## **CONSTRUCTED WETLAND – THE SERBIAN EXPERIENCE**

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**Abstract:** Constructed Wetland System (CWS) in the Gložan settlement is the first such system for wastewater treatment constructed in Vojvodina (Serbia), aimed at treating local municipal wastewaters. The common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), naturally growing on the CWS location, is used as biofiltering vegetation. The CWS efficiency was monitored based on the water balance, removal of suspended matter, reduction of the amount of organic matter, removal of nitrogen compounds and total phosphorus, bioaccumulation of nutrients in the plant organs, as well as via the number of coliform bacteria. The water balance of the CWS indicates that water is always present. The system appeared to be capable to remove 94% of suspended matter, while the decrease in organic matter, expressed via biological oxygen demand (BOD<sub>5</sub>), in the investigation period has been 80.9%. The efficiency of removal of nitrogen compounds ranged from 47.3% for nitrates, 47.5% for ammonium, to 78.3% nitrites, while the efficiency of total phosphorus removal was 29.1%. The results of microbiological analyses confirmed the sanitary safety of the effluent. The results of the chemical composition of the reed confirmed the bioaccumulation of nutrients in the plant organs. Best results were achieved at the end of the growing period (autumn).

Keywords: Wastewater, Constructed Wetland, Pollutants, Microorganisms, *Phragmites australis*, Purification

#### **1. INTRODUCTION**

Wetlands have been recognised as a natural resource throughout human history. Their importance is appreciated in their natural state by such people as the Marsh Arabs around the confluence of the rivers Tigris and Euphrates in southern Iraq, as well as in managed forms; e.g., rice paddies, particularly in South East Asia (Mitsch & Gosselink, 2000). Also, natural wetlands exist during the centuries on the Pannonian Basin (Szucsne-Murguly, 2006). The natural water purification processes occurring within these systems have become increasingly relevant to practical use of constructed or even semi-natural wetlands for water and wastewater treatment (Scholz, 2006).

Wastewaters are often discharged into the recipients without any pretreatment, which represents a serious threat to the quality of water and its further usability. As a consequence, many water resources have been brought to the state of unsatisfactory water quality, which affects the health of people and all living world in the environment. A

first step in preserving a satisfactory quality of water resources is certainly an appropriate treatment of wastewaters. Various methods have been used to achieve this, and a special place among them occupy constructed wetland systems (CWS). These systems represent a combination of biological, chemical and and their remarkable physical processes. characteristics are practicality, economicity, and simplicity of exploitation (Lakatos, 1998). The essence of the CWS method is in the utilization of phytofiltration and phytoaccumulation capacities of marsh plants (Brix, 1994a; Ellis et al., 1994; Urbanc-Berčič, 1997; Nikolić et al., 2003; Nikolić et al., 2007). Besides, due to the favorable conditions, the root zones of these plants are amply populated with microorganisms, which are of essential importance in the transformation of nitrogen and its compounds, through the processes of ammonification. nitrification and denitrification (Greenway, 2007), as well as in the mineralization of organic matter (Jarak & Čolo, 2007).

Nuttall et al. (1997) presented a list of aquatic plants that are suitable for the application in the CWS,

and among them a special place occupy the emerse species like *Phragmites australis* (Cav.) Trin. ex Steud, *Typha latifolia* L. and *T. angustifolia* L., as the species which bear well high organic loads. Brix (1994a, 1994b) also mentioned the importance and manifold functions of reed in the functioning of CWSs.

CWSs can be applied in the different stages of water treatment (primary, secondary, tertiary), to treat municipal wastewaters, wastewaters from the different industries and farms, runoffs from the agricultural, urban and traffic areas, as well as of landfill leachates. According to Kadlec & Knight (1996), the CWS in the USA are mostly used to treat municipal and industrial wastewaters, then mine drainage waters, storm waters and landfill leak waters. Lakatos (1998) pointed out that CWSs are most suitable to treat wastewaters of smaller rural settlements.

CWSs have no negative influence on the air, water, soil, population, climate, and ecosystem (Belić & Josimov-Dunđerski, 2007; Josimov-Dunđerski & Belić, 2007). Just contrary, they contribute to the preservation and improvement of the quality of the environment. It is a clean technology; solar energy is used to obtain purified water and usable biomass as final products.

The objective of this study was to examine the efficiency of the wetland system constructed to treat municipal wastewater of the Gložan settlement, the first such system built in Vojvodina (northern province of the Republic of Serbia), with the Danube as the outflow recipient and common reed, *Phragmites australis*, as dominant emerse species. The CWS efficiency was assessed on the basis of the water balance, removal of suspended matter, reduction of the organic load, removal of nitrogen compounds and total

phosphorus, bioaccumulation of nutrients in the reed plant organs, all measured in the investigation period of several years. The investigation encompassed seasonal variations of these parameters in particular parts of the system. Besides, the sanitary safety of the effluent was assessed via the count of the coliform bacteria and *E. coli* under the eco-hydrological conditions of a moderate continental climate.

## 2. STUDY AREA

Since the autumn of 2004, the municipal wastewaters of the Gložan settlement have been treated in the CWS with common reed as phytofilter. The geographic coordinates of Gložan are 45°17' North latitude and 19°33' East longitude, while the altitude is 80-82 m.a.s.l. The regional surface water is the Danube River and land drainage canals. The settlement is located at a distance of 6 km from the Danube, which at the place forms an oval meander (Fig. 1).

Between the settlement and the Danube there is an inundation area which is protected from high waters by a dike, along with the constructed drainage system. According to its geographic position, Gložan is in the belt of moderate continental climate: autumns are warmer than springs, and temperature transition from the winter to summer is less sharp than vice versa. Precipitation has the distribution features characteristic of the Danube coastal area. The favorable geographic position and fertile agricultural soil have influenced the formation of the other elements of the environment, which has been affected significantly by the anthropogenic activities.



Figure 1. Aerial photograph

Gložan is an urbanized settlement of Pannonian type, with 2275 inhabitants. The waterworks are constructed in 1973, and to the construction of the sewerage system in 2004 use was made of septic tanks.

The CWS is located in the inundation zone of the Danube alluvial plane, south of the Gložan settlement, between two land drainage canals. The terrain is almost horizontal, and its altitude is 76.50m.a.s.l. The location itself has characteristics of a marshy landscape.

The CWS consists of three cells of a total area of 9400 m<sup>2</sup>. The constructed CWS is a horizontal subsurface flow. The substrate consists of gravel beds, 0.6 m wide and 0.6 m thick, with alternate in succession of the belts of natural soil, 1.0 m wide (Fig. 2). The surface layer consists of gravel mixed with earth, along with the reed vegetation. The lining is made of an impermeable clay layer that existed on the wetland location. Treatment of wastewater is carried out by passing it through three cells, the residence times being 24, 48 and 33 hours in Cell I, II, and III respectively. The technological process encompasses the collecting and conducting of used waters, their treatment in the CWS and discharge into the recipient, a canal connected to the Danube (Belić et al., 2004). The system has a section for treatment of raw sewage prior to entering the wetlands. The water from a lagoon comes to a coarse grid (Fig. 3) for the separation of large suspended particles and flowing material. After that, the settled sewage is introduced to Cell I. The CWS has to be constantly controlled and maintained, which primarily assumes timely harvest of the reed, i.e. to remove all above-ground plant parts in order to prevent their decay and secondary pollution of the system.

Dry plant parts (primarily stems) may be collected and used, for example, in the civil engineering industry. A timely harvest of the reed has a favorable effect on the development of new young shoots from the rhizome buds in the subsequent growing season. However, it should be pointed out that reed harvest in the given CWS has not been practiced.

## **3. METHODS AND PROCEDURES**

The investigation of the CWS operation was carried out in the 2005-2008 period. The average hydraulic load was determined by volumetric method, and it was 1.5 l/s. Based on the characteristics of the substrates in Cells I, II and III the flowing time of wastewater through the wetlands was determined by tracer method.



Figure 2. CWS in construction (2004) and after several years of operation (2008)



Figure 3. Appearance of the lagoon and of the coarse grid

The overall residence time, from the inflow to outflow, was found to be 105 h. Regarding the hydraulics, the wetlands are a stationary linear system through which water flows at a constant rate under the gravitation force. Since the coefficient of daily nonuniformity in the summer period amounts to 2 (Jahić, 1990), the average yearly flow for the Gložan CWS working period amounts to 2.25 l/s.

The water balance was calculated on the basis of monthly values in the investigation period, using equation (1), while the evapotranspiration was obtained from Penman's equation.

$$|W_{O\text{-effluent}}|_T = |W_{O\text{-influent}} \pm (P - ET)|_T$$
 (1)

where:

 $W_{Q\text{-effluent}}$ - CWS effluent (mm) $W_{Q\text{-influent}}$ - CWS influent (mm)P- Precipitation on CWS (mm)ET- Evapotranspiration (mm)T- Water residence time (days)

The measurements encompassed chemical analyses of the influent wastewater, bioaccumulation of nutrients in the reed organs, and tests of sanitary safety (total counts of coliforms and *E. coli*). Characteristic sites in the CWS were chosen as sampling (measuring) points (Fig. 4): the inflow to Cell II – sampling site 1; the inflow to Cell II – sampling point 2; the inflow to Cell III – sampling point 3, and the outflow from the cell immediately prior to the discharge to the canal – sampling point 4.

Chemical analyses were carried out by the following methods: Suspended matter was measured according to the standard SRPS H.Z1.160, BOD<sub>5</sub> according to the SRPS ISO 5815, ammonium (NH<sub>4</sub>-N) according to the ISO 7150-1, nitrite (NO<sub>2</sub>-N) and nitrate (NO<sub>3</sub>-N) by the SEV 1973, total phosphorus (P) by the ISO 6878/1. Numerical characteristics of the samples were expressed as mean values.

The total coliforms and *E. coli* were determined on the McConkey medium (Torlak) using the plate count method. Ten millilitres of the sample (water from wetlands) was transferred into 90 ml sterile water and decimal dilutions were prepared. One millilitre of each dilution was introduced into petri dishes and overflowed with

nutrient medium. The characteristic colonies were counted after incubation.

Analyses of the chemical composition of reed encompassed the determination of nutrients, nitrogen and phosphorus, expressed on the dry mass basis. Plant material from all three CWS cells was collected and analyzed at the end of the vegetation period according to the standard methods (APHA, 1995). Contents of nutrients (N and P) were determined in both the vegetative and generative reed parts. Total nitrogen in the dry mass was determined by standard micro-Kjeldahl method (Nelson & Sommers, 1973). Phosphorus was determined after the ignition and treatment with HCl, by spectrophotometric method using ammonium molybdate-vanadate reagent (Gericke & Kurmies, 1952).

#### 4. RESULTS AND DISCUSSION

Previous literature results (Kadlec & Wallace, 2008) indicated the differences in the efficiency of wetland functioning, which can be ascribed to a great number of variables. These are: natural conditions (geographic, climatic, hydrological) of the region, amount and quality of treated water, required quality of the effluent, characteristics of the substrate, hydrological system, type and characteristics of the aquatic plants, etc.

related to the typical pollution If in concentrations household wastewater (Ljubisavljević et al., 2004), the measured parameters of the Gložan municipal wastewater are: high concentration of suspended matter, extremely high organic load, high content of inorganic nitrogen, and high content of total phosphorus.

The measurement carried out on the Gložan system gave very good results regarding the chemical indicators (Tables 2 and 3), characteristic of the level of primary and secondary water treatment.

The results of chemical composition of the reed confirm the bioaccumulation of nutrients in the plant organs (Table 4), while the results of microbiological analyses confirm the sanitary safety of the effluent (Table 5).

Inflow to CWS - Sampling point $1 \rightarrow$	Cell I Sampling point 2 $\rightarrow$	Cell II Sampling point 3 $\rightarrow$	Cell III Sampling point 4 →	Effluent before discharging to the canal

Figure 4. Schematic of sampling points in the CWS

## 4.1. Water balance

The eco-hydrological effects on the Gložan CWS were analyzed via the water balance as the basic ecological factor in the wetland system. The results indicated that despite of an extreme nonuniformity of precipitation during the day, month, or year, and extreme temperature conditions, the wastewater treatment in the CWS has been efficient.

The water balance results show that both the dilution caused by precipitation and water loss due to intensive evapotranspiration occurred in the CWS. The changes in the amount of the effluent were in the range from 5 to 12% due to dilution and the loss from 15 to 25% (Table 1). However, it is evident from the water balance that wastewater has always been present in the substrate, ensuring thus the necessary conditions for microbiological processes and reed growth.

# 4.2. Effects of the municipal wastewater treatment

Table 2 shows the average values of chemical parameters for the particular measuring points.

Based on the influent (measuring point 1) and effluent (measuring point 4) data it was possible to calculate the overall removal efficiencies, and they were used to estimate the effects of the treatment

#### 4.2.1. Removal of suspended matter

Settling in the CWS is facilitated by the relatively low water flow (1.91 m/h), as well as by the effect of filtration through the substrate, so that the removal of suspended mater is realized mainly by this process.

Based on the average values, the efficiency of suspended matter removal in the four-year period of CWS operation was 94% (Table 2). The most intensive reduction in the content of suspended matter of 84.8% occurred in Cell I, while the reductions in the subsequent cells were much lower: 5.3% in Cell II and 3.9% in Cell III. At the system outflow, in 75% cases of measurement the effluent concentration was  $\leq$  30 mg/l, in 44% it was  $\leq$  15 mg/l, and in 31% of measurements  $\leq$  10 mg/l.

The study on a CWS with subsurface flow in France (Molle et al., 2004) also showed that it was possible to reduce the content of suspended matter to a level of 15 mg/l.

Month	Ι	II	III	IV	V	VI	VII	VIII	IX	Χ	XI	XII
	2005/ PRECIPITATION 750.1 (mm) AND EVAPOTRANSPIRATION 889.2 (mm)											
W <sub>Q</sub> -influent	61	61	91	91	91	121	121	121	91	91	91	61
W <sub>Q</sub> -effluent	62.0	66.2	89.9	81.9	77.4	116.7	123.2	125.1	88.6	83.8	90.2	68.0
Comment			C	hange o	f the eff	luent am	ount fron	n <b>+ 12%</b>	to - 15	%		
	2006/ PRECIPITATION 672.1 (mm) AND EVAPOTRANSPIRATION 905.4 (mm)											
W <sub>Q</sub> -influent	61	61	91	91	91	121	121	121	91	91	91	61
W <sub>Q</sub> -effluent	65.0	63.4	94.4	92.2	82.6	116.0	103.3	125.4	79.1	84.9	88.8	63.5
Comment	Change of the effluent amount from $+7\%$ to $-15\%$											
	2007/ PRECIPITATION 764.2 (mm) AND EVAPOTRANSPIRATION 1014.1 (mm)											
W <sub>Q</sub> -influent	61	61	91	91	91	121	121	121	91	91	91	61
W <sub>Q</sub> -effluent	63.8	62.8	91.8	74.0	85.4	113.4	101.2	105.5	92.7	97.3	104.5	64.0
Comment	Change of the effluent amount from $+ 12\%$ to $- 19\%$											
	2008/ PRECIPITATION 484.8 (mm) AND EVAPOTRANSPIRATION 1026.7 (mm)											
W <sub>Q</sub> -influent	61	61	91	91	91	121	121	121	91	91	91	61
W <sub>Q</sub> -effluent	62.1	56.0	88.9	82.2	68.2	112.3	103.3	106.6	91.4	85.2	94.2	64.0
Comment	Change of the effluent amount from $+5\%$ to $-25\%$											

Table 1. Water balance in the Gložan CWS (2005-2008)

Table 2. Effects of wastewater treatment in the Gložan CWS (2005-2008)

Parameter/indicator of	Meas. point 1	Meas. point 2	Meas. point 3	Meas. point 4	Treatment
water quality	(mg/l)	(mg/l)	(mg/l)	(mg/l)	efficiency, %
Suspended matter	321.44	48.81	31.69	19.31	94.0
BOD <sub>5</sub>	501.96	187.12	135.94	95.96	80.9
Ammonium	86.25	56.98	51.54	45.28	47.5
Nitrite	0.092	0.244	0.182	0.020	78.3
Nitrate	0.23	0.41	0.21	0.12	47.3
Total phosphorus	14.46	10.58	8.72	10.25	29.1

Also, the authors of the study pointed out that the age of the system of several years had no significant effect on the concentration of suspended matter in the effluent. The results obtained for the Gložan CWS showed small variations in the concentration in the four-year period of study.

Therefore, the Gložan CWS gave excellent results in this respect. According to the recommendations of USEPA (2000), the suspended matter removal is effective at the loads  $\leq 20$  g/m<sup>2</sup>·day, calculated on the monthly maximum of total suspended matter. For the Gložan CWS the load was 9 g/m<sup>2</sup>·day at the monthly maximum for the summer period, which certainly determined the high removal efficiency of suspended matter.

## 4.2.2. Reduction of BOD<sub>5</sub>

The average reduction of the BOD<sub>5</sub> value amounted to 80.9% (Table 2). The oxidation in the CWS takes place due to the activity of aerobe microorganisms. It was most intensive in Cell I – 62.7%, and much lower in Cell II – 10.2%, and Cell III – 8.0%. These findings are in agreement with those by Reed et al. (1995) and Kadlec & Knight (1996), showing a great initial decrease of BOD<sub>5</sub> when the organic load is high, and a marked decrease in the subsequent stages, when the organic matter concentration is lowered.

The values measured at the inflow to the system amounted even up to 838.40 mg/l. According to Jahić (1990), the BOD<sub>5</sub> value for sanitary wastewater is up to 400 mg/l. Therefore, the organic load, as assessed via BOD<sub>5</sub> as its indicator, is extremely high. In the 75% of cases the influent concentrations exceeded the average concentrations in municipal wastewaters.

The highest BOD<sub>5</sub> values were measured for the effluent in the winter time, at a low hydraulic load of 1.5 l/s. The count of microorganisms depends of the organic load and ecological factors, first of all, temperature (Jarak & Čolo, 2007). Relatively low air temperatures in the winter (mean 5.9°C, mean minimal -4,7°C) decelerated the microbiological processes, which can be one of the reasons for the high BOD<sub>5</sub> in the effluent.

The BOD<sub>5</sub> values measured in the other seasons were significantly lower. Best results were achieved in the summer-autumn period, when the decrease in BOD<sub>5</sub> amounted to above 95%, with the value in the effluent not exceeding 10 mg/l.

The wastewater residence time in the system was relatively short, 4.4 days. The mean organic load in the influent, as expressed via the BOD<sub>5</sub>, was 501.96 mg/l. It is probable that these hydraulic characteristics of the system and high influent load

may be one of a number of causes of high  $BOD_5$  values in the effluent.

## 4.2.3. Removal of nitrogen

Raw municipal wastewater of the Gložan settlement has a high content of inorganic nitrogen, mostly in the form of ammonium, but nitrates and nitrites are also present. The efficiency of ammonium removal in the CWS was 47.5% (Table 2). While the content of ammonium in Cell I decreased, in Cells II and III it was transformed in the other forms and/or taken up by plants, which was significantly less pronounced. Nitrates were present in the majority of water samples, while nitrites were found only in a smaller number of them.

The processes occurring most in the CWS are nitrification and ammonification. The former process takes place in Cells I and II, while ammonification is characteristic of Cell III. Hence, transformations of nitrates and nitrites, participating in these processes, take place just in concordance with these observations (Table 2).

Nitrogen transformation in wetlands is closely associated with the activity of bacteria in the water and substrate. The activity of particular bacteria groups depends on whether the conditions are aerobic or anaerobic (Jarak & Čolo, 2007). Based on the monitoring of the contents of ammonium, nitrate and nitrite, it can be concluded that the nitrification is the main process, but denitrification and ammonification can also take place (Belić et al., 2006).

On the basis of the average values, the mass load of total inorganic nitrogen was 8.0 kg/day, i.e. 2924.0 kg/year. It was found that a certain portion of nitrogen was taken up by reed and accumulated in its particular parts, mostly in the leaves, where nitrogen content may be up to 42 g/kg DM (Table 4).

## 4.2.4. Removal of phosphorus

The efficiency of phosphorus removal is 29.1% (Table 2). Phosphorus sedimentation and sorption of soluble phosphorus into particles are two physical processes of its removal. The phosphorus cycle in the CWS substrate is mainly characterized by sedimentation. As reported by Molle et al. (2004), the efficiency of phosphorus removal in CWSs does not exceed 40%. According to Wang & Mitsch (2000), previous investigations have shown that the most important pathway of phosphorus removal is just its physical sedimentation. Studies of Reddy et al. (1999) pointed out the tendency of phosphorus to precipitate with some ions, depending on the acidity/alkalinity of the substrate.

Based on the average values, the mass load of total phosphorus in the Gložan CWS is 0.8 kg/day,

that is 292.0 kg/year. Chemical composition of the reed showed that a certain portion of phosphorus was taken up by plants. The reed bloom accumulated phosphorus most, where its content was up to 2.1 g/kg DM (Table 4).

As has been observed in the pilot wetlands in the North-East Illinois, total phosphorus content in water increased in the presence of macrophytes in the inactivity and initial growth periods (Wang & Mitsch, 2000). The majority of the phosphorus taken up by the present macrophytes is returned back to the substrate as dead plant material. By harvesting of macrophytes at the end of vegetation period it is possible to remove phosphorus from its internal wetland cycle. In order to increase the efficiency of phosphorus removal it would be necessary to harvest the reed in the Gložan CWS, which has been lacking in the investigation period.

## 4.2.5. Seasonal and spatial variations in treatment efficiency

Table 3 shows the seasonal variation of treatment efficiency, which was highest in the autumn. The BOD<sub>5</sub> value of the influent was 403.3 mg/l, and of the effluent only 8 mg/l, the reduction being >95%. Content of suspended matter decreased from 230 mg/l to 19 mg/l, i.e. by 91.7%. The decrease in total phosphorus content was >95% (from 17 mg/l to 0.42 mg/l). The ammonium content in the influent was 93.23 mg/l and in the effluent 0.22 mg/l, the decrease being >95%.

The decrease in organic load in Cell I was 70%, in Cell II 96% and in Cell III 21%. Maximum reduction of suspended matter content of 91% occurred in Cell I, followed by stagnation in the subsequent sections. Content of total phosphorus in Cell I was reduced by 21%, in Cell II by 46% and in Cell III by 94%. The effect of ammonium removal in Cell I was 26%, in Cell II 51%, and in Cell III 99% (Table 2).

The obtained results show that, judging from the values of chemical parameters, the CWS is very efficient in treating the given municipal wastewater. The values of the analyzed parameters confirm the expected significant degree of purification in constructed wetlands. The observed variations in the influent parameters observed in the course of system operation indicate the nonuniformity of the wastewater composition, which is, normally, reflected on the span of measured parameters of the effluent. The highest treatment efficiency was obtained in autumn.

#### 4.3. Contents of nutrients in the reed

The reed leaf, as could be expected on the basis of plant physiology, had the highest content of nitrogen, which ranged from 29.3 g/kg DM (Cell II, 2005) up to 42.7 g/kg DM (Cell I, 2007) - Table 4. The obtained data are in agreement with those reported by Dinka (1986), but they are somewhat higher than those found for the reed leaf from the Kovilj Marsh, a natural wetland in Vojvodina (Pajević et al., 2002).

Parameter	Spring	Summer	Autumn	Winter
BOD <sub>5</sub>	84	83	>95	66
Suspended matter	>95	93	92	94
Total phosphorus	74	18	>95	28
Ammonium	50	50	>95	46

Table 3. Seasonal variations in treatment efficiency (%)

Plant organ	Cell	2005		2006		2007	
		Ν	Р	Ν	Р	Ν	Р
	Ι	24.7	1.6	28.2	2.1	27.1	1.9
Bloom	II	25.8	1.7	30.1	2.0	27.6	1.5
	III	24.8	1.5	32.9	1.6	23.9	0.9
Leaf	Ι	34.2	1.1	31.0	1.3	42.7	1.2
	II	29.3	0.8	36.5	1.4	32.1	0.9
	III	34.2	0.9	36.7	1.4	28.3	0.7
Stem	Ι	10.5	0.4	9.4	0.7	11.7	0.3
	II	7.0	0.3	10.2	0.7	9.9	0.2
	III	8.6	0.2	10.5	0.9	8.0	0.5
Rhizome + root	Ι	11.5	0.4	8.5	0.4	21.5	0.8
	II	8.7	0.2	11.4	0.4	10.2	0.2
	III	8.2	0.2	13.9	0.6	8.5	0.2

Maasuring	Summe	r (July)	Autumn (October)		
point	Total coliforms $(10^3 \text{ml}^{-1})$	<i>E. coli</i> ( $10^1$ ml <sup>-1</sup> )	Total coliforms $(10^3 \text{ml}^{-1})$	<i>E. coli</i> $(10^{1} \text{ml}^{-1})$	
1	420	287	340	104	
2	220	21	60	65	
3	181	7.5	35	40	
4	51	3.5	75	3	

Table 5. Average number of coliform bacteria and E. coli in the CWS water

An increased nitrogen content in the leaf of the reed grown in the wetland systems for wastewater treatment may be explained in terms of the nature of the influent, which is characterized by a high organic load (Josimov-Dunđerski & Belić, 2007). Significant concentrations of nitrogen accumulate in the bloom and rhizome, while the lowest contents are found in the reed stem.

The lowest phosphorus contents (0.2 g/kg DM) were found in the rhizome with the root and in the stem (Cells II and III, 2005 and 2007), whereas the highest values were found in the bloom (2.1 g/kg)DM, Cell I, 2006) - Table 4. Such distribution is characteristic for the phosphorus accumulation in the particular parts of reed (Pajević et al., 2002; Nikolić et al., 2003). In regard of the content of phosphorus, which in the wetland substrate occurs in the form of soluble and insoluble organic and inorganic complexes, the reed acts as а good phytoaccumulator, especially in autumn.

#### 4.4. Removal of coliform bacteria and *E. coli*

High number of coliform bacteria, many of them being pathogenic, are present in municipal wastewaters. If such waters are used for irrigation or are directly discharged to water bodies, this may have negative consequences to the health of humans and animals. In order the wastewater would be acceptable from sanitary point of view it is necessary to reduce the count of coliform bacteria to a most possible extent. The mechanisms that affect pathogen reduction in wetlands include different factors. The negative effect on the survival coliforms in wetlands have the dissolved oxygen in association with the growing season (Toet et al., 2005), chemicals released from plant roots (Brix, 1997), sediment deposition (Karim et al., 2004), etc. Flint (1987) found the E. *coli* survival progressively decreased from 15°C to 37<sup>o</sup>C. Song et al. (2008) reported that the constructed wetlands could effectively reduce E. coli by 99.9%, and total coliforms by 99.8%. The results of Baeder-Bederski et al. (2004), McHenry & Werker (2004), etc. suggested that the application of CWSs is one of the most effective methods for reducing the number of coliform bacteria.

In the examined CWS, coliform bacteria and *E. coli* have been found in all the samples. Coliform bacteria are heterotrophic microorganisms that use organic sources of carbon and nitrogen, which explains their highest number in the raw wastewater (measuring point 1). The number of microorganisms decreased in passing from one cell to another, which indicates the efficiency of the treatment. This is evident from table 5, giving the counts of examined microorganisms at the highest hydraulic loads and under conditions of their maximum activity (summer and autumn).

## 5. CONCLUSIONS

The Gložan CWS has been designed to receive local municipal and household wastewaters. It consists of three cells with subsurface horizontal flow, covering an area of about 1 ha. The results obtained on this CWS can be summarized as follows:

• The CWS efficiency in removing suspended matter in the investigation period was 94%, while the organic matter content, expressed via BOD<sub>5</sub>, was reduced by 80.9%. The removal of nitrogen compounds was also significant, and it was 47.3% for nitrate, 47.5% for ammonium, and 78.3% for nitrite. The efficiency in the removal of total phosphorus was 29.1%.

• The efficiency of the system depends also on the activity of microorganisms and reed vegetation, optimum conditions for both being observed in summer and autumn.

• Due to the presence of high populations of heterotrophic microorganisms, organic matter present in the influent is degraded effectively, the rate of microbiological processes being determined by aeration conditions, temperature, chemical composition content of organic matter, etc. Under optimal conditions, these microorganisms mineralize organic matter, which results in decrease of their counts, and, if good aeration is ensured, they are being fully replaced by oligotrophs and later by autotrophs. This process was most efficient in autumn, which was also evidenced by the decrease of  $BOD_5$  of >95 %, which was highest in this season. The presence of the reed bed has certainly contributed to faster mineralization through the substrate aeration, facilitating thus functioning of aerobic microorganisms.

• The system was capable of retaining 293 kgP/year and 2924 kgN/year, taken up by the plants. Ammonium and nitrate are also accumulated in the reed, the highest nitrogen content being found in the leaf. Chemical analysis showed also certain uptake of phosphorus, the major portion of it being accumulated in the reed bloom. To increase the system's efficiency in the removal of nutrients, it would be necessary to harvest the reed each year.

• Microbiological investigations showed that the CWS was efficient in reducing the number of coliform bacteria and *E. coli*, ensuring thus the sanitary safety of the effluent.

• The CWS water balance confirmed the permanent presence of wastewater as the necessary ecological factor for the activity of microorganisms and vegetation, which yields the transformation and cycling of nutrients in the system.

• The effluent from the CWS is returned again to the hydrological cycle. Therefore, such system could be effectively used to treat wastewaters of smaller settlements prior to their treatment in a centralized plant.

• Serbian water quality standards for discharge treated wastewater are in coordination with Directive EU (91/271/EEC). According to limit concentration values and taking into consideration number of residents the quality of effluent are satisfied. Exception is only present in case of total phosphorus.

The Vojvodina's population of more than two millions of inhabitants live in 467 settlements. Of this number, 404 are settlements with less than 6000 inhabitants, having no systems for collecting and treating local wastewaters. The main reason for such a situation is the shortage of financial resources. Hence, in these settlements use is made of septic tanks, so that their leaks contaminate ground waters, whose level in this region is rather high. Contents of septic tanks are disposed on the fertile soil or to water bodies. Thus the soil and water resources are constantly contaminated. A promising solution to this problem would be to construct low-pressure sewerage systems for collecting used waters and treating them in CWSs. The results presented for the Gložan CWS demonstrate the usability of such systems under the conditions of moderate continental climate, which could be an essential contribution to the sustainable development and environmental protection.

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