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# The use of constructed wetlands for the treatment of agro-industrial wastewater – A case study in a dairy-cattle farm in Sicily (Italy)

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**ABSTRACT**

Wastewaters generated by agro-industrial operations often represent an unsustainable cost for farms due to high wastewater-treatment management costs. The wastewater produced by dairies, wineries or oil mills may vary in quantity and in quality depending on the time of the year, making the use of a conventional treatment system less efficient and more costly. Constructed wetland systems (CWs) provide low-cost technology and an efficient solution in the treatment of a number of wastewaters from agriculture. They are simple to build, have low maintenance costs and are sustainable compared to conventional treatment methods. This paper shows a case study that was carried out on a dairy-cattle farm, located in the West of Sicily (Italy). The aim of the study was to evaluate the pollutant removal efficiency of an horizontal subsurface flow system (HSSFs) for the treatment of dairy parlor wastewater (DWWs). An HSSFs was planted with Phragmitesaustralis (Cav.) Trin. ex Steud. DWWs were initially pre-treated with a degreaser and two Imhoff septic tanks were used for the removal of suspended solids. The results showed a significant removal rate of organic pollutants by the HSSFs. The system was efficient in the treatment of DWWs and represents an artificial engineering system that corresponds well to Italian legislation requirements concerning the management of agricultural wastewaters from dairy-cattle farms.

*Keywords:* Dairy wastewater; Horizontal subsurface flow system; Pollutant removal efficiency; Sustainable wastewater management

## 1. Introduction

The dairy sector is of fundamental importance for the agri-food industry in Sicily (Italy): the area has a large number of dairy farms and produces a great variety of products. Milk processing is usually carried out using standard methods in order to guarantee a high quality. However these processes determine the production of large quantities of contaminated wastewaters that farms must then treat. These wastewaters differ according to the product obtained and their qualitative composition is also affected by wastewater management, climate, operating conditions and methods as stated by Prazeres et al. [1] and Pattnaik et al.[2]. Dairyparlor wastewaters (DWWs)mainly contain disinfectant/cleaning products used for washing and disinfecting rooms and equipment, residues of milk, fats and by-products of milk processing, such as whey and buttermilk. Whey is considered the most important pollutant in DWWs, this is due not only to the high organic load but also to the volumes that are generated [3]. The waters contains all the soluble components of milk,such as lactose, proteins, minerals and other minor components, as stated by Prazeres et al. [1] and Carvalho et al. [3]. With regard to the chemical composition, DWWs generally do not have high heavy metal content but are characterized

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by high organic matter content, nutrients and salts [3–6]. Agronomic literature highlights that whey wastewaters can be re-used in the production of horticultural crops and can lead to increases in crops yield due to a significant supply of nutrients and organic matter [6–8]. However, the direct application of these wastewaters can determine negative effects on the chemical and physical characteristics of agricultural soils [9,10]. Nutrients, organic matter and salt concentrations in DWWs are significantly higher than urban wastewaters, they tend to vary through the course of the year and can negatively affect soil characteristics and crop requirements as stated by Healy et al. [11] and Johnson et al. [12]. Moreover, the high organic loads of DWWs can be a source of pollution for surface waters if these wastewaters are discharged directly into water bodies, leading to eutrophication [13,14]. These are the main reasons why the use of sustainable technologies for the treatment of DWWs is encouraged. DWWs are usually treated using physicochemical methods (e.g. coagulation, flocculation, precipitation) and/or biological methods (e.g. anaerobic and aerobic digestion) [1,3]. In Sicily and other Mediterranean countries, small and medium dairy farms are often isolated from conventional treatment plants and are located close to areas with high agricultural and ecological importance, such as lakes, lagoons, ponds and open fields. In these areas, the direct application of DWWs can determine significant environmental risks for the agro-ecosystems, as claimed by Carvalho et al. [3]. In recent years, the use of constructed wetlands (CWs) in the agricultural sector for the treatment of DWWs has been gaining popularity due to their relatively low capital costs, low maintenance requirements and high pollutant removal efficiency [13–18]. CWs are engineering systems which use plant species and microbial communities to eliminate organic, inorganic and microbial pollutants from wastewaters. Horizontal sub-surface flow system (HSSFs) is a CW system that can be used for the treatment of various agricultural wastewaters [18]. As stated by Mantovi et al. [16], dairy farms can benefit from a HSSFs for the treatment of wastewaters which have low organic matter and nutrient content,such as those deriving from washing operations of milking areas little trampled over by cows or other animals. However, taking into account the standard characteristics of DWWs, a HSSFs has to be combined with an effective pretreatment technology to obtain the highest treatment performance: the accumulation of a significant amount of organic matter in the substrate can contribute to the clogging of substrate porosity, reducing the pollutant removal efficiency as noted by Nguyen [17]. In Sicily, HSSFs have been used to treat different types of wastewaters mainly for re-use in crop irrigation [19–30]. However, little attention has been paid to the use of HSSFs for the treatment of DWWs . The aim of this study was to evaluate the pollutant removal efficiency of an HSSFs used to treat the DWWS produced by a Sicilian dairy-cattle farm.

## 2. Materials and methods

### 2.1. Test site

The study was carried out in the two years 2014–2015 on a dairy-cattle farm located in central-western Sicily (750 m a.s.l.). The farm produced milk for cheese-making (Pecorino, Caciocavallo and Ricotta cheeses). The farm had two sheds and an average of 120 lactating cows. During the tests, the waiting area heavily trampled on by the cows, was periodically washed and cleaned in order to dispose of the solid wastes. Wastewaters from this waiting area were collected in a storage tank also used for shed wastes. DWWs used in the study were composed of wastewaters from the waiting area (following solid liquid separation), milking room and then mixed with domestic wastewaters produced by the staff of the dairy farm.

### 2.2. Description of the HSSFs

The HSSFs was built in 2013 and located downhill from the dairy-cattle farm. The system included 2 units (A, B) each 12.5 m long and 6.0 m wide, providing a total surface filter bed area of 75 m$^2$ (Fig. 1). The two units were designed in series. Filter bed depth was 0.9 m to allow for greater root development and to create a larger rhizosphere. The considerable bed depth was due to the low temperatures usually recorded in the area during the winter season. The slope was 2%, needed to obtain regu-

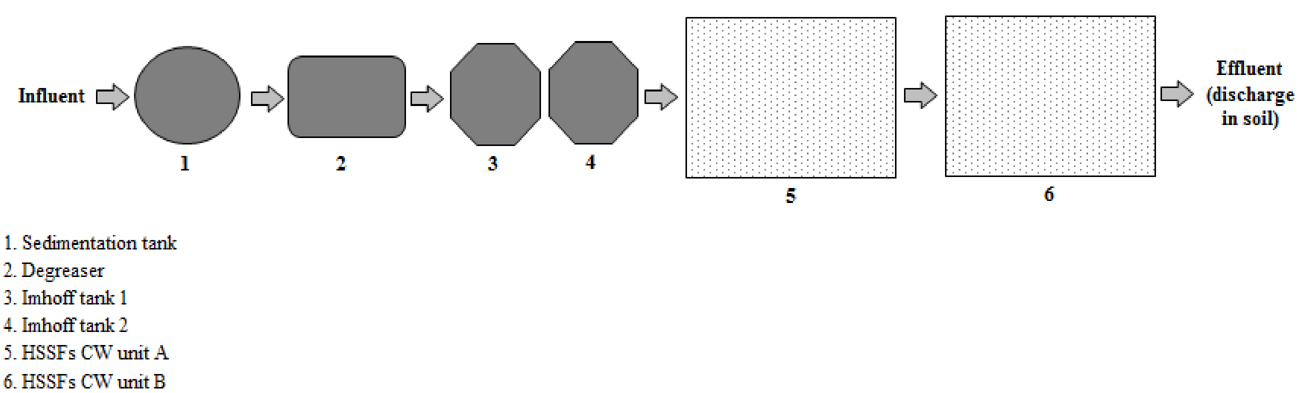


Fig. 1. Layout of the system for the treatment of the DWWs mixed to domestic wastewaters.

lar flow. The units were filled with a washed substrate of evenly-sized 4–16 mm silica quartz river gravel. A layer of coarse gravel (30–40 mm) was laid close to the inflow and outflow points of both the units in order to facilitate the flow of the incoming and outgoing wastewater. Each unit was lined with a PVC geomembrane which was put over a layer of straw and was covered with a layer of nonwoven fabric. Unit A and B were planted with *Phragmites australis* (Cav.) Trin. ex Steudel (common reed). The DWWs, mixed with domestic wastewaters,were initially fed into a sedimentation tank in order to remove suspended solids. Then they were treated with a static degreaser to separate fats, soaps and food wastes. Both flotation and settling processes were carried out inside the degreaser. Monthly sediment removal was carried out monthly by a trained member of staff; the fatty films on the surface of the degreaser was also removed. Residues were disposed in a landfill. Two Imhoff septic tanks were also used for the removal of suspended solids. Subsequently, the pretreated wastewaters were pumped through a 120 mm-diameter perforated pipe into unit A to ensure even distribution of the wastewater throughout the filter bed section and to reduce the risk of hydraulic short-circuiting. The pipe was placed 15 cm from the surface of the substrate. The pre-treated wastewaters then flowed into unit B for further treatment. The homogeneous distribution of wastewater was ensured through a timer-controlled pumping system. The pumping was continuous throughout the day without variations in time. The HSSFs was connected to a subsurface irrigation system that was used to disperse the treated wastewaters (TWWs) into the soil. The subsurface irrigation system was designed taking the number of equivalent inhabitants, soil permeability, porosity and texture into consideration. The two planted HSSFs units operated under the same hydraulic conditions and were tested under a hydraulic loading rate (HLR) of 10 cm $d^{-1}$.

### 2.3. Plant species material

In 2013, *Phragmites australis* plants were collected from natural wetland areas close to the dairy-cattle farm and the rhizomes used for propagation in a small nursery. Fertigation was applied to the *plantulae* in order to increase plant vegetative vigour. An automatic fertigation system was used for water and fertilizer irrigation. The fertigation unit was composed of a fertilizer tank for the concentrated solution, valves, a main filter and water meter. For injection of the fertilizer solution into the irrigation system an injection pump was used. The time and rate of fertilizers was regulated to ensure correct application of nitrogen (N) and phosphorus (P) for *plantulae* growth; nitrogen was applied in the form of ammonium nitrate and phosphorus in the form of phosphoric acid. Potassium was not applied due to high soil K content. Two injectors were used to introduce the fertilizers into the irrigation water: one for N application and the other one for P application .Fertigation was applied 4 times per month from December to January. Subsequently, the root rhizomes were planted in the two units in February 2014 with a plant density of 5 rhizomes $m^{-2}$. Plant height, stem density, and dry weight of the aboveground plant parts (leaves and stems) were used to determine plant growth. Biometric measurements were taken from April to September for each year. Plant height was determined fortnightly by measuring the maximum height of 10 plants selected randomly from the initial, the middle and the end sections of each unit. Maximum height was measured from the surface of the filter bed to top leaf insertion and only plants in good vegetative and phytosanitary condition were selected [31]. Leaf number per plant and the length of root systems were determined monthly by making a leaf count and measuring the roots length of 10 plants selected randomly for each unit. Stem density was determined monthly on an area of 1 $m^2$ for each unit. According to Allen et al. [32], four crop growth stages were identified in 2014 only: a) initial stage: from green up 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; d) late-season stage: from canopy senescence to plant harvest. In October 2014, the plants were cut back to a height of 50 cm above the gravel bed. The fresh aboveground (stems and leaves) and below ground (roots and rhizomes) weights were determined on a representative sample of 10 plants from each unit. The above and below ground biomass dry weight was then calculated by drying the collected plant material in an oven at 62°C for 72 h. Nitrogen levels in the aboveground biomass parts of the plants were determined using a CHN analyzer, in full compliance with plant biomass basic analysis standards. This process was repeated following the next cutting, after 12 months.

### 2.4. Dairy wastewater analysis

Wastewater samples were taken monthly during the period April–September for both years, amounting to a total of 48 times. Hourly sampling always occurred at the same time, usually coinciding with milking. The samples were collected at the inflow and outflow of each unit. A litre of wastewater was collected from each of the two points at each sampling. There was only one influent sampling point for each of the units. The influent sample was taken close to the pipe while the effluent sample was collected at the mouth of the outflow pipe. The influent and effluent samples were instantaneous samples. The pH was determined directly on site using a portable Universal meter (Multiline WTW P4).Using Italian water analytical methods [33], total suspended solids (TSS), biochemical oxygen demand ($BOD_5$), chemical oxygen demand (COD), total nitrogen (TKN), organic nitrogen (Organic N), ammonia nitrogen ($NH_4$–N), total phosphorus (TP), chloride (Cl), calcium (Ca)and heavy metals (Cu, Ni, Pb and Zn) were determined. Total coliforms (TC), faecal streptococci (FS) and *Escherichia coli* (*E. coli*) levels were determined by membrane filter methods, based on standard methods for water testing [34]. Removal efficiency (RE) of the HSSFs was based on pollutant concentrations and was calculated according to IWA [35]:

$$RE = \frac{C_i - C_0}{C_i} * 100$$

where $C_i$ and $C_0$ are the mean concentrations (mg/L) of the pollutants in the influent and effluent.

### 2.5. Climatic data

Data on rainfall, temperature and potential evapotranspiration were collected from a meteorological station belonging to the Sicilian Agro-Meteorological Information Service situated close to the pilot HSSFs. The station was synchronized with GMT in order to operate using synoptic forecast models. It was equipped with a MTX datalogger (model WST1800) and various sensors. This equipment provided data on the wind speed (m/s), minimum daily relative moisture levels (%), average daily soil temperature (°C), average daily air temperature (°C), total daily solar irradiance (MJ/m$^2$), total daily rainfall-frequency [days mm > 1] (%), and rainy days per year [days mm > 1] (%).

### 2.6. Statistical analysis

An estimate of variability in the data populations was determined using mean ± standard error calculations. Statistical analysis was performed with the software MINITAB release 17.1 for Windows.

## 3. Results and discussion

### 3.1. Plant growth and biomass production

Common reed, although not native to Sicily, is considered to be the most commonly used macrophyte in the world of CWs [36,37]. It was chosen as the plant component in the HSSFs units on the dairy-cattle farm due to the need for a macrophyte which was known to adapt well, was resistant, and had a good phyto-extractive capacity and tolerance to high wastewater loads [38]. During the testing period, growth of this macrophyte was affected by the climate. In the study area, temperature trends were consistent with the ten-year average. Maximum average temperatures were 21.4°C and minimum average temperatures were 11.2°C. The most significant rainfall events were recorded in the 2$^{nd}$ 10-d period of February 2015. Dry periods were observed between July and August. For both the years, plants reached maximum plant growth during the summer months at the same time as high temperatures and relative humidity levels. Growth rates of common reed were also affected by the propagation method used. The use of the rhizomes ensured good and uniform plant establishment in the two units and produced good biomass levels from as early as the first year compared to other propagation methods [37]; *Phragmites australis* usually takes three or four growing seasons to reach maximum standing-crop, as stated by Vymazal and Krőpfelová [36]. If we look at growth rates for common reed over the year, the species reached maximum height in August 2014 at 197 cm (Table 1). Stem density during the two years varied from 111 to 321 stems m$^{-2}$ and was similar to those of other studies. Stem density decreased over the period due to a self-thinning process which is common in plant monocultures, as said by Vymazal and Krőpfelová [36]. For example in unit A, stem density was 209.7 stems m$^{-2}$ in 2014 and 188.2 m$^{-2}$ in 2015. Consequently, the number of stems can vary over time and this could affect the pollutant removal rates of the macrophyte. In this study, plant cover of the species was high in both units at 91% for unit A and 89% for unit B on average. When comparing the two units, however, plants grew faster in unit A than unit B and this could be attributed to the greater amounts of organic and inorganic nutrients received by the first unit, as compared with the second one. However, when considering the duration of all the growth stages of the species in 2014 (Fig. 2), the initial stage was found to be the shortest whilst crop development and mid-season stages were the longest and were found to be between the beginning of April and the end of September. The flowering stage for common reed occurred at the beginning of July and at the end of August but it was not found abundant in terms of number of flowers emitted. The senescence for the above-ground biomass occurred on average in mid-October. Climate conditions in the area, temperatures in particular, allowed plant growth to continue up to late Autumn, delaying the dormancy period of the species. Harvesting was carried out when plant growth slowed down considerably and when the uptake capacity of the macrophyte decreased significantly. In Table 1, the results of above and below ground biomass production of common reed are also shown. Plant biomass is highly variable depending on climate, hydraulic and operative characteristics of CWs, type of wastewaters and interactions between these factors, as claimed by Vymazal [39]. In this study, the average dry matter for the aboveground parts (leaves and stems) of the common reed was 3847.5 g m$^{-2}$ y$^{-1}$, and 3336.8 ± 203.6 g m$^{-2}$ y$^{-1}$ for the below ground parts (roots and rhizomes). With comparing the two years, we observed that plant biomass increased from 2014 to 2015 despite the decrease in stem density. For example in Unit B, aboveground biomass was 3540 g m$^{-2}$ in 2014 and 3810 g m$^{-2}$ in 2015 and stem density was 174.6 and 169.1 stems m$^{-2}$ in 2014 and 2015 respectively. Plant biomass

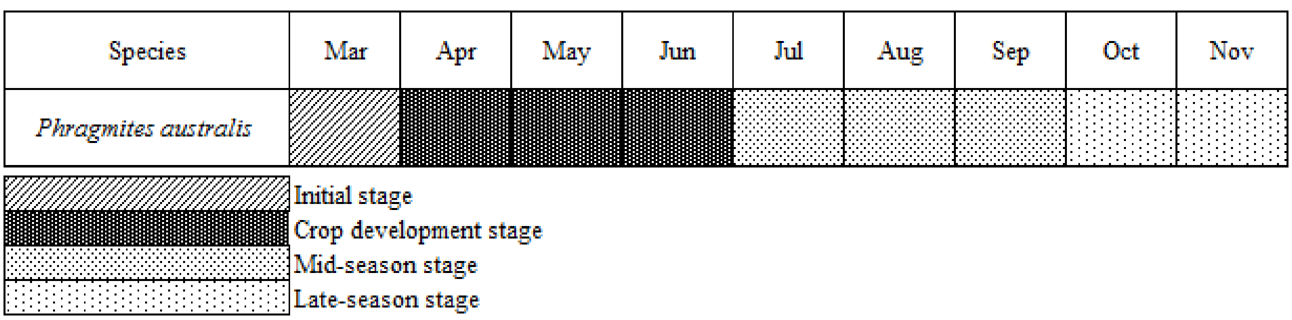


Fig. 2. Evolution of the main growth stages of *Phragmites australis* in 2014.

Table 1
Biometric and productive parameters of *Phragmites australis* plants in the HSSFs. Two-year average (± standard error), minimum and maximum values are shown

| Parameters | Average values (± standard error) | | |
|---|---|---|---|
| | Unit A | Unit B | Unit A/Unit B (%) |
| Stem density (no. m$^{-2}$) | 188.2 ± 13.6 | 169.3 ± 10.2 | 111.6 |
| | (127–321) | (111–298) | |
| Stem height (cm) | 177.7 ± 2.3 | 170.3 ± 2.2 | 104.3 |
| | (161–197) | (159–185) | |
| Number of leaves per plant | 15.2 ± 1.0 | 14.3 ± 0.9 | 106.9 |
| | (9–21) | (8–21) | |
| Root length (cm) | 26.7 ± 1.1 | 22.5 ± 0.9 | 118.6 |
| | (17–32) | (14–27) | |
| Dry matter of aboveground parts (g m$^{-2}$ year$^{-1}$) | 4020 ± 310 | 3675 ± 135 | 109.3 |
| Dry matter of belowground parts (g m$^{-2}$ year$^{-1}$) | 3452 ± 207 | 3221 ± 234 | 107.1 |

continued to increase from the first to second year of tests due to an increase in plant weight, subsequent to nutrient uptake. The results of plant biomass were consistent with other studies carried out in different climatic areas. In their review, Vymazal and Krőpfelová [36] report the range of dry matter between 413 and 9.860 g m$^{-2}$ for 12 natural stands from Europe, Asia and Australia. The same authors highlight that the biomass of *Phragmites australis* is similar in HSSFs with a values of dry matter between 788 and 5.070 g m$^{-2}$. In an HSSFs, in Italy, for the treatment of DWWs, the dry matter of culms and root systems of common reed were on average 0.87 and 0.66 kg m$^{-2}$ respectively [16]. Differences in the biomass production levels of common reed led to important consequences concerning the removal of the principal components of the wastewaters. In particular, the greater the production of biomass the greater the nutrient uptake by the plants, as stated by Leto et al. [22]. Literature highlights that, in a HSSFs, pollutant removal by the plants is usually lower than that performed by microorganisms in the gravel substrate [35,40,41]. Many studies show that the aboveground and belowground parts of the macrophytes provide large surface areas for the development of biofilm, which is responsible for most of the microbial processes occurring in the wetlands. It has also been widely documented [42–44] that macrophytes release oxygen into the rhizosphere, thereby influencing sediment biochemical cycles through the effect the vegetation has on redox conditions in the sediment. As claimed by Bialowiec et al.[45], oxygenation carried out by plant roots has a very important effect on wastewater treatment in CWs, including the effects it has on redox potential, which is fundamental in nutrient removal and on microbial activity. In this study, nitrogen levels detected in plant tissues were due to high total N concentration in the DWWs (Fig. 3). On the contrary, heavy metal content in the plant biomass was not analysed due to a low concentrations in the DWWs. A comparison between plants from the two units did not show great differences in N concentrations in plant tissues. Similar results were obtained by Tanner [37] who compared the growth and nutrient uptake of eight emergent plant species using milking parlor wastewaters. The same author stated that common reed showed the highest concentration of N, of the 8 test species, highlighting the fast establishment and growth of this species in experimental wetland mesocosms. In a comparative analysis of CWs with different plant species, Tomoko et al. [46] observed that the unit planted with common reed performed better in $NH_4$–N, $NO_3$–N, $NO_2$–N and TKN removal than those planted with other species. These results highlight the high N-uptake potential of common reed when it is used for phytoremediation purpose in CWs. However, in a CWs, the choice of species influences pollutant removal efficiency and, in this case, the adaptability of the species to its environmental and management conditions is crucial; albeit a consideration which is not universally accepted by all researchers.

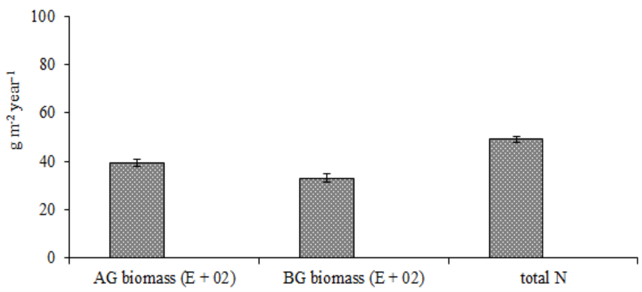


Fig. 3. Aboveground (AB), belowground biomass (BG) and nitrogen content (N) in total biomass of the *Phragmites australis* plants. Bars indicate standard error of the means.

### 3.2. Dairy wastewaters characteristics

The main chemical and microbiological composition of the different influent wastewaters are shown in Table 2. Generally, pollutants in milking and washing wastewaters are significantly higher than in domestic wastewaters and concentrations tend to vary throughout the year. In this study, the organic and nutrient concentrations in the three types of wastewater varied significantly throughout the seasons and this was in agreement with Brewer et al. [47]. The highest concentrations organic and nutrients parame-

Table 2
Main chemical and microbiological composition of the different influent wastewaters. Two-year average values (± standard error) are shown

| Parameters | Domestic wastewater | Milking wastewater | Washing wastewater |
|---|---|---|---|
| pH | 7.81 ± 0.04 | 7.92 ± 0.06 | 7.31 ± 0.03 |
| TSS (g $L^{-1}$) | 0.05 ± 0.01 | 0.41 ± 0.01 | 1.13 ± 0.01 |
| $BOD_5$ (mg $O_2$ $L^{-1}$) | 55.3 ± 0.6 | 512.4 ± 2.9 | 564.6 ± 8.2 |
| COD (mg $O_2$ $L^{-1}$) | 93.1 ± 1.5 | 1005.8 ± 30.8 | 1670.1 ± 51.8 |
| Total N (mg TKN $L^{-1}$) | 30.8 ± 0.4 | 75.2 ± 1.7 | 68.3 ± 0.6 |
| N–$NH_4$ (mg $NH_4$ $L^{-1}$) | 12.8 ± 0.4 | 30.8 ± 0.3 | 21.7 ± 0.7 |
| Organic N (mg N $L^{-1}$) | 17.5 ± 0.1 | 43.3 ± 0.8 | 49.0 ± 0.4 |
| TP (mg P $L^{-1}$) | 7.6 ± 0.1 | 10.2 ± 0.3 | 16.9 ± 0.4 |
| Chloride (mg Cl $L^{-1}$) | 117.0 ± 1.2 | 67.5 ± 0.8 | 43.3 ± 0.2 |
| Ca (mg $L^{-1}$) | 85.6 ± 1.4 | 729.4 ± 5.5 | 484.7 ± 5.0 |
| Cu (mg $L^{-1}$) | 0.104 ± 0.003 | 0.070 ± 0.001 | 0.080 ± 0.002 |
| Ni (mg $L^{-1}$) | 0.016 ± 0.001 | 0.025 ± 0.001 | 0.036 ± 0.001 |
| Pb (mg $L^{-1}$) | 0.072 ± 0.001 | 0.019 ± 0.006 | 0.022 ± 0.001 |
| Zn (mg $L^{-1}$) | 0.190 ± 0.001 | 0.393 ± 0.004 | 0.481 ± 0.008 |
| Total coliforms (CFU 100 $ml^{-1}$) | $2.1 \times 10^4$ ± 0.01 | $2.4 \times 10^5$ ± 0.01 | $1.7 \times 10^5$ ± 0.02 |
| Faecal streptococci (CFU 100 $ml^{-1}$) | $7.1 \times 10^3$ ± 0.02 | $5.3 \times 10^4$ ± 0.03 | $6.6 \times 10^4$ ± 0.02 |
| *Escherichia coli* (CFU 100 $ml^{-1}$) | $1.2 \times 10^3$ ± 0.01 | $1.4 \times 10^5$ ± 0.01 | $1.1 \times 10^5$ ± 0.02 |

ters were found in spring and summer. Variation in chemical parameters were greatest in milking wastewaters and this was mainly due to dairy farm management practices. Domestic wastewaters did not vary as much as washing and milking wastewaters in the course of each of the years. Average pH values were between 7.3 (washing wastewater) and 7.9 (milking wastewaters). Highest TSS, $BOD_5$, organic N and TP levels were found in washing wastewater. Milking wastewaters were found to have the highest average values of total N (75.2 mg $L^{-1}$), ammonia nitrogen (30.8 mg $L^{-1}$) and calcium (729.4 mg $L^{-1}$). Domestic wastewaters had lower organics and nutrient concentrations despite the presence of chlorides (117.0 mg Cl $L^{-1}$). Heavy metals were found at low levels in all three types of wastewaters and this was in agreement with Mantovi et al. [16]. However, no significant variations in heavy metals concentrations were determined over the seasons. With regard to the microbiological parameters, milking wastewaters were found to have the highest average TC and *E. coli* levels whilst the highest average FC levels were found in milking wastewaters. Microbiological parameter results varied over the tests period in the same way as the chemical parameters. According to international literature, DWWs tend to vary depending on dairy farming activities and practices and their composition is not necessarily stable over the time.

### 3.3. Removal of pollutants in the pilot-scale HSSFs

Results of pH variation and chemical pollutant removal levels of the wastewaters (DWWs + domestic wastewaters) are shown in Table 3. At the inlet of the HSSFs units, pretreated wastewater pollutant concentrations were significantly lower than prior to pre-treatment. The intensive pretreatment (1 sedimentation tank + 1 degreaser + 2 Imhoff tanks) provided efficient treatment of the wastewaters through physical, chemical and biological processes. Odours were not unpleasant and at the inlet of HSSF no coarse matter was found in the wastewaters. The pH value at the inflow pipe was slightly alkaline whilst at the outflow it was less alkaline in both of the planted units with a greater decrease in unit B. This phenomenon was in agreement with findings from [48]. As claimed by Gerardi [49], the decrease in pH value in a HSSFs is due to the production of $CO_2$ caused by the decomposition of plant residues, by the removal of various components of the wastewaters retained in the root area and by the nitrification of ammonia. TSS, $BOD_5$ and COD removal efficiencies were on average close to 70%. TSS removal efficiency was found to be similar in the two planted units. At outflow of unit B, TSS concentrations were on average higher than 70% and were consistent with values found in literature regarding the use of CWs for DWWs treatment. High TSS removal efficiency was due to filtration and sedimentation processes which were carried out by substrate and root systems of the macrophyte, as affirmed by Cooper et al. [50]. These physical actions allowed the DWWs to flow easily in the two planted units without causing blockages or preferential flow channels. Throughout the two test years, hydraulic short-circuiting occurred only twice, during plant dormancy. $BOD_5$ and COD removal rates varied on average from 64.2% to 70.9% and stayed within limits consistent with findings from other authors for HSSFs [51]. In general, the organic matter removal efficiency was facilitated mainly by the large surface area of the HSSFs and the high root density of common reed. As stated by Brix [38], part of the oxygen released by the macrophyte roots and rhizomes is usually used to aid organic matter aerobic biodegradation through heterotrophic bacteria. However, it is not possible to consider this quantity of oxygen as sufficient to guarantee high organic

Table 3
Main chemical and physical composition of the wastewaters from the inflow to outflow of the HSSFs units. Removal efficiency (RE) from April to September 2014/2015. Average (± standard error), minimum and maximum values are shown

| Parameters | | Unit A (24 samplings) | | Unit B (24 samplings) | | |
|---|---|---|---|---|---|---|
| | Main inlet | *Phragmites australis* | | *Phragmites australis* | | |
| | | Outlet | RE (%) | Outlet | RE (%) | Threshold values for Italian Legislative Decree 152/2006 |
| Colour | P[a] | NP[b] | | NP | | – |
| Odour | NU[c] | NU | | NU | | – |
| Coarse matter | Present | Absent | | Absent | | Absent |
| pH | 7.61 ± 0.06 (7.22–8.02) | 7.3 ± 0.04 (7.04–7.51) | | 7.41 ± 0.04 (7.04–7.61) | | 6–8 |
| TSS (mg $L^{-1}$) | 250 ± 0.01 (230–270) | 80 ± 0.01 (70–100) | 66.1 | 23 ± 0.01 (10–40) | 71.3 | 25 |
| $BOD_5$ (mg $O_2$ $L^{-1}$) | 214.5 ± 4.2 (183.1–243.9) | 64.6 ± 1.1 (51.2–69.4) | 69.7 | 21.3 ± 0.3 (19.2–23.5) | 67.0 | 20 |
| COD (mg $O_2$ $L^{-1}$) | 384.4 ± 4.4 (357.9–421.3) | 111.6 ± 2.4 (98.0–132.1) | 70.9 | 39.7 ± 0.7 (35.2–46.5) | 64.2 | 100 |
| Total N (mg TKN $L^{-1}$) | 45.9 ± 0.6 (42.9–51.2) | 27.0 ± 0.8 (22.5–34.2) | 41.4 | 14.0 ± 0.2 (12.9–15.2) | 47.5 | 15 |
| $N–NH_4$ (mg $NH_4$ $L^{-1}$) | 16.9 ± 0.3 (14.2–19.6) | 10.2 ± 0.3 (8.5–13.2) | 39.6 | 6.6 ± 0.2 (5.2–8.6) | 35.0 | – |
| Organic N (mg N $L^{-1}$) | 29.6 ± 0.3 (27.1–31.5) | 18.7 ± 0.2 (17.5–19.6) | 36.7 | 11.1 ± 0.3 (9.2–12.7) | 40.7 | – |
| TP (mg P $L^{-1}$) | 10.2 ± 0.1 (9.4–11.2) | 6.0 ± 2.9 (5.2–6.9) | 40.8 | 3.6 ± 1.2 (2.7–4.7) | 40.1 | 2 |
| Chloride (mg Cl $L^{-1}$) | 54.5 ± 0.8 (48.1–60.2) | 44.1 ± 1.1 (37.5–51.1) | 19.1 | 36.1 ± 1.0 (31.2–46.5) | 18.3 | 200 |
| Ca (mg $L^{-1}$) | 374.1 ± 2.5 (361.2– 401.3) | 317.3 ± 4.3 (291.2– 341.2) | 15.2 | 269.8 ± 3.7 (238.9– 297) | 15.0 | – |
| Cu (mg $L^{-1}$) | 0.066 ± 0.001 (0.058–0.074) | 0.044 ± 0.001 (0.038–0.051) | 33.5 | 0.030 ± 0.001 (0.028–0.033) | 30.4 | 0.1 |
| Ni (mg $L^{-1}$) | 0.019 ± 0.001 (0.017–0.021) | 0.011 ± 0.001 (0.009–0.016) | 43.1 | 0.007 ± 0.001 (0.004–0.007) | 39.9 | 0.2 |
| Pb (mg $L^{-1}$) | 0.027 ± 0.001 (0.022–0.045) | 0.015 ± 0.001 (0.010–0.018) | 44.7 | 0.008 ± 0.004 (0.006–0.011) | 39.2 | 0.1 |
| Zn (mg $L^{-1}$) | 0.24 ± 0.002 (0.23–0.25) | 0.09 ± 0.004 (0.08–0.12) | 58.3 | 0.04 ± 0.023 (0.03–0.06) | 46.3 | 0.5 |

[a] perceptible; [b] not perceptible; [c] not unpleasant

matter removal rates. Based on the constructional and functional characteristics of HSSFs, the high organic matter removal efficiency in the two planted units was also due to additional anaerobic biodegradation processes carried out by localized bacteria around the root system of the plants and the substrate particles, as confirmed by IWA [35]. Total N and TP removal efficiency was found to be extremely similar in units A and B. In a HSSFs, nitrogen removal usually depends on physical settlement, denitrification and plant/microbial uptake. In this study, nitrogen removal rates were lower than organic matter removal in both the planted units due to low oxygen levels in the system. This had a significant effect on the ammonium nitrification process, believed by many researchers to be one of the most important nitrogen removal mechanisms in the system. The contribution of common reed to N removal was significant in terms of total N levels in the above/belowground biomass although, according to literature [50,51], this is only part of all the N removed. Moreover, plant uptake does not represent continual N removal unless plants are routinely harvested, as stated by Healy et al. [11]. As regards P removal, the same authors affirm that, in a CWs, P can be removed through short-term or long-term storage. Macrophytes and bacteria provide an initial removal mechanism of P but this is a short-term P storage. The authors also state that the only long-term P storage in a CWs is via peat accumulation and substrate fixation. In our study, P removal was found close to 40%, probably due to a gradual filling of the sorption sites by the plant root systems, by the presence of under composed plant material around the substrate surface and by the adsorption properties intrinsic to the substrate. Literature highlights that P removal is efficient only in the short-term, immediately after the introduction of the wastewater, even in low plant density conditions and when the gravel adsorption processes are highly active [52] whilst, in the long-term, the burial capacity of the P biogeochemical cycle is limited [53]. However, according to Maehlum et al. [54], adsorption capacity in HSSFs can be used to obtain significant P removal rates, although adsorption would seem to decrease over time. Both planted units showed low Ca removal rates. Our findings were consistent with literature [4] and the reasons for the low removal rates of alkali metals, such as calcium, have been explained by Kadlec and Wallace [55]. The removal efficiency of heavy metals was on average high for Ni, Pb and Zn in unit A in each of the year. Alternatively, low Cu removal rates were observed in both the units despite the low concentration of this heavy metal in the DWWs inflow. Heavy metal accumulation in macrophytes can vary significantly,depending on the plant part as well as the type of element, as stated by Stoltz and Greger [56]. Low heavy metal concentrations in the aerial parts of common reed are reported by Baldantoni et al. [57], although other authors [56] highlight that Zn accumulation in plant tissue can be considerable. Literature indicates a great variation in heavy metal concentrations, such as Cu, Ni and Zn, in aerial parts and roots of common reed over the seasons. In our research, we did not determine heavy metal concentrations in plant tissues but it would be of great interest to investigate how, when and to what extent heavy metals accumulate in above and belowground plant parts; Bragato et al. [58] claim that greatest heavy metal content is found in late autumn after senescence. Bacterial load removal was particularly effective from as early as the initial sampling stages (Table 4). At the outflow of unit B, maximum removal levels were found to be 78.7% for *E. coli*, 79.3% for FC and 83.5% for TC. No *Salmonella* spp. was reported either in the DWWs inflow or outflow of the two planted units. The high *E. coli*, TC and FC removal rates were due to a combination of physical, chemical and biological processes carried out by the plants, nematodes, virus and bacteria, such as filtration and adsorption, chemical oxidation, sedimentation, predation by nematodes and protists, and viral and bacterial activity, as stated by various authors [38,51]. The aerobic conditions in the planted units, partly due to atmospheric air circulation and partly to the translocation of oxygen from the root system of common reed, favoured the production of a greater bacterial biofilm and promoted pathogen load removal efficiency, as noted by El-Khateeb et al. [59]. In Italy, the discharge of TWWs into the soil is regulated by Legislative Decree 156/2006. Regarding chemical contamination, the above mentioned Decree establishes threshold values as being lower than those found in international guidelines. Furthermore, the Decree establishes threshold values for *Escherichia coli* in relation to the environmental context, soil hygiene and soil use. In this research, average chemical parameter results at the outflow were not all within the legal limits of the Italian Decree.

### 3.4. Worldwide DWWs treatment with the use of CWs

In Italy, in a HSSFs planted with common reed and used for the treatment of dairy parlor wastewaters and domestic wastewaters, with an initial pretreatment using an Imhoff tank and plastic filter, $BOD_5$ and COD removal rates were 93.7% and 91.9% respectively, with highly significant FC, TC and *E.coli* removal rates [16]. The same authors state that CWs can be considered appropriate technology in the treatment of DWWs produced on isolated rural farms, leading, thereby, to a reduction in pollutant levels which are then acceptable for discharge into soil and surface waters. In Lithuania, in a HSSFs planted with common reed for the treatment of domestic wastewaters and wastewaters from a dairy farm, the authors [60] claim that this system provides efficient removal of total N and T, they also state that organic pollutant and nutrient removal depends on filter loads. Total N removal rates were similar to those found in our study in percentage terms. In Vermont (USA), Lee et al. [61] used hybrid CWs with horizontal and vertical flows planted with *Schoenoplectus fluviatilis* to treat DWWs and affirmed that CWs showed high potential for contaminant removal in DWWs, even under cold climate conditions, although greater ammonia reduction being observed

Table 4
Main microbiological composition of the wastewaters from the inflow and outflow of the HSSFs units. Removal efficiency (RE) from April to September 2014/2015. Average values (± standard error) are shown

| Parameters | | Unit A (24 samplings) | | Unit B (24 samplings) | | |
|---|---|---|---|---|---|---|
| | Main inlet | *Phragmites australis* | | *Phragmites australis* | | |
| | | Outlet | RE (%) | Outlet | RE (%) | Threshold values for Italian Legislative Decree 152/2006 |
| Total coliforms (CFUs 100 ml$^{-1)}$) | 4.97 ± 0.02 | 4.17 ± 0.01 | 83.5 | 3.46 ± 0.01 | 81.0 | – |
| Faecal streptococci (CFUs 100 ml$^{-1)}$) | 4.49 ± 0.01 | 3.73 ± 0.01 | 82.3 | 3.08 ± 0.00 | 77.2 | – |
| *Escherichia coli* (CFUs 100 ml$^{-1}$) | 4.78 ± 0.02 | 3.98 ± 0.01 | 84.0 | 3.20 ± 0.01 | 83.2 | 3.69 |
| *Salmonella spp.* (CFUs 100 ml$^{-1}$) | absent | absent | | absent | | – |

*the microbiological values are shown as units of $Log_{10;}$

**it represents a suggested threshold value.

in late summer. In New Zealand, in a study carried out on a HSSFs partially planted with *Schoenoplectus validus* and used for the treatment of dairy parlor wastewaters, total BOD removal increased from 50 to 80% and faecal coliform removal increased from 90–95% to > 99% with increasing wetland retention time over the tests period [62]. In the UK, in a study on a dairy farm, Cooper et al. [50] noted that the use of integrated CWs: 2 VSSFs (vertical subsurface flow system), 1 lagoon and 1 HSSFs, provided high organic and TSS removal rates but the system also limited nitrification. In Germany, in an experimental HSSFs planted with *Spartina pectinata*, *Phragmites australis* and *Carexacuti formi*, organic N removal rates were 80.6% higher than those found in our research [63]. In Japan, a real scale hybrid HSSFs and VSSFs CWs was used to treat milking parlor wastewaters under cold climate conditions. Researchers [64] found high removal rates for TSS (> 95%), $BOD_5$ and COD (> 89%), TN (>76%) and TP (>72%). In Ontario (Canada), in a hybrid CWs treating DWWs designed with a facultative pond for pretreatment followed by two FWSs (free water surface system) planted with *Typha latifolia* and *Typha angustifolia* and a vegetated filter strip, plant uptake for TKN and TP played a significant role in overall removal, taking into consideration the wetland age and nutrient loading [65]. In Ireland, in an integrated CWs used to treat contaminants and nutrients from dairy farmyard wastewater water, the phosphorus retention varied with the season (5–84%) with lowest amounts being retained during winter [66]. Further examples of CWs for the treatment of DWWs in various countries around the world were also reported by a number of researchers [11,18]. On comparison with data from our research, similar results were found concerning the removal of the main chemical and microbiological pollutants, even when parameters varied, such as the type of pretreatment, the system size and slope, the concentration levels of DWWs, the flow rates and the daily hydraulic load.

## 4. Conclusions

Constructed wetland systems represent a low-cost technology that can offer an efficient solution for the treatment of dairy parlor wastewaters. These systems are easy to build and to manage, and contribute to obtaining efficient removal of organic, inorganic and microbial pollutants from wastewaters. Dairy farms can benefit from the use of CWs as they are isolated from centralized conventional treatment plants and, in some cases, are located near to ecologically sensitive areas, where any type of risk to the environmental must be reduced to a minimum. However, DWWs need pretreatment technologies for prior contaminant removal due to higher organic loads and nutrient content than domestic wastewaters. The pretreatment of DWWs may increase TSS and COD removal rates to 90% and 50%, respectively, as confirmed by literature. The use of CWs to treat DWWs combined with other wastewaters, such as domestic wastewaters, is also possible, depending on the complexity of the pretreatment system and wastewaters volumes . In CWs, it is important to highlight the essential role plant species play in wastewaters treatment; furthermore, all the agronomic practices, such as transplanting and harvesting, are also vital in order to optimize the contribution of the plants in chemical and physical processes. In this research, results of 2-year tests in a Sicilian dairy-cattle farm highlight the high removal efficiencies of the HSSFs regarding the main chemical and microbiological parameters. At the outflow of the system, the average concentration values of the TWWs were within Italian Legislative limits (Decree 156/2006) regarding discharge of TWWs into the soil,with the exception of $BOD_5$ and TP. *Phragmites australis* showed significant nitrogen uptake and good tolerance to high wastewaters loads, confirming its role as a macrophyte with high phyto-extractive capacity. Currently, the use of CWs in DWWs treatment appears to be limited in Sicily and, in general, in the Mediterranean region, and one the main reasons for this is the system's inconstancy in obtaining high contaminant removal rates under different design and hydraulic conditions. The combination of various types of CWs, such as HSSFs combined with VSSFs, may increase the removal efficiency of organic and inorganic contaminants and also allow treated DWWs to be discharged into soils and surface waters.

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