for drinking water purposes and requires some kind of purification. Half of this water is found in the ice caps of the poles, which makes the availability of drinking water sources deficient. Additionally, the water remains unevenly distributed around the globe, making about 25% of the population (nearly 2 billion people) with no access to freshwater – it is evident that this vital resource is the worst managed globally [4]. The Earth's population is expected to reach 10 billion by 2050, which not only will increase the drinking water scarcity but will also increase the amount of wastewater released back into nature [1, 12]. Whenever used, water becomes more polluted and its quality deteriorates. About 80% of the water used is discharged to the natural water bodies without treatment. There are various contaminants in water – viruses and bacteria, potentially toxic elements, compounds of nutrients (nitrogen and phosphorus) and other organic compounds such as pesticides, petroleum products, hydrocarbons and metabolites. Therefore, the use of natural resources must be improved to reduce the number of pollutants released. The World Health Organization [22], the United Nations Assembly [20] and the European Commission [10] have adopted a series of documents in this direction. The Water Framework Directive and its sub-directives – 91/271/EEC [8] and 98/83/EC [9] lay down requirements for discharged wastewater and drinking water quality, respectively. The aim is to achieve environmental sustainability [16, 21]. Nevertheless, almost 20 years after the adoption of the Water Framework Directive [10], receiving water bodies that have improved their ecological status by 2015 are about 10% [21].

Maritsa River is the longest river on the territory of the Republic of Bulgaria (480 km in total, 309 on the territory of Bulgaria) with the largest catchment (53,000 km^2, of which 66.2% in Bulgaria, 27.5% in Turkey and 6.3% in Greece). The chemical status of Maritsa River according to the set limits of Directive 2013/39/EC [11] is assessed based on the analysis of the priority substances in twelve surface water samples collected annually. According to the published reports by the East Aegean River Basin Directorate for Water Management [13], the status changed from bad in 2011 for the entire river on the Bulgarian territory after the town of Plovdiv to good from the spring to the country's border in the period 2014-2018. In 2012 and 2013 only the part of the river between the towns of Plovdiv and Dimitrovgrad showed bad chemical status.

According to the Water Framework Directive and the national legislation, the general approach to the classification of the ecological status includes assessment of biological elements for quality, physicochemical parameters and priority pollutants. The hydromorphological quality elements are also included in ecological status assessment. The ecological status of Maritsa River was assessed based on the analysis of the supporting physicochemical parameters, set in the Water Framework Directive [10], adopted and transposed in Ordinance H-4/2012 [19] – pH, electrical conductivity (EC), orthophosphate (PO_4^{3-}–P), total phosphorus (TP), ammonium nitrogen (NH_4^+–N), nitrate-nitrogen (NO_3^-–N), total nitrogen (TN) and biological oxygen demand after 5 days (BOD_5). According to the published reports [13], the ecological status changed from "very bad" in 2011 to "moderate" in 2012 and 2013 between the towns of Pazardzhik and Stamboliyski and from "very bad" in 2011 to "bad" in 2012 and 2013 after the town of Plovdiv. The ecological status of the entire river on the Bulgarian territory improved to "moderate" in the period 2014-2018.

In the present study, the analysis of the selected physicochemical parameters from Ordinance H-4/2012 [19] were used as the basis for determining the water quality of the Maritsa River and the impact of wastewater treatment plants' (WWTPs) discharges on the surface water quality. The assessment is performed by estimation of the contribution of the WWTPs' discharged loads to the total river loads for the mandatory monitoring parameters and by analysis of broader range of pollutants at the WWTPs' outlets and in the surface water. Additionally, a battery of biotests is used for water quality assessment [17, 18] as it could provide an informative indication of the integral effect of environmental pollutants, including new and emerging pollutants. Such a water quality assessment of the Maritsa River surface waters was carried out as part of the process for establishing the ecological status. The article does not discuss or analyze the results of the biological quality elements that are a priority in the preparation of the overall assessment of the ecological status of the body of water.

Materials and methods

Sampling and data acquisition

Mandatory monitoring data according to Directive 91/271/EEC [8] for 2017 of three WWTPs (Pazardzhik, Plovdiv and Svilengrad, Fig. 1) was used for calculation of the monthly average flow rates and the monthly average concentrations of COD, BOD_5, TN, and TP at the outlet of each WWTP (Fig. 2). Using these averages, monthly loads were calculated.

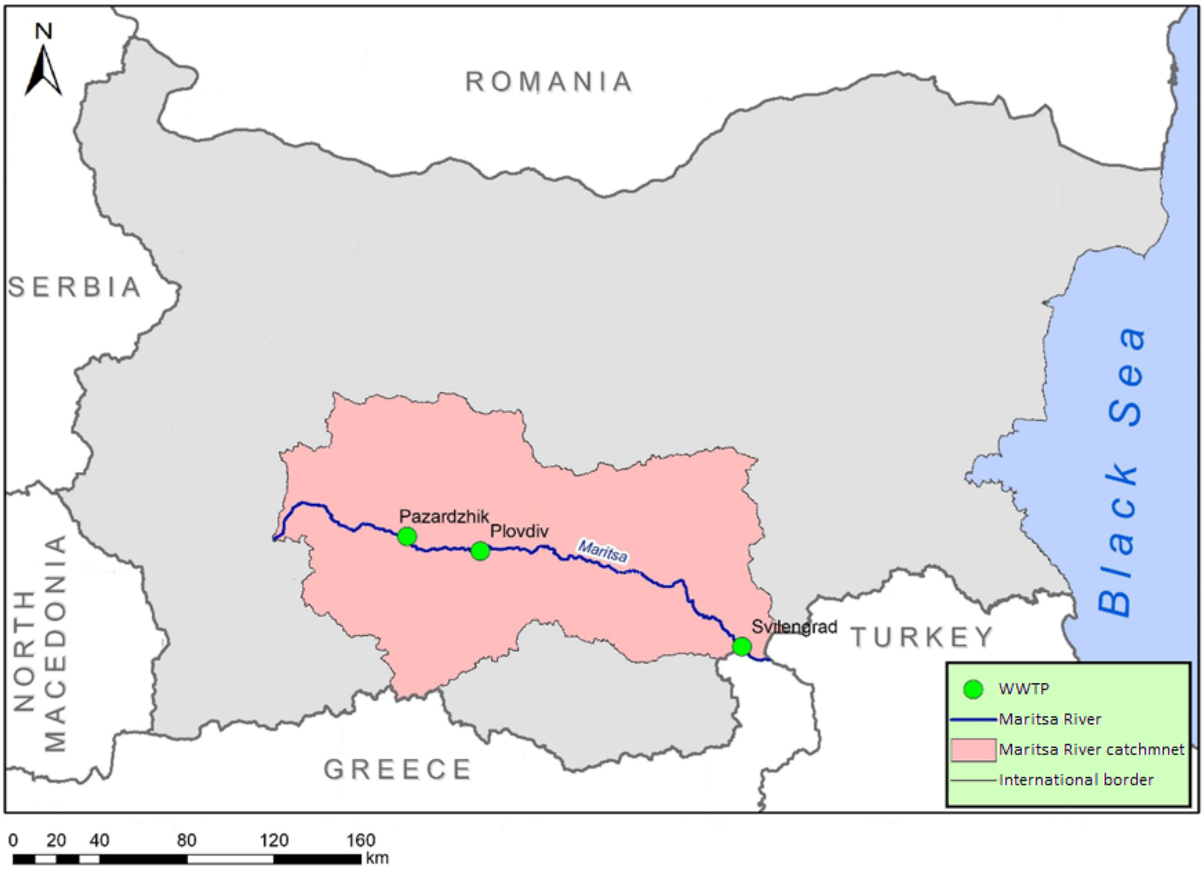

Fig. 1 Sampling locations of the WWTPs on Maritsa River

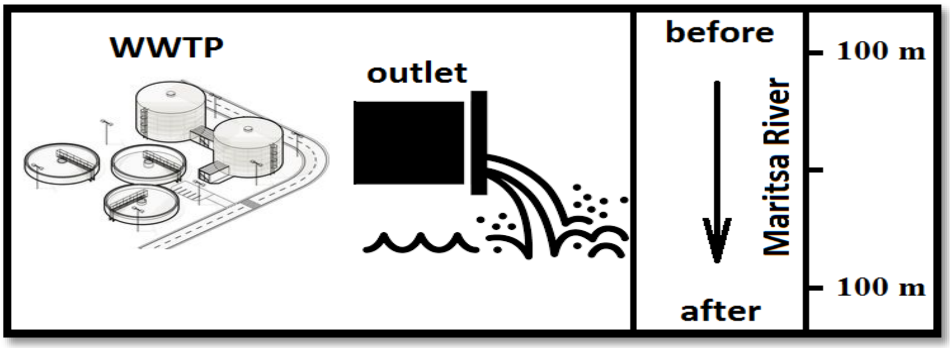

Fig. 2 Sampling scheme

Mandatory monitoring data according to Directive 75-440-EEC [7] for 2015-2017 at three sampling points (Pazardzhik, Plovdiv and Svilengrad), located upstream the WWTPs, was used to calculate loads of COD, BOD_5, TN and TP in the surface water of the Maritsa River.

Chemical and ecotoxicological analysis

Additionally, surface water samples from Maritsa River and wastewater samples at the outlets of WWTPs – Pazardzhik, WWTPs – Plovdiv, and WWTPs – Svilengrad were collected according to the scheme, presented in Fig. 2 in August 2018. Water samples were collected in glass bottles and stored at 4 °C before transportation to a laboratory. Fifty milliliters of the sample intended for ICP-MS analysis were filtered through 25 mm PES sterile syringe filters (0.45 μm) and 1.5 mL of concentrated suprapur nitric acid was added. Two hundred and fifty milliliters of the sample intended for ecotoxicological analysis was filtered through a 25 mm PES sterile syringe filters (0.2 μm) and frozen.

All the analyses were performed in triplicate. The relative standard deviations were less than 5 % for all the measured parameters.

The samples were analysed for COD, BOD_5, TN and TP. For the determination of the BOD_5, a standard methodology was used [15], based on the measurement of the dissolved oxygen in the sample on the first and the fifth day. Between the measurements, the samples were stored in thermostat Friocell FC 222 (Friocell, Germany) at 20 ± 1 °C in darkness. All steps of the standard procedure were followed. The methods for the spectrophotometric determination used cuvette tests – LCK 1414 for COD, LCK 138 for TN and LCK 348 for TP; a portable spectrophotometer DR 3900 (Hach Lange GmbH, Berlin, Germany); and thermo-reactor LT 200 (Hach Lange GmbH, Berlin, Germany) in case of COD, TN and TP. The detailed procedure is described elsewhere [23].

Trace elements analysis of the acidified water samples (67-69% suprapur HNO_3, Fisher Chemicals) was carried out with an ICP-MS PerkinElmer SCIEX – ELAN DRC-e (MDS Inc., Concord, Ontario, Canada). The spectrometer was optimized (RF power, gas flow, lens voltage) to provide minimal values of the ratios CeO^+/Ce^+ and Ba^{2+}/Ba^+ as well as the maximum signal intensity of the analytes. External calibration by a multi-element standard solution was performed. The calibration coefficients for all calibration curves were at least 0.99. Single element standard solutions of Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, Zn and U (Fluka, Germany) with an initial concentration of 10 μg/mL were mixed and used for calibration after appropriate dilution to obtain the following concentrations: 0.5, 1.0, 5.0, 10.0, 25.0 and 50.0 ng/mL. All solutions were prepared with double deionized water (Millipore purification system Synergy, France). The accuracy of the proposed method was checked by analyzing standard reference material NIST 1640a (Trace Elements in Natural Water). The obtained values for analytical recovery varied between 95% and 108%, which was considered satisfactory.

A battery of selected biotests was applied to assess the ecotoxicity of the collected samples. The selected species belong to different trophic levels in the food chain, as follows – producers: *Sorghum saccharum*, *Lepidium sativum*, and *Sinapis alba*; consumers: *Daphnia magna*, and reducers: *Vibrio fischeri*. The Phytotoxkit F™ biotest (MicroBioTests Inc. Ghent, Belgium) measures the change of the seed germination (SG) and the root growth (RG) of the higher plants *Sorghum saccharatum* (SS), *Lepidium sativum* (LS) and *Sinapis alba* (SA) after 3 days of exposure to the analyzed samples, compared to a control sample [2]. The biotest Daphtoxkit F™ (MicroBioTests Inc. Ghent, Belgium) is an acute toxicity test, which utilizes dormant eggs (ephippia) of *Daphnia magna* [5]. The ecotoxicological effect (%) is calculated as a ratio between the number of dead test organisms in the studied sample and the number of alive organisms in the control sample. The Microtox® biotest (ModernWatern, Cambridge, UK) registers the slowdown of the metabolism of *Vibrio fischeri* bacteria when exposed to toxic substances, based on the reduction of the intensity of the emitted by the bacteria light compared to a control sample [6].

Results and discussion

Loads

The flow rate of Maritsa River at the investigated three sampling points (Pazardzhik (PAZ), Plovdiv (PDV), Svilengrad (SVG)) for the period 2015-2017 is presented in Fig. 3. There is a clear downstream increase of the flow rate from PAZ to SVG. Water quantities decrease with time, but have higher values between January and March for each year.

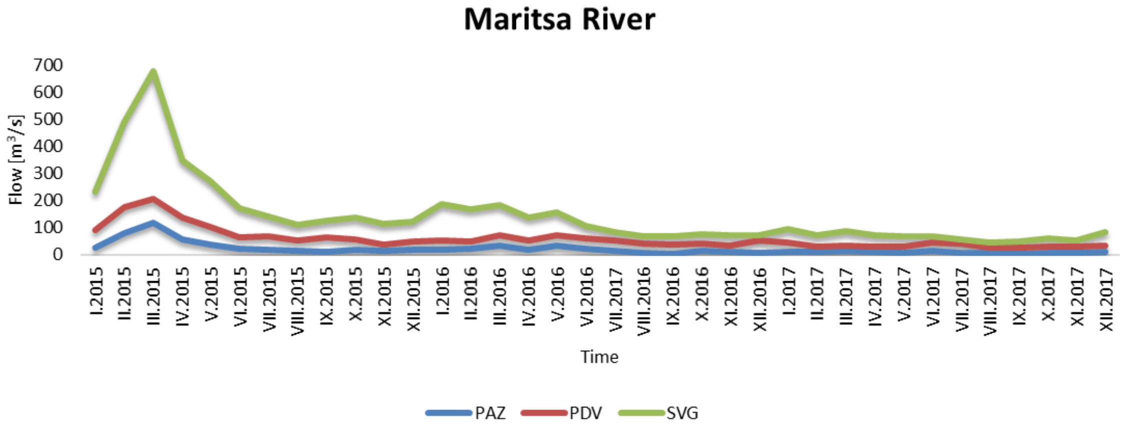

Fig. 3 Comparison of river flows at Pazardzhik, Plovdiv and Svilengrad for 2015-2017

Mandatory monitoring data according to Directive 75-440-EEC [7] for 2015-2017 at three surface water sampling sites (Pazardzhik, Plovdiv and Svilengrad) is presented in Fig. 4. The sampling sites are located upstream of the respective WWTPs. To calculate the loads of COD, BOD_5, TN and TP in the surface water of the Maritsa River, average monthly flows (m^3/s) and average monthly concentrations (mg/L) of the parameters were used. In general, the calculated loads follow the flow rates profiles presented in Fig. 3. The increase in the average monthly flow, while the concentrations of the COD, BOD_5, TN and TP in the surface water remain relatively constant, is the reason for the great increase of the loads downstream.

Mandatory monitoring data according to Directive 91/271/EEC [8] for 2017 at the outlets of three WWTPs (Pazardzhik, Plovdiv and Svilengrad) was used for calculation of the monthly average flow rates (m^3/month) and the monthly average concentrations (mg/L) of COD, BOD_5, TN, and TP. The load profiles are presented in Fig. 5.

Contrary to the surface water loads' profiles, the monthly averages for the loads at the outlets of the WWTPs have different order. The reason is the size and the technological scheme of each plant. The biggest WWTPs is Plovdiv (design capacity 596,000 p.e., real capacity 372,398 p.e. for 2017), the smallest is Svilengrad (design capacity 20,903 p.e., real capacity 19,488 p.e. for 2017) and Pazardzhik being somewhat in the middle (design capacity 150,000 p.e., real capacity 67,036 p.e. for 2017).

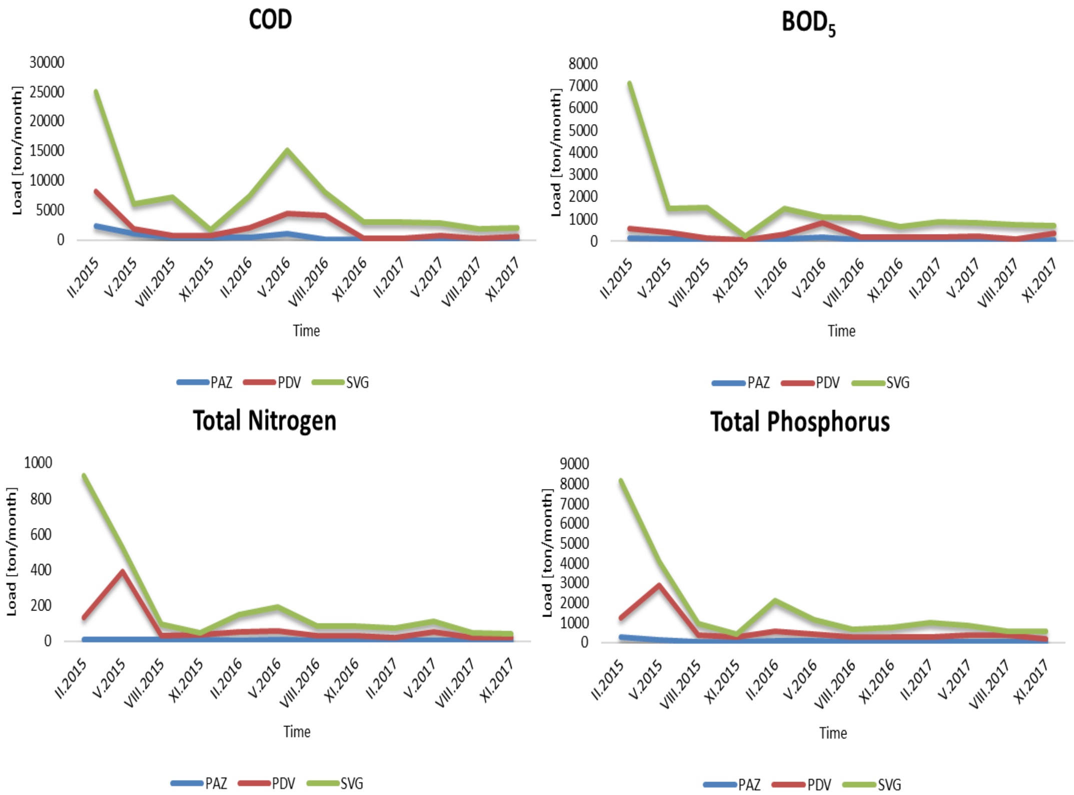

Fig. 4 Load profiles of COD, BOD_5, TN and TP in Maritsa River for 2015-2017 (n = 12) in Pazardzhik, Plovdiv and Svilengrad sampling points

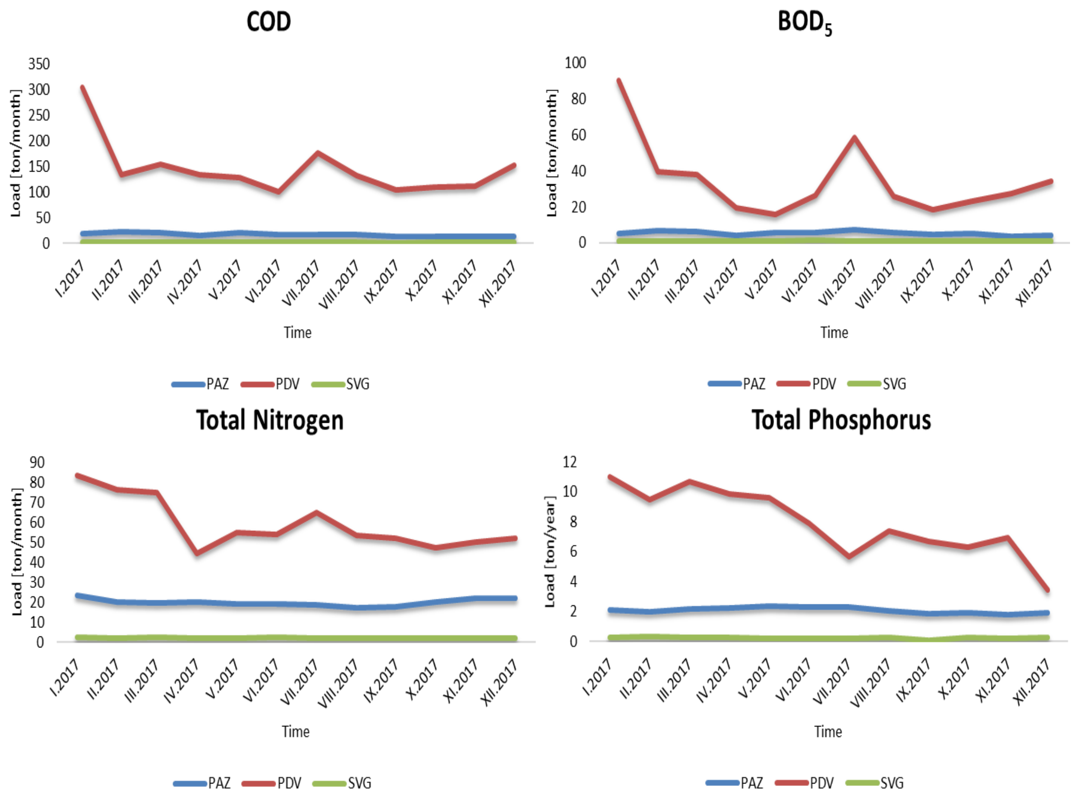

Fig. 5 Load profiles of COD, BOD_5, TN and TP for 2017 (n = 12) in outlets of WWTP – Pazardzhik, WWTP – Plovdiv and WWTP – Svilengrad

The average monthly loads of the WWTPs were compared to the average monthly loads of the surface waters in the Maritsa River and their contributions are presented in Table 1.

Table 1. Contributions of the WWTPs' loads to the loads in Maritsa River at the respective sampling points

Month	Sampling point	COD, %	BOD_5, %	TN, %	TP, %
II.2017	Pazardzhik	21.1	14.3	29.7	38.7
V.2017		23.2	7.2	32.1	35.7
VIII.2017		25.1	23.0	49.6	60.5
XI.2017		20.3	8.1	47.2	45.1
II.2017	Plovdiv	37.0	19.8	23.8	36.1
V.2017		15.4	9.6	13.8	16.5
VIII.2017		37.1	25.9	13.1	30.3
XI.2017		16.3	7.9	24.4	26.1
II.2017	Svilengrad	0.1	0.1	0.3	0.6
V.2017		0.1	0.2	0.4	0.4
VIII.2017		0.1	0.1	1.1	1.1
XI.2017		0.2	0.2	0.5	1.1

The relatively moderate discharge flow rates of WWTP – Pazardzhik, compared to the relatively low flow of the Maritsa River in this sampling point, results in a relatively high contribution of the plant to the surface water loads for all the parameters, especially for the nutrients – TN and TP.

The high discharge flow rates of WWTP – Plovdiv, compared to the moderate flow of the Maritsa River in this sampling point, results in similar contributions of the plant to the river loads for all the parameters. Another reason for the high contribution to the total nutrients' (TN and TP) loads is the lack of chemical precipitation of phosphorus and biological nitrogen in the removal facilities of both WWTPs. On the other hand, the WWTP of Svilengrad employs all the treatment facilities, including mechanical and biological steps, phosphorus and nitrogen removal steps. The low discharge flow rate of the WWTP – Svilengrad compared with the high flow rate of the Maritsa River in this sampling point results in a very low contribution of the plant to the total river loads for all the parameters.

Distinctive seasonality with summer maximum for nutrient loads of WWTPs of Pazardzhik and Svilengrad is observed. The oxygen demanding loads (COD and BOD_5) do not show specific seasonal behavior. As both BOD_5 and COD are important water quality indicators reflecting the presence of oxygen demanding pollutants, the calculated BOD_5 to COD (BOD_5/COD) ratio in the Maritsa River surface water and WWTPs' outlets is presented in Fig. 6.

The BOD_5/COD ratio represents the biodegradable organic fraction of oxygen demanding pollutants, which decreases on the Maritsa River from annual average of 0.68 at Pazardzhik monitoring station to 0.30 at Svilengrad. This fact is an indication for increasing quantity of refractory organics and inorganic pollutants downstream on the river. The ratio decreases downstream at the sampling sites located upstream from the WWTP, suggesting that the river water quality may be deteriorated by various uncontrolled pollution loads from the industrial and residential areas [3]. The BOD_5/COD ratio trend in WWTPs' effluent is reversed to the size of the WWTPs. The smallest WWTP at Svilengrad has annual average value of 0.39 while the biggest at Plovdiv has value of 0.21. This trend could be explained with the water quality of incoming wastewaters and the abovementioned differences in WWTPs' technological schemes. It should be mentioned that during the investigated period no exceeding of maximum permissible BOD_5 and COD limits according to the Directive 91/271/EEC [8] (25 mg/L for BOD_5 and 125 mg/L for COD) in WWTPs' outlets was observed. The BOD_5/COD ratio tendencies in Maritsa River and WWTPs' outlets lead to the higher outlet value than river water ratio at Svilengrad. The results for Pazardzhik and Plovdiv are in line with the expected low ratios in the WWTPs' effluent loads [14].

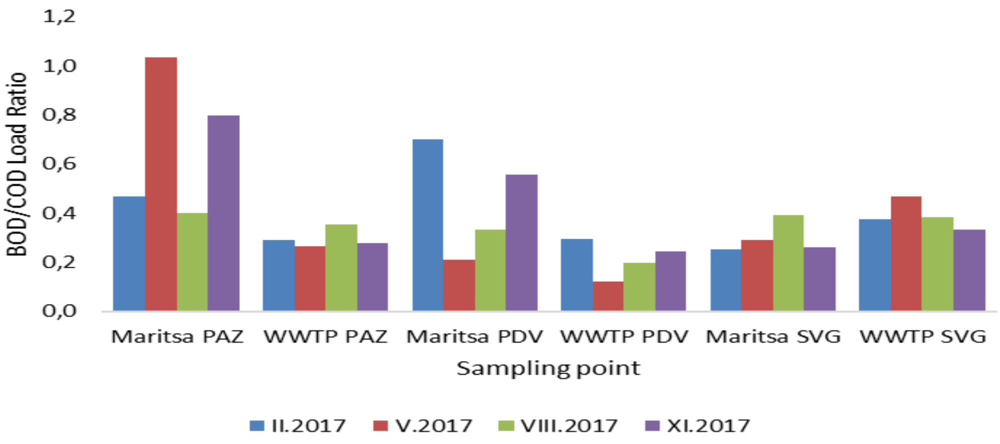

Fig. 6 BOD_5/COD ratio in surface water and wastewater

The combined total loads of the three WWTPs compared to the total surface water load of the Maritsa River after Svilengrad for 2017 is presented in Table 2.

Table 2. Contributions of the combined total WWTPs' loads to the total loads in the Maritsa River after Svilengrad

Month	**COD, %**	**BOD_5, %**	**TN, %**	**TP, %**
II.2017	5.9	6.8	13.7	69.1
V.2017	6.9	3.5	16.7	21.1
VIII.2017	9.1	5.0	35.7	36.4
XI.2017	9.3	8.9	18.5	43.3

The contribution of the oxygen demanding WWTPs' loads to the total load of the Maritsa River is in the range 3.5 and 9.3%. The contribution of WWTPs' nutrient loads is significantly higher than the COD and BOD_5 loads, with annual averages of TN and TP 21.2% and 42.5%, respectively. The remaining part of the river loads is due to numerous other point and non-point sources located in the large Maritsa River catchment area.

Surface water and compliance with Directive 75/440/EEC

The next assessment of the impact of WWTPs on the river surface water quality is based on the comparison of the concentrations of the physicochemical parameters and trace elements to the limits set in Directive 75/440/EEC. Such comparisons are made to show whether the surface water quality in the river is deteriorated by the discharged wastewater from WWTPs in ways to change its category or make it unsuitable for drinking water abstraction. The results of the surface water sampling from the Maritsa River in August 2018 are presented in Table 3. The standard methods for treatment of surface waters for categories described in Directive

75/440/EEC [7] to meet the requirements for drinking water abstraction, as described in Directive 98/83/EC [9], are the following: for A1 – needing simple physical treatment and disinfection; for A2 – requiring normal physical, chemical treatment, and disinfection and for A3 – with intensive physical, chemical treatment and extended disinfection.

Table 3. The concentration of the physicochemical parameters and the trace elements in the surface water of the Maritsa River before and after the outlets of the WWTPs

Parameter	WWTP – Pazardzhik		WWTP – Plovdiv		WWTP – Svilengrad		Limits for A1 / A2 / A3
	Before	After	Before	After	Before	After	
pH	7.88	8.24	8.25	8.33	8.35	8.21	8.5 / 9 / 9
EC, [μS/cm]	125	142	136	160	443	452	1000
COD, [mg/L O_2]	11.1	8.32	6.71	7.7	20.4	18.6	30 (A3 only)
TSS, [mg/L]	8.2	8.8	12.6	16.5	36.2	42.8	25 (A1 only)
NO_3^-–N, [mg/L]	< 0.23	0.97	0.7	3.1	1.8		
TN, [mg/L]	1.2	1.1	0.5	**3.7**	1.9	1.8	1 / 2 / 3
TP, [mg/L]	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	0.4 / – /0.7*
Al, [μg/L]	153.5	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
As, [μg/L]	2.84	2.28	1.15	1.12	2.96	3.23	50 / 50 / 100
Ba, [μg/L]	23.10	25.38	23.38	37.57	43.42	46.21	100 / 1,000 / 1,000
Cd, [μg/L]	0.03	0.02	0.02	0.02	0.23	0.24	1
Co, [μg/L]	0.48	0.41	0.32	0.35	0.65	0.68	20 (A1 only)
Cr, [μg/L]	0.69	0.74	0.85	1.00	2.05	1.84	500
Cu, [μg/L]	9.76	8.74	3.74	4.20	7.61	8.23	20 / 50 / 1,000
Fe, [μg/L]	327.8	366.5	517.0	549.0	**1360**	**1550**	100 / 1000 / 1000
Mn, [μg/L]	75.87	80.26	31.63	41.80	103.53	115.63	50 / 100 / 1000
Ni, [μg/L]	2.76	2.75	3.60	4.30	7.16	7.86	20 (A1 only)
Pb, [μg/L]	6.60	6.80	1.37	3.40	8.77	8.20	50
Se, [μg/L]	0.25	0.38	0.13	0.75	1.00	0.38	10
U, [μg/L]	2.61	2.75	2.14	3.73	5.38	4.86	
V, [μg/L]	1.14	1.50	1.90	2.68	4.47	4.68	10 (A1 only)
Zn, [μg/L]	7.18	8.00	3.47	20.65	17.69	19.12	500 / 1000 / 1000

*Expressed as P_2O_5

The concentration of TN in the surface water of the Maritsa River before the discharge of WWTP – Pazardzhik is higher than the limit for category A1. Although this concentration increased by 50% after the discharge of WWTP – Svilengrad, it remained within the limit for category A2. The concentration of TN after the discharge of WWTP – Plovdiv rose even above the limits for category A3, but apparently, the self-cleaning effect of the river is sufficient to reduce this concentration before the point of the next discharge. The concentration of TSS in the surface water of Maritsa River is below the set limits for category A1 before the discharge of WWTP – Pazardzhik but increased thereafter at all sampling points to exceed the limits for category A1 at Svilengrad. The concentrations of Fe and Mn in the surface water before the discharge of WWTP – Pazardzhik exceeds the set limit for category A1. The concentration of Fe steadily increases to exceed the limit for A3 at Svilengrad, while the Mn concentration in the surface water before and after the discharge of WWTP – Plovdiv complies with the set limit for A1, before exceeding this category at the last sampling point. For all the other studied physicochemical parameters and trace elements, the surface water quality satisfies the limits set for category A1.

The assumption is that the concentrations of all parameters in the surface water before the discharge of the first plant (WWTP – Pazardzhik) are not affected by discharges of wastewaters and significant anthropogenic activities, so they can be used as reference level. Any difference in the concentration of the same parameters, measured after the discharge of the last plant along Maritsa River on the Bulgarian territory (WWTP – Svilengrad), could be used for indirect assessment of the anthropogenic impact of all pollutant sources in Maritsa River catchment including WWTPs on the river surface water quality.

The total phosphorus concentrations before and after the discharge points of the WWTPs along the Maritsa River are below the limit of quantification (LOQ) of the method of choice (< 0.5 mg/L), so the impact of the plants on the river surface water quality cannot be determined using this parameter. All other physicochemical parameters studied showed an increase in concentration to different extend, only for the concentrations of Al and Cu a decreasing tendency is observed (Fig. 7).

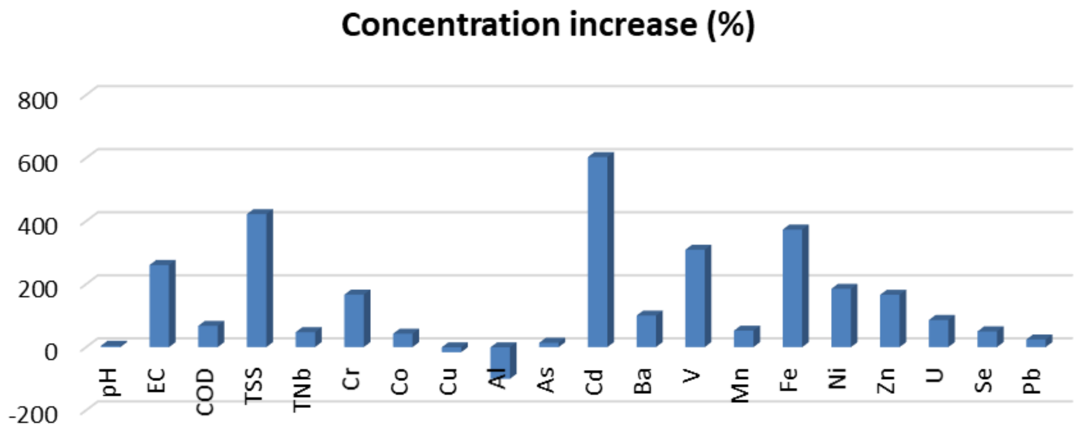

Fig. 7 Comparison of concentrations of the studied parameters in the surface waters of the Maritsa River after the discharge of WWTP – Svilengrad and before WWTP – Pazardzhik

Ecotoxicity

The most commonly used biotest for assessing the toxicity of water samples is Daphtoxkit F. Even though it does not show a significant change along the Maritsa River. The reason could be found in the low ecotoxicity of the studied surface waters which is shown by all the three biotests used. The Microtox® test shows decreasing of the ecotoxicity after WWTP – Pazardzhik and WWTP – Plovdiv. Increasing of the ecotoxicological effect is observed only after WWTP – Svilengrad but it is still lower than the sample before WWTP – Pazardzhik. The ecotoxicological parameters LS-SG, SA-SG, SS-SG do not show significant changes in the surface water samples along the Maritsa River (Table 4). The reason could be found in the low ecotoxicity of the surface water, which doesn’t reflect on the germination of the seeds of the three plant species.

Table 4. Ecotoxicological test results for Maritsa River and at the outlets of the WWTPs, August 2018 (B – Before; O – Outlet; A – After)

Ecotoxico-logical test, [%]	**WWTP – Pazardzhik**			**WWTP – Plovdiv**			**WWTP – Svilengrad**		
	B	**O**	**A**	**B**	**O**	**A**	**B**	**O**	**A**
LS-SG	0.00	0.00	0.00	3.3	5.00	6.7	0.00	3.3	0.00
LS-RG	-28.2	-15.7	-15.3	-15.9	-20.85	7.3	-75.4	-57.6	-69.7
SA-SG	-3.5	-3.5	0.00	-3.5	-3.45	-3.5	-3.5	0.00	-3.5
SA-RG	-28.3	-44.6	-23.0	-22.7	-19.40	-31.7	-20.9	-6.4	-47.6
SS-SG	0.00	0.00	0.00	6.9	6.90	3.5	3.5	10.3	-3.5
SS-RG	12.9	-3.9	-2.4	37.7	40.94	40.4	18.8	6.5	22.2
Daphtoxkit F	6.7	33.3	13.3	0.00	6.67	13.3	13.3	20.0	13.3
Microtox	45.4	19.9	14.5	32.0	20.30	9.0	17.9	32.8	29.1

More noticeable changes are observed in the other phytotoxicity parameter – the root growth. The negative ecotoxicological effect of most of the samples shows that the average root length in the tested sample is longer than the average root length in the control sample. Even that most of the results show that the observed ecotoxicity after the discharge of WWTPs' outlets is higher, an increase of the root length for the dicotyledonous species *Lepidium sativum* and *Sinapis alba* along the river could be generally outlined.

The reason may be due to the nutrients present in the surface waters, which concentration increases along the river as a result of the WWTPs discharges and favors the development of these plants. There is no clear tendency in the results for the monocotyledonous species *Sorghum saccharatum* – the ecotoxicity decreases after WWTP – Pazardzhik, increases after WWTP – Plovdiv and a slight decrease is observed after WWTP – Svilengrad, compared to the samples before the WWTPs.

Conclusion

The influence of WWTPs along the Maritsa River on the territory of Bulgaria has been investigated. The contributions of the discharged loads assessed through the basic physicochemical parameters (COD, BOD_5, TN and TP) to the total loads of the Maritsa River, are presented and compared. The total loads of the oxygen demanding pollutants (expressed by BOD_5 and COD) is lower than 10% while the total loads of nutrients (TP and TN) are 21.2% and 42.5%, respectively. The main part of the loads from WWTPs comes from the relatively big WWTPs of Pazardzhik and Plovdiv (design capacities of 150,000 and 596,000 p.e., respectively) which do not employ nitrogen removal facilities and chemical precipitation of phosphorus in their technological schemes. The large catchment area of Maritsa River poses big differences in the flows at the different sampling points, which suggests that substantial part of the loads' increase is due to numerous other point and non-point sources such as inflowing rivers and streams.

All investigated physicochemical parameters (except Cu, Al) show an increase in concentration after the last WWTP (Svilengrad) compared to the water properties before the discharge of the first WWTP (Pazardzhik). Based on the results obtained for TN, the category of the surface water is deteriorated after discharge of the WWTP – Plovdiv.

The ecotoxicity of the surface water samples is low as the test organisms respond differently to the changes in water quality along the Maritsa River, with the lowest response being observed for the ecotoxicological indicator associated with the germination of plant seeds.

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