Phytoremediation potential of Vetiveria zizanioides and Oryza sativa to nitrate and organic substance removal in vertical flow constructed wetland systems.

Adelaide Almeida[a,b], Carlos Ribeiro[a], Fátima Carvalho[a,b], Anabela Durao[c], Piotr Bugajski[d], Karolina Kurek[d], Patrycja Pochwatka[e], Krzysztof Józwiakowski^*

[a] Department of Technologies and Applied Sciences, Polytechnic Institute of Beja, Beja, Portugal
[b] FibEnTech - Materiais Fibrosos e Tecnologias Ambientais, Covilhã, Portugal
[c] Department of Engineering, Polytechnic Institute of Beja, Beja, Portugal
[d] Department of Sanitary Engineering and Water Management, University of Agriculture in Kraków, Kraków, Poland
[e] Department of Environmental Engineering and Geodesy, University of Life Sciences in Lublin, Lublin, Poland

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ABSTRACT

The aim of this work was to determine the potential of phytoremediation of Vetiveria zizanioides and Oryza sativa to nitrate ($NO3^-$-N) and organic matter (COD - Chemical oxygen demand) removal in subsurface vertical flow constructed wetland systems (VFCWs). The tests were carried out in models of 2 beds with a surface of 0.24 m^2 and depth 0.70 m each at various hydraulic loads (HL) and COD constant in influente wastewater. The VFCW-1 bed was planted with the Vetiveria zizanioides and the VFCW-2 bed with the Oryza sativa. During the 22 weeks of research, the hydraulic load of the analyzed beds was: H_{LI} = 148dm^3^m^{-2}^d^{-1}, H_L2 = 239$dm^3m^{-2}d^{-1}$, H_L3 = 350dm^3^$m^{-2}d^{-1}$, H_L4 = 473dm^$m^{-2}d^{-1}$. The VFCWs were fed from two reservoirs, one with mineral medium and nitrate, and the other one with fructose as organic matter. Based on the conducted tests, it was found that in both analyzed systems (VFCW-1 and VFCW-2) the highest $NO3^-$-N removal rate was found under HL2 - the median is 59 and 42% respectively. The most effective removal of COD in the VFCW-1 system was obtained at a HL2 - 55%, while in the system VFCW-2 at a HL1 - 43%. The research shows that the low HL and high COD/$NO3^-$-N ratio ensures good effects of nitrate and organic matter removal in VF type constructed wetland systems. It has been shown that the analyzed plants, especially Vetiveria zizanioides have good phytoremediation potential in the field of nitrate nitrogen and organic substance removal and can be recommended for use on a full scale of technology.

1. Introduction

Several nitrogen-containing compounds, such as nitrate, after nitrite and ammonium have been found as common pollutants in drinking water and various wastewaters. Especially nitrate causes several problems, such as: eutrophication and diseases, for example, cyanosis and esophagus cancer (Ozturk and Bektas, 2004). Although the nitrogen removal via conventional solutions (e.g. centralized wastewater treatment plants) have been recognized as inefficient; biological denitrification is considered a cost-effective method (Chatterjee and Woo, 2009).

Constructed wetlands can be an alternative for nitrate removal from water and wastewater, through the mechanisms of microbial denitrification and direct uptake by plants and microbes (Lin et al., 2008, Pawetek and Bugajski, 2017). In recent decades, vertical flow constructed wetland (VFCWs) have been designed and engineered to utilize the natural processes of nitrogen removal. Thus, VFCWs design and operation could be optimized to provide sufficient oxygen for ammonia oxidation or create anoxic condition for denitrification. This one is the major nitrate removal process in constructed wetlands (Matheson and Sukias, 2010). It is an anoxic process where bacteria use nitrate ($NO3^-$) or nitrite (NO_2^-) as an electron acceptor, and organic carbon as an electron donor to obtain energy for growth and maintenance, and produce nitrogen gas (N_2), nitrous oxide (N_2O) or nitric oxide (NO) (Saeed and Sun, 2012). There are some factors that influence denitrification, including, appropriate redox potential (Eh), temperature, pH, soil type, moisture saturation degree, availability of nitrate and carbon, absence of oxygen, and plants species of the wetland system (Chatterjee and Woo, 2009; Vymazal, 2007). Denitrification is an anaerobic dissimilative pathway in which the synthesis of the enzymes involved in each denitrification step and the corresponding denitrification rates are greatly repressed by the presence of dissolved oxygen (DO). DO should be maintained below 1mg-dm^{-3}; however denitrification was observed in systems with DO in the range of 0.3-1.5 mg-dm^{-3} or higher (Metcalf and Eddy, 2003; Kadlec and Wallace, 2008). In addition, carbon source is a controlling factor in the process of denitrification. It is usually supplied by wastewater, soil, plant root exudates or can be improved in occasions throughout un- treated influent bypass or step-feeding (Torrijos et al., 2016; Vymazal and Kropfelová, 2008; Songliu et al., 2009), but an externai source, such as glucose, sodium acetate and fructose may be needed when influents contain low C/N ratios (Chatterjee and Woo, 2009).

Hydraulic load (HL) and hydraulic retention time (HRT) are some of the most important factors that control the performance of subsurface flow constructed wetland systems. High HL promotes a quicker passage of wastewater through the substrate, thus reducing the optimum con- tact time, and the denitrifying bacteria receive large amounts of nitrate. Also, high H_L may have a negative impact on the nitrate removal, e.g. through oxygenation of the sediment surface and resuspension of or- ganic material (Spieles and Mitsch, 1999; Kjellin et al., 2007). High oxygen concentrations can restrict denitrification to occur in the upper sediments rather than on plants, litter and other surface structures in the water column. Thus, it is important to identify the critical HRT or H_L, to obtain substantial nitrate removal. The effect of hydraulic loads or nitrate loading rate on nitrate removal has received great attention by some researchers. A H_L increase leads to an increase of the applied nitrate load, contributing to the rising denitrification efficiencies (Lin et al., 2008; Spieles and Mitsch, 1999; Gajewska et al., 2015; Mucha et al., 2018; Torrijos et al., 2016; Almeida et al., 2010). Furthermore, the effect of H_L varies, depending on e.g. the dominant plant commu- nity and shape of the basin, as those factors can affect the hydraulic efficiency of constructed wetlands, as well as the type of wastewater to be treated. The wastewater chemical composition plays an important role for such differences. Kantawanichkul et al. (1999) employed two separated VF systems for the treatment of swine and domestic waste- water, which were subjected to H_L increments. A H_L increase reduced nitrogen and organic removal performances of the swine wastewater wetland reactor. However, the other VF wetland reactor, employed for the treatment of domestic wastewater, did not exhibit reduction performances when H_L increased. Plants are considered an indispensable component in constructed wetlands. They take up the nitrate as nu- trient, provide energy and carbon to fuel denitrification either from biomass or root release, and produce biomass for energy purposes (Gizinska-Górna et al., 2016). Numerous studies have reported that plants stimulate nitrate removal in constructed wetlands; however, the effects are often divergent with regard to different plant species (Kadlec and Wallace, 2008; Vymazal and Kropfelová, 2008; Lin et al., 2002). Plants may also influence soil microenvironment by excreting enzymes, exudates, and oxygen, which can influence microbial diversity or can indirectly affect the rhizosphere enzymatic activity (Kong et al., 2009). Some assays with planted and unplanted plots were performed, and it was observed that nitrate reductase activity was significantly higher in the planted plots than in the unplanted plots (Kong et al., 2009; Zhang et al., 2010).

In this experimental study, Vetiveria zizanioides was selected due to some evidences; for instance, this plant has been used successfully in wastewater VFCW treatment. Additionally, Vetiveria zizanioides presents a great ability to remove total nitrogen, ammonium and nitrate (Kantawanichkul et al., 1999; Almeida, 2012; Kantawanichkul and Duangjaisak, 2011; Almeida et al., 2008). Additionally, it was observed that Vetiveria zizanioides can uptake nitrogen till 10 g N m^{-2}-d^{-1} when treating swine wastewater (Almeida et al., 2008). This plant was first recognized in 1995 for wastewater treatment in Australia. Vetiveria zi- zanioides is a perennial grass species belonging to the Poaceae family,
with short rhizomes and a massive and finely structured root system that grows very quickly. In this sense, it can be referred that in some applications, the root depth reaches 3-4 m in the first year. This deep root system makes the vetiver plant an extremely drought tolerant species and very difficult to dislodge when exposed to a strong water flow, presenting a good performance for wastewater treatment (Truong, 2000). Oryza Sativa was selected for this study because is a very abundant plant in Portugal, and was also used by Kantawanichkul and Duangjaisak (2011) and Ying-Hua et al. (2006) to treat domestic was- tewater in VFCWs, obtaining high removal efficiencies. The utilization of wastewater for growing rice can not only provides irrigation water, but also acts as fertilizer. Oryza sativa was used in a pilot-scale surface- flow wetland in Japan for treating nutrient-polluted river water (Zhou and Hosomi, 2008). This plant was also used in horizontal subsurface flow constructed wetlands in Brasil to the treatment of water from urban stream polluted with sewage (Meira et al., 2013).

This work intends to study of phytoremediation potential with using Vetiveria zizanioides and Oryza sativa to removal of nitrate nitrogen and organic substance (COD - chemical oxygen demand) in subsurface vertical flow constructed wetland systems (VFCWs). The research was made in a model conditions with different hydraulic load (H_L) and variable COD value in treated wastewater.

2. Materials and methods

2.1. Characteristics of experimental setup

The experimental work was carried out by 22 weeks in two identical pilot-scale VFCWs with an area of 0.24 m^2 and a depth of 0.70 m each, filled with light expanded clay aggregates (Leca®NR 10/20) and planted with Vetiveria zizanioides (VFCW-1) and Oryza sativa (VFCW-2) (Fig. 1), using a plant density between 120 and 130 plants-m^{-2}. Bat- ches of 125 dm^3 of nitrogen and carbon aqueous solutions were homogenized by two submersible pumps and VFCWs were fed through a network of equidistant sprinklers. The medium was not saturated due to effluent drainage type and substrate material used, allowing the oxygen diffusion into the beds. The flooding levels were maintained at 14% through a siphon in the outlet.

In order to allow the use of low C/N ratio and keeping nitrate concentrations constant as well as to minimize variability in experiment, the VFCWs were fed with synthetic wastewater, prepared by mixing in tap water potassium nitrate and fructose as nitrogen and carbon sources, respectively, as described in Table 1. It was also added a mineral medium composed of CaCl2 - 28 mg-dm^{-3}, MgSO4-7H2O - 52 mg-dm^{-3}, KH_2PO_4 - 17.40 mg-dm^{-3}, K_2SO_4 - 11 mg-dm^{-3}, CuCl2-2H2O - 0.03 mg-dm^{-3}, MnCl2-4H2O - 0.18 mg-dm^{-3}, ZnCl2 - 0.08 mg-dm^{-3}, $FeSO_4$-$7H_2O$ - 1.7 mg-dm^{-3} diluted in tap water (Hunter et al., 2001).

2.2. Experimental conditions

This study was carried out in four successive experimental trials - with different hydraulic load (H_{L1} = 148$dm^3m^{-2}d^{-1}$, H_{L2} = 239$dm^3m^{-2}d^{-1}$, H_{L3} = 350dm^3-m^{-2}-d^{-1}, H_{L4} = 473$dm^3m^{-2}d^{-1}$), lasting about 38days each. The experimental conditions applied to the wetlands during each trial are presented in Table 1.

2.3. Sampling collection and analysis

The wastewater samples were taken for analysis for 22 weeks, every day at 9.00 a.m. from Monday to Friday. The whole examination was divided into four experiments (Table 1). So, each means that appears in Figs. 2 and 3, corresponds to an experiment (according to Table 1), done with a different hydraulic load. The influent and effluent were the same for both VFCWs.

The electrical conductivity (EC), pH, redox potential (Eh) and

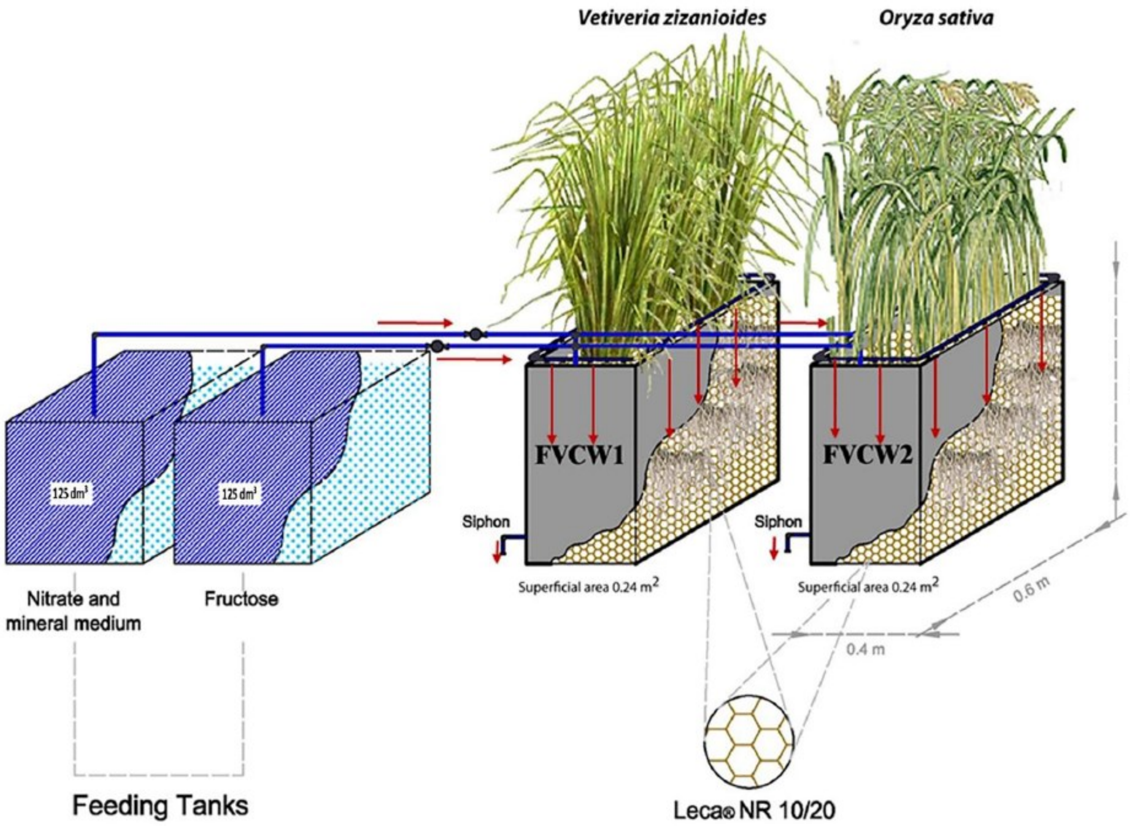


Fig. 1. Schematic representation (not at scale) of the VFCW system, composed by two pilot beds (VFCW-1 and VFCW-2 planted with *Vetiveria zizanioides* and *Oryza sativa*, respectively).

dissolved oxygen (DO) were immediately measured. Aliquots were frozen at -20 °C to determine the other parameters. Chemical oxygen demand (COD), nitrite ($NO2^{-}$-N) and nitrate ($NO3^{-}$-N) were de- termined according to Standard Methods (APHA, 2013). COD was used to indirectly quantify carbon content (Table 1) (Metcalf and Eddy, 2003). Plants were visually inspected for toxicity signals, such as chlorosis, leaf curl, early senescence stages and plant root. In the aboveground plant tissue, the Total Kjeldahl Nitrogen (TKN) con- centration was determined (Campbell and Plank, 1998; Miller, 1998).

2.4. Hydraulic retention time (HRT) and hydraulic load (HL)

The HRT and HL were established from the following Eqs. (1) and (2):

$$HRT = AYp/Qi \quad (1)$$

$$HL = Qi/A \quad (2)$$

where:

Q_i (dm^3-d^{-1}) - the influent flow rate (measured daily at inlet of the bed);

A - the surface area of the bed (m^2);

Y - the flow depth (m),

p - the porosity (which expresses the space available for the water to flow through the media, roots and other solids in constructed wet- land system).

It was also determined the HL in effluent for both VFCWs and used to determine the nitrate nitrogen and COD removal efficiencies (Crites et al., 2006).

2.5. Data analysis

Results were statistically verified using software "Statistica 8.0" (StatSoft, Inc., USA). Differences in wastewater quality between in- fluent and effluent of the constructed wetlands were determined using ANOVA at the significance level of $p < 0.05$. Post-hoc (a posteriori) Tukey's test was used to determine differences between means of spe- cific variables. Each trial lasted three weeks at least, and, as stated above, samples were taken daily from Monday until Friday each week; so a minimum of ten samples were taken from influent and effluent wastewater in each trial from each VFCW. The means and the standard deviation (S.D.) were calculated with $n > 10$.

Table 1

Inlet operating conditions of the trials: hydraulic load (H_L), nitrate load (NO_3^--N_{load}), total nitrogen concentration [TN], nitrate concentration [NO_3^--N], nitrite concentration [NO_2^--N], chemical oxygen demand (COD) and C/N ratios, air and soil temperatures (T_{air}, T_{soil}), (mean ± SD, calculated for a confidence level of 95% and $n \geq 10$).

Exp	H_L	NO_3^--N_{load}	[TN]	[NO_3^--N]	[NO_2^--N]	COD	C/N	T_{air}	T_{soil}
	($dm^{-3} \cdot m^{-2} \cdot d^{-1}$)	($g \cdot m^{-2} \cdot d^{-1}$)	($mg \cdot dm^{-3}$)					(°C)	
Exp 1	148 ± 9	3 ± 0.1	19 ± 1.9	19 ± 1.6	0.3 ± 0.1	91 ± 11	0.33 ± 0.10	28 ± 3	24 ± 3
Exp 2	239 ± 9	5 ± 0.4	19 ± 1.9	19 ± 1.8	0.1 ± 0.1	82 ± 13	0.30 ± 0.10	25 ± 3	20 ± 3
Exp 3	350 ± 8	7 ± 0.4	19 ± 2.5	19 ± 2.5	0.1 ± 0.1	93 ± 6	0.34 ± 0.10	26 ± 2	22 ± 2
Exp 4	473 ± 9	9 ± 0.2	18 ± 1.3	18 ± 1.3	0.1 ± 0.1	94 ± 9	0.34 ± 0.10	24 ± 2	20 ± 3

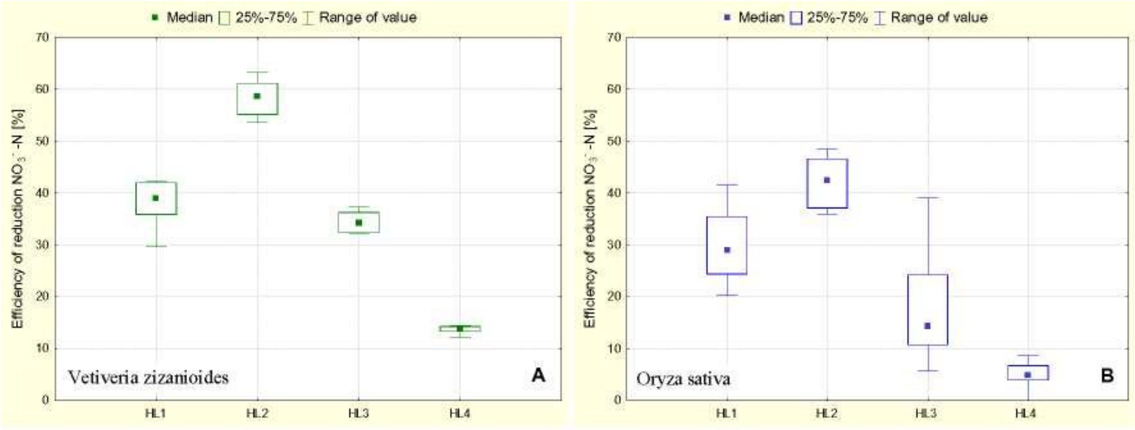


Fig. 2. Effectiveness of nitrate nitrogen removal in the VFCW-1 (A) and VFCW-2 (B) at different hydraulic loads.

3. Results and discussion

3.1. Efficiency of NO3⁻-N removal

The assumption of the experiment was a variable volume inflow of wastewater (H_L) (Table 1) with a constant concentration of nitrate nitrogen. The median NO3⁻-N concentration in the wastewater flowing into the VFCW-1 and VFCW-2 was 19 mg-dm^{-3}. In addition, it was also assumed that in inflowing wastewater COD values vary from 70 to 125 mg O2-dm^{-3} (median 90 mg O2-dm^{-3}). The same amounts of wastewater with the same concentration of NO_3^--N and COD were inflow to both analyzed VFCW-1 and VFCW-2. This allowed to compare the effectiveness of nitrate nitrogen and COD removal at variable hydraulic load and at a variable dose of organic pollutants expressed using COD indicator.

In the first stage of the analysis of the results, the influence of hydraulic load (H_{L1} - H_{L4}) on the efficiency of nitrate nitrogen removal was determined. Fig. 2 shows the median, quartile range 25%-75%, in which the part of the range variation of the NO_3^--N, removal was determined. In this part, there is 50% of the “middle” reduction amounts along with the range of non-outgoing values.

In the case of the VFCW-1 bed with the Vetiveria zizanioides, the highest efficiency of NO_3^--N removal at the level of 59%, was obtained with the hydraulic load H_{L2}. While, with the hydraulic load H_{L1} a ni- trate nitrogen reduction of 38% was obtained. The lowest effects of NO_3^--N removal in the VFCW-1 bed were found at the highest hydraulic load: HL3 and HL4 -34 and 14% respectively (Fig. 2A). In the case of the second analyzed VFCW-2 bed with the Oryza sativa, the highest nitrate nitrogen removal efficiency was found with the hydraulic load HL2 and HL1 - 42 and 30% respectively. Significantly lower effects of NO_3^--N removal in the VFCW-2 bed were found at the highest hydraulic load: HL3 and HL4 - 19 and 7% respectively (Fig. 2B).

Based on the results, it was found that in both analyzed VFCW-1 bed with Vetiveria zizanioides and VFCW-2 with Oryza sativa, the highest efficiency of NO_3^--N removal was obtained with the hydraulic load HL2. It was found, that in the case of the VFCW-1 bed the efficiency of NO3⁻-N removal was higher by 16% than in the VFCW-2 bed. In the case of other analyzed hydraulic loads a similar tendency was observed. Throughout the experimental period, which is composed of four ex- periments, H_L increased and also as a consequence the mass load of nitrate in the influent. Thus, in the first two tests performed with H_{L1} and HL2 (the hydraulic retention time (HRT) was 240 min ± 0.5; 140 min ± 0.5) it was observed that to the nitrate mass load applied increase the system showed an increase in the nitrate mass load re- moved too (according to a first-order kinetics). In the H_{L3} and H_{L4} tests, although the nitrate mass load was also increased, the mass load re- moved decreased, most probably because the low retention time at which these tests were performed (90 min ± 0.5 and 70 min ± 0.4). Reducing the contact between nitrate, plants and microorganisms and simultaneously nitrate load applied increased too.

As a result of the conducted research, it was found that the variable efficiency of nitrate nitrogen removal with different hydraulic loads had an influence on the concentration of this indicator in treated waste- water. The concentration of NO3⁻-N in the outflow from the VFCW-1 bed ranged from 8 mg^dm^{-3}, with hydraulic load H_{L2} up to 16 mg^dm^{-3} with hydraulic load HL4 (Fig. 3A). In the case of VFCW-2 bed, con- centration of NO_3^--N in the outflow ranged from 11mg^dm^{-3}, with hydraulic load H_{L2} to 19mgdn^3 with hydraulic load H_{L4} (Fig. 3B).

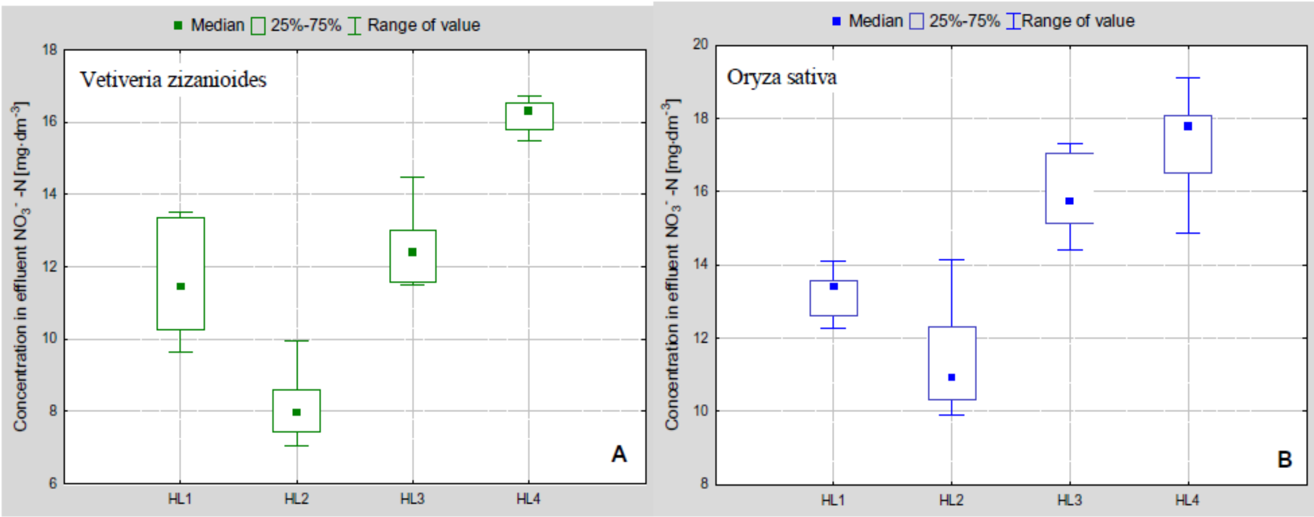


Fig. 3. Characteristic concentrations of nitrate nitrogen in the outflow from the bed VFCW-1 (A) and VFCW-2 (B) at different hydraulic loads.

In the next stage of the analysis of the results, the influence of the COD/NO3$^-$-N dependence was determined in the wastewater flowing into the VFCW-1 bed with Vetiveria zizanioides and VFCW-2 with Oryza sativa, with different hydraulic load on the efficiency of NO3$^-$-N removal. In this part of the analysis, a partial correlation analysis was used, which allows determining the influence of two or more in- dependent variables on one dependent simultaneously. For this analysis, the dependent variable is the percentage efficiency (%) of NO_3-N. removal. The first independent variable is the amount of incoming sewage HL1-L4, and the second independent variable is the COD/N-NO3 ratio in the sewage flowing into the filter bed. This part of the analysis uses the program “Statistica 8.0" (StatSoft, Inc., USA).

Based on the results of the correlation analysis, in the case of the VFCW-1 bed it was found that the influence of the amount of inflowing wastewater on the effectiveness of elimination of nitrate nitrogen according to the Stanisz (1998) scale is on a high level as evidenced by the value of the correlation rx,y = -0.71. On the other hand, it was determined that the effect of COD/NO_3^--N ratio in wastewater inflowing influencing the efficiency of elimination of nitrate nitrogen according to the mentioned scale (Stanisz, 1998) is on the average level, where $r_{x,y}$ = -0.30. The significance of the calculated correlation coefficients was tested with the Student's í-test at the significance level of a = 0.05. In both cases, the significance of the studied relationships was found. Based on the results of the partial correlation, the model presented in Fig. 4 was developed. Thus the percentage efficiency of NO_3^--N removal in the VFCW-1 bed can be predicted. The created model can be described by the equation R of NO_3^--N (%) = 81.9133-0.0907H_L
-3.7947COD/NO3$^-$-N (Fig. 4).

In the case of the VFCW-2 bed, it was found that the influence of the amount of inflowing wastewater on the efficiency of elimination of NO3$^-$-N according to Stanisz (1998) scale is also high, as evidenced by the value $r_{x,y}$ = -0.70. While the influence of the COD/NO_3^--N ratio in the wastewater inflowing on the efficiency of NO3$^-$-N removal is on the average level, where rx,y = -0.38. Thus, in comparison to the analyzed dependencies obtained in the VFCW-1 bed, the correlation between the inflow (HL) and efficiency (%) was practically at the same level. The dependence of the effectiveness (%) of nitrate nitrogen removal on the COD/NO_3^--N ratio was higher in the VFCW-2 bed than in the VFCW-1 bed, but in the proposed Stanisz (1998) scale is in the same range. The significance of both calculated correlation coefficients was tested with the Student's t-test at the significance level of a = 0,05. In both cases, the significance of the studied dependencies was found. Based on the results of the partial correlation, a model was developed

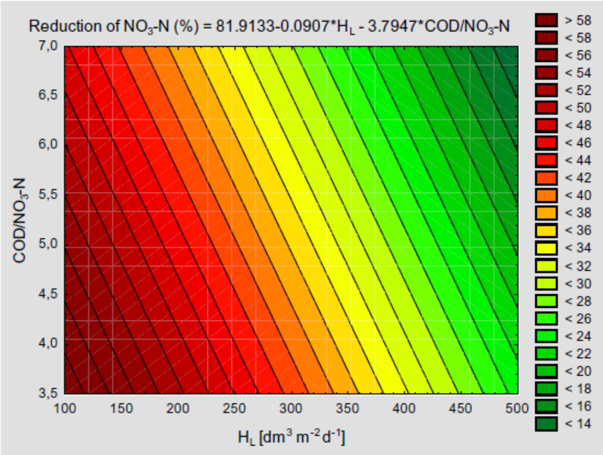


Fig. 4. Nomogram to determine the effectiveness of NO_3^--N removal (%) depending on the amount of inflowing wastewater H_L and the COD/NO_3^--N ratio in inflowing wastewater to VFCW-1 bed.

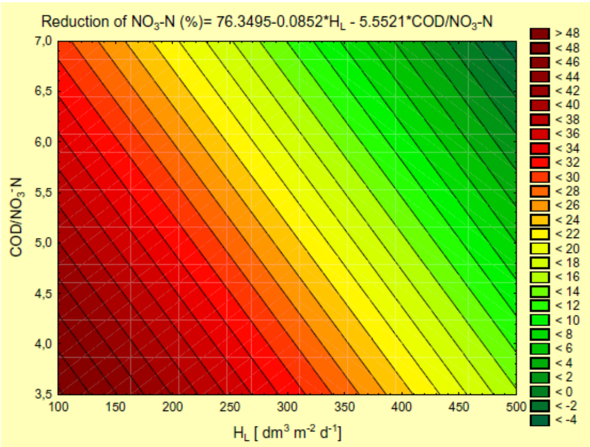
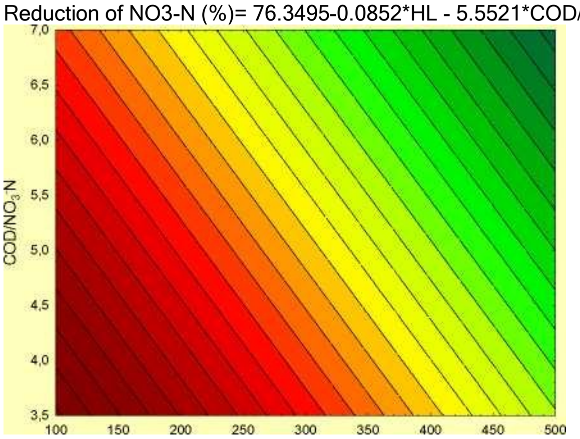


Fig. 5. Nomogram to determine the effectiveness of NO_3^--N removal (%) depending on the amount of inflowing wastewater H_L and the COD/NO_3^--N ration inflowing wastewater to VFCW-2 bed.

Reduction of NO3-N (%)= 76.3495-0.0852*HL - 5.5521*COD/NO3-N



(Fig. 5), from which the percentage efficiency of NO3⁻-N elimination in the VFCW-2 bed can be predicted. The created model can be described by the equation R of NO_3^--N (%) = 76.3495-0.0852 H_L-5.5521COD/ NO3⁻-N (Fig. 5).

According to Gagnon et al. (2010) and Kozub and Liehr (1999) the content of organic compounds, in particular organic carbon, is a factor determining the removal of nitrates from wastewater in constructed wetland systems. Misiti et al. (2011) observed that with a COD/N ratio below 3:1 in the wastewater inflowing into bed, the reduction efficiency of nitrates decreases. While, the results of the research presented by Gagnon et al. (2010), Misiti et al. (2011) and Narvaez et al. (2011) indicate that with the COD/N ratio, in the proportions of 3:1 and 7:1 respectively, occurs increased denitrification. This fact was also con- firmed by Zhang et al. (2016) and Zhu et al. (2014), who observed that with the increase of the COD/NO3⁻-N ratio in the treated wastewater, the efficiency of nitrates removal increased. Also, own research carried out in the VF Vetiveria zizanioides and Oryza sativa constructed wetland systems showed that the low hydraulic load and the high COD/NO3$^-$-N ratio ensure good nitrates removal. The best effects of NO_3^--N removal in the VFCW-1 and VFCW-2 beds were obtained at the lowest hydraulic load: HL1 and HL2. The effects of NO3$^-$-N removal in the VFCW-1 bed with Vetiveria zizanioides, with a hydraulic load at H_{L2} level equals 59% and were similar to those obtained by Chang et al. (2013) (56.2%) in two replicated, pilot-scale integrated vertical-flow constructed wetland systems under a relatively low influent COD:N ratio of 1.67. Also from model research made by Seo et al. (2010) it follows that the effectiveness of elimination of nitrates in beds with willow may reach to 58.9%. While research carried out in a hybrid constructed wetland system VF-HF type with reed and a basket willow showed that the efficiency of NO3$^-$-N removal in the second HF bed was 54.6% and the concentration of nitrate nitrogen decreased from 23.8 to 10.8 mg^dni^{3} (Józwiakowski and Wielgosz, 2010). Significantly lower effects of NO3$^-$-N removal (16.6 and 46.7%) were obtained in two HF beds with a basket willow, occurring after VF-type bed with reed in in a hybrid constructed wetland system VF-HF type operating in full technical scale (Józwiakowski, 2012).

3.2. Efficiency of COD removal

In the next stage of analysis, the influence of hydraulic load (H_{L1} - H_{L4}) on the efficiency of organic matter removal expressed by the COD indicator in the examined systems was determined. Fig. 6 shows the median, quartile range 25%-75%, in which the part of the range

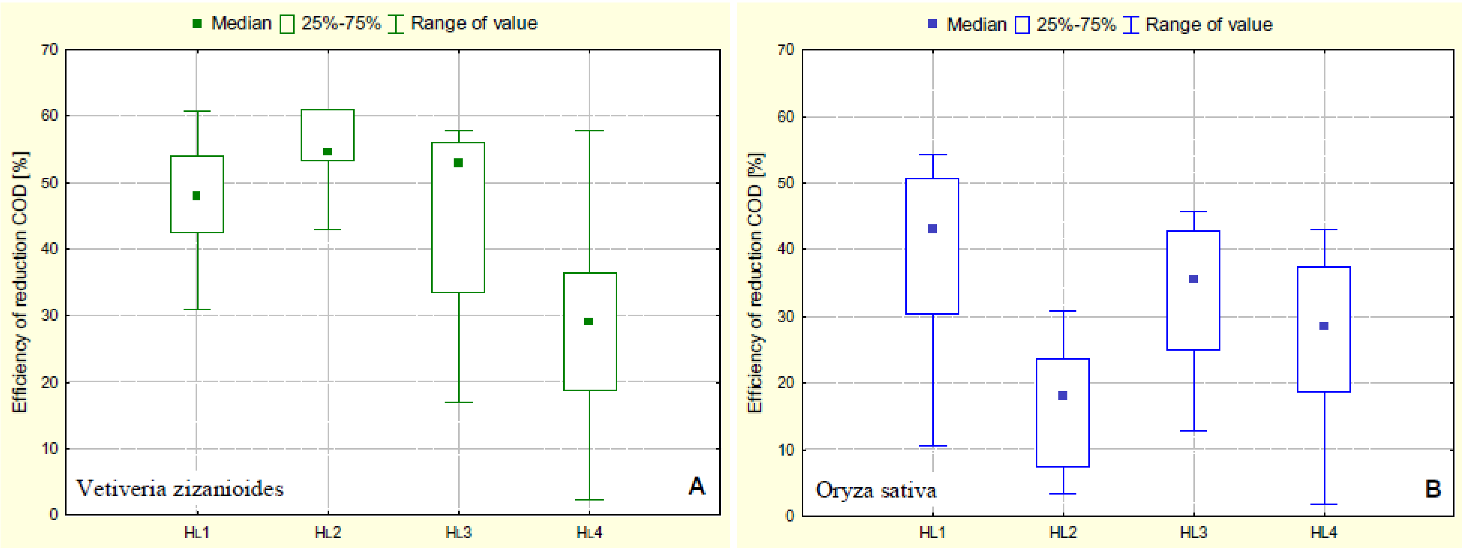


Fig. 6. Effectiveness of COD removal in VFCW-1 (A) and VFCW-2 (B) at different hydraulic loads.

variation of the nitrate nitrogen removal was determined. In this part, there is 50% of the “middle” reduction amounts along with the range of non-outgoing values.

In the case of VFCW-1 bed with Vetiveria zizanioides, the highest removal efficiency of COD on the level 55%, was obtained in the same way as in the case of NO3$^-$-N removal under hydraulic load HL2. While, with the load HL1 and HL3 COD reduction on the level 48% and 53% respectively was achieved. The lowest effects of COD removal in the VFCW-1 bed were found at the highest hydraulic load: H_{L4} - 29% (Fig. 6A). In the case of the second analyzed VFCW-2 bed with Oryza sativa, the highest COD removal efficiency was found with the hydraulic load H_{L1}, which was 43%. Slightly lower results were obtained with a hydraulic load HL3 and HL4 - 36 and 29% respectively. The lowest ef- fects of COD removal in the VFCW-2 bed were found under hydraulic load HL2 - 18% (Fig. 6B).

Studies have shown that the efficiency of COD removal at various hydraulic loads in the VFCW-1 bed with Vetiveria zizanioides and VFCW- 2 bed with Oryza sativa was varied. The VFCW-1 bed most effectively removed COD under hydraulic load HL2, and the VFCW-2 bed at load. It was found that the effectiveness of COD removal, like in the case of NO3$^-$-N removal in the VFCW-1 bed (at all hydraulic loads tested) was much higher than in the VFCW-2 bed (Fig. 6).

Based on the obtained results, it was found that the variable COD removal efficiency at different load hydraulic had an influence on this ratio in treated wastewater. It has been shown that the median value of COD in the outflow from the VFCW-1 bed ranged from 36 mg O_2^dm^{-3}, under hydraulic load H_{L2} to 63 mg O/dm^{-3} under hydraulic load H_{L4} (Fig. 7A). In the case of VFCW-2 bed COD value in the outflow ranged from 52 mg Oydm^{-3}, under hydraulic load H_{L1} to 68 mg Oydm^{-3} under hydraulic load H_{L2} (Fig. 7B).

In the next part of the analysis, the influence of the COD/NO_3^--N dependence in the wastewater inflowing into the VFCW-1 and VFCW-2 bed was determined, with different hydraulic loads on the efficiency of COD removal. In this case, as in the case of NO3-N removal, the ana- lysis of partial correlation in the aspect of COD removal was used. The percentage (%) removal of COD was assumed as a dependent variable, while two independent variables were considered as the amount of incoming sewage Q_{1-4}, and the COD/N-NO_3 ratio in the effluents flowing into the filter bed.

Based on the results of the correlation analysis, in the case of VFCW- 1 bed, it was found that the influence of the amount of inflowing wastewater on the efficiency of COD removal according to Stanisz (1998) scale is at a high level, where $r_{x,y}$ = -0.52. On the other hand, it was determined that the effect of the COD/NO3⁻-N ratio in wastewater inflowing on COD elimination efficiency according to the aforemen- tioned scale (Stanisz, 1998) is at a faint level, where rx,y = -0.09. The significance of the calculated correlation coefficients was tested with the Student's í-test at the significance level of a = 0.05. The statistical significance of the influence of the amount of inflowing wastewater on the level of COD, reduction was found, while the influence of the COD/ NO3⁻-N on the reduction of COD in VFCW-1 bed on the significant level a = 0.05 was not found.

Based on the results of the partial correlation, the model presented on Fig. 8 was developed, from which the percentage efficiency of COD re- moval in the VFCW-1 bed can be predicted. The created model can be described by the equation R COD (%)= 64.108-0.067HL+ 0.0964COD/

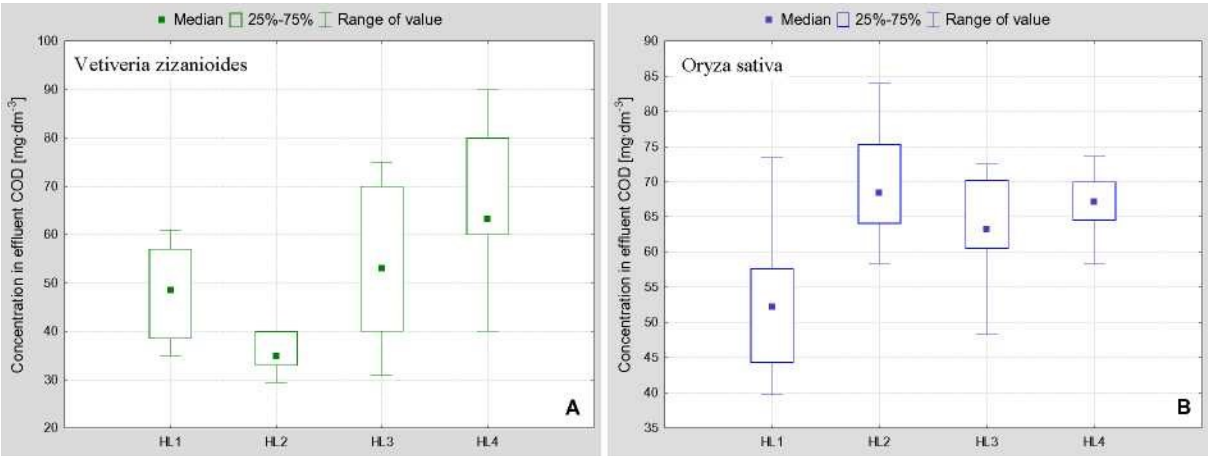


Fig. 7. Characteristic COD values in the outflow from the VFCW-1 bed (A) and VFCW-2 bed (B) at various hydraulic loads.

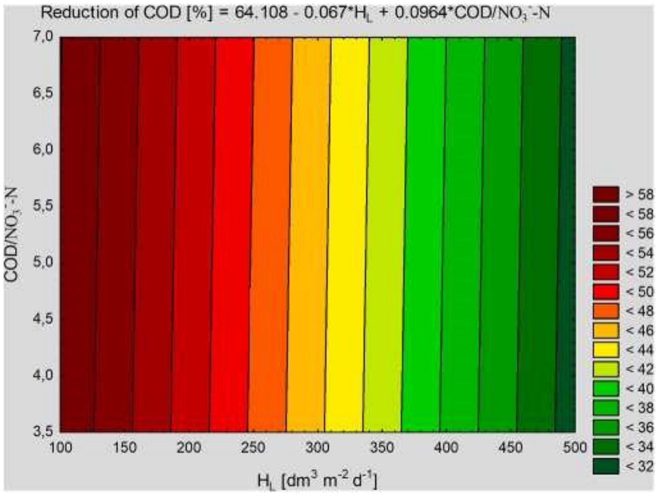


Fig. 8. Nomogram to determine the effectiveness of COD removal (%) de- pending on the amount of inflowing wastewater HL and the COD/N-NO3 ratio in inflowing wastewater to VFCW-1 bed.

NO3⁻-N (Fig. 8).

In the case of VFCW-2 bed, it was found that the influence of the amount of inflowing wastewater on the efficiency of COD removal ac- cording to the Stanisz (1998) scale is also at the weak level, as evidenced by the value of rx,y = -0.16. While, the effect of the COD/NO3⁻- N ratio in inflowing wastewater affecting COD removal efficiency is on the average level, where gdzie $r_{x,y}$ = -0.46. There was no statistical significance of the amount of inflowing wastewater on the level of COD reduction, while the significance of the effect of the COD/NO_3^-N quotient on the COD reduction in the VFCW-2 bed at the level of a = 0.05 was found.

It was found that in the case of the VFCW-1 bed, the impact on the efficiency of removal of organic compounds expressed as COD on the load of the bed with wastewater, while no such dependency in the case of the VFCW-2 bed. However, the influence of the COD/NO3⁻-N quotient on the COD reduction rate in the VFCW-2 bed was found, and no such dependency for the VFCW-1 bed.

Based on the results of the partial correlation, a model was developed (Fig. 9), from which the percentage efficiency of COD removal in the VFCW-2 bed can be predicted. The created model can be described by the equation R COD (%) = -10.8898-0.0316H_L + 10.2924COD/ $NO3^-$-N (Fig. 9).

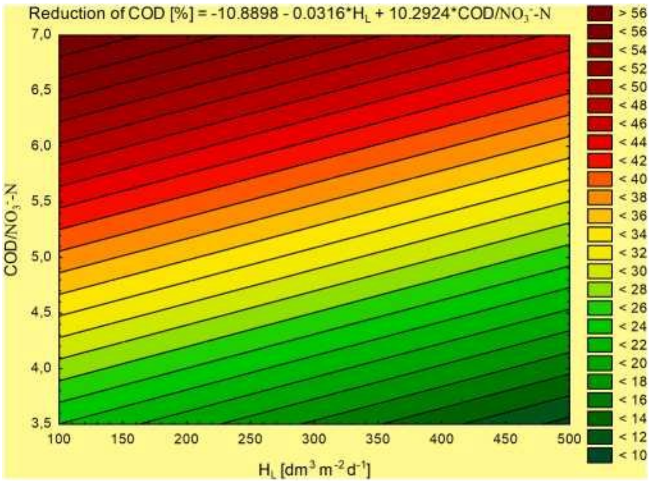


Fig. 9. Nomogram to determine the effectiveness of COD removal (%) de- pending on the amount of inflowing wastewater HL and the COD/$NO3^-$-N ratio in inflowing wastewater to VFCW-2 bed.

As mentioned above, the best effects of COD removal in the own research were obtained in the VFCW-1 bed with Vetiveria zizanioides under the hydraulic load H_{L2} - 55%, while the smaller ones in the VFCW-2 bed with Oryza sativa under the hydraulic load H_{L1} - 43%. The obtained effects of COD removal in the tested beds are much lower than those obtained in VF type CWs operating in full technical scale in the world. During research carried out in the 11-year CWs exploitation with common reed, it was found that the average effects of COD removal equals 78,0%, and the concentration of this indicator decreased from 323 to 62 mg O_2^dm^{-3} (Józwiakowski, 2012). While Chen et al. (2008) at Londgdao River in China in object type VF located in Beijing noted an average efficiency of reducing COD at the level of 82%. While Haberl et al. (1995) reported that the average efficiency of reducing COD in four VFs with a reed at Oaklands Park, Bausen, Wolfern, Wartberg was 87%. The higher efficiency of removal of COD in above mentioned fa- cilities is related to the fact that wastewater contained much higher concentrations and COD loads than those found in the wastewater flowing into the tested beds with Vetiveria zizanioides and Oryza sativa (Table 1). From the research of Gajewska et al. (2004) and Maehlum and Stalnacke (1999) show that the efficiency of removing pollutants in CWs depends mainly on the hydraulic load and pollutant load, but also on air temperature, bed oxygenation, vegetation used, season of the year and wastewater treatment method (vertical or horizontal flow).

3.3.Plants growth

The studies of described dependencies were carried out over a period of 22 weeks (151 days), with air temperature ranging from 24 to 28 °C (Table 1). At the start of the experiment, the height of both species Vetiveria zizanioides and Oryza sativa was 20 cm. In the case of both species, which were planted in the analyzed beds, different dynamics of their growth were observed during the research period. The growth of the species tested: Vetiveria zizanioides and Oryza sativa are shown in Fig. 10.

The growth period of Vetiveria zizanioides can be divided into three stages. The first stage, covering about 36 days, is a period of slow growth, in which the growth was 20 cm to a height of 40 cm. The second stage, lasting about 50 days, was the period of dynamic growth of the discussed plant. During this period, the growth of Vetiveria zizanioides was recorded by approximately 85 cm. In the third stage, lasting about 35 days, there was no significant growth of this plant, and its maximum height was 130 cm.

In the case of the second analyzed species Oryza sativa, the dynamics of its growth in the whole period was at the same (linear) level. During the 151 days of the study, there were no characteristic periods of greater and lower growth dynamics, as was the case with Vetiveria

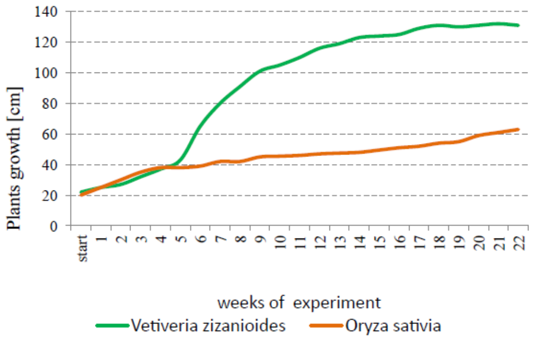


Fig. 10. Data on the plant growth of *Vetiveria zizanioides and Oryza sativa* during 22 weeks of reaserch.

zizanioides. In the analyzed period, the Oryza sativa increased to 63 cm from the initial state, which indicates the growth of this plant by about 43 cm.

During the whole study period all plants grew very well, without obvious symptoms of toxicity signals, such as chlorosis, leaf curl, early senescence stages and deficit of nutrients. However, the height reached by the plants was different, being higher in Vetiveria zizaniodes than in Oryza Sativa, presenting a maximum of 130 ± 9 cm and 63 ± 7 cm respectively (Fig. 10). Almeida et al. (2010) and Almeida et al. (2008) in Vetiveria zizanioides assays obtained a similar height when working with synthetic wastewater (130 cm), but higher when a swine waste- water was treated (204 cm). For Oryza Sativa, the available data from the Portuguese rice producers demonstrate that in rice fields the plant height can vary from 60 to 180 cm.

Plants exposure to increasing nitrate load resulted in a total nitrogen rise in the plant leaves tissues, once the nitrogen content increased from the beginning until the end of the trials ($p < 0.05$). In Vetiveria ziza- nioides, the total nitrogen at the beginning of the trial was 10.9 ± 0.3 mg g^{-1} and at the end of the trial was 14.4 ± 0.3 mg g^{-1}, and in Oryza sativa was 9.6 ± 0.4 and 12.4 ± 0.5 mg g^{-1}. It occurred an increase of 3.5 ± 1.1 mg g^{-1} and 2.8 ± 1.0 mg g^{-1}, respectively in Vetiveria zizaniodes and Oryza Sativa. Then, it can be concluded that some nitrogen was removed from the effluent through uptake by plants. When nitrate is the main form of nitrogen, plants uptake it, although if ammonium is present, they prefer the ammonium one (Ying-Hua et al., 2006; Britto and Kronzucker, 2002; Peterson and Hanna, 2016; Brauer et al., 2015).

At the end of experiment, the Vetiveria zizanioides showed a completely different root system from that of Oryza sativa. The Vetiveria zizanioides root system created a network that extended for the whole volume of the VFCW squeezing and holding the light expanded clay aggregates, being impossible to separate the roots from aggregates without breaking the roots; on the contrary, the Oryza sativa root system was more defined and localized in a small region of the VFCW in such way that it was easy to pluck the entire plants without breaking their roots. These distinct root systems may also have contributed to the result differences observed between the two VFCWs. The spreading of the Vetiveria zizanioides root system through the whole VFCW may have created a bigger extension of microzones where denitrification could occur.

4. Conclusions

It can be concluded that the removal efficiency of nitrate nitrogen in the tested VFCW-1 bed with Vetiveria zizanioides and VFCW-2 with Oryza sativa was primarily dependent on the hydraulic load. The best effects of NO_3^--N removal were obtained with a hydraulic load of H_{L2} = 241 $dm^3m^{-2}d^{-1}$, which equal 59 and 42% for the VFCW-1 and VFCW-2 respectively. In the case of other analyzed hydraulic loads, i.e. H_{L1} 148 $dm^3m^{-2}d^{-1}$), H_{L3} (350 $dm^3m^{-2}d^{-1}$) and H_{L4} (473$dm^3m^{-2}d^{-1}$) efficiency of NO_3^--N removal in both beds was significantly lower. It was also found that the ratio of COD/NO_3^--N in inflowing wastewater affected the efficiency of nitrate nitrogen removal in both tested beds. In order to predict the effectiveness of NO_3^--N removal for both VFCW-1 and VFCW-2 studied beds, nomograms were developed. Thus, knowing the amount of inflowing wastewater and the ratio of COD/NO_3^--N it is possible to predict the effectiveness (%) of nitrate removal.

Moreover, it was shown that the best effects of COD removal in the VFCW-1 bed were obtained under hydraulic load H_{L2} - 55%, while in the VFCW-2 bed, under hydraulic load H_{L1} - 43%. However, the obtained effects are significantly lower than those obtained in VF type constructed wetlands systems operating in full technical scale in the world, with higher load and COD values.

The research shows that the analyzed species, especially Vetiveria zizanioides have good phytoremediation potential in the area of nitrate nitrogen and organic substance removal and can be recommended for use on a full technical scale.

Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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