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## SEASONAL RESPONSE OF VEGETATION ON POLLUTANTS REMOVAL IN CONSTRUCTED WETLAND SYSTEM TREATING DAIRY WASTEWATER

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| **Abstract:** | In Sicily (Italy), the dairy sector is mostly composed of small and medium farms. These farms are often isolated from conventional treatment plants and this makes the treatment of dairy wastewater (DWW) very difficult. Constructed wetland systems (CWs) represent the ideal solution because can be built close to the farm and are easy to manage and use. However, their perfomance is significantly affected by vegetation activity during the year. The aims of the present study were to assess the DWW treatment by a horizontal subsurface flow system (HSSFs) and the effect of plants on removal efficiency (RE) of $BOD_5$, COD, total N (TN) and total P (TP). The HSSFs had a total surface area of 100 $m^2$ and treated 6 $m^3$ per day of wastewater produced by a small dairy farm, subsequent to biological treatment. The system included two units which were separately planted with giant reed (*Arundo donax* L.) and umbrella sedge (*Cyperus alternifolius* L.). During two-year study, 108 DWW samples were collected and analysed to determine the main chemical and microbiological characteristics as well as pollutant RE. Plant growth analysis was carried out and the biomass production was determined. All DWW parameters showed significant differences between inlet and outlet. For $BOD_5$ and COD, average RE values were 77.8% and 61.61%, respectively. Removal percentages for TN (52.3%) and TP (41.5%) was lower than those of organic compounds. *Escherichia coli* levels were found to be higher than 85.0%. Giant reed produced greater biomass than umbrella sedge for both above- (4240.3 g $m^2$ /year) and below-ground (6996.3 g $m^2$ /year) plant parts. A seasonal variation in RE of $BOD_5$, COD, TN and TP was recorded due to plant growth rate. Our findings indicate the use of HSSFs as an appropriate system to reduce pollutants in DWW and highlight that the contribution of plants in the pollutants RE tends to vary seasonally. |

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## Abstract

The dairy sector in Sicily (Italy is mostly composed of small and medium farms. These farms are often located far from conventional water treatment plants, making the treatment of dairy wastewater (DWW) extremely demanding. Constructed wetland systems (CWs) provide the ideal solution as they can be built close to the farm and are easy to manage and use. However, their perfomance is significantly affected by vegetation activity during the year. The aims of the present study were to assess the treatment of DWW by a horizontal subsurface flow system (HSSFs) and the effect of plants in the removal efficiency (RE) of $BOD_5$, COD, total N (TN) and total P (TP). The HSSFs had a total surface area of 100 $m^2$ and treated 6 $m^3$ per day of wastewater produced by a small dairy farm subsequent to biological treatment. The system included two units which were separately planted with giant reed (*Arundo donax* L.) and umbrella sedge (*Cyperus alternifolius* L.). During a three-year study, 108 DWW samples were collected and analysed to determine the main chemical and microbiological characteristics as well as pollutant RE. Plant growth analysis was carried out and biomass production was determined. All DWW parameters showed significant differences between inlet and outlet. For $BOD_5$ and COD, average RE values were 77.8% and 61.6%, respectively. Removal percentages for TN (52.3%) and TP (41.5%) was lower than those of organic compounds. *Escherichia coli* levels were found to be above 85.0%. Giant reed produced greater biomass than umbrella sedge for both above- (4240.3 g $m^2$/year) and below-ground (6996.3 g $m^2$/year) plant parts. A seasonal variation in RE of $BOD_5$, COD, TN and TP was recorded due to plant growth rates. Our findings indicate the use of HSSFs as an appropriate system to reduce pollutants in DWW and highlight that the contribution of plants in pollutant RE tends to vary seasonally.

**Key-words**: dairy wastewater, constructed wetland, *Arundo donax*, *Cyperus alternifolius*, seasonality

## 1. Introduction

The dairy sector is of primary importance to the agri-food industry in Italy, ccounting for over 15% of the food business (Ismea, 2020). Italy is one of the largest milk and dairy-product producers in the European Union (Milk Market Observatory, 2000) with an annual average total milk yield of more than 11,000,000 tons over the last ten years (Istat, 2020). Milk processing commonly requires high quantities of water, estimated by

Rossi *et al*. (2013) as 1 litre of water per kilogram of raw milk produced by the dairy. Sanna *et al*. (1982) reported an average water consumption ranging from 2 to 30 L of water per kg of raw milk during butter and cheese processing. Due to various activities, including milk processing, disinfecting and the washing of equipments and rooms, dairy farming annually generates considerable amounts of wastewater (WW). This water is a source of pollutants, often resulting in high costs for the farm and potential damage to the environment (Schierano *et al*., 2020). Several authors (Vourch *et al*., 2008; Matos *et al*., 2010; Custodio *et al*., 2022) report that the dairy industry could have a significant impact on the environment due to high effluent production, estimated approx. 0.2-10 litres of wastewater per litre of processed milk. Pattnaik et al. (2010) and Prazeres *et al*. (2012) sustain that the composition of dairy wastewater (DWW) can be affected over time by various factors, such as type of dairy-product produced, seasonality of dairy activities, operating conditions and methods, and WW management. In general, DWW contains high amounts of suspended and dissolved solids, organic components, lactose, nutrients, fats, sulphates and chlorides, and it is generally characterized by high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Sarkar *et al*., 2006; Carvalho *et al*., 2013; Prazeres *et al*., 2016; Ahmad *et al*., 2019). Shi *et al*. (2021) affirm that the pH of DWW is generally neutral or slightly alkaline, with a tendency to become acidic due to lactose fermentation. In previous studies, typical characteristics of DWW under different operational conditions have been reported and clearly explained by several authors (Masi *et al*., 2016; Akratos *et al*., 2018). Although literature reports the application of DWW as a fertiliser in agricultural land (Torr, 2009; Gogoi *et al*., 2021), it is worth noting that the long-term use of this practice negatively affects the chemical and physical soil characteristics, leading to a reduction in soil fertility, as explained by Healy *et al*. (2007). Furthermore, the high organic load of DWW is a source of pollution for surface waters if it is discharged directly into water bodies, resulting in their eutrophication (Ibekwe *et al*., 2003; Dunne *et al*., 2005). Ahmad *et al*. (2019) affirm that globally every year, approx. 4-11 million tonnes of DWW are released into the environment, causing severe hazard to all biodiversity. All these reasons highlight the need to treat DWW effectively before its release into natural ecosystems and/or agroecosystems. Generally, DWW is treated by physical-chemical and/or biological methods. There are a number of studies (Farizoglu *et al*., 2004; Prazeres *et al*., 2012; Carvalho *et al*., 2013; Ahmad *et al*., 2019) related to DWW treatment which demonstrate the benefits of each method compared to others. However, as reported by Schierano *et al*. (2019), these methods entail high initial investment costs, operation and maintenance costs and energy consumption. As a consequence, the use of low-cost technologies for DWW treatment should be highly encouraged. In Sicily (Italy), the dairy sector is one of the most productive and comprimes a large number of small and medium farms specialised in the production of milk and various types of dairy products, such as high quality butter, cheese and yoghurt. These farms are often located close to areas of considerable ecological importance (lakes, lagoons and ponds) and in many cases, due to their specific size and business, have no funds to build conventional plants to treat DWW (Licata *et al*., 2017). In order to avoid any negative impact of DWW on natural ecosystems, the use of constructed wetland systems (CWs) provides an ideal solution for dairy farms due to a series of reasons. CWs provide effective WW treatment and can be considered an environmentally friendly technology since their installation has low environmental impact, they do not consume large amounts of chemical or energy, and they require low operational and maintenance expenditure (Stefanakis, 2019). CWs have been successfully used in DWW treatment, for example, in Italy (Mantovi *et al*., 2003; Comino *et al*., 2011; Gorra *et al*., 2014; Masi *et al*., 2016; Licata *et al*., 2017), Greece (Akratos *et al*., 2018; Sultana *et al*., 2016), Argentina (Schierano *et al*., 2019), Ireland (Dunne *et al*., 2005) and Japan (Kato *et al*., 2013) becoming one of the most valued nature-based systems for the treatment of this source of WW. Plants are essential structural components of CWs and greatly contribute to WW treatment due to their multiple functions and interactions with microorganisms and substrates. However, their action greatly depends on various factors, including climate which plays an important role. Air temperature and solar radiation, in particular, significantly affect vegetation growth and various plant physiological processes. When air temperature stimulates plant growth the performance of vegetation in the CWs increases together with the growth and activities of bacteria (Karathanasis *et al*., 2003; Zhu *et al*., 2018). In general, increasing temperatures enhance the growth of macrophytes, the metabolism and activities of bacteria and, consequently, the pollutant removal efficiency (RE) of CWs, as proved by Akratos and Tsihrintzis (2007), and Yan and Xu (2014). In the case of DWW treatment, understanding how plant growth can seasonally vary with temperature is interesting for exploration of pollutant RE under changing temperatures and the creation of effective CWs for dairy farms over the year. In Sicily, there is growing interest in CWs to treat effluents from dairy farm however, information on the potential application of this green technology in the treatment of DWW is still largely little-known by farmers. Pilot scale studies, therefore, are very useful to provide scientific and technical information on CWs in this Mediterranean area.

The aims of this study were to assess: i) the pollutant RE of a pilot-scale horizontal subsurface flow system (HSSFs) for treatment of WW produced by a dairy farm, ii) the effect of plants on removal of $BOD_5$, COD, total nitrogen (TN) and total phosphorus (TP).

## 2. Materials and Methods

### *2.1. Experimental setup*

The study was carried out from 2019 to 2021 on a pilot HSSFs CW in Raffadali, in the West of Sicily (37°24'N – 1°05'E, 446 m a.s.l.). The pilot CW system was used for the treatment of a portion of the wastewater produced by a dairy farm located in the surrounding area. The farm specialised in milk production for cheese-making (caciocavallo, pecorino and ricotta cheeses). The number of lactating cows on the farm was, on average, 85 at the time of the three-year study. The production capacity of milk was approximately of 1,600 L/day. The wastewaters from the various sectors of the dairy farm (holding area, milking system, milking parlor and milk room) were mixed with domestic WW produced by the staff.

### *2.2. The HSSFs CW*

The HSSFs CW had two separate, parallel units each 50 m long and 1 m wide (Fig. 1). The units were made of concrete and lined with sheets of ethylene and vinyl-acetate. They were designed to receive a total of 6 $m^3$ of wastewater per day. The units had a depth of 0.5 m and a slope of 2%. The substrate was made of evenly sized 30 mm silica quartz river gravel (Si 30.02%; Al 5.11%; Fe 6.10%; Ca 2.65%; Mg 1.05%) with a porosity of 35-40%. In February 2008, the two units were separately planted with giant reed (*Arundo donax* L.) at a density of 4 rhizomes $m^{-2}$ and umbrella sedge (*Cyperus alternifolius* L.) at a density of 5 stems $m^{-2}$. In subsequent years, plants which had died in the two units were replaced in order to maintain the same plant density.

*2.3. Description of the wastewater treatment*

On the dairy farm, an equalization tank and two Imhoff septic tanks were used to treat WW in order to remove total suspended solids (TSS). The pre-treated DWW was, subsequently, collected into a 15.0 $m^3$ storage tank at the HSSFs CW area. The storage tank was equipped with a submerged electric pump to feed water into the CW units, and with a litre gauge and outlet valve for periodic cleaning of solid sediments. The WW was fed into a static degreaser to separate fats, soaps and food wastes, and pumped through a 1.0 m wide perforated polyvinylchloride pipe into the two CW units. The WW was distributed homogeneously in each unit through a timer-controlled pumping system. In each unit, the pipe was placed 10 cm from the surface of the substrate. The treated dairy wastewater was collected using a perforated drainage pipe system placed at the bottom of the unit and conducted downhill into a system of four interconnected tanks each of 5.0 $m^3$. The two units had a hydraulic loading rate (HLR) of 6.0 cm/day and hydraulic retention time (HRT) of 8.3 days. The treated wastewater was generally discharged into the soil using a subsurface irrigation system connected to the last of the four tanks. The layout of the system for treatment of wastewater is shown in Fig. 2.

*2.4. Sample collection and analytical methods*

WW sampling was carried out on a monthly basis from March to November of each year.  A total of 108 WW samples were taken at inlet and outlet of the CW units. 1.0 L of WW was collected from each of the two points at each sampling. The influent sample was collected close to the pipe while the effluent sample was taken at the mouth of the outflow pipe. Sampling always occurred at the same time, according to operations carried out on the dairy farm. PH and electrical conductivity ($EC_w$) were measured directly on site using a portable Universal meter (Multiline WTW P4). TSS, BOD in five days ($BOD_5$), COD, TN, ammonia nitrogen ($NH_4$-N), organic nitrogen (ON), TP and heavy metals (Cu, Ni, Pb and Zn) were determined according to Italian water analytical methods (APAT-IRSA-CNR, 2004). Microbiological analyses were conducted according to Standards Methods for the Examination of Water and Wastewater (APHA, 1998). Total coliforms (TC), faecal streptococci (FS), *Escherichia coli* (E. coli) and *Salmonella* spp. levels were examined. RE of the HSSFs CW was based on pollutant concentrations and calculated in accordance with the International Water Association (Kadlec *et al*., 2000):

$$RE = (C_i - C_o)/C_i \times 100 \quad (1)$$

where $C_i$ and $C_0$ are the mean concentrations of the pollutants in the influent and effluent.

The qualitative characteristics of the CWs effluent were assessed in accordance with the guidelines of the Italian Decree no.152/2006.

## *2.5. Plant measurements*

The main detected morphological parameters were: plant height, culm/stem density, and root-system diameter and length. Plant measurements were taken from March to

November for each year. Plant height was calculated fortnightly by measuring the maximum height of 10 plants, randomly selected from the initial, middle and end sections of each unit. Root-system diameter and length were determined monthly by measuring the root diameter and root length of 10 plants selected randomly from each unit. Culm/stem density was calculated monthly on three 1.0 $m^2$ areas randomly selected, from the initial, middle and end sections of each unit. Four crop growth stages were identified (Allen *et al*., 1998): a) initial stage: from greenup to the beginning of stem elongation; b) crop development stage: from stem elongation to initial flowering; c) mid-season stage: from flowering to initial canopy senescence; and d) late-season stage: from canopy senescence to plant harvest.

In November of each year, the plants were cut back to a height of 50.0 cm above the gravel unit. Fresh above- (leaves and stems) and below-ground (roots and rhizomes) weights were determined on a representative sample of 10 plants from each unit. The above- and below-ground dry weights were, then, calculated by drying the collected plant material in an oven at 62.0 °C for 72 hours. TN levels in the above- below-ground parts of the plants were determined using a carbon (C), hydrogen (H), nitrogen (N) elemental analyser (CHN), in full compliance with plant biomass basic analysis standards. TP levels in the plant parts were, instead, determined using spectrophotometric method based on molybdenum blue colouration (Murphy and Riley, 1962). Nutrient standing stock in vegetation was calculated by multiplying the nutrient concentrations in the plant tissues by plant biomass per unit area, as suggested by Vymazal (2011).

*2.6. Climatic data*

A weather station belonging to the Agro-Meteorological Information Service of Sicily (2022) was used to collect climate data. It was located close to the pilot HSSFs CW. The station was equipped with a MTX datalogger (model WST1800, Padova, Italy) and sensors which provided data on various climate parameters.

*2.7. Statistical analysis*

Statistical analyses were performed using the package MINITAB 19 for Windows. A paired *t*-test was used to compare the mean levels of each chemical and microbiological parameter at influent and effluent. A level of $p < 0.01$ was used for all comparisons. For DWW composition, all the representative values were presented using mean ± standard deviation calculations.

**Results and discussion**

3*.1. Microclimatic conditions at the HSSFs CW area*

The study area is characterized by a warm temperate climate with dry summers in accordance with the Köppen–Geiger climate classification (Kottek *et al.*, 2006). Annual average rainfall is 650 mm, mainly distributed between October and April. The annual average temperature is 17.5 °C, the average maximum temperature is 23.5 °C, and the average minimum temperature is 11.2 °C.

In this study, between March and November of each year, average air temperatures never fell below 11.0 °C. The maximum average air temperature (45.5 °C) was recorded in the second 10-day period of August 2021 and the minimum average air temperature (2.2 °C) in the first 10-day period of March 2020. Air temperature trends were similar over the three years. During the study period, total rainfall was 660 mm (2019), 310 mm (2020) and 601 mm (2021). The highest rainfall levels (103 mm) occurred during the third 10-day period of October 2021. Rainy days were highly concentrated between September and November. In the summer period, average monthly rainfall was 44 mm (2019), 17 mm (2020) and 21 mm (2021). Relative humidity trends varied over the two years due to different air temperature and rainfall values. In particular, maximum daily average relative humidity was 95.2% in 2019, 94.8% in 2020 and 95.4% in 2021. From March to November of the three years, average total solar radiation was 19.4 $MJ/m^2$. For each year, the highest total solar radiation was recorded in July.

When considering the microclimatic conditions, in general, the highest performance of the system in terms of pollutants removal was observed between June and August when air temperature and solar radiation, in particular, had positive effects on plant growth and stimulated bacterial activities in the substrate. At the beginning of autumn, mild climatic conditions delayed the dormancy period of plants, which contributed to maintaining a high pollutant RE of the system in this season.

### *3.2. Removal of pollutants in the pilot HSSFs CW*

Data showing chemical variations and pollutant removal relating to DWW are shown in Tables 2 and 3. Variations in the main chemical parameters were observed each year in the study due to seasonal changes in dairy activities. The use of two Imhoff tanks and a static degreaser ensured the effective removal of TSS, fats and organic components from DWW.

For pH measurements, significant differences were found between influent and effluent values: in both units, influent values were found to be higher than outlet. Mantovi et al.

(2003) and Schierano *et al*. (2020) reported average pH values of the effluent ranging from 7.5 to 8.7 despite different operating conditions at the dairy farm and type of DWW pre-treatment. Brix *et al.* (2001) and Chen *et al*. (2019) highlighted the production of carbon dioxide ($CO_2$) from the decomposition of plant residues, the nitrification of ammonia and the removal of some WW components in the root area, among the factors which can determine a decrease in pH. In this study, differences in $EC_w$ were significant ($p \leq 0.001$). When comparing the two units, average $EC_w$ of the CW effluent was 598.8 µS/cm in the giant reed-unit and 535.2 µS/cm in the umbrella sedge-unit. These differences were mainly due to morphological aspects of the species. The two macrophytes had different root apparatus and foliage systems which greatly affected daily evapotranspiration; giant reed consumed more water than umbrella-sedge due to higher evapotranspiration rates that led to an increase in salt levels in the effluent. A number of authors agree with this explaination highlighting the need to be aware of the water consumption of plants in an CW and how evapotranspiration can affect pollutant removal (Headley *et al.,* 2012; Pedescoll *et al*., 2013; Beebe *et al*., 2014; Tuttolomondo *et al*., 2015; Licata *et al*., 2017; La Bella *et al*., 2017)). As expected for dairy effluent, fluctuations in the levels of TSS, $BOD_5$ and COD were observed in the two units, with peaks during milk processing and various washing operations of equipments at the dairy farm.

TSS values showed significant differences between influent and effluent. The giant reed-unit produced RE value for TSS which were almost identical to those of the umbrella sedge-unit. Previous studies conducted on HSSFs CW for DWW treatment reported TSS RE ranging from 75.0 to 85.0% (Masi *et al*., 2016; Schierano *et al*., 2020; Vymazal, 2014). In Italy, Mantovi *et al*. (2003) obtained RE for TSS, COD and $BOD_5$

consistently above 90.0% during the operation period. Although the removal rate of TSS in a CW is usually associated with physical processes such as filtration and sedimentation, it is worth noting that, in the case of DWW, the choice of the type of pre-treament and CW system (e.g. hybrid systems) greatly influences the removal process of suspended solids.

For $BOD_5$ and COD, significant differences were found between influent and effluent levels. The average removal rates of $BOD_5$ and COD were found to be similar to those observed by other studies. In southern Europe, average $BOD_5$ RE ranging between 70.0 and 94.0% was reported by some authors (Mantovi *et al*., 2003; Licata *et al*., 2007; Sultana *et al*., 2016; Akratos *et al*., 2018). In Argentina, Schierano *et al*. (2020) obtained average RE for $BOD_5$ and COD of 57.9% and 68.7%, respectively. In Japan, Kato *et al*. (2013) found average values of COD RE to be higher than 90.0%. In Vermont (USA), Lee *et al*. (2010) used an integrated system consisting of various combinations of HSSFs and vertical sub-surface flow system (VSSFs) to treat DWW, obtaining a $BOD_5$ removal rate of 89.0%. As stated by Kadlec *et al*. (2000) and Vymazal (2005), the main removal processes of $BOD_5$ and COD in the HSSFs CW are anaerobic degradation, filtration and sedimentation, which depend on the activities of plants, microorganisms and substrates and their interaction. However, the high performance of this system is related to the type of DWW pretreatment, size of the CW and presence of monocultures or polycultures in the CW, for example. In our study, the combination of a specific pre-treatment system with a CW separately planted with two emergent macrophytes produced high organic compound removal rates.

As regards TN, significant differences were recorded between influent and effluent values. TN RE was found to be similar in the two planted units but lower than that of

$BOD_5$ and COD. However, the findings were consistent with those obtained by Mantovi *et al*. (2003) and Gorra *et al*. (2014) in Italy, which were in the range of 40-50.0%. Vymazal (2005) sustains that the most important removal mechanism of N in an HSSFs is nitrification/denitrification. However, the same author affirms that oxygenation of the rhizosphere is often insufficient and, therefore, incomplete nitrification leads to limited N removal. It has been demonstrated that N uptake by plants is usually low and play a smaller role in N removal: for example, Chan *et al*. (2008) found that plant uptake could account for 10–15.0% of N removal in CWs. As a consequence, high TN RE cannot be expected in a CW.

TP effluent levels were significantly lower than influent. The two planted units produced similar TP RE, also within the range (30-60.0%) of those observed in HSSF CWs in many studies. The cause of the low TP removal rate was not investigated in this study but was probably due to gradual filling of the sorption sites in the long-term and the presence of undercomposed plant material around the substrate surface, in accordance with the findings of Lin *et al*. (2002) and Lu *et al*. (2006).

Regarding heavy metals, average RE, in general, was lower than other nutrients. Significant differences were observed between influent and effluent values. In general, heavy metals levels in the influent were low.

In this study, bacteria were constantly present in the effluent as DWW was combined with domestic WW. No *Salmonella* spp. was reported either in the DWW at inlet or outlet of the two planted units. Bacteria levels in the DWW varied over the year, depending on dairy farming activities and quality of domestic WW. Bacterial removal was particularly effective during the 3-year test, as shown in Table 4. Average RE values were high for each parameter and recorded as over 80.0%. The giant reed-unit showed higher patogen removal than the umbrella sedge-unit in all samples. As well explained in previous studies (Kadlec *et al*., 2000; Vymazal, 2005; El-Khateeb *et al*., 2009; Wu *et al*., 2016), the high E. coli, TC, FC and FS removal rates in the HSSFs CW can be explained by considering the combination of physical, chemical and biological processes carried out by the plants, nematodes, viruses, bacteria and the effect of high oxygen levels in the root area.

In Italy, the discharge of treated wastewater into soils is regulated by Legislative Decree 156/2006. The Decree reports threshold values for various chemical parameters and for E. coli in relation to the environmental context and soil use. In our study, average chemical and microbiological parameter results at the outlet of the HSSFs CW were not all within the limits of the Decree. E. coli levels in some cases were found to be above threshold limits, mainly due to the composition of the DWW which varied over the seasons. This could prove problematic in the long-term because of considerable accumulation of pathogens in the soil. To avoid this risk, a good solution could be the use of a hybrid CW exploiting the effect of different hydraulic retention times on pathogen removal (Stefanakis and Akratos, 2016; Wu *et al*., 2016) or the application of a polyculture system with different macrophytes (Licata *et al*., 2019, Abou-Kandil *et al*., 2021).

### *3.3. Characteristics of the planted units*

During the three years, intense plant growth occurred during spring when air temperatures were above 20 °C, and reached maximum growth in summer at approximately 30-35 °C. When considering the morphological parameters examined, the two macrophytes showed great differences as regards plant height, average culm/stem density, root diameter and length (Table 5). Giant reed obtained highest average height in comparison with umbrella sedge plants. It was noted that, in both planted units, culm/stem density decreased over the study period. Vymazal and Krőpfelová (2005) affirmed that the decrease in culm/stem density could be due to a self-thinning process which is common in monocultures. The same authors explained that, in dense populations, total density decreases due to mortality, usually caused by light deficiency. It was also seen that the root system was uniformly distributed in the two planted units, as detected by Leto *et al*. (2013). Root lenght, however, was greater in the giant reed plants due to larger growth of the aboveground plant parts (Fig. 3). Observing the growth stages of the two species (data not shown), crop development and mid-season stages were found to be longer than initial and late season stages for both the macrophytes in each year. In general, intensive crop development was recorded between May and July while senescence for the above-ground plant parts occurred at the beginning of October, mainly due to decreasing air temperatures and solar radiation. This affected the contribution of plants in nutrients removal. Harvest occurred in November, when dormancy started and nutrient uptake by plants decreased greatly.

Above-ground dry matter production was found to be different in the two planted units. The giant reed-unit produced greater above- and belowground biomass than the umbrella sedge-unit because of specific morphological and production characteristics of the species. Giant reed is considered one of the most high-yielding biomass species of all macrophytes used in CWs (Avellan *et al*., 2007) and shows a higher growth rate during the vegetation period. In a recent study on biomass production by plants in CWs, Ennabili and Radoux (2021) compared four riparian plants grown separately in mesocosms and found that giant reed had a large harvestable biomass equal to 118-134 t dry weight/ha. On the contrary, for umbrella sedge, Cui *et al*. (2009) and Soda *et al*. (2012), in tropical and subtropical areas, obtained average biomass production levels which were much higher than those detected in this study. Average dry matter for the above-ground parts of the giant reed plants was 4240.3 g $m^2$/year and 6996.3 g $m^2$/year for the below-ground parts (Fig. 4). Concerning umbrella sedge, dry matter for the above-ground parts varied between 3457.5 and 3789.1 g $m^2$/year, with an average value of 3635.3 g $m^2$/year; dry matter for the below-ground parts ranged from 4230.2 to 4650.3 g $m^2$/year with an average value of 4446.7 g $m^2$/year. A comparison of the three years, showed a small increase in above- and below-ground dry matter production between 2019 and 2021 for the giant reed and umbrella-sedge plants. Previous studies conducted in CWs for the treatment of various sources of WW reported no similar findings for biomass production concerning giant reed and umbrella sedge due to a series of factors such as growing season, environmental conditions, plant age, type of CW system used, CW configuration and source of wastewater (Cui *et al*., 2009; Idris *et al*., 2012; Soda *et al*., 2012; Ennabili and Radoux, 2021). Regarding the amount of nutrients uptaked and stored in plant parts, more N and P accumulated in the aerial parts than rhizomes and roots and this was consistent with results of Kantawanichkul *et al*. (2009) and Schierano *et al*. (2020). This demonstates that macrophytes have mechanisms for translocate and accumulate nutrients in various plant parts. Average N levels in the above-ground parts were found to be 72.5 g $m^2$/year for giant reed and 57.1 g $m^2$/year for umbrella sedge. In contrast, average N content in the below-ground parts was 44.7 g $m^2$/year for giant reed and 37.2 g $m^2$/year for umbrella sedge. Concerning P, average P levels in the above-ground parts were recorded to be 5.7 g $m^2$/year for giant reed and 3.6 g $m^2$/year for umbrella sedge. In the below-ground parts, average P levels were 2.6 g $m^2$/year for giant reed and 2.1 g $m^2$/year for umbrella sedge. Our findings were consistent with values found in literature regarding the uptake of N and P by plants in CWs. For HSSF CWs and for various macrophytes, Vymazal (2020) states that the N and P standing stocks could vary between 30-80 g N/$m^2$ and between 2-6 g P/$m^2$, respectively. Futhermore, the same author affirmes that, in stands with high biomass, standing stocks could exceed 150 g N/$m^2$ and 180 g P/$m^2$.

Our results highlight that the greater the production of biomass, the greater the nutrient uptake by the plants (Leto *et al.*, 2013). In fact, giant reed plants stored greater N and P levels in the roots and subsequently translocated it to aerial parts than umbrella sedge.

### *3.4. Effect of vegetation on $BOD_5$, COD, TN and TP removal efficiencies*

Average $BOD_5$ and COD levels at different dates at the inlet and outlet of the two planted units are shown in Figs. 5 and 6, respectively. In both years, at the outlet of the giant reed and umbrella sedge-planted units, $BOD_5$ and COD varied seasonally. The highest RE values were found in summer while the lowest values in winter. A number of factors may be able to explain this, such as the hydraulic condition of the CW, the type of substrate, the plant species and the influent load, as reported by Zhu *et al.* (2018). However, the role of plants in the CW can be considered more important than others. It is clear that vegetation affects organic compound removal through various chemical and physical mechanisms; however, its level of contribution may differ can be different during the seasons mainly as results of plant growth rate. In this study, environmental factors, such as temperature and solar radiation, greatly influenced the development of the above- and below-ground plant parts of the two macrophytes which reached maximum growth during spring and summer. Based on this, it is worth noting that as plant growth increases, the RE of $BOD_5$ and COD also increases. This positive relationship (Figs. 7 and 8) can be explained by considering the effect of vegetation on microbial activities in CW. Literature (Kadlec *et al*., 2000; Sultana *et al*., 2006; Wu *et al*., 2016) affirms that plants provide adequate surface areas for microbial growth and allow the bacteria to degradate organic compounds, thus increasing dissolved oxygen in the rhizosphere. However, the rate of oxygen released by roots is not uniform during the year (high in spring/summer due to intense plant growth and low in winter due to senescence). As a consequence, in spring/summer, when plants grow fast and oxygen levels in the root zone are greater than in other seasons, conditions are more favourable for bacterial growth and the oxidation of organic compounds by bacteria is higher. Thus, seasonal variations in RE of these compounds can be expected in a HSSFs CW.

Similarly, N levels varied seasonally at the outlet of the planted units (Fig. 9). The highest N levels were recorded in winter while the lowest in summer. This fact was, in part, due to farm activities which had a direct effect on DWW composition. However, it is also be related to plant growth and different plant N uptake over the seasons. It is well known that plant uptake could contribute to at least 10-15% of N removal in CW based on literature (Chan *et al*., 2008; Zhu *et al*., 2018). In addition, as explained by Akratos and Tsihrintzis (2007), plant roots provide oxygen for complete nitrification and release organic carbon as an energy source for heterotrophic bacteria, such as denitrifying bacteria. This means that plants contribute greatly to N removal in a CW but their contribution tends to vary over the seasons. The seasonal response of plants in N removal can be explained taking into consideration the way in which air temperature affects N removal, as it has, a direct effect on plant growth and bacterial metabolism. The influence of air temperature on the performace of a CW is more evident when a monoculture system is used. Literature reports that a severe fall in air temperature in winter induces plant senescence and reduces the metabolism and activity of denitrifying bacteria. Yan and Xu (2014) reported that bacterial activity tended to decrease when temperatures fell below 10 °C and the denitrification process stopped when temperature dropped below 6 °C. The same authors affirmed that the decrease in temperature suppressed nutrient removal efficiency in the CW during the cold season. On the contrary, an increase in air temperatures in spring/summer determines plant regrowth and favours the bacterial metabolism, enhancing the nutrient removal efficiency of the CW. As a consequence, understanding how plant and bacterial activities change with variations in temperature is fundamental in order to obtain a more effective CW. To avoid or reduce the effect of air temperature on plant and bacterial activities, the use of a polyculture system could be the best solution. A mix of species would provide greater pollutant RE as the various species provide a range of adaptive capacities to changes in WW composition in the short- and long-term. For example, Zhu *et al*. (2018), examining the influence of vegetation type and temperature on performance of CWs, found that a polyculture system showed best performance in the removal of N when the average temperature dropped to 19.8 °C. However, a polyculture system requires a preliminary evaluation of the interspecific competition for nutrients and water in order to maintain a stable state over time. Furthermore, most polyculture systems are composed of species which start senescence at the same period and show the same sensivity to variations in temperature. As a consequence, a mix of warm and cool-season plant species, with low interspecific competition, would seem fundamental in order to minimize the effect of seasonality and to obtain constant pollutant RE.

In the case of P, the levels of TP showed low seasonal variation at the outlet of the planted-units and no large differences were found between warm and cold periods (Fig. 10). Furthermore, removal percentages of TP in the giant reed- and umbrella sedge-planted-units were found to be comparatively stable over the seasons. This means that plants contributed to TP removal but their activity was less affected by the season than TN removal. Our findings were in contrast with results obtained by Mesquita *et al*. (2017), who found that removal efficiency was marked by seasonality for P compounds with highest percentage removal occurring in the spring-summer period when plants were in exponential growth phase. In our study, the fact that the effect of seasonality was less evident for TP removal was probably due to a range of factors, including plant age, the gradual filling of the sorption sites by the plant roots over time, the presence of undecomposed plant parts around the substrate surface over time and the adsorption properties intrinsic to the substrate itself. On this basis, it is possible to affirm that plants usually uptake available phosphorus, translocate it to the aerial parts for growth but the removal of TP tends to be less affected by the effect of the season in the long term. However, this does not mean that plants contribute little in TP removal. Although Liang *et al*. (2017) state that the dominant pathway for TP removal in CWs might be sediment storage and/or substrate adsorption, the contribution of plants is evident. For example, Tanner *et al*. (1995) reported an increase in TP RE of up to 38.0 % with planted-units. Mucieri *et al*. (2020) demonstrated that plant uptake represents one of the ways to improve P removal efficiency and that plant species can play a different contribution to P removal over time.

When comparing our findings with literature, similarities were found in various studies. In Japan, Sharma *et al*. (2013) found that, in a hybrid CW for milking parlor wastewater treatment, RE for TSS, COD, TN, total carbon and total coliform increases during warm periods. In China, Zhou *et al*. (2017) found that the use of a polyculture system and fall in temperature had significant effects on RE of $NH_4$, $NO_3$, TN and TP. In Portugal, Mesquita *et al*. (2017) observed a seasonal variation in N and P removal in a full-scale HSSFs CW and highlighted that the higher removal rates were detected when plant growth was more intense. In Ireland, Dunne *et al*. (2005) found that, in an integrated CW used to treat contaminants from DWW, phosphorus retention varied with the season (5–84 %), with the lowest amounts being retained during winter. In Vermont (USA), Lee *et al*. (2010) used hybrid CWs planted with *Schoenoplectus fluviatilis* to treat DWW and affirmed that greater ammonia reduction was observed in late summer. Similar results were also found in the USA by Karathanasis *et al*. (2003). Although these studies were conducted using different parameters, such as pretreatment type, system size, pollutant levels, flow rates and daily hydraulic loads, they agree with the fact plants play an important role in the removal of organic compounds and nutrients and that a seasonal response of vegetation to pollutant RE can be expected when using a monoculture system, whilst the effect is not as pronounced in a polyculture system.

## Conclusions

This study reveals that a constructed wetland provides an efficient nature-like technology to treat dairy wastewater produced by a small dairy farm. This system provides a great deal benefits in terms of pollutant removal efficiency and environmental restoration. These benefits seem to be more evident for small and medium dairy farms which are located far from conventional water treatment plants and often near to ecologically sensitive areas. The constructed wetland system used in this study, combined with an appropriate pretreatment system, performed well in the treatment process of dairy wastewater and led to an improvement in the chemical and microbiological quality of wastewater. Two monoculture systems were compared under the same operational conditions and the results showed that the giant reed-planted unit outperformed the umbrella sedge-planted unit, both in terms of biomass production and nitrogen and phosphorus uptake and storage because of greater growth of the above and belowground plant parts. However, despite differing performances, both planted units showed seasonal variations in $BOD_5$, COD, TN and TP levels mainly due to a seasonal contribution of vegetation in pollutant removal efficiency. The removal of organic and nutrient compounds was found to increase during warm periods and decrease in cold periods, thus demonstrating a clear link plant growth rates which varied over the year. As a consequence, it is possible to state that the seasonal response of a monoculture system could represent a point of weakness for dairy wastewater treatment using constructed wetlands since this system needs to ensure high levels of efficiency throughout all seasons, especially for dairy farms which produce wastewater all year. We could conclude that a polyculture system would obtain costantly high pollutant removal efficiency values, reducing the seasonal response of plants; however, this would only be possible if the system consisted of warm and cool-season plant species with low interspecific competition.

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**Table captions**

Table 1. Climatic conditions at the experimental site during the test period.

Table 2. Variation of pH and $EC_w$ in the planted units of the HSSFs CW from March 2019 to November 2021. Three-year average (± standard deviation), minimum and maximum values are shown (*n = 81*).

Table 3. Main chemical composition of the dairy wastewater from the inlet and outlet of the planted units. Removal efficiency from March 2019 to November 2021. Three-year average (± standard deviation), minimum and maximum values are shown (*n = 81*).

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Table 5. Morphological parameters of giant reed and umbrella sedge plants in the HSSFs CW. Three-year average values (± standard deviation), minimum and maximum values are shown (*n = 16*).

**Figure captions**

Fig. 1 - A view of the pilot-scale HSSF constructed wetland: (a) refers to initial storage tank and CW area, (b) refers to final storage tank, (c) refers to giant reed-unit while (d) refers to umbrella sedge-unit.

Fig. 2 - Layout of the system for treatment of dairy wastewater.

Fig. 3 - Root system of the two species: (a) refers to giant reed while (b) refers to umbrella sedge plants.

Fig. 4 - Aboveground (AG) and belowground (BG) biomass, total nitrogen (TN) and total phosphorus (TP) contents of *Arundo donax* and *Cyperus alternifolius*. Bars indicate standard error of the means.

Fig. 5 - Times series charts for $BOD_5$ removal with influent and effluent concentrations in the two planted units.

Fig. 6 - Times series charts for COD removal with influent and effluent concentrations in the two planted units.

Fig. 7 - Correlations between plant growth and $BOD_5$ (a), and COD (b) removal efficiency based on concentration in the giant reed-unit.

Fig. 8 - Correlations between plant growth and $BOD_5$ (a), and COD (b) removal efficiency based on concentration in the umbrella sedge-unit.

Fig. 9 - Times series charts for TN removal with influent and effluent concentrations in the two planted units.

Fig. 10 - Times series charts for TP removal with influent and effluent concentrations in the two planted units.

**RESEARCH HIGHLIGHTS**

- Constructed wetland is efficient to treat dairy wastewater produced by small dairy farm.
- Pollutants removal efficiency tends to vary depending on plant species.
- Vegetation seasonally affects removal processes depending on plant growth rate.
- The effect of seasonality can be less evident for total phosphorus removal.
- The influence of temperature is more marked when monoculture system is used.

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Sincerely yours,

Nicolò Iacuzzi on behalf of the authors.

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**COVER LETTER**

Dear Editor,

Please find enclosed the manuscript: "Seasonal response of vegetation on pollutants removal in constructed wetland system treating dairy wastewater" by Mario Licata *et al*. to be submitted as a new full length article to Special Issue in Ecological Engineering entitled "Wetlands for ecosystem restoration & management" edited by Thomas Hein & Gabriele Weigelhofer.

All co-authors have seen, read and agreed with the contents of the manuscript and there is no financial interest to report. The paper has not been published in whole or in part elsewhere. It is not currently under consideration in another journal. We attest to the validity and legitimacy of the data and its interpretation and we agree to its submission to the mentioned journal.

This paper reports three-year study on the performance of a pilot-scale horizontal subsurface flow system adopted for dairy wastewater treatment in Sicily (Italy). Treated dairy wastewater was discharged in soil. All wastewater parameters showed significant differences between inlet and outlet. A high removal efficiency of the main chemical and microbiological pollutants was found during the tests period. Giant reed performed better than umbrella sedge in terms of growth and nitrogen uptake.
A seasonal variation in RE of $BOD_5$, COD, TN and TP was recorded due to plant growth rate. Our findings indicate the use of constructed wetland as an appropriate system to reduce pollutants in dairy wastewater in small/medium farms and highlight that the contribution of plants in the pollutants removal efficiency tends to vary seasonally. We believe that our findings could be of interest to the readers of Ecological Engineering as they confirm the suitability of constructed wetland systems for dairy wastewater treatment and highlight their interest for dairy farms which are often located close to areas of high ecological importance such as lakes, lagoons and ponds and in many cases have no funds to build conventional treatment plant.

We confirm that the authors used the Ecological Engineering style template and the manuscript meets now the journal's style requirements. Finally, I attest that the manuscript has been revised by a native English lecturer (Lucie Branwen Hornsby MCIL, CL, DipTrans, Chartered Institute of Linguists, http://www.ciol.org.uk, contact e-mail: lbhornsby@gmail.com) for professional language editing.

We hope the editorial board will agree on the interest of this study.

Sincerely yours,

Nicolò Iacuzzi on behalf of the authors.

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Dairy industry produces approx. 0.2-10 litres of wastewater per litre of processed milk.
Which "green" solution for the environment?

A constructed wetland system..!

Efficiency

Sustainability

- CW is an efficient natural-like technology to treat dairy wastewater produced by small dairy farm.
- Vegetation seasonally affects removal processes depending on plant growth rate.
- The combination of various type of constructed wetland systems and/or the use of polyculture system could improve the treatment efficiency.

Table 1 Click here to access/download;Table;Table 1.xls

Table 1. Climatic conditions at the experimental site during the test period.

| Year | Min. air temperature (°C) | Max air temperature (°C) | Average air temperature (°C) | Total rainfall (mm) | Average relative humidity min. (%) | Average relative humidity max (%) | Average total solar radiation (MJ/m$^2$) |
|---|---|---|---|---|---|---|---|
| *2019* | | | | | | | |
| March | 5.43 | 21.42 | 11.59 | 74 | 51.05 | 98.69 | 17.60 |
| April | 7.85 | 20.67 | 14.38 | 61 | 48.16 | 97.41 | 17.95 |
| May | 9.65 | 28.37 | 18.38 | 52 | 43.61 | 96.23 | 17.63 |
| June | 16.84 | 38.30 | 27.46 | 72 | 25.03 | 84.31 | 14.22 |
| July | 18.82 | 37.71 | 26.75 | 0 | 30.54 | 85.76 | 22.35 |
| August | 19.63 | 37.63 | 25.34 | 0 | 39.58 | 95.58 | 22.32 |
| September | 16.81 | 31.27 | 21.63 | 50 | 45.56 | 99.56 | 20.18 |
| October | 13.55 | 28.63 | 17.04 | 110 | 59.69 | 99.76 | 15.87 |
| November | 9.24 | 21.70 | 12.34 | 241 | 65.73 | 100.00 | 8.93 |
| *2020* | | | | | | | |
| March | 5.70 | 17.45 | 11.28 | 111 | 52.93 | 99.23 | 15.45 |
| April | 8.22 | 21.56 | 16.60 | 12 | 46.50 | 98.22 | 19.58 |
| May | 12.63 | 28.13 | 21.06 | 6 | 35.28 | 90.95 | 25.12 |
| June | 14.89 | 30.36 | 25.02 | 2 | 30.43 | 88.22 | 29.09 |
| July | 18.48 | 34.35 | 26.94 | 35 | 31.75 | 88.51 | 29.28 |
| August | 19.88 | 35.24 | 26.74 | 0 | 34.68 | 92.14 | 24.78 |
| September | 17.60 | 30.44 | 21.82 | 29 | 43.96 | 96.43 | 18.03 |
| October | 11.62 | 24.75 | 15.30 | 66 | 48.25 | 99.91 | 15.28 |
| November | 9.24 | 21.74 | 12.59 | 49 | 66.58 | 100.00 | 11.59 |
| *2021* | | | | | | | |
| March | 2.81 | 22.06 | 10.72 | 58 | 50.12 | 98.25 | 17.98 |
| April | 4.76 | 27.83 | 15.62 | 39 | 47.45 | 98.56 | 20.64 |
| May | 9.26 | 32.53 | 20.35 | 9 | 42.18 | 97.13 | 24.07 |
| June | 12.32 | 37.76 | 27.62 | 3 | 31.11 | 87.12 | 25.70 |
| July | 16.23 | 39.11 | 28.19 | 8 | 32.12 | 86.19 | 28.66 |
| August | 17.92 | 36.22 | 26.25 | 25 | 35.17 | 94.16 | 24.33 |
| September | 15.23 | 33.13 | 22.17 | 38 | 44.18 | 97.14 | 19.26 |
| October | 8.56 | 27.26 | 17.02 | 182 | 50.11 | 99.88 | 13.21 |
| November | 6.37 | 21.13 | 11.12 | 239 | 65.19 | 100.00 | 8.96 |

Table 2. Variation of pH and $EC_w$ in the planted units of the HSSFs CW from March 2019 to November 2021. Three-year average (± standard deviation), minimum and maximum values are shown (*n = 81* ).

| Parameter | Treatment | | | | | Discharge in soil[1] | *t* -Test[2] |
|---|---|---|---|---|---|---|---|
| | Main inlet | *Arundo donax*- planted unit | | *Cyperus alternifolius* -planted unit | | | |
| | | Outlet | Variation (%) | Outlet | Variation (%) | | |
| pH | 7.9 ± 0.4<br>(6.9 - 8.3) | 7.4 ± 0.3<br>(6.6 - 7.8) | 7.1 | 7.3 ± 0.4<br>(6.2 - 8.1) | 8.4 | 6-8 | * |
| $EC_w$ (µS/cm) | 446.3 ± 150.4<br>(225.5 - 811.7) | 598.8 ± 153.7<br>(298.2 - 897.6) | - 37.2 | 535.2 ± 133.9<br>(274.2 - 822.5) | - 24.4 | – | * |

[1] Threshold values for Italian Decree 152/2006.
[2] Significant differences between influent and effluent values (p ≤ 0.01).

Table 3

Click here to access/download;Table;Table 3.xls

Table 3. Main chemical composition of the dairy wastewater from the inflow and outflow of the planted units. Removal efficiency from March 2019 to November 2021. Three-year average (± standard deviation), minimum and maximum values are shown (*n = 81* ).

| Parameter | Treatment | | | | | Discharge in soil[1] | *t*-Test[2] |
|---|---|---|---|---|---|---|---|
| | Main inlet | *Arundo donax*- planted unit | | *Cyperus alternifolius* -planted unit | | | |
| | | Outlet | Removal efficiency (%) | Outlet | Removal efficiency (%) | | |
| TSS (mg/L) | 154.9 ± 17.1<br>(133.6 - 184.5) | 16.7± 6.4<br>(19.2 - 43.2) | 82.8 | 27.9 ± 7.1<br>(18.4 - 45.6) | 82.0 | 25 | * |
| $BOD_5$ (mg/L) | 87.4 ± 7.1<br>(72.2 - 97.9) | 17.9 ± 6.2<br>(10.3 - 28.9) | 79.6 | 21.1 ± 7.2<br>(12.4 - 35.1) | 76.1 | 20 | * |
| COD (mg/L) | 219.4 ± 20.2<br>(188.2 - 253.3) | 84.2 ± 14.9<br>(61.2 - 113.3) | 61.8 | 87.3 ± 11.2<br>(62.3 - 104.5) | 61.4 | 100 | * |
| TN (mg/L) | 91.4 ± 6.7<br>(82.5 - 112.4) | 44.2 ± 4.5<br>(37.8 - 53.2) | 51.5 | 46.0 ± 4.0<br>(34.9 - 50.2) | 53.1 | 15 | * |
| N-$NH_4$ (mg/L) | 56.1 ± 3.5<br>(50.4 - 63.2) | 30.7 ± 3.1<br>(26.5 - 39.1) | 45.2 | 32.7 ± 3.7<br>(27.1 - 40.3) | 41.7 | – | * |
| ON (mg/L) | 29.6 ± 1.4<br>(27.1 - 31.5) | 18.7 ± 0.8<br>(17.2 - 19.9) | 36.7 | 11.1 ± 1.2<br>(9.2 - 12.7) | 40.7 | – | * |
| TP (mg/L) | 14.6 ± 0.6<br>(13.4 - 15.9) | 8.5 ± 0.7<br>(7.5 - 9.8) | 41.8 | 8.6 ± 0.5<br>(7.4 - 9.3) | 41.1 | 2 | * |
| CU (mg/L) | 0.066 ± 0.0<br>(0.059 - 0.074) | 0.033 ± 0.0<br>(0.028 - 0.042) | 49.8 | 0.017 ± 0.0<br>(0.014- 0.017) | 45.7 | 0.10 | * |
| Ni (mg/L) | 0.019 ± 0.0<br>(0.018 - 0.021) | 0.011 ± 0.0<br>(0.016 - 0.011) | 43.2 | 0.007± 0.0<br>(0.005 - 0.009) | 39.9 | 0.20 | * |
| Pb (mg/L) | 0.027 ± 0.0<br>(0.023 - 0.049) | 0.015 ± 0.0<br>(0.010 - 0.018) | 44.7 | 0.008 ± 0.0<br>(0.006 - 0.011) | 39.3 | 0.10 | * |
| Zn (mg/L) | 0.23 ± 0.1<br>(0.22 - 0.25) | 0.09 ± 0.0<br>(0.08 - 0.12) | 58.3 | 0.04 ± 0.0<br>(0.03 - 0.07) | 46.3 | 0.50 | * |

[1]Threshold values for Italian Decree 152/2006.

[2] Significant differences between influent and effluent values ($p \leq 0.01$).

Table 4. Main microbiological composition of the dairy wastewater from the inflow and outflow of the planted units. Removal efficiency from March 2019 to November 2021. Three-year average (± standard deviation), minimum and maximum values are shown (*n = 81* ).

| Parameter | Treatment | | | | | Discharge in soil[1] | *t* -Test[2] |
|---|---|---|---|---|---|---|---|
| | Main inlet | *Arundo donax*- planted unit | | *Cyperus alternifolius* -planted unit | | | |
| | | Outlet | Removal efficiency (%) | Outlet | Removal efficiency (%) | | |
| TC (CFUs/100 ml) | 4.45 ± 3.70[3] | 3.53 ± 3.08 | 88.1 | 3.89 ± 3.10 | 83.2 | – | * |
| | (4.34 - 4.57) | (3.19-3.73) | | (3.16 - 3.80) | | | |
| FS (CFUs/100 ml) | 4.02 ± 3.32 | 3.24 ± 2.81 | 83.9 | 3.45 ± 2.84 | 81.3 | – | * |
| | (3.86 - 4.13) | (2.93 - 4.46) | | (2.93 - 4.46) | | | |
| *Escherichia coli* (CFUs/100 ml) | 3.04 ± 2.37 | 2.10 ± 1.65 | 88.3 | 2.16 ± 2.58 | 86.9 | ≤ 3.69 | * |
| | (2.95 - 3.19) | (1.85 - 2.36) | | (1.93 - 2.34) | | | |
| *Salmonella* spp. (CFUs/100 ml) | Absent | Absent | | Absemt | | – | |

[1] Threshold values for Italian Decree 152/2006.
[2] Significant differences between influent and effluent values ($p \leq 0.01$).
[3] The average concentration values are shown as units of $Log_{10}$.

 

Table 5. Morphological parameters of giant reed and umbrella sedge plants in the HSSFs CW. Three-year average values (± standard deviation), minimum and maximum values are shown (n = *16* ).

| Parameter | *Arundo donax* -unit | *Cyperus alterifolius* -unit |
|---|---|---|
| Height of culm/stem (cm) | 147.4 ± 40.1 | 126.1 ± 46.3 |
| | (72 - 201) | (52 - 184) |
| Number of culms/stems (no./$m^2$) | 24.7 ± 2.01 | 81.2 ± 2.87 |
| | (18 - 29) | (61 - 95) |
| Diameter of root system (cm) | 42.3 ± 1.58 | 35.1 ± 1.78 |
| | (36 - 49) | (25 - 47) |
| Length of root system (cm) | 33.1 ± 1.77 | 29.1 ± 1.87 |
| | (25 - 35) | (22 - 34) |

Figure 1



Figure 2



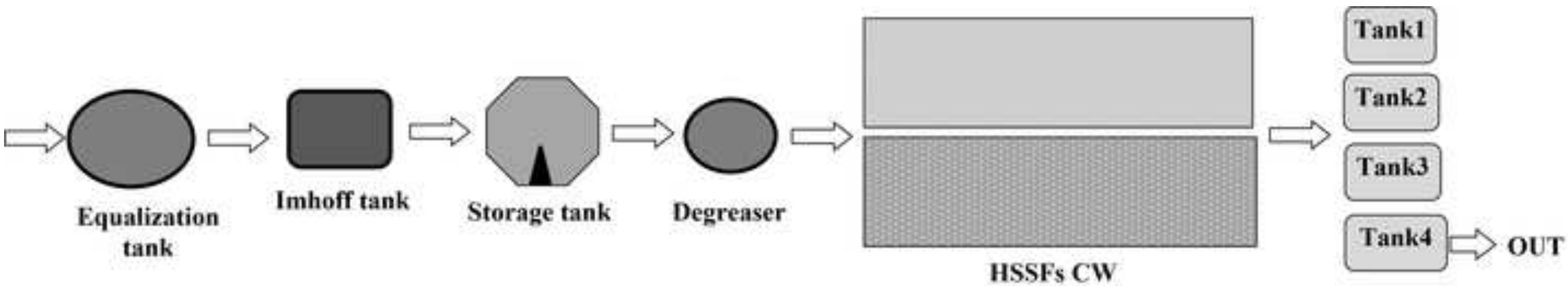
Equalization tank
Imhoff tank
Storage tank
Degreaser
HSSFs CW
Tank1
Tank2
Tank3
Tank4
OUT

Figure 3



Figure 4

Click here to access/download;Figure;Figure 4.tif

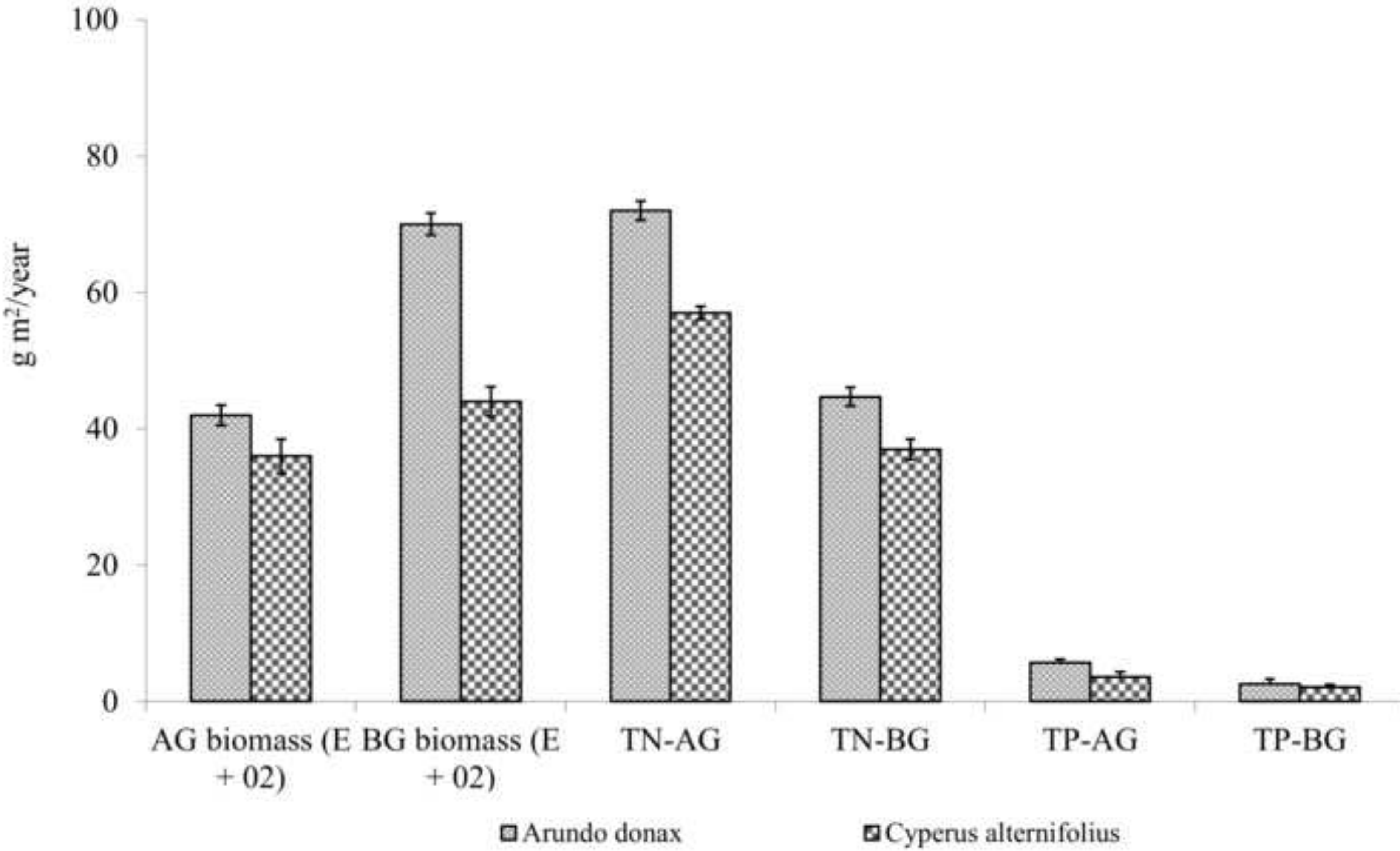

Figure 5

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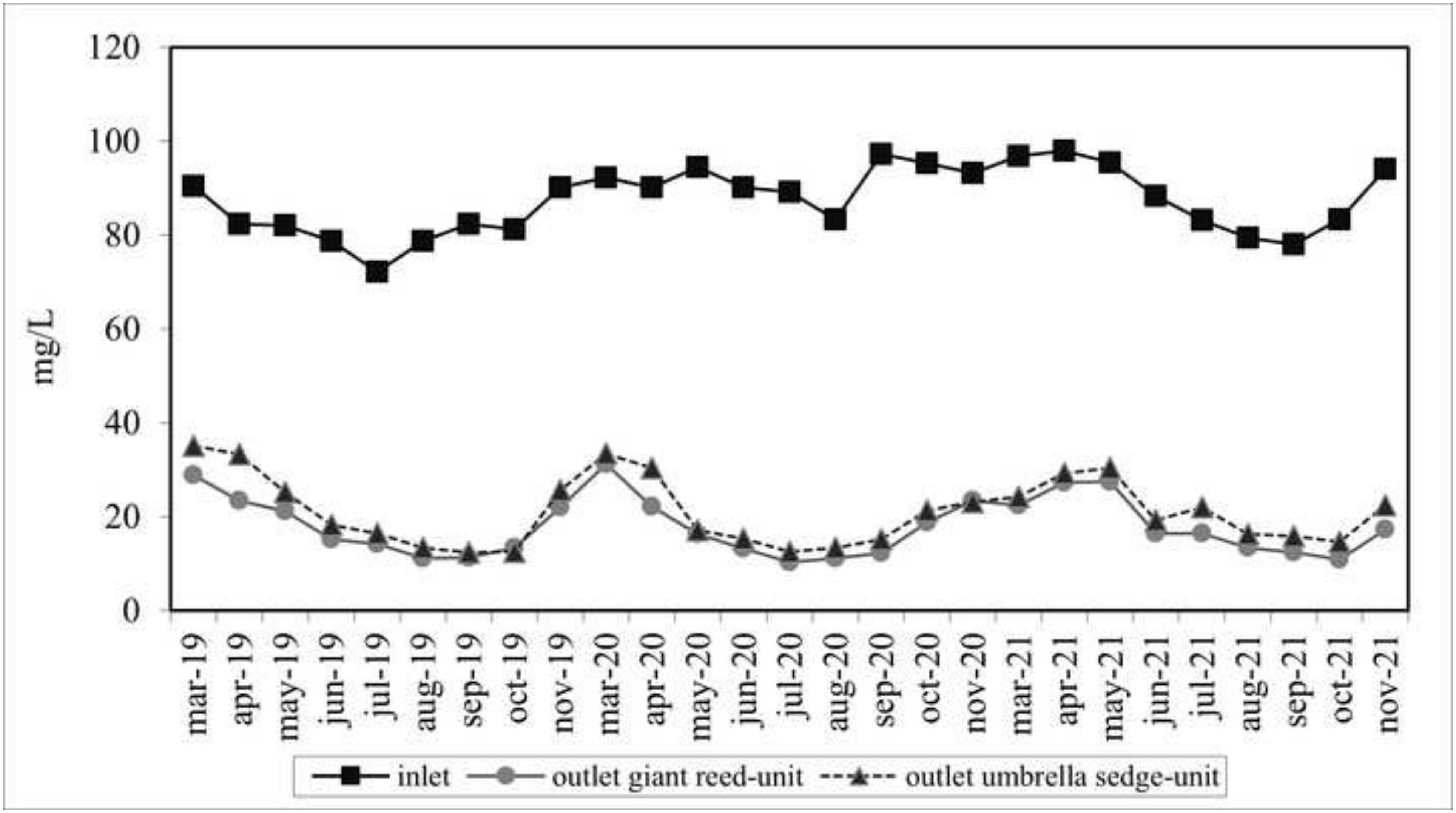
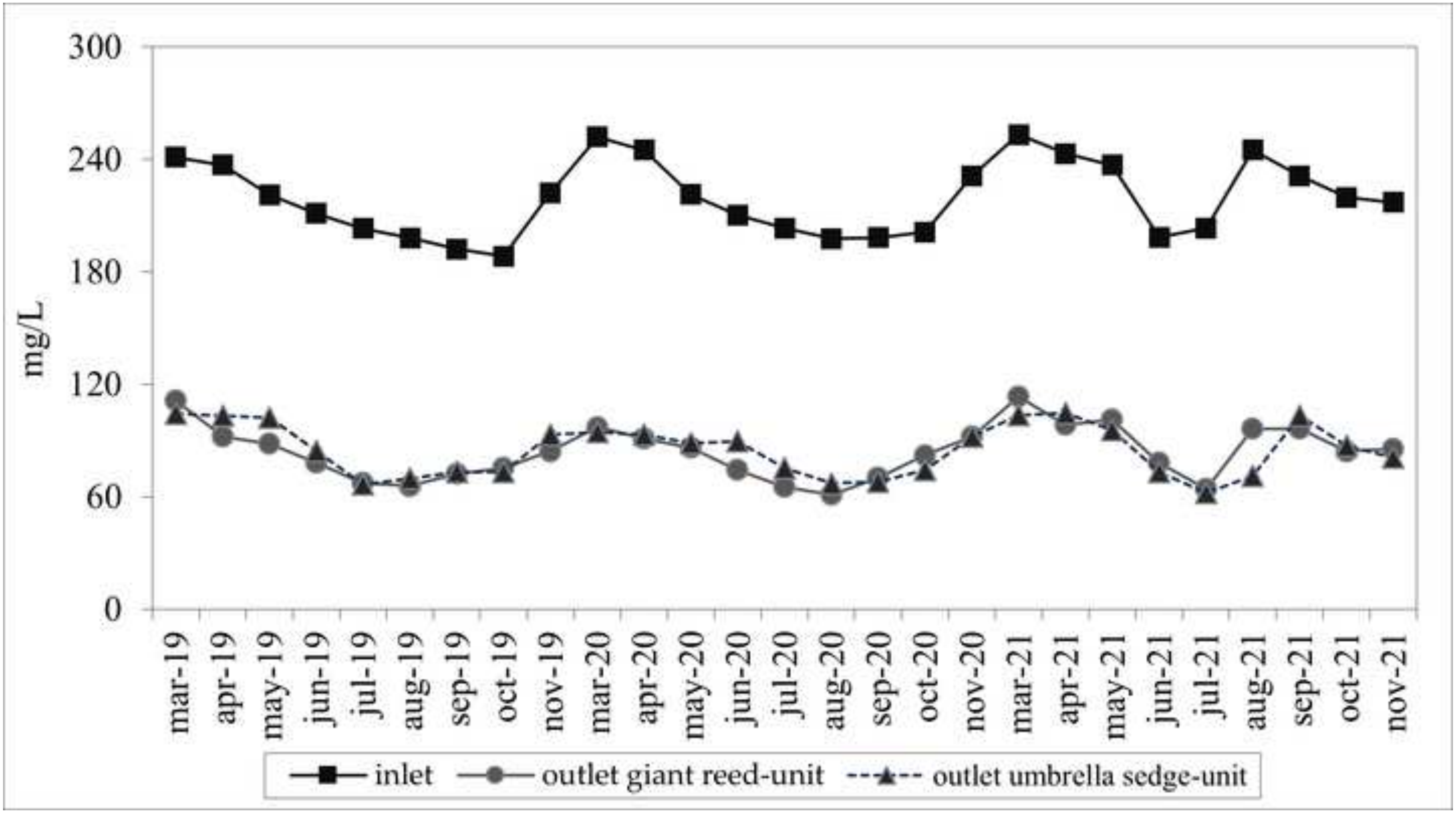


Figure 6





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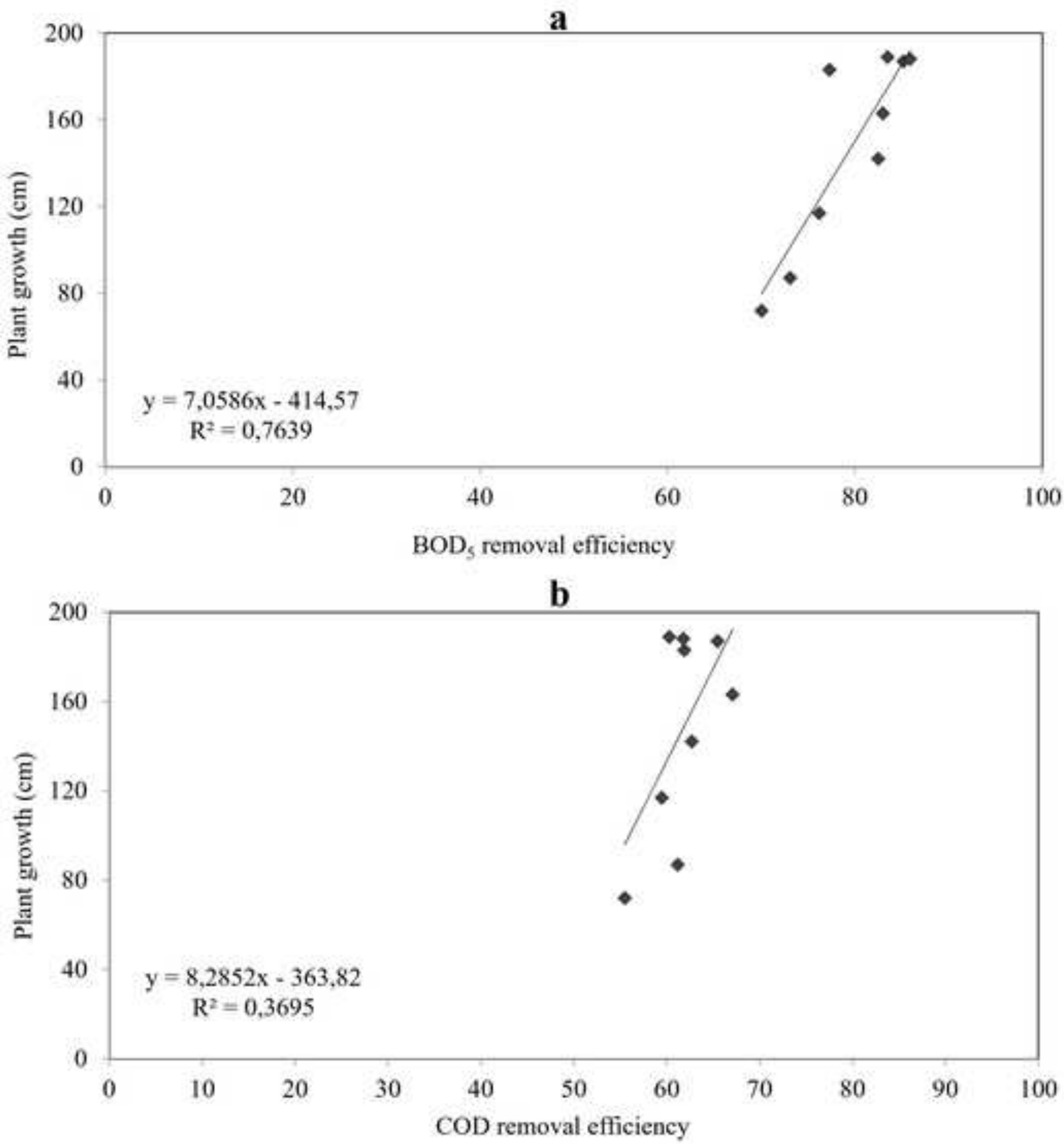
nov-21
inlet
outlet giant reed-unit
outlet umbrella sedge-unit

Figure 7

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200
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Plant growth (cm)
y = 7,0586x - 414,57
R² = 0,7639
0
20
40
60
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BOD5 removal efficiency
b
200
160
120
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40
0
Plant growth (cm)
y = 8,2852x - 363,82
R² = 0,3695
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10
20
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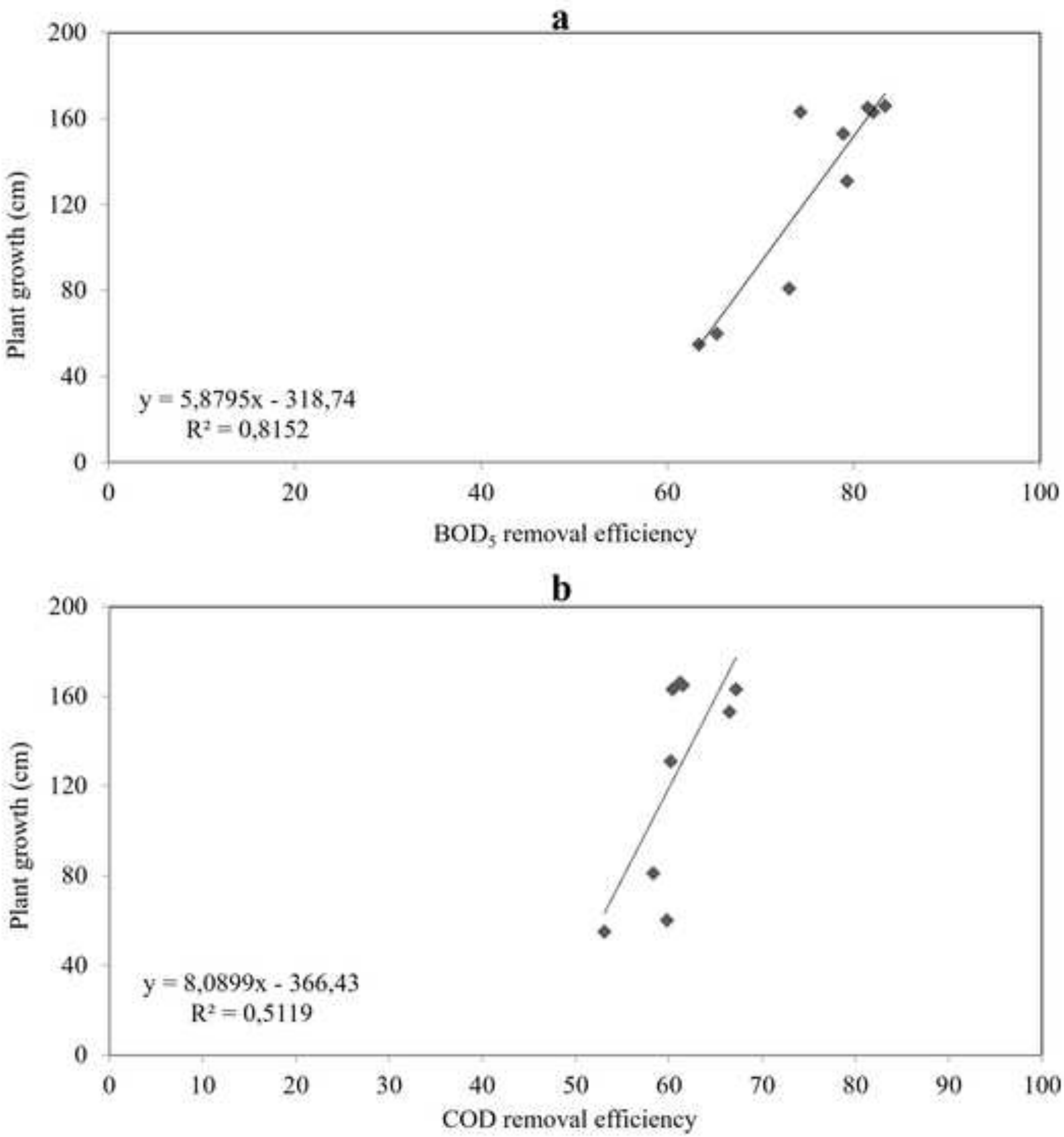
90
100
COD removal efficiency

Figure 8

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Plant growth (cm)
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80
100
BOD5 removal efficiency
b
200
160
120
80
40
0
Plant growth (cm)
y = 8,0899x - 366,43
R² = 0,5119
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100
COD removal efficiency

Figure 9 Click here to access/download;Figure;Figure 9.tif

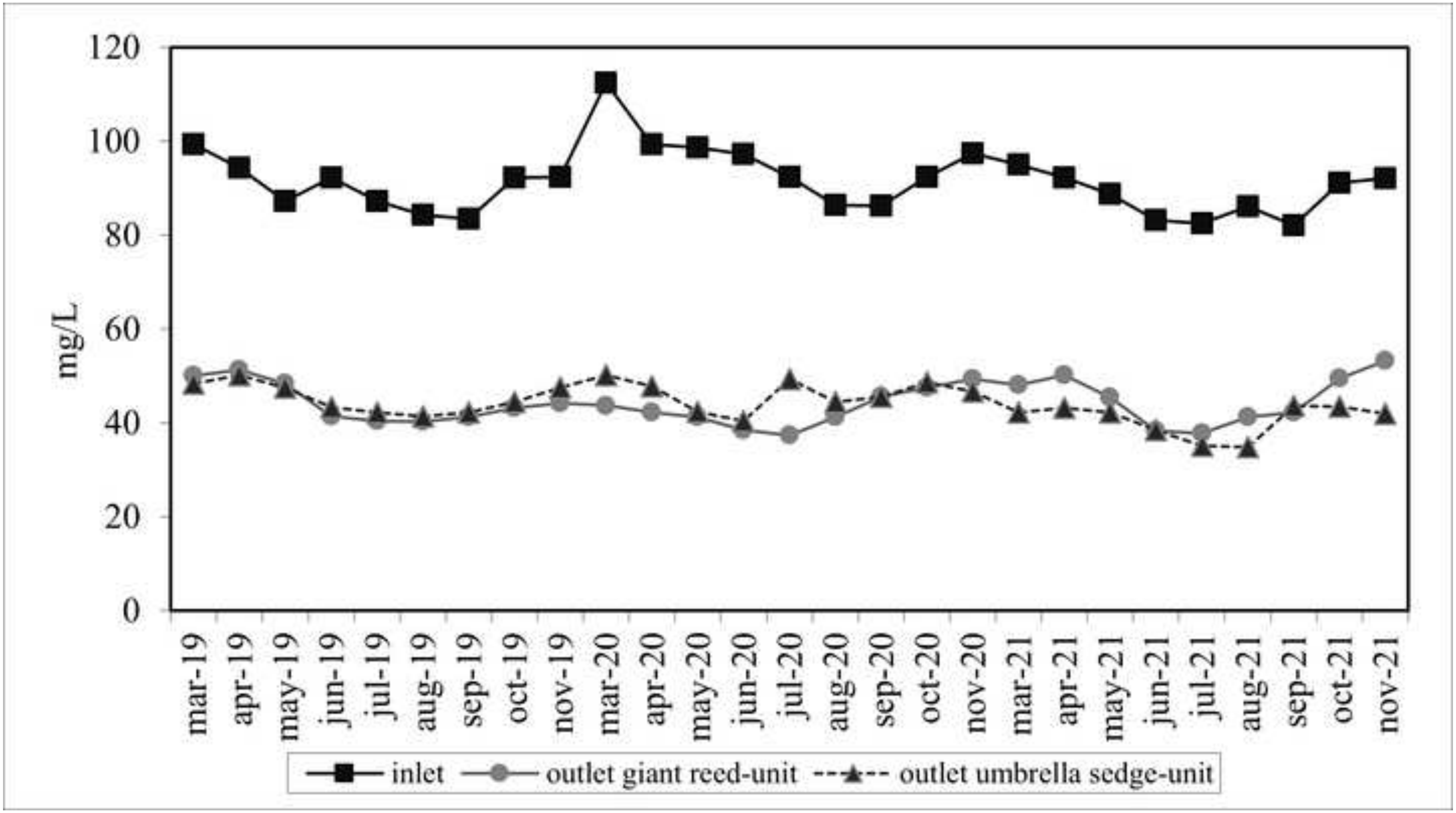

Figure 10

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