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CICLO XXVII

Evaluation of phytodepuration intensified systems for the treatment of agricultural and livestock wastewaters

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Alla mia famiglia

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Riassunto

Il processo di intensificazione dell'allevamento suino in Italia ha portato negli anni ad un alta concentrazione di animali in alcune parti del paese, specialmente al nord, accrescendo i rischi di inquinamento ambientale. I potenziali danni sull'ambiente causati da queste attività riguardano l'eutrofizzazione delle acque superficiali e sotterranee, le emissioni di ammoniaca con conseguenti problemi di deposizioni acide in seguito a precipitazioni e di altri gas, gli odori, e il rischio di accumulo di metalli pesanti e di sali nei suoli agricoli. Inoltre la forte crescita negli ultimi anni del biogas nel settore agro-zootecnico ha generato un altrettanto rapido ed importante incremento dei quantitativi di digestato prodotto. I reflui derivanti da queste attività devono distribuiti secondo la normativa italiana (Direttiva CE 91/676), che si traduce spesso in un aumento della richiesta di superfici disponibili per la distribuzione dei reflui. Tuttavia, quando la superficie per lo spandimento è insufficiente, c'è la necessità di trovare altre soluzioni, che l'allevatore ovvia delocalizzando i reflui in altri terreni, sostenendo costi di trasporto non esigui. In questi ultimi anni la fitodepurazione si è rivelata di considerevole interesse come sistema di trattamento della frazione liquida dei reflui animali, rappresentando una valida soluzione per il loro smaltimento quando la superficie è insufficiente.

Questo lavoro ha esaminato sistemi intensivi di fitodepurazione per il trattamento di reflui agro-zootecnici, basati sull'economicità e sulla riduzione della superficie per la depurazione. La ricerca ha riguardato principalmente lo studio di sistemi pilota filtranti per il pretrattamento di refluo suino e digestato, allo scopo di depurare il refluo prima del trattamento di fitodepurazione, in cui sono utilizzati materiali sostenibili e di scarto. Inoltre è stato investigato un sistema di fitodepurazione sviluppato verticalmente, ove sono state testate diverse specie vegetali per il trattamento del refluo suino. Infine, una minor parte della ricerca è stata dedicata allo studio dell'idraulica di filtri, usati per il trattamento del fosforo,e alla crescita di alghe su refluo suino pretrattato per analizzare la rimozione chimica e l'eventuale accumulo di astaxantina. I sistemi di pretrattamento si sono rivelati abili nella depurazione del digestato e del refluo suino, con maggiori abbattimenti per N-NH_4, COD e TN, rispetto agli altri composti chimici. La fitodepurazione ha risaltato le potenzialità di alcune piante e le debolezze di altre per il trattamento del refluo suino, mostrando i maggiori abbattimenti di concentrazione per N-NH_4 (69-99%) e più bassi per gli altri parametri chimici.

Summary

The intensification of Italian pig breeding over the years has resulted in high livestock concentrations in some parts of the country, especially in the north, increasing environmental pollutions threats. The potential environmental hazards caused by these activities regard surface water or groundwater eutrophication, emission of ammonia with consequent problems of acid deposition due to rainfall, other gases, odours, and accumulation risk of heavy metals and salts in agricultural soils. In addition, the proliferation over time of biogas plants in the livestock and agricultural field has led to a rapid and significant increase in the digestate quantities produced. The wastewaters from these activities are distributed on fields according to the European Directive (91/676 EC), which often results in an increase in the demand for available surfaces for the distribution of the effluent. However, when the land for spreading is not sufficient, other solutions must be found, that breeders solve by relocating the wastewater in other areas, incurring high transport costs. In recent years phytodepuration has proved to be of substantial interest as a system for the treatment of animal wastewater liquid fraction, representing a valid solution for its disposal when the surface area is not adequate.

This work examined phytodepuration intensified systems for the treatment of agricultural and livestock wastewaters, based on low-cost solutions and reduction of the area required for treatment. The research concerned in particular the study of pilot filter systems using recycled and sustainable materials for the pretreatment of piggery wastewater and digestate, in order to purify wastewater for the phytodepuration treatment. A vertically arranged phytodepuration system was also investigated, where both wetland and halophytic plant species have been tested for the treatment of piggery wastewater. Lastly, a secondary part of this research was devoted to the hydraulics study of filters used for phosphorus treatment, and to the growth of algae on pretreated piggery wastewater to analyze the chemical removal and possible accumulation of astaxanthin. The pretreatment systems proved to be suitable for digestate and piggery wastewater depuration, with higher removal for NH_4-N, COD and total N than other chemicals. The phytodepuration highlighted the potential of several plants and the weaknesses of others for the treatment of piggery wastewater, showing highest concentration abatements of NH_4-N (69-99%) and lower abatements of the other chemicals.

Chapter I

GENERAL BACKGROUND AND OBJECTIVES OF THE THESIS

INTRODUCTION

Organic wastewaters in agriculture and nitrogen pollution

The adoption of increasingly intensive cropping and livestock systems, combined with progressively more sophisticated and industrialized transformation techniques of agricultural production, have resulted in increasing use of natural resources accompanied by significant problems in terms of environmental compatibility of the entire production model.

The reuse of animal effluents in agriculture is becoming more frequent because they represent a good fertilizer, supplying valuable nutrients to crops and improving soil properties. The agronomic use of effluents requires an adequate knowledge of the weather, local soil and cropping conditions, as well as the characteristics of the wastewater. Factors such as rainfall and temperature, soil texture and porosity, crop rotation and processing techniques adopted, are fundamental elements to be able to rationally define the correct utilization of effluents and to report any necessary "contraindications", in relation to the particular composition of wastewater.

Animal wastewaters used in agriculture can cause numerous pollution problems, related to nutrients, pathogens, heavy metals and salinity. The effects of environmental pollution from these effluents include the loss of nutrients in surface water and groundwater by leaching and scrolling phenomena (Hoonda et al., 2000). The most dangerous nutrients are the nitrogen and phosphorus compounds, both involved in the process of eutrophication of freshwater and seawater (Levine and Schindler, 1989). Among inorganic nitrogen forms, nitrate is lost by leaching with the risk of reaching groundwater, even causing toxicity for humans. The principal health hazards associated with the chemical constituents of wastewaters arise from the contamination not only of groundwater, but also of crops. The possible accumulation of certain toxic elements in plants results in the intake of toxic materials through eating crops irrigated with contaminated wastewater (Pescod, 1992).

In Italy the number of farms recorded in the last census was 1,620,884 (-32.4% compared to 2000), with an average size of 7.9 hectares of utilized agricultural area (UAA) (+ 44.2%). The UAA amounts to 12.9 million hectares (42.8% of the national territory, -

2.5% compared to 2000), with an average of 5.4 farms per square kilometre, while the total farmland area amounts to 17.1 million hectares (-9.0%) (ISTAT, 2012). The majority of farms are concentrated in the regions of Southern Italy, particularly Puglia, Campania, Calabria and Sicily are the first four regions with almost 48% of Italian farms. More than half of the UAA is cultivated as arable land (54.5%), a low percentage is used as permanent meadows and grassland (26.7%), tree crops (18.5%), and also as family vegetable gardens (0.2%).

The number of livestock farms in Italy amounts to 217,449, mostly cattle (44%), pigs (25%) and poultry (22%), mainly raised in Northern Italy. Compared to 2000, in general, the number of farms decreased, but the number of animals per farm augmented, meaning that breeding has become more intensive in the last 10 years. In particular pigs, with 9.6 million (+11.6%), and poultry, with 195 million (+14.1%), are the most common. Pig farming is mainly concentrated in Northern Regions, with almost 8 million animals in Piedmont, Lombardy, Veneto and Emilia Romagna (on average 356 pigs per farm, 6 times more than in 2000), representing 85% of the national stock (**Table 1.1**). Specifically, in the Veneto Region more than 798,000 pigs are raised, producing an average of 1,805,000 m^3/year of wastewater (ISTAT, 2012).

Piggeries produce large volumes of wastewater and have higher animal densities compared to other livestock types (Kumm, 2003; Bassanino et al., 2007; Healy et al., 2007; Harrington and Scholz, 2010). Piggery wastewater is characterized by high organic loading (organic N, organic P and particularly COD and BOD), with high contents of nutrients, such as N and P (Petersen et al., 2007; Lee et al., 2010), suspended solids and a high level of microbial population (Chelme-Ayala et al., 2011), Na, K, Cl and SO_4 (Krapac et al., 2002), trace metals such as Zn and Cu (Petersen et al., 2007), and Ca, Mg, Fe, Mn (Sánchez and González, 2005). Cu and Zn are essential micronutrients for pig metabolism and their feed is supplemented with these elements. Due to their poor bioavailability, Cu and Zn are added at levels that largely exceed physiological requirements (Jondreville et al., 2003). As a consequence, most of the dietary supply is excreted so the slurries contain high concentrations. The characteristics of liquid pig manure vary highly depending on the amount of water used to clean the stalls and the kind of pits used to collect the slurries (Sánchez and González, 2005), livestock feeding habits, zone climatology (González-Fernández et al., 2008), as well as the number of animals on the farm, their health state, feed composition (Suzuki et al., 2010), means used for cleaning, washing and disinfecting, and the sort of drugs used for animal treatment and disease prevention.

With its content of nutrients, therefore, pig slurry is often used as a cheap fertilizer (Meers et al., 2008) and is commonly recommended as an inorganic fertilizer (Li-Xian et al., 2007). Piggery wastewater is mainly composed of liquid manure, which has a low dry matter content (0.5-2%), implying more expensive transport costs and difficult land and crops application. Land spreading is often used where there are large areas of farmland (Harrington and Sholz, 2010); however when there isn't sufficient land, farmers must find a way to dispose of the surplus wastewater. The application of organic effluents to agricultural soils is regulated by the Nitrates Directive (91/676/EEC), which limits the amount of nitrogen that can be spread on farmland with the aim of reducing water pollution caused or induced by nitrates from agricultural sources. The maximum quantity imposed in Nitrate Vulnerable Zones (NVZ) is 170 Kg of N per hectare per year, raised to 250 Kg during the 2012-2015 period in Regions of the Padana Plain (Piedmont, Lombardy, Veneto and Emilia-Romagna) for crops with high nitrogen demand and long growing season (European Derogation No. L 287/36), and 340 Kg for other zones (Official Journal of the European Union, 2011). In Veneto Region NVZ include almost the entire plain area, and correspond to a UAA of 475,109 hectares (61% of the Padana plain). These limits pose particular challenges that cannot be solved just by the regulation of land spreading. Although biogas production systems allow the exploitation of livestock effluent as a source of income, they do not diminish the nitrogen load.

Table 1.1 Number of piggeries and pigs, with variation (%), detected in Italy in years 2000 and 2010, and mean number of pigs per farm (ISTAT, 2012).

Region	**Farms (N°)**			**Pigs (N°)**			**Pigs/farm (N°)**		
	2000	2010	Var. (%)	2000	2010	Var. (%)	2000	2010	Var. (%)
Lombardy	6,481	2,642	59	3,839,077	4,758,963	-24	592	1,801	-204
Emilia-Romagna	4,438	1,179	73	1,555,344	1,247,460	20	351	1,058	-201
Piedmont	3,120	1,197	62	923,700	1,112,083	-20	296	929	-214
Veneto	8,431	1,793	79	699,374	798,242	-14	83	445	-436
Other Regions	134,348	19,386	86	1,585,646	1,414,566	10	12	73	-508
Italy	156,818	26,197	83	8,603,141	9,331,314	-8	55	356	-547

In the EU, current issues such as global warming, demand for renewable energy, landfill tax on organic waste, demand for organic fertilizer, high fossil fuel prices, environmental pollution and legislation relating to the treatment and disposal of organic wastes are all important factors influencing increasing investments in Anaerobic Digestion (AD) that occurs in biogas plants (EurObserv'ER, 2010).

The number of Italian biogas plants in the agricultural and livestock field has, in fact, increased exponentially in the last years, from 50 pre-2000, to 994 in 2012, in which the installed electric power has also strongly augmented (756 MWe in 2012 versus 3MWe pre-2000) (Fabbri et al., 2013). The average installed power of these plants is 0.5 MWe with an average daily digestate production of 100 m^3 per installed MWe (Balsari et al. 2013), corresponding to an estimated 35,000–40,000 m^3/day of digestate. 77% of biogas plants have been built in Northern Italy, mainly in Lombardy (37%), followed by Veneto (15%), Emilia Romagna (14.4%) and Piedmont (10.7%).

Biogas plants used in the agricultural and livestock field are fed with animal manures, agricultural crops, energy crops, food residues and agro-industrial by-products. The feedstock, sometimes referred to as substrate, can be either a single input (e.g. animal manure) or a mixture of two or more types (this is termed co-digestion). In Italy the majority (62%) of biogas plants use a mixture of animal wastewaters and energy crops or agro-industrial by-products as input matrix, while 18% are powered with only animal manure. Biogas plants fed with animal wastewaters represent interesting solutions for livestock farmers, not only because they are an environmentally sustainable way to manage organic waste (Tambone et al., 2010) but also for incentives that the Italian government allocates to them.

In a biogas plant the AD process (carried out by anaerobic microorganisms) converts organic wastewater into biogas (a gas mixture of approximately 70% CH_4 and 30% CO_2) that can be used to generate heat and/or power. Digestate is the AD by-product and is considered a highly valuable biofertilizer (Lukehurst, 2010). Digestate, constituted by 5-10% of dry matter, comes from a complex set of reactions that do not significantly change the amount of total nitrogen introduced, but change the composition of nitrogen forms present in the starting matrix (Fabbri and Piccinini, 2010). During the AD process the organic nitrogen forms are broken down to produce biogas, while the amino group is released in solution as mineral nitrogen (ammonium). The magnitude of these reactions depends on the type of nitrogen compound and on the efficiency of the AD process. In order that the biological process can ensure the complete degradation and transformation of organic matter introduced into the biogas plant, important factors influencing the activity of bacteria and archaea should be monitored and kept steady. Anaerobic conditions, substrate temperatures, pH, solid biomass and liquid by-products quality, content of acids and ammonia nitrogen, development of substances such as sulphur compounds and presence of microelements are factors that directly affect the degrading activity of microorganisms (Reale et al., 2009). In the case of animal wastewater as input matrix, nitrogen is mainly in the ammonium form (up to 65-70% of the total nitrogen in piggery wastewaters) and after AD it increases to 80-85% of total nitrogen. Conversely, if the input matrix is constituted by crops or agro-industrial by-products, nitrogen is mainly in the organic form, and the quantity of ammonium-nitrogen produced after AD depends on the process efficiency, but in general represents 50-60% of the total nitrogen (Mantovi et al., 2009; Mantovi et al., 2012). The NH_4-N content in digestate can increase from 10 to 33% compared to undigested matrix (Mantovi et al., 2009). In addition to nitrogen, digestate contains other macro-nutrients (such as phosphorus), micro-nutrients and heavy metals, as well as persistent organic compounds that are not biodegradable (Adani and D'Imporzano, 2009). The P content of digestate is lower compared to the undigested matrix, because while passing through the biogas plant, small amounts of P (<10%) are lost (Field et al., 1984; Schievano et al., 2011). A few papers indicate much higher P losses up to 25% (Massé et al., 2007) or even 36% (Marcato et al., 2008). The probable causes are partial retention in the digesters due to precipitation processes (Field et al., 1984; Massé et al., 2007; Marcato et al., 2008; Schievano et al., 2011). Most of the heavy metals in manure are introduced through the livestock diet, and they are especially high in pig slurry, particularly Zn (403 mg/Kg dry matter) and Cu (364 mg/Kg dry matter) (Lukehurst et al., 2010). Digestates from feedstocks with a high degradability (e.g. cereal grains, manure from poultry and pigs with a diet high in concentrates) are characterized by high NH_4-N:total N ratios and narrow C:N ratios (Pötsch et al., 2005; Emmerling, and Barton, 2007; de Boer, 2008; Fouda, 2011). Cattle manures or fibrous feedstocks low in N (e.g. silage maize) lead to a low NH_4^+-N:total N ratio (Möller et al., 2010; Fouda, 2011). BOD_5, dissolved organic C, and the corresponding organic C:total N ratio of dissolved substances can be considered the most reliable indicators in describing digestate biodegradability (Alburquerque et al., 2012c). Higher BOD_5 values are found in digestate when cattle manure or solid lignocellulosic materials are used as biogas feedstocks and indicate a lower degree of microbial stability (more biodegradable material). Highly biodegradable digested materials are not suitable for agricultural use as they cause a high CO_2-C production and lead to N-immobilization in soil, thus greatly limiting their N-fertilizing potential in the short-term (Alburquerque et al., 2012c). When digestate is recycled to land as a biofertilizer, most of these micro-nutrients are fully utilized, as they are essential for plant and microbial growth. However, any heavy metals and persistent contaminants can cause problems (Marcato et al., 2008). For this reason, to reduce pollutants, holding tanks are the most common storage solution for digestate before the disposal or the spreading on farmland (Meers et al., 2008). Sørensen and Møller (2009) demonstrated that AD of animal slurries, as it increases the mineral N to organic N ratio, enhances the efficiency of N assimilation by crops. The efficiency obtained is comparable with that of mineral fertilizers (Sørensen and Møller, 2009). Ammonium-N can be easily lost by ammonia volatilization during storage and land spreading due to the alkaline pH of digestate (Sommer and Husted, 1995b). It is known that the digestate pH is higher than undigested slurry (on the range 7.0-8.0; Mantovi et al., 2009; Mantovi, 2012) for several factors that occur during AD process, and can increase from more than 0.5 to more than 2 units (Webb et al., 1985; Pain et al., 1990; Kirchmann, and Witter, 1992; Pötsch, 2005; Möller et al., 2008; Chantigny et al., 2009). A pH increase is usually due to formation of ammonium carbonate ($(NH_4)_2CO_3$) (Georgacakis et al., 1982; Webb et al., 1985) and the removal of CO_2 (Sommer and Husted, 1995a) as a result of the transformation of CO_3^{2-} and $2H^+$ to CO_2 and H_2O. Furthermore, the fatty acid contents of feedstocks are reduced by AD (Pain et al., 1990). Digestate pH is also affected by the concentration of basic cations (e.g. Ca, K), which increase digestate pH because the electric charge balance of the solution has to be neutral, thus decreasing the concentration of H^+ (Hjorth et al., 2010). Mineralization and reduction of multivalent ions in feedstocks (e.g. SO_4 $FeIII(OH)_3$) increase pH. Also the reaction between Mg, NH_4 and PO_4 ions causes the release of H^+ ions in solution (Le Corre et al., 2009). In addition, NH_4-N is nitrified rapidly in soil under favourable conditions, this form being highly available to crops but also subjected to leaching through the soil profile, which may result in groundwater pollution. Therefore, storage and land spreading operations with digestates must be carefully controlled to avoid negative environmental impacts.

Italian legislation (Article 52 paragraph 2-bis; Article 184 of Legislative Decree 152/06) defines digestate as a by-product if it comes from livestock effluents or residues of vegetable origin and residues of agro-industrial transformation and it is used for agronomic purposes. Digestate is then equated to livestock effluents, as well as animal wastewaters (Legislative Decree 152/09, with supplements and amendments). To facilitate the management and agricultural use of livestock effluents, many proposals for treatment systems have emerged in the last years. Concerning nutrients content (mainly nitrogen and phosphorus), treatments can be conservative or reductive. Conservative treatments operate by nutrients displacing in a concentrated fraction to a small volume, which can be exported and sold on the organic fertilizers market, or it can have an agronomic use, with the advantage of having reduced transportation costs. This type of treatment does not eliminate nutrients, but wastewater management is easier from technical and environmental points of view. There are several types of conservative treatments: liquid/solid separation, membranes filtration, evaporation under vacuum, stripping, anaerobic digestion (Chiumenti et al., 2012; Moscatelli and Fabbri, 2008). Digestate is usually separated into liquid and solid fractions, which have different organic matter and ammonia nitrogen compositions. The reductive treatments transform the nitrogen in molecular form and free it in the air. Among these, biological nitrogen removal is used for not-shovelable effluents and waste to energy for shovelable effluents treatments. In biological processes, the effluent volume remains unchanged, while in the case of the energy treatment the volume reduction amounts to 80-90% of the initial mass (Piva and Anselmi, 2014).

Conventional conservative treatments adopted by farmers have high cost for realization, maintenance, energy consumption and often do not solve the problem related to land when the nitrogen load coming from livestock effluents exceeds the amount that can be spread on fields. Particularly, in the case of digestate, the problem of the liquid fraction coming from the solid from liquid separation treatment usually used, rich in nutrients and nitrates, cannot be solved only by land spreading. Indeed, spreading the excess slurry over arable land may result in groundwater contamination and eutrophication of surface waters (Smith et al., 2000; Martinez et al., 2009).

Solutions that can be adopted to solve problems of nitrogen excess, but also of other nutrients and pollutants, also with economic and aesthetic advantages, are constructed wetlands (CWs). CWs are engineered systems designed and constructed to utilize natural processes and remove pollutants from contaminated water within a more controlled environment (Faulwetter et al., 2009; Vymazal, 2010).

Application of CWs in Italy is encouraged by the legislation (''Water Framework Regulation'', D.Lgs 152/99 and modifications) that introduced the concept of ''adequate treatment'' (Annex 5, paragraph 3), by advising that: ''for all settlements with population equivalent (p.e.) between 50 and 2000 p.e. the application of techniques for natural depuration such as long-term storage or CW is thought to be favourable…'' (Mantovi et al., 2003). The diffusion of CWs only after 1999 is one of the main reasons why this technology has spread much less in Italy than in other European countries, despite its good climatic conditions. CWs are concentrated in Central and Northern Italy. Out of a total of145 systems, 106 (74%) are located in Veneto, Emilia-Romagna and Tuscany. In these regions, the higher distribution is strictly influenced by favourable local conditions.

Constructed wetlands (CWs) treating agricultural and livestock wastewaters

CWs are eco-friendly solutions with low external energy requirements and are easy to manage and maintain. CWs have been used in several wastewater treatments since the 1990s, for domestic and municipal wastewater, animal and industrial waters, urban and agricultural stormwaters, mine waters, groundwater remediation, landfill leachate (Vymazal and Kröpfelová, 2008; Kadlec and Wallace, 2009). At the early stage of CW development, their application was mainly for the treatment of traditional tertiary and secondary domestic/municipal wastewater (Kivaisi, 2001).

Processes that take place in CWs are related to wetland vegetation, soils, and the associated microbial populations, and therefore their efficiency on contaminants removal depends on a combination of numerous mechanisms: settling of suspended particulate matter, filtration and chemical precipitation, chemical transformation, adsorption and ion exchange on surfaces of plants, substrate and litter, or breakdown, transformation and uptake of pollutants and nutrients by micro-organisms and plants, and predation and natural die-off of pathogens (Haberl et al., 2003). Treatment efficiency is also related to the type of CW used, its design, feeding mode, hydraulic retention time (HRT), hydraulic loading rate (HLR), and age of the system (Karpiscak et al., 1999).

The basic types of CWs are based on hydrological modes, and are classified (**Figure 1.1**) in: 1) free water surface (FWS) wetlands that have areas of open water with a similar appearance to natural marshes; 2) subsurface flow (SSF) wetlands, which can be horizontal subsurface flow (HSSF) wetlands that typically have a gravel bed planted with wetland vegetation, and the water, kept below the surface of the bed, flows horizontally from the inlet to the outlet, and vertical subsurface flow (VSSF) wetlands where water is distributed across the surface of a sand or gravel bed planted with wetland vegetation and percolates through the plant root zone (Kadlec and Wallace, 2009). There are also hybrid wetland systems in which the various types may be combined to achieve higher treatment effect, especially for nitrogen. Hybrid systems most frequently comprise VSSF and HSSF systems arranged in a staged manner to create processes of nitrification-denitrification, so effluent has a much lower total-N concentrations (Cooper, 1999).

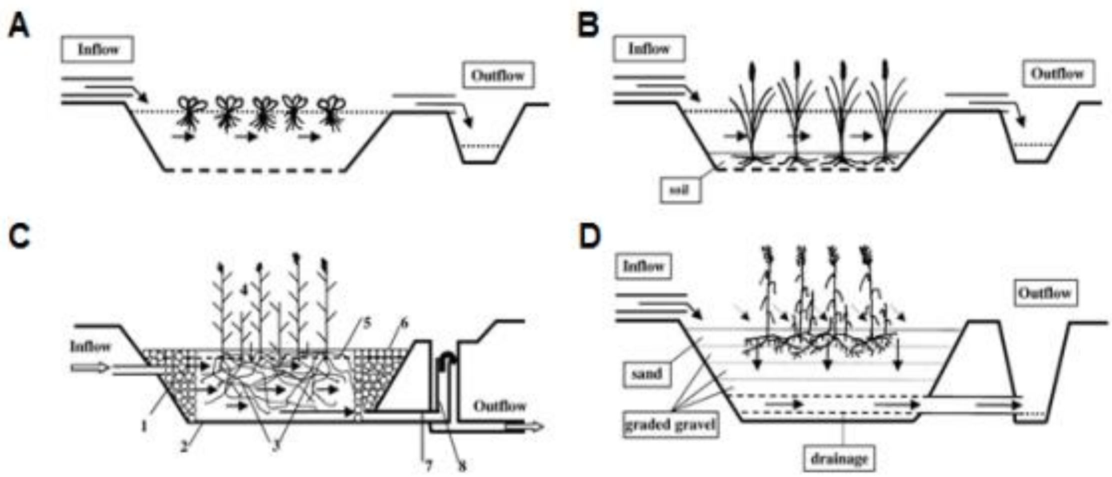

Figure 1.1 Basic types of CWs (Vymazal, 2007). Free water surface (FWS) CW with free-floating plants (A) and emergent macrophytes (B); horizontal sub-surface flow (HSSF) CW (C); vertical sub-surface flow (VSSF) CW (D).

Depending on the type of CW, the vegetation can be different and usually consists of emergent, submerged, floating leaved or floating plants. The aquatic plants growing in CWs (macrophytes) are adapted for growing in water-saturated soils and have many functions related to the treatment of wastewater in CWs. They are aquatic vascular plants (angiosperms and ferns), aquatic mosses, and some larger algae that have easily visible tissues. Macrophytes, like all other photoautrophic organisms, use solar energy to assimilate inorganic carbon form the atmosphere to produce organic matter, which consequently provides the energy source for heterotrophs (animals, bacteria, fungi) (Vymazal et al., 1998). The role of the macrophytes in treatment wetlands is well-documented by several authors (Brix, 1997; Wood, 1994; Armstrong et al., 1990; Burka and Lawrence, 1990). The plants are often claimed to provide adequate oxygen via their root zones to degrade the organic and nitrogen compounds present in the wastewater, stimulating growth of nitrifying bacteria.

The use of CWs for treating concentrated animal wastewaters gained a measure of popularity in the early 1990s. Animal wastewater contains high suspended solids and high concentrations of nutrients and ammonia. High ammonia levels can kill aquatic plants in wetlands. Given the nature of these types of wastewaters, it would seem that FWS systems have distinct management advantages, with less operational demand and greater capacities for more comprehensive treatment, especially for the removal and retention of phosphorus (Kadlec and Knight, 1996). In the case of piggery wastewater and digestate, another important parameter to consider in CWs use is salinity, as it can cause negative effects in not tolerant or resistant plants, such as productivity loss, reduced growth, damage to the aerial part and root system, and also plant death (Parida and Das, 2005; Shannon and Grieve, 1999). Salinity can be higher than 30 mS/cm (as electrical conductivity) for digestate (Alburquerque et al., 2012-*Assessment*), and can exceed 25 mS/cm in pig slurries (Moral et al., 2008), depending on the production stage.

For the treatment of piggery wastewater, CWs have the potential to remove organic compounds and nutrients contained therein (Szogi et al., 1999). A recent study (Borin et al., 2013) summarized results on chemical parameters of 18 CW case studies used worldwide for the treatment of piggery wastewater. From these, the greater concentration abatements were for ammonia-nitrogen (75.2%), COD (69.1%) and TKN (60.6%); lower abatements were detected for total phosphorus (51%) and vice versa an increment in nitrate-nitrogen concentration of piggery wastewater was achieved after CW treatment. FWS systems are the most frequently used for the depuration of pig farm effluents (Hunt et al., 1993; Poach et al., 2003; Poach et al., 2004), and have generally been configured either as a continuous marsh or marsh-pond-marsh (m-p-m). The m-p-m system consists of a continuous-marsh design bisected by a deeper, open-water or pond section. The pond section is added to the wetland design to promote the input of oxygen to the wastewater (Hammer, 1994; Reaves, 1996). The promotion of oxygen input is expected to improve the ability of CWs to treat animal wastewater. FWS systems have proved able to achieve removal efficiencies between 18 and 80% for phosphorus and between 16 and 95% for nitrogen (Hunt et al., 2001; Poach et al., 2003; Lee et al., 2009). Also SSF wetlands are used for piggery wastewater depuration, although less often than the previous systems, and they are primarily in China and Europe (Lithuania and Spain). However, in the case of HSSF systems the high TKN concentrations can cause problems with respect to oxygen supply; for this reason VSSF systems have also been piloted (Kantawanichkul et al., 1999; Sezerino et al., 2003). Based on several studies (Strusevičius and Struseviciene, 2003; Lee et al., 2004; Meers et al., 2005), SSF systems showed median abatement of 63.7% for TKN and 41% for phosphorus. There have been few studies on the use of hybrid systems for the treatment of piggery wastewaters. The literature reports mainly pilot-scale and meso-scale experiments (Harrington and Scholz, 2010; Dong and Reddy, 2010; Meers et al., 2008), and only two full scale experiments (Kato et al., 2010; Borin et al., 2013). In Kato et al. (2010) the system was composed of four VSSF wetlands followed by one HSSF wetland, which were filled with volcanic pumiceous gravel and planted with *Phragmites australis*. In Borin et al. (2013) the HCW system was formed of three VSSF wetlands in parallel, followed by one HSSF wetland, connected in series, in which different filling media (gravel, zeolite, coarse sand, coarse-rock) and plant species (*Canna indica, Symphytum officinale, Phragmites australis*) were used. In these experiments the systems concentration reduction efficiencies were 70-79% for COD, 36-64% for TN, 36-63% for NH_4-N, and 61-77% for phosphorus.

Few researches have been carried out up to now on the use of CWs for the treatment of digestate. Among these, an integrated constructed wetland (ICW) was examined for the effective treatment of separated anaerobically digested swine wastewater with emphasis on nutrient removal (Harrington et al., 2012). The plant was made of sixteen mesocosm ICW systems, vegetated with typical Irish wetland plants and differently fed to achieve high and low hydraulic loading, effluent recycling, and high and low nutrient loading rates. The removal efficiency yielded substantial results over an 18 month period and highlighted high mass removal of ammonia–nitrogen (98.1–99.9%) from the influent and low mass removal for nitrate-nitrogen (0-79.1%). Comino et al. (2013) studied the effectiveness of hybrid

CWs in the treatment of agricultural and livestock diluted digestate. The hybrid CW platform had three units (two vertical subsurface flow and one horizontal subsurface flow) filled with river gravel and sand media and planted with halophytes and *Typha latifolia*. Two inlet flows were used: 50 L/d and 200 L/d. The hybrid CW was able to remove COD, nitrate, ammonia and phosphorus. Mean removals referred to the lower inlet flow (50 L/d) organic load were 76% for COD, 86% for nitrate, 87% for ammonia and 87% for phosphorus, while those referred to the higher inlet flow (stress conditions 200 L/d) organic load were 88% for COD, 73% for nitrate, 98% for ammonia and 99% for phosphorus. Another Italian study (Pavan et al., 2014) examined a pilot FWS wetland for the treatment of diluted digestate liquid fraction (DLF) coming from an anaerobic digester. The plant consisted of three separate square pools (depth 0.6 m, and surface areas between 27 and 33 m^2) where eighteen halophyte species were planted in floating elements. This study reported that not all plants were able to survive DLF, and two halophytes in particular, *Puccinellia palustris* and *Elytrigia atherica* presented the highest potential to be used to treat DLF in floating wetlands.

Innovative solutions of CWs

One of the disadvantages of basic types of CWs is the requirement for a large area. Area requirements for different configurations and different treatment purposes (BOD removal, nitrification, etc.) have been given in the range of 1.3±10.3 m^2/p.e. (1 m^2/p.e. for BOD removal and 2 m^2/p.e. for BOD removal and nitrification) by Cooper and Findlater (1990). In addition, the treatment by CWs of high organic wastewaters, like agricultural and livestock wastewater or digestate, require different and innovative solutions to be found to enhance wetland performance.

In the last years "intensified" wetlands have been developed, where intensification represents a range of design or operational modifications that can be applied to any treatment wetland (TW) types (Kadlec and Wallace, 2008; Vymazal, 2010). Intensification is generally achieved through increased inputs of electricity (e.g. aeration or recirculation pumping), physicochemical amendments (e.g. dosing with coagulants or flocculants, or the use of highly sorptive media), or added operational effort or complexity (e.g. frequent plant harvesting, cyclical resting or recirculation of flow).

Among sorptive media, expanded clay, zeolites, bauxsol, or chitinous material can be used. These types of intensification can be considered Unit-based forms of intensification because they relate specifically to the design or operation of individual TW units. Another form of intensification can be identified as System-based, which relates to the various ways in which different TW units can be coupled together to form a treatment chain with the aim of optimizing the efficiency of the overall system. Such systems are commonly referred to as “hybrid” or “integrated” systems, and can consist of multiple units in series or multiple units in parallel (Kadlec and Wallace, 2008; Vymazal, 2010).

The intensification of CWs has led to a great variety of designs and configurations (Wu et al., 2014), such as aerated subsurface-flow CWs (Nivala et al., 2007, 2013), baffled subsurface-flow CWs (Tee et al., 2012), and combinations either of various types of CWs (Vymazal, 2013a) and/or with other technologies, to enhance the performance of CWs for wastewater treatment, e.g., microbial fuel cell (MFC) and electrochemical oxidation (Grafias et al., 2010; Yadav et al., 2012). Another design was proposed by Ye and Li (2009), for a towery hybrid CW to enhance N removal.

Other innovative solutions of CWs have been developed in recent years to also reduce the surface required for the treatment, especially in urban areas where usable space has become valuable. For this purpose there are recent examples of CWs used as green roof systems (Song et al., 2013; Chen, 2013), or as cascade systems (Machate et al., 1999; Tanner et al., 2002; Yang and Tsai, 2007). A vertically arranged CW can therefore be an interesting solution to reduce surface, but also to improve performance on pollutants removal. It can also have an aesthetic purpose when positioned close to buildings. However in these systems devices must be different from the basic types of CWs. Specifically, CWs arranged vertically require:

- lightweight structures;
- lightweight plant growing medium;
- typical plant species used in CWs or other marsh plants of limited size.

Lightweight structures are intended as tanks made of plastic or similar material. Lightweight medium for plant growth must be different from the soil, sand or gravel usually used in CWs. Among lightweight materials, zeolite, pelleted clay, lighweight expanded clay aggregates (LECA) are widespread, because they are porous materials with high specific surface area compared to other substrates and widely used for the removal of chemical compounds.

Plant species usually grown in CWs are persistent emergent macrophytes (Vymazal and Kröpfelová, 2008) such as bulrushes (*Scirpus*), sedges (*Carex* spp.), rushes (*Juncus* spp.), common reed (*Phragrnites australis*), cattails (*Typha* spp.), and reed canarygrass (*Phalaris arundinacea*) (Davis, 1994; Stottmeister et al., 2003; Brisson and Chazarenc, 2009). Obviously their use depends on a lot of variables (environment, wastewater type, biomass production, management, aesthetic function, etc.). When they have to be used to vegetate a vertically arranged CW, a limited size is required, and regards both aboveground, to avoid covering and shading the other plants, and belowground parts, because if they are grown in tanks the space is limited. Several wetland plants are suitable, e.g, *Carex* spp., *Phalaris arundinacea, Juncus* spp., and others, depending on the environment (Vymazal and Kröpfelová, 2005; Salvato and Borin, 2010; Tamiazzo et al., 2014).

For the treatment of livestock wastewater, it is necessary to carefully choose the species, because the high content of nitrogen (ammonia), phosphorus, organic compounds, and high salinity, as previously reported, can cause serious damage to plants, threatening the entire system. Considerable research has been conducted on systems planted with glycophyte plant species for remediation of freshwater effluents, which is not directly transferable to saline systems (Webb et al., 2013). There is growing interest in the potential of CWs planted with facultative or obligate halophytes used for the remediation of saline effluent and intensive land-based marine aquaculture wastewaters (Brown et al., 1999; Calheiros et al., 2012; Lin et al., 2002a, 2003, 2005; Lymbery et al., 2006; Sousa et al., 2011; Webb et al., 2012; Pavan et al., 2014). Recently these types of plants have also been studied for the phytoremediation of heavy metal contaminated soils (Van Oosten and Maggio, 2015) and in a HSSF CW to treat municipal wastewater to enhance the quality and reduce salinity (Freedman et al., 2014).

Halophytes are plants with an adapted salt tolerance that allows them to prosper in salinities that are harmful for other plants (Glenn et al., 1999; Waisel, 1972). In order to survive in saline conditions halophytes utilize several mechanisms such as ion exclusion, secretion and compartmentalization of ions in their vacuoles (Manousaki and Kalogerakis, 2011). NaCl is a dual stressor, as it challenges osmotic regulation and sodium is toxic to enzyme systems. Salt marsh halophytes cope with salt by excluding entry into roots, sequestering salts intracellularly (leading to succulence), and excreting salt via glands, usually on leaf surfaces (Zedler et al., 2014).

Halophytes were originally grown for food, fuel or fibre or for the stabilization and/or rehabilitation of degraded land. They are distributed in a variety of saline habitats, which include inland (playa) or coastal (sabkha) salt-marshes, dunes and deserts. Not only are the habitats occupied by halophytes varied, but also their habits, from ephemerals to shrubs and trees. Halophytes that are distributed in temperate habitats are either annual or perennial herbs (that die down over winter) with a few woody species, while those of subtropical salt marshes are primarily perennial shrubs (Gul et al., 2013). The hallmark of temperate salt marsh halophytes is the tolerance of seeds to high concentrations of salts with an ability to germinate at the first opportunity (Ungar, 1995) and they can often remain in highly saline water for months. Seeds of species from this region usually maintain a large persistent seed bank and germinate readily during early spring (Khan and Ungar, 1986a) when soil salinity is reduced and the temperature is warmer. Early germination in the presence of enough water confers an ecological advantage to these seedlings. Their size increases rapidly and they produce a long and deep root system that protects them in the late spring and early summer when the frequency of rains decreases and drought may set in (Khan and Ungar, 1986b).

Research is limited regarding CWs planted with emergent macrophytes (e.g. *Phragmites* spp., *Typha* spp.) for the treatment of saline/brackish wastewater (Klomjek and Nitisoravut, 2005; Lin et al., 2002a, 2003, 2005; Lymbery et al., 2006). There are fewer studies on the use of CWs planted with halophytes for saline wastewater remediation (Brown et al., 1999; Calheiros et al., 2012; Sousa et al., 2011). Their use in CWs generally regards the treatment of mariculture or highly saline aquaculture wastewaters, and in only one case the treatment of high salinity tannery wastewater. For saline wastewaters as digestate, only two Italian studies (Comino et al., 2013; Pavan et al., 2014) were recently carried out using halophytic plants, which showed interesting results on plant survival and growth and treatment of this high organic content wastewater. However, for the treatment of livestock wastewaters, swine in particular, there are no previous studies on the use of halophytes.

Pretreatment systems for CWs

CWs are not capable of holding at all levels all pollutants present in wastewater, because they are biological systems that can only exist in a defined potential concentration of certain contaminants. They must therefore be designed according to the capacity to treat these pollutants, or the systems may suffer injury or destruction. Inflows often require pretreatment to allow a functioning ecosystem to exist (Kadlec and Wallace, 2009). The factors that can damage CW systems include excessive contents of solids (that cause clogging), phosphorus, nitrogen (especially ammonia), and sulphide (which represent a threat for wetland plants). The treatment of piggery wastewater in a CW has usually required the application of pretreatment operations or even influent dilution (Kadlec et al., 2000; Hunt and Poach, 2001). Suspended solids removal is considered essential even for the treatment of low strength municipal sewage (Álvarez et al., 2008). Facultative or anaerobic ponds and settling basins have been used as pretreatment for livestock wastewater prior to entering a CW (Knight et al., 2000), to reduce chemical content and avoid clogging by solids. Prolonged clogging leads to a decrease of CW performance and causes the reduction in the percolation for different reasons:

- blocking of the pore space by deposition of organic and inorganic particles (Börner, 1992; Ellis and Aydin, 1995; Müller and Lützner, 1999; Nguyen, 2000);
- precipitation and deposition of $CaCO_3$ (Baveye et al., 1998);
- clogging of the pores from microbial biomass (Baake, 1985; Vandevivere and Baveye, 1992; Ronner and Wong,1994; Bihan and Lessard, 2000);
- root influence (Börner, 1992);
- mechanical compaction of the soil (Otis, 1985);
- entrapped gas blocking the pores (Soares et al., 1989).

However, in the case of livestock and agricultural wastewaters, CWs can require other pretreatment types, because they not only have high contents of chemical compounds and solids, but also high salinity, pathogens, heavy metals, as previously described. Among the possible solutions to the treatment of piggery manure, CWs are in general used as tertiary treatment, namely in combination with previous treatment in stabilization ponds (Dong and Reddy, 2010, 2012), vermifiltration (Morand et al., 2011), anaerobic digesters (Harrington and Scholz, 2010) or even activated sludge reactors aiming for nitrogen reduction by nitrification and denitrification (Meers et al., 2008). In these experiments, pretreated swine manure either has low organic matter and nitrogen concentrations or is subjected to dilution with water, prior to being applied to CWs. Among pretreatments, new microbial processes (SHARON, ANNAMOX and CANON) are been studied in remediation of nitrogen pollution from wastewaters, with interesting results (Khin and Annachlatre, 2004).

A pretreatment that can save area and be economical and easy to manage is filtration. Some studies have previously been done on filters but few of them on CWs pretreatment (Park, 2009; Arias et al., 2003; Kim et al., 2013, 2014). Most of them deal with the treatment of municipal wastewaters for phosphorus removal. There are different filtration technologies and substrate types. Traditional methods of wastewater filtration are classified as aerobic (either aerated or non-aerated), anaerobic and hybrid (anaerobic/anoxic and aerobic), and consist of trickling filters, rotating biological contactors, intermittent sand filtration and infiltration percolation systems (Loupasaki and Diamadopoulos, 2013). Attached growth processes are classified as aerobic (either aerated or non-aerated), anaerobic and hybrid (anaerobic/anoxic and aerobic). Trickling filters consist of a non-submerged fixed-film biological reactor using rock or plastic packing over which wastewater is distributed continuously (Metcalf and Eddy, 2003). Rotating biological contactors (RBC) are called disc, surface, media and biofilm reactors and provide an alternative to the activated sludge process. The RBC have a solid medium that encourages microbial growth in a static biofilm (Singh and Mittal, 2012). The RBC medium is arranged in a series of plates or discs that are rotated on a shaft through a biozone trough by motor or air drive (Patwardhan, 2003). The RBC combines bacterial growth and substrate utilization with a natural biomass separation system; however effluent quality and process stability is contingent on a distal sedimentation zone (Hassard et al., 2014). Using intermittent sand filtration, sand filters may be operated either in single-pass or recirculation mode. In single-pass mode, following primary sedimentation, the wastewater is intermittently dosed onto a stratified sand filter (Gross and Mitchell, 1985). Infiltration percolation systems are a low technology process based on the intermittent application of sewage on buried sand filters or permeable native soils and are used to treat primary or disinfect secondary effluents. The infiltrated water percolates through an unsaturated porous medium. The treated water is collected by a drainage system or percolates down to the underlying aquifer (Salgot et al., 1996; Mottier et al., 2000).

Filters could also be considered attached growth systems (Loupasaki and Diamadopoulos, 2013), because they form substrates for bacteria growth. In an attached growth treatment process, a biofilm consisting of microorganisms, particulate material, and extracellular polymers is attached and covers the support packing material. Several support materials have been tested: glass (Zhifei and Graham, 2006; Horan and Lowe, 2007), peat (Zhifei and Graham, 2006), powdered minerals (Lee et al., 2002), natural zeolite (He et al., 2007), expanded clay polystyrene sheets (Clifford et al., 2010), polyurethane foam cubes (Kargi and Karapinar, 1997), and fibrous carriers (Li et al., 2003). The packing material provides a large surface area per unit volume for biofilm development, therefore material selection plays an important role in maintaining high amounts of active biomass and a variety of microbial populations (Yu et al., 2008) .

Among the substrates tested for filtration, sand layers are quite common (e.g. Roseth, 2000; Liu et al., 2003; Nakhla and Farooq, 2003; Healy et al., 2004; Tao and Wang, 2009; Zheng et al., 2012). For the treatment of organic wastewater, sand filters have been used as a cost-effective alternative to conventional septic tank/soil adsorption systems for domestic waste (Furman et al., 1955; Sauer et al., 1976; Piluk and Hao, 1989). The treatment methodology involves the intermittent dosing of wastewater following primary sedimentation onto a stratified sand filter (Gross and Mitchell, 1985). On a single pass through the system, organic carbon removal, ammonification and nitrification of organic nitrogen occurs. Virus removal has also been estimated to occur in the first 30 cm of filter sand (Gross and Mitchell, 1985; Gross, 1990) and BOD removal rates are higher than 90%, with the total nitrogen mainly present as NO_3–N after filtration (Schudel and Boller, 1990). Intermittent sand filter can also remove phosphorus (Søvic and Kløve, 2005), although the phosphorus adsorption capacity in sand is controlled by the interaction of redox potential, pH, native iron, calcium and aluminium minerals, and the iron to P ratio (Rodgers et al., 2005). Sand filtration is largely used also for the treatment of dairy parlour wastewaters (e.g. Healy et al., 2004; Healy et al., 2007a; Healy et al., 2007b).

A wide range of materials for filtration purposes, other than sand, are used for wastewater purification, and according to the aim of the treatment, the type of substrate can be different. For example, a biological filter filled with semi-soft plastic media can give a good result in COD removal (Wei et al., 2010). Yasuda et al. (2009) demonstrated that rock wool could be a possible substrate for ammonia removal from manure composts. Laterite material has also been shown to be a suitable medium to reduce COD, BOD, ammonia, nitrite and turbidity (Kadam et al., 2009). Filters filled with organic media, such as a mix of wood chips/wheat straw or wood chip/coarse sawdust showed good performances in treating high nitrate content wastewaters (Saliling et al., 2007; Schipper et al., 2010), or as bamboo, revealed high reductions of COD from agro-industrial wastewater (Colin et al., 2007).

For the treatment of piggery wastewater, a wide variety of filtering materials is reported in literature, from inert materials to those plastic and organic, and their use is related to the type of pollutant to be removed. Zheng et al. (2012) studied a lab-scale biological sand filter system for an anaerobic ammonium oxidation (anammox) process in treating anaerobically digested effluent of swine wastewater. They reported an average efficiency of NH_4-N removal of 61.34% and conversion efficiency of NH_4-N to NO_2 -N of 79.77%. Wei et al. (2010) investigated an aerobic biological filter filled with semi-soft plastic media to treat piggery wastewater, studying the influence of temperature, pH value and recirculation rate on the treatment effect of the filter, and showing an overall COD reduction of 63.0-89.3%. Another research (Buelna et al., 2008) on the use of organic bed biofiltration using BIOSOR^{TM} technology for pig manure liquid fraction, made of a mixture of peat moss (30%) and wood chips (70%), reported 90% pollutants removal and 80% odour intensity reduction. Studies have been conducted using natural or modified zeolite medium in the treatment of piggery wastewater, with the purpose of removing organic compounds and ammonium. Among these, Nguyen and Tanner (1998) studied two natural New Zealand zeolites (clinoptilolite and mordenite) to remove NH_4 from several wastewaters (dairy and piggery wastewaters, and synthetic solutions) under both batch and flowthrough conditions. Another research (Huang et al., 2014) used magnesium modified zeolite to enhance NH_4-N and P removals from swine wastewater, achieving good results (82% for NH_4-N; 98% for P). Nikolaeva et al. (2002) studied the performance of zeolite bed filters preceded by an anaerobic rubber tires filter, where zeolite was mixed with gravel at different grain sizes. The average removal efficiencies were 23% for COD and 33% for BOD. A recent study (Reddy et al., 2013) in which a comprehensive treatment system consisting of geotextile bag, zeolite bed and constructed wetland was devised to remove solids and nutrients from pig waste, reported the capacity of the zeolite bed to reduce 95% of COD, and to adsorb 50% of NH_4-N.

Zeolites are microporous crystalline hydrated aluminosilicate minerals and can be structurally considered as inorganic polymers; they have a framework structure that contains pores occupied by water and alkali and alkaline earth cations and are well-known ion exchangers all over the word (Tsitsishvili et al., 1985). Moreover their low cost, high occurrence and easy availability in large quantities represent additional benefits in zeolite application for the purpose of eliminating or reducing many pollution problems (Lee et al., 2001; Caputo and Pepe, 2007). These minerals are widely used for different applications: animal nutrition (e.g. Poulsen and Oksbjerg, 1995; Hu et al., 2013; Kanyılmaz et al., 2014), wastewater depuration (García et al., 1991; Kalló et al., 1995; Alvarez-Ayu et al., 2003; Dabrowski et al., 2004¸ Tiwari et al., 2008), aquaculture (e.g. Mwale and Kaiser, 2001; Tacon and Forster, 2003; Zhou and Boyd, 2014) and various purposes in agriculture (Ramesh et al., 2010), taking advantage for their exchange capacity and selectivity for ammonium ions and other cations. The capacity of zeolites to remove NH_4 from wastewaters depends on the type used, particle size, and wastewater anion-cation composition (Koon and Kaufman, 1975; Jorgensen et al., 1976) . Moreover, the structural characteristics (e.g. porosity, density and channel length) and the composition and exchangeability of Ca, Na, K, and Mg cations in zeolites are also important factors that influence their capacity to remove NH_4 from wastewaters (Czaran et al., 1988; Curkovic et al., 1997). The most common zeolites are chabazite, heulandite-clinoptilolite, and phillipsite (Passaglia and Laurora, 2013). Clinoptilolite is the most abundant zeolitic form and frequently investigated in water treatment processes in order to achieve cheap and efficient ammonium removal (Štembal et al., 2005; Juan et al., 2009). Besides clinoptilolite, other natural zeolites, such phillipsite, chabazite, mordenite and erionite, have been proposed in the removal of ammonium (Klieve and Semmens, 1980; Amicarelli et al., 1988; Ciambelli et al., 1985a; Ciambelli et al., 1985b; Colella et al., 1983, 1984). The natural Italian zeolites that are of greater interest from the application point of view are phillipsite and chabazite, which can be found in large deposits of tuff, widespread in Southern and Central Italy (Sersale, 1978).

RESEARCH STRUCTURE AND OBJECTIVES

The main objective of this PhD dissertation is to identify a method for the treatment of agricultural and livestock wastewater using innovative treatment systems for phytodepuration.

Specifically this research aims to develop and study an intensified phytodepuration technology to be potentially applied to both swine wastewater and digestate, using rarely or never previously used solutions in order to make the treatment of these wastewaters easier to manage and more economical.

The purpose of these systems is to reduce polluting load, in particular nitrogen, to achieve an effluent with low chemical contents that allows easier management by the farmer.

The sub-objectives for achieving the main goal are:

I. to detect economical, easily available and disposable, N selective materials to use for filtration of piggery wastewater and digestate;

II. to study the treatment performances of the selected filter materials, with the aim of reducing the high polluting load of wastewater and to prepare it for the following phytodepuration treatment;

III. to use a phytodepuration system capable of withstanding filtered wastewaters, identifying and examining plant species suited to living in a water environment that are resistant to high polluting load and high salinity.

In the first two years of this PhD the research was based on the study of filtering materials for the pretreatment of digestate, piggery wastewater and synthetic P-rich wastewater, and of an intensified system of phytodepuration. Different plant species were tested for the depuration of piggery wastewater, starting with typical wetland plants, and at a later stage salt resistant plants, rarely used for this purpose. For the treatment of filtered piggery wastewater, a study based on algae cultivation with valuable astaxanthin production was also performed.

The last year of this PhD regarded final observations on the filtering materials and the phytodepuration system of the experimental plant studied the previous years, in order to obtain an overview of what has been studied in these years.

Chapter II

FILTER SYSTEMS FOR THE PRETREATMENT OF DIGESTATE

THE CASE STUDY

The research started in March 2012 and ended in May 2013 with the aim of depurating digestate for the reduction of nitrogen and organic load through a pretreatment system composed of filtering systems to prepare it for a potential subsequent phytodepuration treatment. This research was part of a project named “Sviluppo di sistemi innovativi per il trattamento di digestato, reflui zootecnici e rifiuti ceramici”, funded by the Italian funder for business training FONDIMPRESA (project number AVI/26/11). Four partners were involved in this project: the Department of Agronomy Food Natural resources Animals Environment of the University of Padova (DAFNAE) for the scientific contribution, two private companies of Emilia-Romagna region (Pirani srl and New Plant srl) for equipment construction and marketing, and the biogas plant company (Bioenergy Parks situated in Bondeno) for the digestate supply. As subcontractor the training centre SINERGIE SOC. CONS A R.L. (Reggio Emilia) was part of the project.

With the purpose of increasing the sustainability of an economical system, this research was based in the use of recycled refractories as filtering media provided by the Pirani srl company and metal material for filters manufactured by the New Plant srl company. Through the use of these waste materials (low-cost and easily available) and a simple system capable of depurating the digestate, the project aspired to find an economical and easily managed treatment system of practical interest for farmers.

The first months of the research regarded the analysis of the digestate to be used and preliminary tests to evaluate refractory materials efficiency towards digestate, never previously studied for this type of research. At the beginning of the research, it was planned to use raw digestate (solid and liquid fractions together) in order to identify a possible treatment system able to remove the digestate solid part. For this reason an economical pretreatment system to be placed before filters was also designed, built and tested.

The real pilot system of filters was set up in 2013 and monitored from March to May. It was located in the premises of New Plant srl, at S. Agostino di Finale Emilia (FE). As effluent to be purified, the digestate liquid fraction derived from the Bondeno biogas plant was used to feed the system.

MATERIALS AND METHODS

Preliminary tests for identification and selection of materials to use as media

Percolation tests

In the initial phase of the research, the choice of filtering media was based on percolation efficiency of the digestate. Different percolation tests were done: the first using tap water, and the other two using digestate.

To contain the media, five small stainless steel cylinder-shape filters (height 306 x diameter 153 mm) with perforated base (holes of 3 mm diameter) were produced.

<u>Tests with digestate liquid fraction (DLF)</u>

Three media with different grain sizes were tested after washing with water to remove powder, obtaining five material types (Table 2.1): brick 6-12 mm, porous refractory 3-12 mm, porous refractory 0-6 mm, porous refractory 0-3 mm, gravel 4-8 mm.

Before percolation tests, each medium was placed in a cylinder-shaped stainless steel container (height 172 mm x diameter 165 mm; weight 2.94 Kg) to calculate the porosity, applying tap water until substrate saturation. The material porosity (ø, %) was calculated as:

$$\emptyset = \frac{V_v}{V_t} \times 100$$

Where V_v is the void-space volume and V_t the total volume of material.

Table 2.1 Materials used for the first percolation tests.

Material	Grain size (mm)
Brick (A)	6-12
Porous refractory (B)	3-12
Porous refractory (C)	0-6
Porous refractory (D)	0-3
Gravel (E)	4-8

Each filter was filled with a medium and the percolation tests were then performed (Figure 2.1). Two percolation trials were performed using the digestate liquid fraction (DLF) as input, the first with a volume load per filter of 1 L, and the second with a higher volume (2 L) for a better study. For both tests the percolation start time (PST, seconds) and output volume (L) were measured. The PST was defined as the start time of the effluent from the filter.

Figure 2.1 Four of the five materials used for the first percolation tests: brick 6-12mm (A), porous refractory 3-12mm (B), porous refractory 0-6 mm (C), porous refractory 0-3 mm (D)

<u>Test with raw digestate (RD)</u>

Another percolation trial was conducted to select efficient materials in the treatment of raw digestate (RD). To avoid clogging, media with larger grain size were chosen (Table 2.2): brick 8-12 mm, tiles 8-80 mm, porous refractory 8-100 mm, gravel 4-8 mm.

Each filter was washed and filled with a medium up to 8 cm from the top (Figure 2.2). A load volume of 1 L of RD per filter was then poured manually to measure the percolation efficiency.

In this trial, the dry matter content and physical characteristics of the digestate were sampled at the inlet, to analyze its composition. Dry matter content (DM, %) was obtained from the following computation: DM (%) = (FW-DW) x100, where FW is the digestate fresh weight (g) and DW is the digestate dry weight (g) after drying at 105 °C for 72 h. The physical parameters measured were electrical conductivity (EC, mS/cm), pH, turbidity (NTU), using a Hach Lange HQD 40d multi-parameter with interchangeable probes according to standards methods (APHA, 1998).

Table 2.2 Description of the materials used for the RD percolation test.

Material	Grain size (mm)
Brick (F)	8-12
Tiles (G)	8-80
Porous refractory (H)	8-100
Gravel (I)	4-8

Figure 2.2 Three of the four materials used for the RD percolation test: brick 8-12mm (A), tiles 8-80 mm (B) porous refractory 8-100 mm (C).

Pretreatment prototype

A prototype system for the pretreatment was also developed for the use of RD with the purpose of removing the coarse (solid) fraction composed of a plant matrix before the treatment by filters. As economical solution, a rectangular tank with removable grids (with different diameter holes) was designed as a pretreatment system. The aim was to remove the solid fraction of the digestate through perforated grids with bigger to smaller holes. The prototype was developed as a possible alternative to the filters filled with the materials described above.

The pretreatment prototype was a stainless steel tank (100x50xheight 50 cm) with various stainless steel and iron grids arranged differently inside. The tank base was slightly sloping to allow the effluent to move from the first to the last grid. Three pretreatment tests were done using the same tank and changing layout and type of grids.

In the first test four grids were used (Figure 2.3), placing horizontally as first for the treatment the two with bigger holes (diameter of 8 mm) and vertically the grids with smaller holes (diameters of 5 and 2 mm). A load of 51 L of raw digestate was poured manually from above onto the first horizontal grid. The treatment efficiency of the prototype system was evaluated as grid clogging and effluent quality for turbidity. The turbidity of the outlet digestate was measured on site after every stage using a portable HI83414 (HANNA Instruments) turbidimeter.

The second test was conducted as result of the first trial. In this case the horizontal grids were removed and replaced with one vertical grid (Figure 2.4). A load of 45.3 L of RD was poured manually from the top before the grid with bigger holes. Also in this case the treatment efficiency of the system was evaluated as grid clogging and effluent quality for turbidity. The turbidity of the outlet digestate was measured on site after the last stage.

A third pretreatment test was performed to improve the digestate treatment, avoiding clogging. Grids with bigger holes were used (Figure 2.5). A load of 47 L of digestate was manually poured from the top before the grid with bigger holes.

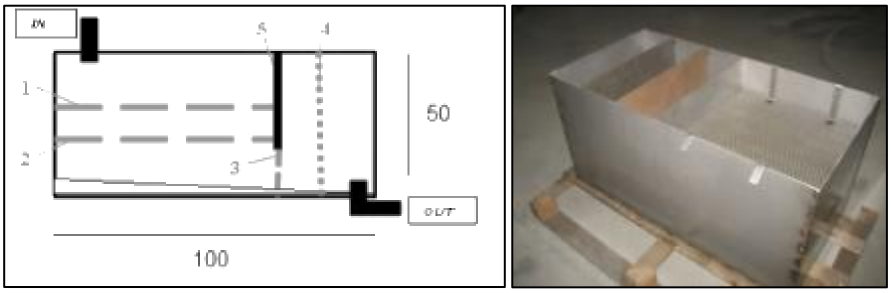

Figure 2.3 Tank used for the first pretreatment test. IN: inlet; OUT: outlet. 1-2: 8 mm holes grids; 3: 5 mm holes grid; 4: 2 mm holes grid; 5: separator panel.

Figure 2.4 Tank used for the second pretreatment test. 1: 8 mm holes grid; 2: 5 mm holes grid; 3: 2 mm holes grid; 4: separator panel .

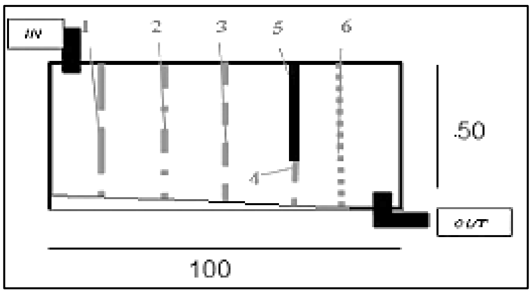

Figure 2.5 Tank used for the third pretreatment test. IN: inlet; OUT: outlet. 1: 30x30mm holes grid; 2: 2 crossed grids with 30x30 mm holes; 3: 8 mm holes grid; 4: 5 mm holes grid; 5: separator panel; 6: 2 mm holes grid.

Preliminary choice of filtering media and filter prototype creation

The preliminary choice of the filtering media was based on the treatment of the DLF and derived from the previous percolation tests. In this case materials with larger particle size were tested for percolation efficiency and digestate treatment. Once the materials had been selected, a filter prototype was designed and created to examine its functionality in terms of plant system. During these trials physical and chemical analyses of the raw and liquid digestate fractions were conducted on site. Density (kg/m^3) was also calculated as ratio weight/volume.

<u>Choice of filtering media</u>

Three stainless steel cylinder-shaped filters (height 600 x diameter 153 mm) with different perforated bases were produced and filled with different washed media (Table 2.3): porous refractory 20-30 mm; brick >40mm; aluminium rollers >8 mm.

A load volume of 2 L per filter was poured manually. Percolation times and outlet volumes were measured and physical parameters (electrical conductivity (EC; mS/cm), wastewater temperature (T; °C), dissolved oxygen (DO; mg/L and %), turbidity (NTU) of digestate at outlet were analyzed (for methods see the section on physical analyses, pages 38).

Table 2.3 Filtering media and characteristics of the base of the filters used for the final tests.

Material type	Material	Grain size (mm)	Holes size of the base (mm)
1	Porous refractory	20-30	20 x 20
2	Brick	> 40	40
3	Aluminium rollers	> 8	8

<u>Filter prototype</u>

The filter prototype was in stainless steel. It was composed of two modules of the same size (0.11 m^3), the upper to contain the filtering medium (with a perforated grid (square holes 30x30 mm) to sustain medium) and the lower to collect the filtered digestate, with a manual valve at the base to allow or block the outflow. The system was supported by three iron pipes about 180 cm long. To allow recirculation a vacuum pump was installed and joined to the filter by a PVC pipe (nominal diameter 50 mm) connected to the base of the lower module (Figure 2.6). The upper module was filled up to 17 cm from the top with porous refractory with grain size >30mm (filtering medium volume 0.054 m^3 and porosity 57%), which was sieved, weighed and washed before use.

A volume load of 40 L of the DLF was poured manually on the top of the filter. The percolation time was checked and the physical parameters (electrical conductivity (EC; mS/cm), pH, wastewater temperature (T; °C) dissolved oxygen (DO; mg/L and %), turbidity (NTU) of the digestate were analyzed at inlet and outlet (for methods see the section on physical analyses, pages 38). A recirculation was then performed to test the pump and functioning of the feeding system.

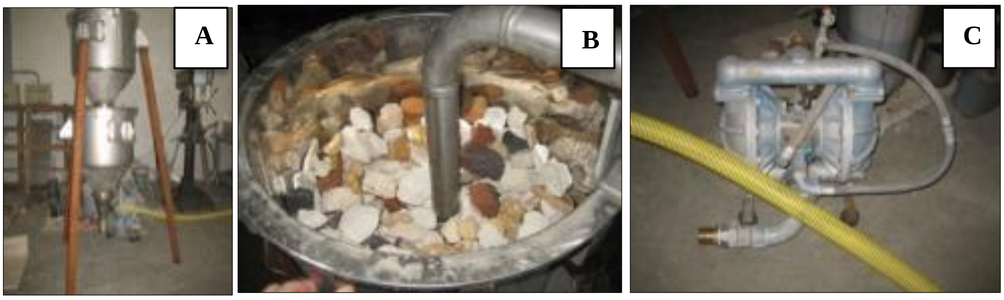

Figure 2.6 Filter prototype details. A: complete system; B: upper module with filtering medium and digestate delivery system; C: vacuum pump used for wastewater recirculation

Filter system description and management

Experimental set-up

<u>Experimental layout</u>

The experimental plant was located in S. Agostino di Finale Emilia (FE) (44°83' N, 11°29 E; 6 m a.s.l., Emilia-Romagna region), inside the New Plant srl industry, where the air temperature was about 20 °C throughout the experimental period.

The plant was set up in February 2013 and monitored from March to May. It consisted of four independent filters (filter 1: F1; filter 2: F2; filter 3: F3; filter 4: F4) in stainless steel positioned next to one another (Figure 2.7). Each filter was supported by three iron pipes about 200 cm long. As in the prototype, each filter was composed of two modules of the same size (0.11 m^3): the upper module was the real filter containing the filtering medium

(medium volume 0.054 m^3), while the lower was the container collecting the filtered wastewater (Figure 2.8). Both modules were composed of a cylindrical container (diameter 475x height 475 mm; volume 0.08 m^3) mounted on a conical container (major diameter 475x minor diameter 150x height 300 mm; volume 0.025 m^3).

To support the filtering medium, a perforated steel base with different holes size (depending on the filter) was fitted inside the upper module (Figure 2.9). A transparent plastic cap with an opening (a vertical pipe 20 cm long, nominal diameter 50 mm) was placed on the top of the filter to avoid the wastewater exit during feeding (Figure 2.10). The closed lower module had a round opening (diameter 100 mm) on the top with removable lid to allow monitoring operations. The lower part of this module had a manual valve to allow or block the outflow exit.

To allow wastewater recirculation, a vacuum pump (MKart 24 Gravity-Easy, MECART S.a.s.®) commanded by an automatic battery electronic controller (GF 36, G.F.®) was installed on each filter and joined to the base by a PVC pipe (nominal diameter 20 mm). For recirculation the filtered wastewater was sent through a plastic pipe (nominal diameter 20 mm) on the top of the filter.

Four materials were chosen as filtering media, based on the preliminary tests. Each filter had a base with different holes size and was filled with one material (Table 2.4). Before use, the filtering media were washed with tap water, sieved (only porous refractory) and weighed.

Afterwards their porosity was measured, applying tap water until substrate saturation (Table 2.4). The material porosity (ø, %) was calculated as:

$$\emptyset = \frac{V_v}{V_t} \times 100$$

Where V_v is the void-space volume and V_t the total volume of material.

Figure 2.7 Experimental plant, with the four filters.

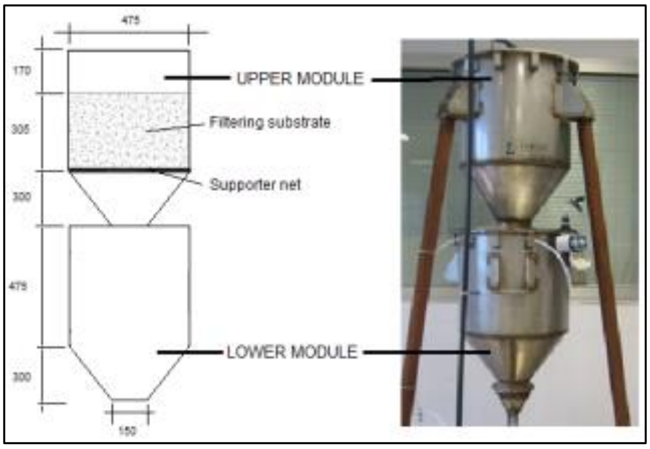

Figure 2.8 Details of the filter with design scheme; measurements are in mm.

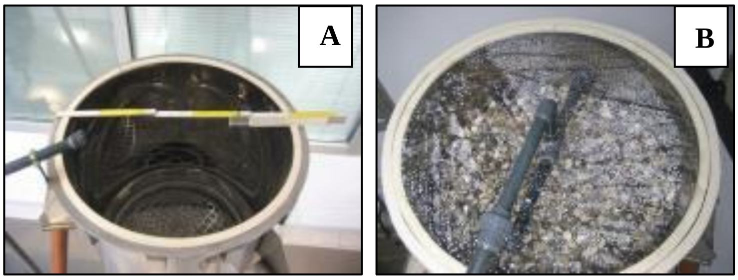

Figure 2.9. Details of the upper module of each filter. A: perforated steel base to support the filtering medium; B: plastic cap situated on the top.

Table 2.4 Composition and characteristics of the filters.

N° filter	Material	Filter acronym	Base holes size (mm)	Grain size (mm)	Material weight (Kg)	Ø (%)
1	Brick	BRICK	40	50-100	59.8	52.6
2	Porous refractory	REFR 30-50	30x30	30-50	60.5	47.4
3	Porous refractory	REFR 20-30	20x20	20-30	40.0	55.2
4	Gravel	GRAVEL	8	8-15	88.9	40.9

The brick was supplied by a demolition recovery company and was a mixture of bricks, roof tiles and pottery. It was used as filtering medium in F1 with grain size between 50 and 100 mm, 52.6% porosity and a weight of about 60 Kg (Figure 2.10).

The porous refractory (Figure 2.10) was a mixture of different kinds of refractory material deriving from blast furnace demolitions. Refractories are products belonging to the ceramic world (inorganic, non-metallic), with the primary characteristic of withstanding high temperatures, but also thermal shock, tension and aggression agents, solid, liquid and gaseous fuels. They are therefore suitable and essential materials for construction and operation of all types of furnaces. This material was used at two particle sizes: the larger (30-50 mm, in F2) had 47% porosity and weighed 60 Kg; the smaller (20-30 mm, in F3) had 55% porosity and weighed 40 Kg (Table 2.4).

Gravel was used as medium to compare the other materials because it is a typical inert material used as substrate for different applications (e.g. for wastewater filtering together with sand (e.g. Zheng et al., 2012), and as filtering medium in HSSF and VSSF wetlands beds (Kadlec and Wallace, 2009). In this study river gravel was used, bought from a building material supplier. The gravel was used as filtering medium of F4 (Figure 2.10) and had a grain size between 8 and 15 mm, total weight of 89 Kg and 41% porosity (Table 2.4).

Figure 2.10 Filtering substrates: brick substrate used in F1(A); porous refractory substrate used in F2 and F3 (B); gravel used in F4 (C).

Feeding system

The system was fed with DLF from the biogas plant of Bondeno, which was fed with a mixture of 8% piggery manure and the rest of plant matrix (mainly shredded maize). It was collected from the biogas plant and transported to the experimental site in small cisterns. Once per week a loading volume of 20 litres was applied per filter. The loading was done manually from the top of the filter after removing the plastic cap. Hence the filtration occurred in the upper module containing the substrate. After filtration the wastewater was accumulated in the lower module and then recirculated 8 times per day (once every 3 hours) for about one week through the pump commanded by the controller.

Monitoring activity

The monitoring was performed from March to May 2013. The first week has not been considered in the data elaboration due to plant engineering problems. A total of 8 monitoring cycles were carried out (Table 2.5). A monitoring cycle referred to about one week of treatment, during which the recirculation varied from 39 to 63 times. Every monitoring cycle all filters were examined before and after the treatment, and for each one this regarded:

- digestate analyses at inlet (IN) and outlet (OUT);
- digestate volume measurement at IN and OUT.

The analyses regarded physical and chemical parameters of digestate measuring the values at IN and OUT. The IN and OUT volumes were checked manually using sized buckets.

Table 2.5 Details of the monitoring cycles performed during experiment

Cycle (n°)	1	2	3	4	5	6	7	8
Period (week)	26th March – 2nd April	2nd-10th April	10th - 17th April	17th - 24th April	24th April - 2nd May	2nd -8th May	8th -14th May	14th - 21st May
Recirculation (n° of times)	54	63	54	49	63*	55	39	56*

*not valid for filter 2

Analyses for filtering media characterisation

X-Ray Powder Diffraction (XRPD)

The X-Ray Powder Diffraction (XRPD) of porous refractory and gravel substrates was done in collaboration with the Department of Geosciences of the University of Padova. The XRDP is a fast and economical technique that allows the various components of a solid sample to be quantified, and also to obtain information on the crystalline structure and size of the crystallites.

The analysis was performed with a PANalytical X'Pert PRO diffractometer in Bragg-Brentano geometry equipped with a Cu X-ray tube operating at 40 kV and 40 mA (CuKa radiation), and an X'Celerator detector. The acquired diffraction patterns were interpreted with X'Pert HighScore Plus 3.0 software.

Cation exchange capacity (CEC) test

The cation exchange capacity (CEC) represents the number of sites of the active complex of the soil, mineral colloids and organic, with density of negative charge, able to adsorb cations that neutralize their charge. The CEC is consequently defined as the total sum of the exchangeable cations adsorbed and is expressed in milliequivalents per 100 g of substrate (meq/100 g).

This parameter is important because ammonium ion is a chemical species that is usually involved in cationic exchange and ammonium abatement is an aim of this study. The CEC test was done for all media.

Standard methods to determinate soil CEC require the use of BaCl, $MgSO_4$ and EDTA titrations. In this study, these procedures have not been used because it regarded materials different from soil. The CEC test was conducted by using ammonium solution with the procedure reported in Gualtieri et al. (1999). Substrates were milled and sieved at 2 mm. Each substrate was washed with a 1g/L NaCl solution (1N) for three hours. This was done to avoid the presence of ammonium nitrogen adsorbed on the substrate yet, in this way the sodium should have occupied all exchange sites. After sodium exchange some washings were done with deionized water to eliminate chlorine ion (that is an analysis interference). The samples were dried overnight in an oven at 105 °C. The samples were then weighed and washed with 500 mg/L NH_4Cl solution for one night by continuous shaking on a rotating shaker (80 rpm) at constant temperature (20 °C). Finally ammonia-nitrogen was photometrically determined using a Hach-Lange DR-2800 spectrophotometer and suitable cuvette test kit (cuvette-test LCK 304) (Hach-Lange, 1989), according to DIN (1985). The pH of the solution was measured before and after the CEC test, using a Hach Lange HQD 40d multi-parameter with interchangeable probes according to standards methods (APHA, 1998).

Digestate analyses

On-site parameters

The parameters of the digestate analyzed on-site were measured at every monitoring cycle. Electrical conductivity (EC, mS/cm), pH, dissolved oxygen (DO, mg/L and %) and temperature (T, °C) were measured, using a Hach Lange HQD 40d multi-parameter with interchangeable probes according to standards methods (APHA, 1998). Before testing, each probe was carefully calibrated according to the manufacturer's procedures. The parameters were measured for IN directly inside the cistern where the untreated digestate was and for OUT directly inside the lower module inserting the probes through the top opening. Turbidity (NTU) was also measured, using a portable HI83414 (HANNA Instruments) turbidimeter. Turbidity is one of the less expensive and easiest to measure methods to determine total suspended solids (TSS), used extensively in many environments, such as lakes (Halfman and Scholz, 1993), streams (Gippel, 1989), wetlands (Mitsch and Reeder, 1992) and tidal saltmarshes (Suk et al., 1998).

Total (TS) and volatile (VS) solids

Total solids (TS) and volatile solids (VS) analyses were done using the gravimetric method (APHA, 1998). TS and VS were determined by drying at 105 °C for 24 h and combustion at 600 °C for 3 h, respectively. TS represent the residual fraction after heating the wastewater samples at 105 °C and VS represent the volatilized fraction, calculated as:

$$\mathrm{TS}\ (\mathrm{w/w}\ \%) = \frac{\mathrm{W105°C - Wc}}{\mathrm{W}}\ \mathrm{X}\ 100$$

$$\mathrm{VS}\ (\mathrm{w/w}\ \%) = \frac{\mathrm{W105°C - W600°C}}{\mathrm{W105°C - Wc}}\ \mathrm{X}\ 100$$

where:
W105°C= gross weight of the sample after drying at 105 °C (g)
Wc= Capsule weight (g)
W = Net weight of the sample (g)
W600°C= Gross weight of the sample after incineration at 550 °C (g)

The digestate was sampled at the inlet and outlet of every filter and stored at -20 °C till analysis, when it was defrosted. The analyses were done for the last seven monitoring cycles.

Chemical parameters

Nitrogen and phosphorous forms and organic chemicals

Chemical parameters of the digestate were analyzed on-site before and after the treatment as concentrations (mg/L). Total nitrogen (TN), nitrate-nitrogen (NO_3-N), ammonia-nitrogen (NH_4-N), total phosphorus (TP), soluble phosphorous (PO_4-P) and chemical oxygen demand (COD) were determined photometrically using a Hach-Lange DR-2800 spectrophotometer and suitable cuvette test kits (cuvette-tests LCK 014, 338, 340, 348, 350, 514) (Hach-Lange, 1989), according to DIN (1985). 5-days biological oxygen demand (BOD_5) was also measured by the respirometric method with the Oxitop® system (WTW). Before chemical analysis adequate sample dilutions were made with a stock supply of deionized water.

Ions

The ions were analyzed via ion chromatography (IC) analysis, performed by a specialized technician using an ICS-900 Ion Chromatography system (Dionex Corporation) equipped with a dual piston pump, a model AS-DV autosample, an isocratic column at room temperature, a DS5 conductivity detector and an AMMS 300 suppressor (4 mm) for anions and CMMS 300 suppressor (4 mm) for cations. Chromeleon 6.5 Chromatography Management Software was used for system control and data processing. A Dionex Ion-Pac AS23 analytical column (4 mm × 250 mm) and a guard column (4 mm×50 mm) were used for anion separations, whereas a Dionex IonPac CS12A analytical column (4 mm x 250 mm) and a guard column (4 mm x 50 mm) were used for cation separations. The eluent consisted of 4.5 mM sodium carbonate and 0.8 mM sodium bicarbonate at a flow rate of 1 mL/min for anions and of 20 mM metansolfonic acid for cations at the same flow rate. Anions (Chloride (Cl), Bromides (Br), Sulphate (SO_4) and cations (Lithium

(Li), Sodium (Na), Potassium (K), Magnesium (Mg), Calcium (Ca) were quantified following a standard calibration method and the calibration curves were generated with concentrations ranging from 0.4 mg/L to 20 mg/L and from 0.5 mg/L to 50 mg/L of standards respectively. The IC was done for digestate analysis just for two monitoring cycles at IN and OUT of each filter. Before the IC analysis samples were centrifuged at 5000 rpm (revolutions per minute) for 2 minutes; afterwards the supernatant was taken and filtered on a 0.20 μm filter.

Data elaboration

Data were elaborated using Microsoft Excel 2010. The statistical analyses were conducted using STATISTICA 8.0 software (StatSoft Inc., 2007). The data series of the parameters did not follow normal distribution even after transformation. Thus, statistical analyses were conducted with the Kruskal-Wallis non parametric test; different letters were used to indicate significant differences (at $p < 0.05$ by Kruskal–Wallis test).

The statistical analysis was performed to determine differences in physical parameters and chemical concentrations between the digestate at the inlet (IN) and outlet of every filter. It was also applied to find differences among the filters on chemical removals and mass abatement.

The depurative performances of the system were studied as:

1. physical parameters reduction (R,%) (for turbidity, EC, TS and VS) calculated on second quartile (Q2; median) values as: $R = [(V_{IN} - V_{OUT})/V_{IN}] \times 100$, where V_{IN} is inlet value and V_{OUT} is outlet value of every filter;
2. chemical concentration reduction (A,%), calculated on second quartile (Q2; median) concentration values as: $A = [(C_{IN} - C_{OUT})/C_{IN}] \times 100$, where C_{IN} is inlet concentration (mg/L) and C_{OUT} is outlet concentration of every filter (mg/L);
3. mass abatement (MA, %) of each filter for all chemical parameters, calculated as: $MA = [(C_{IN} * V_{IN}) - (C_{OUT} * V_{OUT})/(C_{IN} * V_{IN})] \times 100$, where V_{IN} is the digestate inlet volume (L/m^3) and V_{OUT} is the digestate outlet volume of every filter (L/m^3);
4. quantity of chemical element removed by each filter ($g/m^3/d$).

RESULTS

Preliminary tests

Percolation tests

Regarding material porosity (Table 2.6), for the porous refractory 0-3 mm it was not detected because the water did not permeate; this material was therefore discarded for tests. Filters filled with porous refractory 3-12 mm had the highest porosity (35%), while the gravel filter had the lowest (26%).

For the first tests in which a load volume of 1 L of DLF was used, the percolation start time (PST) was slower for porous refractory and faster for the other materials (Table 2.6), due to its feature as a porous material, rather than adsorbing the digestate.

Table 2.6 Porosity (ø, %) of the media, with percolation start time (PST, seconds) and output volume (L) after 3 minutes detected during the percolation test with 1 L of digestate liquid fraction.

Material	ø (%)	PST (s)	Volume (L) at 3 min
Brick 6-12mm (A)	33.1	5	0.60
Porous refractory 3-12 mm (B)	35.0	11	0.41
Porous refractory 0-6 mm (C)	29.4	16	0.25
Porous refractory 0-3 mm (D)	*not detected*	-	-
Gravel 4-8 mm (E)	26.1	6	0.68

The final volume was measured after 3 minutes, when the percolation slowed; brick and gravel showed higher outlet volume than porous refractory because they have less surface adsorption.

The second test with doubled load volume (2 L) of DLF per filter resulted in percolation arrest in filters with A, B, C materials after a load of 0.5 L. The percolation was anyhow observed after 3 days, which revealed clogging of brick and porous refractory 0-6 mm, also deduced visually (Figure 2.11).

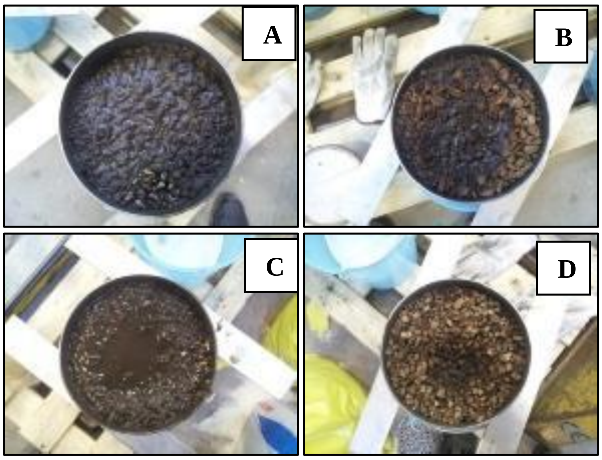

Figure 2.11 Second percolation test using the DLF: brick 6-12mm (A), porous refractory 3-12 mm (B), porous refractory 0-6 mm (C), gravel 4-8mm (D).

In the last percolation test in which a load volume of 1 L of RD was used per filter, the analysis of physical parameters revealed its composition. The digestate had high salinity (EC 29.9 mS/cm) and turbidity (24,820 NTU), pH 9.57 and 6.68% dry matter. Percolation was monitored for 3 hours, during which almost nothing was percolated in all filters. Due the large amount of dry matter in the digestate, which can vary from 3 to 9% depending on the matrices (Mantovi, 2012), a solution for the separation of liquid and solid fractions was necessary. For this reason several pretreatment tests were subsequently conducted.

Pretreatment prototype tests

During the first test, in which 51 L of RD was used, grid clogging problems occurred, especially for vertical ones with smaller holes. However the horizontal grids were able to retain the solid material, corresponding to 9.99 L of fresh weight detected after 1 hour of decanting. Turbidity of the digestate was anyway measured and it clearly reduced after every passage (Table 2.7), decreasing by 50% from the first (holes 8mm) to the second grid (holes 5mm), and by 61% at the last step.

In the second test where grids with bigger holes were used, clogging also occurred, and the turbidity measured at the final stage was 30,580 NTU, double that of the previous test.

In the last pretreatment test the RD was not retained by the grids with bigger holes, and it blocked at the third grid (holes 8 mm), causing clogging.

As a conclusion, the prototype tank was not adequate as pretreatment. For the passage of the digestate from inlet to outlet the prototype tank was efficient, however for a real system to work solutions about grid types and cleaning and obviously mechanization must be found. The hypothesis to continue studying a pretreatment system was dropped after these tests due to the possibility of using clarified digestate in the system.

Table 2.7 Digestate turbidity after every passage detected in the first pretreatment test.

	Turbidity (NTU)
RD	*not detected*
After 8 mm holes grids	43,280
After 5 mm holes grid	21,840
After 2 mm holes grid	16,950

Choice of filtering media and filter creation

In this stage of the research, information about the physical and chemical composition of the digestate was important to understand differences between the raw and liquid digestate. The RD had higher chemical contents (especially total nitrogen and ammonia nitrogen), EC and density, while pH and turbidity were similar between the two samples (Table 2.8).

The use of DLF caused no problems of filter clogging. In the percolation test for porous refractory 20-30 mm, brick >40mm and aluminium rollers >8mm, the digestate started to exit instantly for all media, and it ended 13, 16, 21 minutes later for rollers, brick and refractory, respectively. The whole volume was found at the outlet in brick and refractory, while in rollers it was 50% of the inlet volume. After filtration the EC was similar for refractory and brick (27 mS/cm), while lower for rollers (Table 2.9); the DO was particularly low in refractory (0.3 mg/L), which showed highest turbidity, undoubtedly due to the powdery material release by this medium.

Table 2.8 Physical and chemical parameters of the raw digestate (RD) and digestate liquid fraction (DLF).

	RD	DLF
TN (mg/L)	3,650	3,040
NH_4-N (mg/L)	2,700	1,994
NO_3-N (mg/L)	98.3	97.8
TP (mg/L)	574	434
PO_4-P (mg/L)	545	364
COD (mg/L)	45,970	42,860
pH	9.57	9.48
EC (mS/cm)	29.9	23.6
Density (kg/m^3)	1122 (T 16 °C)	993.9 (T 21 °C)
Turbidity (NTU)	30,910	30,940

Table 2.9 Physical parameters of the digestate measured after the filtering.

	EC (mS/cm)	DO (mg/L)	DO (%)	Turbidity (NTU)	T (°C)
Porous refractory	27.2	0.27	2.3	50,620	8.3
Brick	27.6	2.03	17.3	44,380	8.4
Rollers	24.5	1.89	16.1	35,740	8.1

In the test of the filter prototype filled with porous refractory (particle size 20-30mm), in which 40 L of digestate was poured, the percolation started instantly and after 85 minutes was complete. After the filtering, the EC and OD remained the same and turbidity increased, probably due to the powdery material release by the medium (Table 2.10). The feeding system worked for the recirculation and the pump also proved to be suitable for digestate.

These results were obtained with the selection of porous refractory with grain size of 20-30 mm as one of the filtering media and filters with similar characteristics to the prototype.

Table 2.10 Physical parameters of the digestate measured before (IN) and after (OUT) the filtering.

	EC (mS/cm)	DO (mg/L)	DO (%)	Turbidity (NTU)	T (°C)
IN	25.9	0.35	3.2	48,540	10.7
OUT	24.3	0.33	2.9	56,800	8.9

Features of the filtering media

XRPD analysis

The X-Ray Powder Diffraction analysis of the porous refractory substrate (Figure 2.12-A) revealed a chemical composition constituted by: 51% of Aluminium Silicon Oxide, 19% of Magnesium Aluminium Silicate, 14% of Aluminium Oxide, 8% of Andalusite, 3% of Alpha-quartz, 4% of Cristobalite, 1% of Zirconium Silicate.

The gravel powder diffraction spectrum (Figure 2.12-B) showed the following structure: 51.5% of Dolomite, 17.2% of Potassium Aluminium Silicate Hydroxide, 13.1% of Calcium Carbonate, 8.1% of Albite Calcian, 5.1% of Silicon Oxide, 5.1% of Magnesium Iron Aluminium Silicate Hydrate.

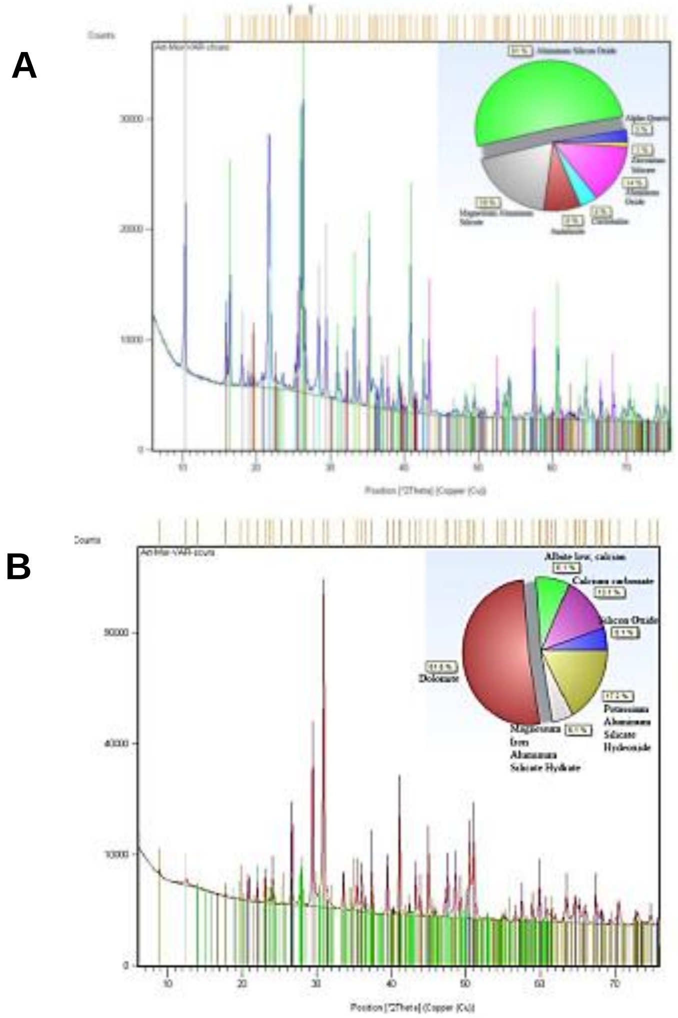

Figure 2.12 Structural characterization (powder diffraction spectrum and quantitative phases ratio) of porous refractory (A) and gravel (B).

CEC test

Results of the CEC test (Table 2.11) revealed that porous refractory did not have ammonium adsorption capacity (-0.5 meq/100g). Gravel showed low ammonium adsorption capacity (1.5 meq/100g) and brick demonstrated higher cationic exchange capacity, of about 19.7 meq/100g.

This analysis revealed that 100 grams of brick could adsorb 355 mg of ammonium. Considering that the filter filled with this substrate contains about 60 Kg of material, the theoretical maximum ammonium adsorption corresponds to about 213 g. Certainly the CEC value found for brick overestimated the real value in field conditions because the grain size used in the filter was much larger than that used for the CEC test (2 mm maximum). It is in fact known that the smaller the particle size distribution (PSD) is, the greater the specific surface area will be and consequently the CEC value increases (Ames, 1960; Hlavay et al., 1982).

High CEC values and selectivity for ammonia removal are generally known for porous adsorbents such as zeolite minerals (Passaglia et al., 1999, Reddy et al., 2013). For recycled materials and gravel no studies with this test have been found. However results showed interesting CEC values to consider brick potentially suitable for ammonia removal.

Table 2.11 CEC test results: NH_4 concentration in solution (mg/L), NH_4 adsorption on the substrate surface (mg), NH_4 adsorption on the substrate surface (mg/g) CEC value (meq/100g) and solution pH.

	NH_4 CONCENTRATION (mg/L)	NH_4 ADSORPTION (mg)	NH_4 ADSORPTION (mg/g)	CEC (meq/ 100 g)	pH
NH_4Cl solution	235.8	-	-	-	6.3
Porous refractory	306.0	-2.3	-0.1	-0.5	
Brick	214.8	88.9	3.6	19.7	6.9
Gravel	296.7	7.0	0.3	1.5	8.4

Digestate treatment performances

During monitoring, malfunctioning of the pump in filter N° 2, filled with porous refractory 30-50 mm, occurred during the 5th and 8th monitoring cycles, so the results for these dates were not considered for data elaboration.

On-site parameters, TS and VS

The electrical conductivity (EC) of the digestate was higher than 20 mS/cm and remained stable over time, ranging between 24.8 and 28.4 mS/cm. In digestate coming from AD of pig slurry (digested alone or co-digested with other matrices) the EC was also higher than 20 mS/cm, even reaching more than 30 mS/cm, much higher compared to digestate derived from cattle slurry (Alburquerque et al., 2012b; Alburquerque et al., 2012c). In the present study, the DLF contained high amounts of salt and was thus classified as very highly saline according to Rhoades et al. (1992). After filtration the EC was lowered, reaching also 15 mS/cm, with in general a constant decrement over time for all filters (Figure 2.13). The EC at the inlet (median 27.5 mS/cm) was significantly reduced by brick, porous refractory 20-30mm, and gravel, with median values of 20.8 mS/cm (R=24%), 18.3 mS/cm (R=33%) and 20.2 mS/cm (R=27%), respectively (Figure 2.13).

the pH of the digestate at IN was also steady over time, with values in the range 7.59-7.76 (median 7.69). Generally, the pH of digestates derived from animal slurries is in the alkaline range, around 8 (Alburquerque et al., 2012c; Chadwick, 2007; Smith et al., 2007). After filtration, the digestate pH was higher compared to the IN with similar trends for all filters except for gravel material, which had a low pH, similar (7.54) to the IN at the 5th monitoring cycle (Figure 2.13). The pH was significantly augmented after filtration by all the recycled materials used, with median values in the range 8.47-8.63 (Figure 2.13). This could be due to their high contents of metal oxides (Al), which, once in contact with water solution, released hydroxide ions. Wang et al. (2013) found a predominance of aluminium (Al-P, 0.413 mg g^{-1}) and iron (Fe-P, 0.125 mg g^{-1}) in bricks substrate, and lower calcium content (Ca-P, 0.030 mg g^{-1}).

The inlet temperature (T) depended on the room temperature where the experiment was located, and increased from the first (9.9 °C) to the fourth cycle (24.7 °C) to then decrease constantly till the end of the experiment (18.5 °C) (Figure 2.13). The trends of temperature after filtration were different from the IN, with higher outlet values in the first three and last two monitoring cycles. Considering median values, significant differences were not observed among IN and filters.

The dissolved oxygen (DO) of the DLF before filtration was very low and values oscillated over time in the range 0.13-0.25 mg/L (1.40-2.30%) (Figure 2.13). The DO trends of the porous refractory were similar between F2 and F3, while filters filled with brick and gravel showed irregular trends, and higher dispersion data, reaching DO values near to 0.6 mg/L (more than 6% of DO) in the middle of experiment. The DO values (mg/L and %) were particularly high in the monitoring period between the 3rd and the 6th cycles. In particular for the filter filled with gravel, the DO concentration reached 0.6 mg/L in the 5th cycle (almost 7% of DO), probably because there were more cycles during the monitoring week. The brick filter increased the outlet DO content from the 4th cycle (0.38-0.62 mg/L; 4.3-6.9%), probably because it was saturated by the wastewater only after this week, releasing a more regular outlet volume and oxygenating the effluent. Considering the entire period, the DO concentration at the inlet (median 0.18 mg/L) remained unchanged after filtration by porous refractory 20-30 mm, while it was significantly increased by the filter filled with brick (median 0.38 mg/L) (Figure 2.13), probably due to the greater water saturation of this medium, releasing digestate more continuously at the outlet.

The turbidity of the inlet DLF was higher than 30,000 NTU, and ranged between 31,540 (1st cycle) and 42,360 NTU (6th cycle) (Figure 2.14). After filtration it was in general lowered until the 7th cycle in the range 30,000-35,000 NTU, while in the last monitoring cycle it climbed in all filters to up to 45,000 NTU. This filter behaviour could be explained as the release of suspended solids previously accumulated inside. No differences were observed for the turbidity among the not filtered and filtered DLFs.

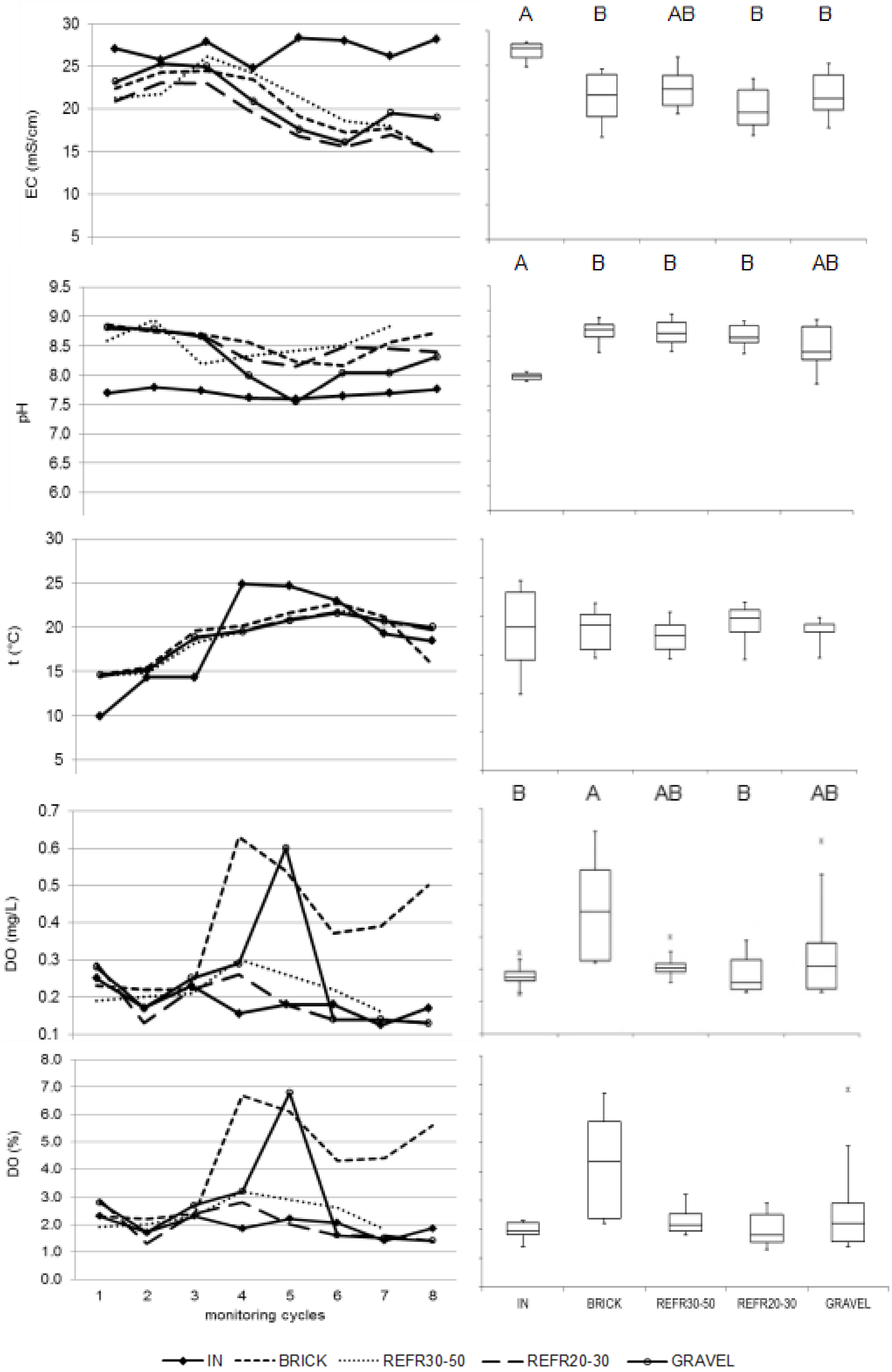

Figure 2.13 Trends and box-plot of the DLF on-site parameters detected before (IN) and after filtration for every filter. Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

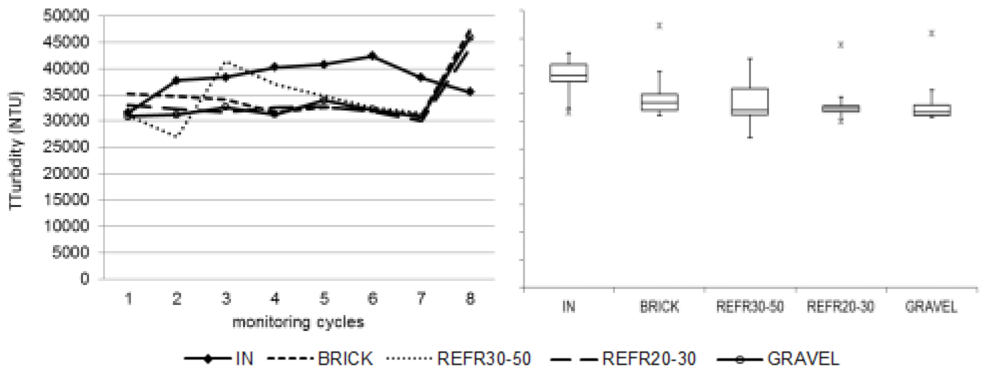

Figure 2.14 Trends and box-plot of DLF turbidity detected before (IN) and after filtration for every filter.

The results of the solids content on the liquid fraction of digestate agreed with Gioielli et al. (2011) for the inlet contents. The analysis revealed that filtration can reduce the total solids (TS) content (Figure 2.15). Specifically the digestate at IN presented between 3.9 and 4.7% of TS (median 4.3%), which was significantly decreased by the brick (median 3.5%) and the refractory 20-30mm (median 3.6%) filters. The volatile solids (VS) content of the IN digestate was 2.8% as median, ranging between 2.4-2.8% (one outlet value of 4.27%) and did not change after filtration.

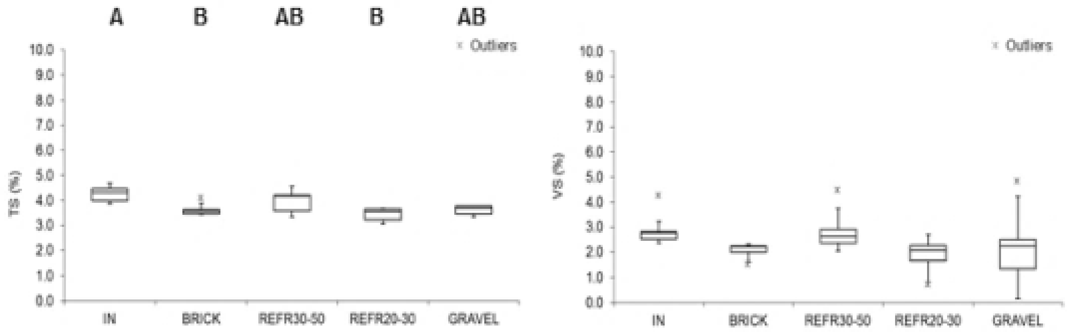

Figure 2.15 Box-plot of the total solids (TS) and volatile solids (VS) contents (%) of DLF detected before (IN) and after filtration for every filter. Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

Chemical contents

Ions

The DLF used for the experiment (IN) had almost 5,500 mg/L of potassium and high salt content (1,276 mg/L of sodium and 900 mg/L of chloride) (Table 2.12). Calcium content was slightly more than 400 mg/L, magnesium close to 200 mg/L, and sulphate around 20 mg/L. Bromide and lithium were not found. After filtration the ions content changed, decreasing or increasing depending on the filter. Cl increased in the filter filled with recycled materials (in particular in brick with 1,134 mg/L), while it decreased after gravel treatment. SO_4 also rose at the outlet in particular on filters with recycled materials, reaching also 191 mg/L. Na was doubled after filtration of brick substrate (around 2,400 mg/L), while it remained similar to the IN in the other filters. On the other hand, brick material showed the lowest K content at outlet, with a median value around 2,900 mg/L, almost the 40% less than the others. Contents of Ca and Mg cations were in general lowered after filtration (Table 2.13). The values of Cl and Na found by the ion chromatography was discordant from the EC results, in which brick, porous refractory 20-30mm and gravel reduced these parameters with filtration. The ions content at inlet was more similar among the two samples analysed, while more differences were detected between the samples at outlet. Probably the analysis of a few samples with different values was unable to provide reliable information. Alburquerque et al. (2012-*Assessment*) reported higher contents of Cl (median 1,606 mg/L) and lower contents of Na (median 698 mg/L) for 12 samples of digestate originating from mixtures of pig slurry with other matrices (slaughterhouse or energy crop residues).

Table 2.12 Median ions concentration (mg/L) of the DLF at inlet (IN) and outlet of every filter. "*n.f.*" indicates that the value was not found.

	Cl	**Br**	**SO_4**	**Li**	**Na**	**K**	**Mg**	**Ca**
					mg/L			
IN	900	*n.f.*	18	*n.f.*	1,276	5,499	194	425
BRICK	1,134	*n.f.*	191	*n.f.*	2,357	2,871	132	343
REFR30-50	907	*n.f.*	69	*n.f.*	1,171	5,133	164	354
REFR20-30	931	*n.f.*	154	*n.f.*	1,021	4,842	198	401
GRAVEL	684	*n.f.*	28	*n.f.*	1,266	5,204	191	387

Nitrogen

The total nitrogen (TN) found in the DLF was formed by 72% of ammonium-nitrogen and 6.8% of nitrate-nitrogen, considering medians. In agreement with this content, Möller and Müller (2012) reported that a total of 45–80% of the N in the liquid phase is present as NH_4-N.

The TN concentration at inlet was higher in the first six monitoring cycles (between 3,080 and 4,980 mg/L) and lower in the last two (3,710 and 3,030 mg/L, respectively), presenting a median value of 4,035 mg/L (Figure 2.16). The filtration decreased the TN concentration especially after the third cycle, with similar downward trends among the filters, till reaching concentrations between 1,368 (gravel) and 2,280 mg/L (refractory 20-30 mm) in the last cycle.

The nitrate-nitrogen (NO_3-N) concentration of the IN digestate had a stable trend throughout the period of study, in the range 200-314 mg/L (median 276 mg/L) (Figure 2.16). Vice versa, the filtration showed up and down trends over time, common for all filters. Specifically, the NO_3-N concentration rose until the 5th cycle, reaching also 500 mg/L (in the filter with gravel substrate), to fall in the last monitoring cycles, achieving similar values to the inlet. The increment in NO_3-N concentration after filtration between the 3rd and 6th cycles was probably due to the increase of dissolved oxygen (as shown at the outlet), which caused the oxidation of the organic and ammonium nitrogen forms, as reported below. Considering the entire period, no significant differences were observed for nitrate-nitrogen between the inlet and outlet values.

The ammonium nitrogen (NH_4-N) content of the inlet DLF ranged between 1,624 and 3,380 mg/L, with an irregular trend over time (Figure 2.16). The trends of NH_4-N content at outlet, similar among the filters, presented higher values with respect to the IN at the beginning of the experiment (first two cycles), and thereafter there was a sharp concentration decline till the last cycle. In particular, there was a strong diminution of the ammonium-nitrogen concentration from the 3rd to the 6th cycles, from 2,770 to 495 mg/L on average, in which higher filters oxygenation occurred. The NH_4-N concentration (median 2,910 mg/L) was significantly reduced after treatment by the filter filled with the porous refractory 20-30 mm (R= 75%), which achieved a median value of 741 mg/L at outlet (Figure 2.16). Aeration in the filter systems provided by intermittent recirculation resulted in the reduction of NH_4-N concentration due to oxidation of this parameter. Recirculation is usually adopted for water treatment in aerobic biological filters. It has many benefits, such as equalizing filter load, enhancing treatment efficiency and reducing influent odour (Wei et al., 2010).

The trends of inlet and outlet nitrogen quantities (Figure 2.17) were similar to the concentration trends previously reported. The total nitrogen quantity at inlet varied from

1,141 to 1,844 g/m^3/filter (median 1494 g/m^3/filter) per cycle, which was lowered over time by all filters, with a final mass abatement of between 28.5% by porous refractory 20-30mm (802 g/m^3 at outlet) and 60% by gravel (456 g/m^3 at outlet) (Figure 2.17). In particular significant differences were found among the median weekly inlet and outlet quantities of the REFR20-30mm filter, which showed a median value of 953 g/m^3 and MA of 37% . The daily TN removal (median) oscillated between 60 (brick) and 90 g/m^3/d (gravel), with MA in the range 13-39%, without significant differences among filters (Table 2.13).

The inlet NO_3-N quantity per cycle was in the range 71-117 g/m^3/filter (median value of 102 g/m^3/filter). In general the nitrate-nitrogen outlet quantity exceeded the inlet quantity in the monitoring period from the 3rd to the 6th cycles, especially in the 5th cycle when it reached about 160 g/m^3 in filters with brick (MA= -51%), refractory 20-30 mm (MA= -54%), and gravel (MA= -61%). In the last monitoring cycle the NO_3-N quantity was reduced by all filters, with mean MA of 11% (Figure 2.17). Considering the entire period, the filtration was shown to increase the quantity of NO_3-N, between 0.3 and 2.3 g/m^3/d, and mass abatement was not observed (Table 2.13).

The NH_4-N quantity at inlet per monitoring cycle ranged between 601 and 1,252 g/m^3/filter (median 1078 g/m^3/filter) (Figure 2.17), which was progressively reduced till less than 400 g/m^3/filter in the last cycle. The ammonium inlet quantity was significantly reduced by the filter with porous refractory 20-30 mm, with a median value of 248 g/m^3 per week and mass abatement of 77%. The filters removed between 24 g/m^3/d (REFR 30-50) and 115 g/m^3/d (REFR 20-30), and provided a mass abatement in the range 9-78%, without significant differences among the filtering substrates (Table 2.13).

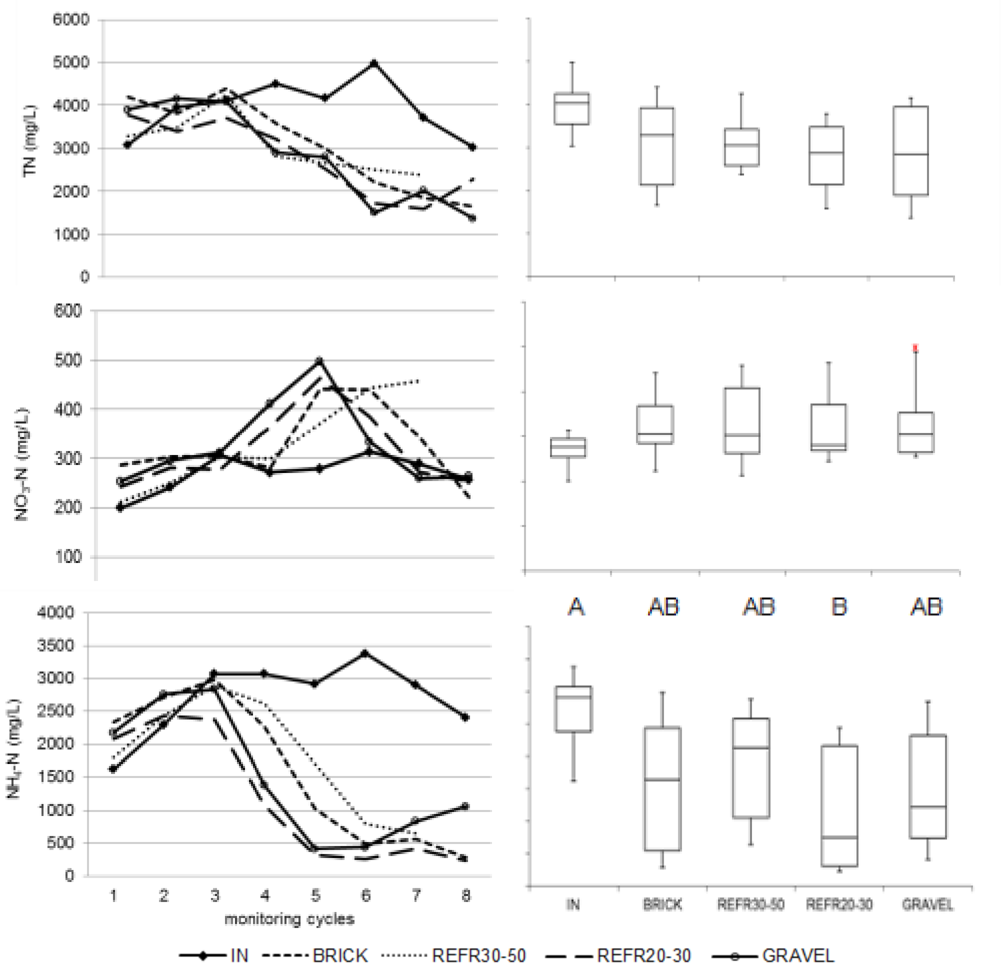

Figure 2.16 Trends and box-plot of the DLF TN, NO_3-N, NH_4-N concentrations (mg/L) detected before (IN) and after filtration for every filter. Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

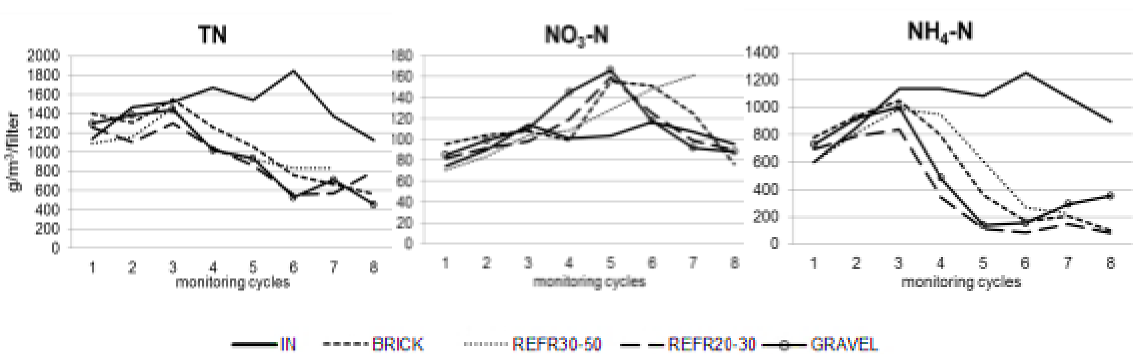

Figure 2.17 Trends of the TN, NO_3-N, NH_4-N quantities (g/m^3/filter) at IN and at outlet of every filter.

Phosphorus

The DLF presented a total phosphorus (TP) content between 266 and 442 mg/L (median 412 mg/L), with lower values at the first and last monitoring weeks (Figure 2.18). Excepting these cycles, the concentration was reduced with filtration by all substrates, except the refractory with larger grain size (REFR 30-50mm), which also presented the highest data dispersion with median values at outlet in the range 342-397 mg/L (Figure 2.20).

The soluble phosphorus (PO_4-P) content of the digestate represented 85% of TP. The trends over time of this chemical element at inlet and outlet were very similar to the TP trends (Figure 2.18) with porous refractory 30-50 mm that exceeded the IN concentration from the 3rd cycle. The PO_4-P concentration detected at IN varied between 204 and 416 mg/L (median 352 mg/L), and was significantly reduced by the filter filled with gravel (R= 71%) with a median outlet concentration of 248 mg/L.

The trends of inlet and outlet phosphorus amounts (Figure 2.19) were similar to the previously shown concentration trends. For each monitoring cycle the inlet TP quantity corresponded to a minimum of 99 g/m^3/filter and a maximum of 164 g/m^3/filter (median 152 g/m^3/filter), and was lowered to approximately 120 g/m^3/filter at the 5th cycle by brick, refractory 20-30 mm, and gravel. The median inlet weekly TP quantity was significantly reduced by gravel substrate, which reached 118 g/m^3 at outlet, with MA of 23%. The daily TP removal ranged between 2.0 (REFR 30-50) and 5.6 g/m^3/d (gravel), which corresponded to the MA of 9% and 25%, respectively (Table 2.13), without significant differences among filtering substrates.

The PO_4-P quantity at the inlet varied from 76 and 154 g/m^3/filter per cycle (130 mg/L as median), while at the outlet it was lower in the filters filled with brick, porous refractory 20-30mm and gravel, which showed up and down trends (Figure 2.19). Also for this parameter, gravel substrate proved to reduce the weekly PO_4-P quantity till 87 g/m^3 with a mass abatement of 34%. The filters proved to remove a PO_4-P quantity between 3.4 and 6.6 g/m^3/d, with MA in the range 19-33% (Table 2.13). However, no significant differences were observed among filtering media for soluble-phosphorus removal.

In sand or gravel substrates, phosphorus is bound to the media mainly as a consequence of adsorption and precipitation reactions with Ca, Al and Fe. As calcium ions can form stable and insoluble products with phosphate, calcium-based materials are considered to be one of the potential sorbents for phosphorus removal (Vohla et al., 2011). At pH levels greater than 6, the reactions are a combination of physical adsorption to iron and aluminium oxides and precipitation as sparingly soluble calcium phosphates. Guo et al. (2005) set up a substrate by using gravels, zeolites, shales and ceramsites to test the effect of phosphorus removal, indicating that gravels were best in the removal of phosphorus in wastewater, with a removal rate that can reach 95%.

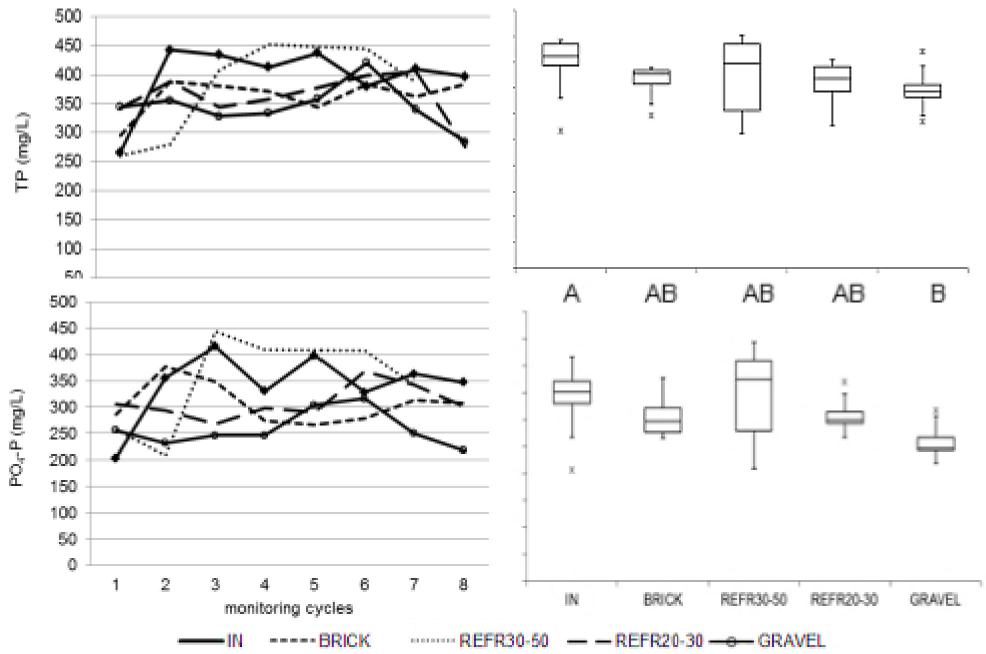

Figure 2.18 Trends and box-plot of the DLF TP and PO_4-P concentrations (mg/L) detected before (IN) and after filtration for every filte. Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

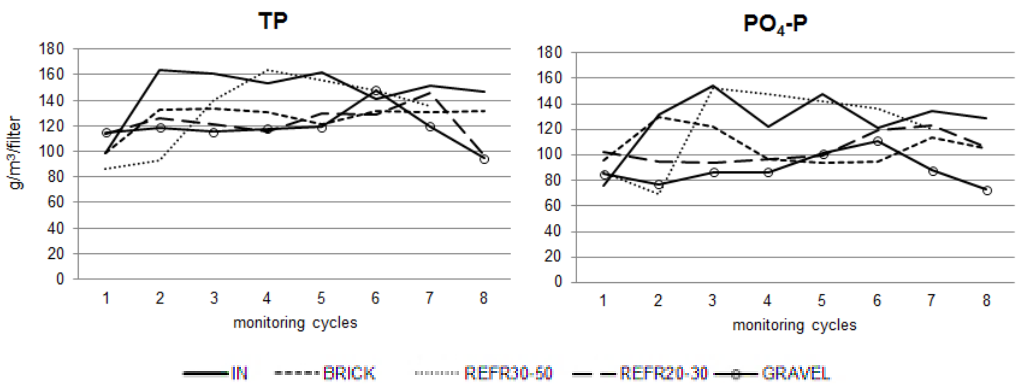

Figure 2.19 Trends of the TP and PO_4-P quantities (g/m^3/filter) at IN and at outlet of every filter.

Organic chemicals

The values of chemical oxygen demand (COD) measured on inlet digestate during the monitoring period ranged between 40,620 mg/L at the 1st cycle to 71,170 mg/L at the 4th cycle (median 59,035 mg/L) (Figure 2.20). In general, the COD outlet concentration was lowered by the filtration, with trends similar among filters filled with brick, gravel and refractory with smallest grain size, which achieved the lowest value close to 30,000 mg/L in the last monitoring cycle. The median outlet COD concentration was in the range 50,190-54,100 mg/L (Figure 2.20).

The 5-day biological oxygen demand (BOD_5) concentration remained stable over time at the inlet, with values close to 3,800 mg/L (Figure 2.20). Similar values were found in Alburquerque et al. (2011) for digestate coming from a co-digestion of pig slurry and agro industrial residues as co-substrates. The filtration reduced this parameter at the filter outlets, but different and irregular trends were seen. Brick and refractory 30-50 mm filters showed the higher data dispersion, anyway medians were similar among filters, in the range of 2,250-2,500 mg/L.

Trends of the COD quantity (Figure 2.21) were very similar to the concentration trends previously shown. Per cycle, the COD quantity at IN was in the range 15,000-26,000 g/m^3/filter, with median value of 21,865 g/m^3/filter. The filters with brick, refractory 20-30mm and gravel presented similar trends, and till 7th cycle they provided a COD quantity at outlet of around 18,000 g/m^3, reaching 10,000 g/m^3 in the last cycle. The filter filled with REFR 20-30 significantly reduced the weekly COD quantity to 17,506 g/m^3 (median), showing MA of 20%. The median removal of this parameter obtained by the filters fluctuated from 408 (REFR 30-50) to 737 g/m^3/d (REFR 20-30), with median mass abatements in the range 12-22%, without significant differences among materials (Table 2.13).

The trends of BOD_5 quantity at outlet were similar to those for the concentration, while the IN trend was different (Figure 2.21). Per cycle, the system received between 1,444 and 822 g/m^3/filter (median 1,407 g/m^3/filter), and showed a quantity less than 1200 g/m^3/filter at outlet. The daily removal quantity varied from 79 and 96 g/m^3/d and the MA was between 17 and 33%, without significant differences among filters (Table 2.13).

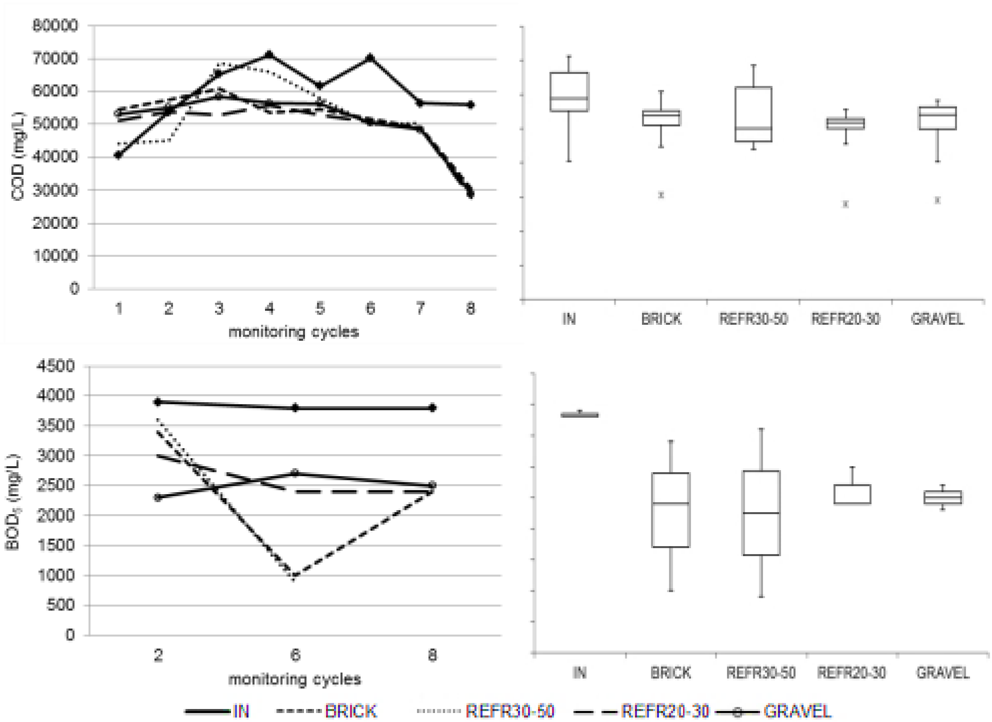

Figure 2.20 Trends and box-plot of the DLF COD and BOD_5 concentrations (mg/L) detected before (IN) and after filtration for every filter. Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

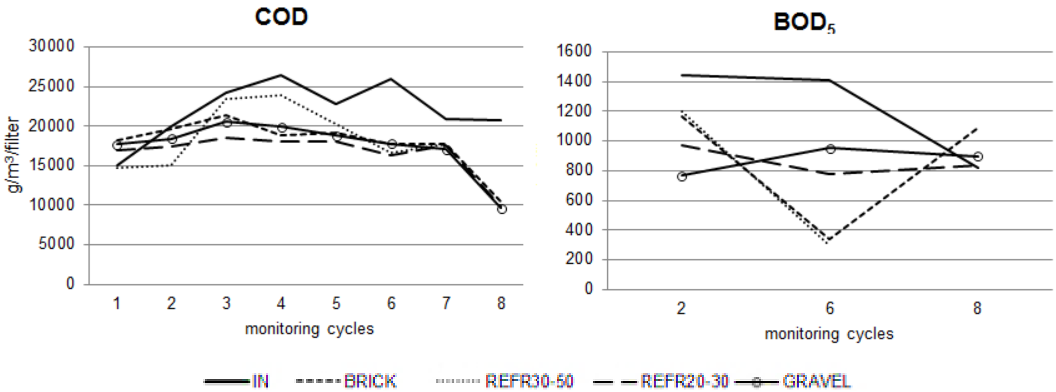

Figure 2.21 Trends of the chemical oxygen demand (COD) and 5-day biological oxygen demand (BOD_5) quantities (g/m^3/filter) at IN and at outlet of every filter: BRICK, REFR 30-50mm, REFR 20-30mm, GRAVEL.

Aeration on filters results in the removal of ammonia and organic compounds, as reported by many authors (e.g. Chang et al., 2009; Westerman et al., 2000; Albuquerque et al., 2012a; Akunna et al., 1994). Albuquerque et al. (2012a) reported that within the applied ranges of loading rates of ammonia between 6 g and 903 g N/m^3 d and organic compounds between 48 g and 2,391 g COD/m^3 d, the results showed that the partially aerated BAF (biological aerated filter) reactor allowed carbon removal, nitrification and denitrification simultaneously, at significant removal rates. The long-term operation of the BAF reactor was characterized by stable and relatively high removal efficiencies in terms of both COD (<80%) and NH_4–N (<75%). Between 50% and 70% of ammonia removal occurred in the upper section of the BAF (MT-P2), where larger biofilm development was observed and DO concentrations remained over 2.1 g O_2/m^3. The applied NLR (nitrogen loading rate) significantly influenced the removal rates of ammonia for NLR above 32.6 g NH_4-N/m^3 d. Wei et al. (2010) reported that COD removal significantly improves at recirculation rate increasing from 1 to 4. When the recirculation rate is above 4, COD reduction enhances slightly with increasing recirculation rate, which indicates the remaining organic matter in the recirculation water is difficult to degrade by increasing recirculation rate and COD removal of effluent is not easily further improved.

Table 2.13 Daily chemical removal quantity ($g/m^3/d$; medians) and mass abatement (MA, %) of each filter. The asterisk indicates significant differences ($p<0.05$ by Kruskal-Wallis test) between the IN and outlet chemical quantity ($g/m^3/d$).

	TN		**NO_3-N**		**NH_4-N**		**TP**		**PO_4-P**		**COD**		**BOD_5**	
	$g/m^3/d$	MA %	$g/m^3/d$	MA %	$g/m^3/d$	MA %	$g/m^3/d$	MA %	$g/m^3/d$	MA %	$g/m^3/d$	MA%	$g/m^3/d$	MA %
BRICK	64.3	28	-2.3	-16	75.9	48	3.1	14	3.5	19	487.4	16	96.0	19
REFR 30-50	60.2	13	-0.3	-8	24.4	9	2.0	9	-0.7	-5	407.7	12	96.6	17
REFR 20-30	70.8*	33	-0.6	-5	114.5*	78	4.9	22	3.4	19	737.0*	22	78.7	33
GRAVEL	90.1	39	-0.7	-6	85.1	59	5.6*	25	6.6*	33	564.7	18	81.1	33

CONCLUSIONS

The general purpose of this research was the development of an easily repeatable and functional filtration system for the pretreatment of clarified digestate originating from biogas plants. On this basis the research focused on the filters design and development, using recycled materials as media, to increase the sustainability and the practical interest of this economical system. The limited duration of the project did not allow to prototypes to be constructed and monitored in a definitive way and led to the use, as far as possible, of materials and opportunities available at the two companies involved in the project. However, this study gave interesting preliminary results for the treatment of a high pollutant wastewater like the digestate liquid fraction, showing an abatement capacity of the recycled materials even greater than gravel medium. The filter systems showed chemical reduction after a feeding period of three weeks, especially for TN, NH_4-N and COD, suggesting that they require an adjustment period to show good performances.

The filters proved to low the electrical conductivity at outlet, but on the other hand they increased the pH (except gravel filter). When digestate is applied to plants for fertilization, these parameters (especially electrical conductivity) can be harmful for plants when present in non-optimal values. Wastewater salinity is an important parameter to be considered according to plants, as it can cause different negative effects in not tolerant or resistant plants, such as productivity loss, reduced growth, damage to aerial part and root system, and also plant death (Parida and Das, 1995; Shannon and Grieve, 1998). In the case of soilless cultivation, for example, undiluted digestates with high electrical conductivity are not suitable (Liu et al., 2009). The key problems obstructing their application in soilless culture are the variability of components and the imbalance in the element composition (Liu et al., 2011). To avoid this, appropriate dilutions of 1:4 to 1:8 (digestate:water) are often used (Liu et al., 2009). Therefore, special care must be taken since excessive doses or continued applications of digestates rich in Cl and Na to soils, could lead to an increase in soil salinity and inhibit plant growth.

In the case of wetland plants, the presence of salt may constrain the type of plants used in a CW for wastewater treatment since excessive salinity contents within the plant root zone has a general deleterious effect on plant growth (Rhoades et al., 1992). Increases in salinity may restrict the ability of macrophytes to mitigate the effects of rising water levels. As salinity increases, rates of photosynthesis and biomass production typically decrease in non-halophytic macrophytes (Sculthorpe, 1985). Biomass allocation patterns can also shift as salinity increases, with lower proportional investment in aboveground tissues and increasing allocation of biomass to roots as changes in osmolality restrict uptake of water and nutrients (Morris and Ganf, 2001). As typical wetland plant, *Phragmites* spp. is commonly found in brackish waters and can tolerate salinity up to 20 ppt (Wallace and Knight, 2006) and it can therefore be used for treatment of saline wastewaters or for CWs in areas with high evapotranspiration, which increases salinity of the wastewater. *Phragmites* spp., together with other species, are therefore widely used in CWs for the treatment of high salinity wastewaters, such as tannery wastewater (EC>16 mS/cm), proving plants resistance and good performance on chemical removals (Calheiros et al., 2008, 2009, 2010, 2012).

Another physical parameter derived from filtration that could damage plants is the alkaline pH. The principal role of macrophytes at high pH is however to provide a continued carbon source for the microbial decomposers responsible for elevating CO_2 in the wetland. There are a number of other potential direct and indirect nutrient cycling changes that can also be of significance for vegetation growth at high pH. These include reduced solubility of micronutrients (particularly Fe, Mn, B, Zn and Cu; Brady and Weil, 1997), reduced microbial activity, reduced availability of potassium (Kinzel, 1983) and direct toxicity of the OH^- (Rowell, 1988). Many treatment bacteria are not able to exist outside the range $4.0 < pH < 9.5$ (Metcalf and Eddy Inc., 1991).The importance of biological activity in lowering the pH of highly alkaline effluents has also been noted by Orupõld et al. (2000) at lagooned oil-shale leachate operations. While there is evidence of diminished vegetation productivity at extreme pH compared to circum-neutral pH reference conditions (e.g. Batty and Younger, 2004), clonal dominants such as *Phragmites australis* and *Typha latifolia* have been documented to maintain vigour at both extremes of the pH spectrum. Successful propagation of vegetation in extreme pH conditions is essential for maintaining readily-available sources of carbon for microbial decomposers.

Besides the values of EC and pH obtained by filtration, for which CWs could tolerate, there are also chemical parameters to consider derived from the DLF filtration. Operating principle of the filters analyzed in this study was the substrate filtration in which the wastewater oxygenation took place through recirculation. Significant results, derived from aeration, have been seen for the reduction in ammonium-N concentration for the filter filled with porous refractory with smallest grain size, where DO concentration at outlet was lowest. This suggests that the feature of this material of being porous adsorbent, combined with smaller grain size, allowed a greater HRT, which is fundamental for pollutant removal. Porous refractory had the lowest value in the CEC test for ammonia adsorption. This could mean that the ammonium removal by this substrate is not related to its ability to remove ammonium (as seen in the CEC test) but to oxidizing processes. It also gave significant reduction of TN and COD quantities compared to the DLF inlet quantities. However, for the other materials, the removal of nitrogen and organic forms did not take place, probably due to not adequate contact time to oxidize the organic matter, made of big and not easily degradable macromolecules. Nevertheless the final values obtained from filtration (in particular the COD) may still be too high for treatment by constructed wetlands. Chemical values of filtered DLF obtained in this study are similar to those of landfill leachate from municipal landfills, which can contain 3,000−10,000 mg L^{-1} of NH_4-N and up to 60,000 mg L^{-1} COD (Tatsi and Zoubolis, 2002). In the USA and Europe, including the temperate and sub-polar climate regions, landfill leachate is depurated by CWs (Kadlec and Wallace, 2009). These systems could thus be able to survive the high chemical contents of these wastewaters, although particular attention should be paid to the other physical and chemical components. Some authors reported that digestate application has no phytotoxic effects (Sànchez et al., 2008; Gell et al., 2011), others found phytotoxic reactions (Salminen and Rintala, 2002; Abdullahi, et al., 2008). Phytotoxicity is related to NH_4-N (Salminen and Rintala, 2002; Drennan and DiStefano, 2010) and organic acid concentrations (Salminen and Rintala, 2002; Abdullahi et al., 2008; Drennan and DiStefano, 2010). Nutrient loading rates, specifically nitrogen species, have been one of the key parameters for wetland design the world over in recent years. This is largely due to the toxic nature of some nitrogen species, such as ammonium (NH_4) and ammonia (NH_3) to macrophyte assemblages (Hill et al., 1997; Hunt et al., 2004). Generally, keeping ammonia to lower levels (100–400 mg NH_3/L) that do not impede macrophyte growth is common practice (Harrington et al., 2005; He et al., 2006). However, there have been pilot scale systems that have examined higher concentrations up to 2,500 mg TN/L (Knight et al., 2000; Hunt et al., 2002).

Therefore, the introduction of strongly ammonium influents to treatment wetlands must be avoided.

Other nitrogen forms, such as nitrate-nitrogen, which remained similar to the inlet DLF (around 300 mg/L), seem to be a not harmful chemical element for wetland plants. In nitrate-rich waters, nitrate may become a more important source of nutrient nitrogen. Nutrients are assimilated from the sediments by emergent and rooted floating-leaved macrophytes, and from the water by free-floating macrophytes (Wetzel, 2001). Plant uptake of nitrate is the main mechanism for its removal in CWs, although the denitrification process plays an important role in its disappearance (Vymazal, 2007). Like nitrate, other nutrients such as phosphorus and potassium, have not been observed to create toxicity to wetland plants. Soluble reactive phosphorus is taken up by plants and converted to tissue phosphorus or may become adsorbed to wetland soils and sediments. Organic structural phosphorus may be released as soluble phosphorus if the organic matrix is oxidized. Most of the phosphorus is taken up by plant roots, absorption through leaves and shoots is restricted to submerged species but this amount is usually very low (Vymazal, 2007). In other environments, phosphate overload can lead to diffuse pollution and excessive P concentrations (eutrophication) of coastal and inland waters.

In general the grain size of the material and the oxygen supply played an important role in this experiment, although the phosphorus abatement (in the filter filled with gravel substrate) was related to precipitation or adsorption processes. On this basis, the studied system can be improved to ameliorate the performance on chemical removal, through several modifications:

- reducing and testing other grain sizes, especially for brick substrate;
- increasing the filter volume;
- raising the recirculation;
- arranging the filters together, to improve removals.

To allow higher retention time and solids removal a smaller grain size is the basic element, but it must be able to avoid clogging. Prolonging the retention time of wastewater increases contact time with microorganisms for wastewater and can induce the increment of the filter volume. A correct computation of the filter volume considers the feed rate and medium filtration rate (Sutherland, 2011); obviously if different media have to be used, the filter volume will differ, so a solution must be found. The increasing of recirculation rate, that results in prolonging the retention time, is another essential factor to contemplate to enhance chemical removal. However, it is important to consider that high recirculation rate increases the operating costs of water treatment projects (Baeza et al., 2004; Xiao et al., 2007).

It is understandable that the results obtained from this study must be considered as preliminary and that further research developments will provide better knowledge on processes and operational repercussions. In particular, in addition to validating the modifications described above for improvements development, the filters behaviour in the medium-long term must be also investigated, to verify, for example, the clogging and types of maintenance operations.

Chapter III

EFFECT OF AGEING ON HYDRODYNAMICS OF STEEL SLAG FILTERS TO UPGRADE PHOSPHORUS REMOVAL FROM WASTEWATER

THE CASE STUDY

This research was part of the SLASORB project (research programme under grant agreement no. RFSPCT-2009-00028 (SLASORB)), where the aim was the use of Electric arc furnace steel slag (EAF-slag) and basic oxygen furnace steel slag (BOF-slag) produced in Europe as substrate in filters designed to remove P from the effluents of small WWTPs. The experiment was realized and started in 2010 at the Ecole des Mines de Nantes (Nantes, France).

The study regarded the hydrodynamics study of filters filled with reactive materials to treat phosphorus by means of tracer tests, spending a period abroad of 3 months at the Ecole des Mines de Nantes.

Premise

The evolution of the European legislation regarding domestic wastewater recently led to a growing need for processes able to remove durably phosphorus at the outlet of small and very small wastewater treatment plants (SBR, waste stabilization ponds, constructed wetlands). Several recent researches have shown evidences that the use of steel slag filters represent a technical and environmental solution (Vohla et al., 2011; Barca et al., 2012a,b; Barca et al. , 2014). The main mechanisms of phosphorus removal in slag filters are related to Ca-P precipitation followed by crystallization (Barca et al., 2012b), therefore there is an accumulation of precipitate in the filtration matrix that could lead to a decrease of the performances.

In this context our work aimed at regularly measuring the hydraulic performances in several pilots scale slag filters from the commissioning period up to 2.5 years after use.

MATERIALS AND METHODS

Pilot scale column system

The experimental system is still in operation and has been the subject of numerous studies (Barca et al., 2012a,b, 2014). Two steel slag types with two different particle sizes on P removal performances were studied. EAF-slag produced in Esch Belval (Luxemburg) and BOF-slag produced in Fos sur Mer (France) were tested in four continuous flow column experiments to determine their capacities to remove phosphorus (P) from a synthetic wastewater containing an inlet concentration of 10 mg P/L as representative of a municipal wastewater.

Two different designs were chosen according to a ratio <0.5width to length, because low ratio of width to length may favor dissipation of initial turbulence and favor an uniform distribution of the flow along the cross section of the columns (Alcocer et al., 2012). Also, the ratio of width of the filter to size of slag was always>10, in order to improve hydraulic performances and limit wall effects (Zeiser et al., 2001). Small size columns had a width of 0.15 m and a length of 0.7 (about 42 L of total volume), thus leading to a ratio of width to length of about 0.21, whereas big size columns had a width of 0.3 m and a length of 0.7 (about 84 L of total volume), thus leading to a ratio of width to length of about 0.42 (Figure 3.1 A). The design of the filters was adapted from a previous study that investigated the effect of different sizes of slag on the hydraulic performances (Anjab, 2009).

Two columns were filled with EAF-slag , one with small-size slag (5-16 mm) and the other one big-size slag (20-40 mm). The other two columns were filled with BOF-slag , everyone with different slag size: 6-12 mm and 20-50 mm (Figure 3.2). These granular sizes were considered large enough to prevent filter clogging, as found in a previous study (Chazarenc et al., 2007). The slag samples used in this study had a bulk density of about 1.8 g/cm^3 (EAF-slag) and 1.6 g/cm^3 (BOF-slag), and a bulk porosity of about 50%.

The experimental plant was monitored in 2013 for 59 days, from October 23th to December 20th . During this period of operation, the columns were continuously fed with a synthetic P solution made of tap water and KH_2PO_4 with a P concentration of about 10 mg/L, which is into the range of the typical P concentrations of municipal wastewaters (4–16 mg P/L, according to Tchobanoglous et al., 2003), at a continuous sub-superficial horizontal-flow (HSSF) (Figure 3.1 B) of 0.6 L/h (small-size columns) and 1.2 L/h (big-size columns), thus resulting in a void hydraulic retention time (HRT) of about 24 h.

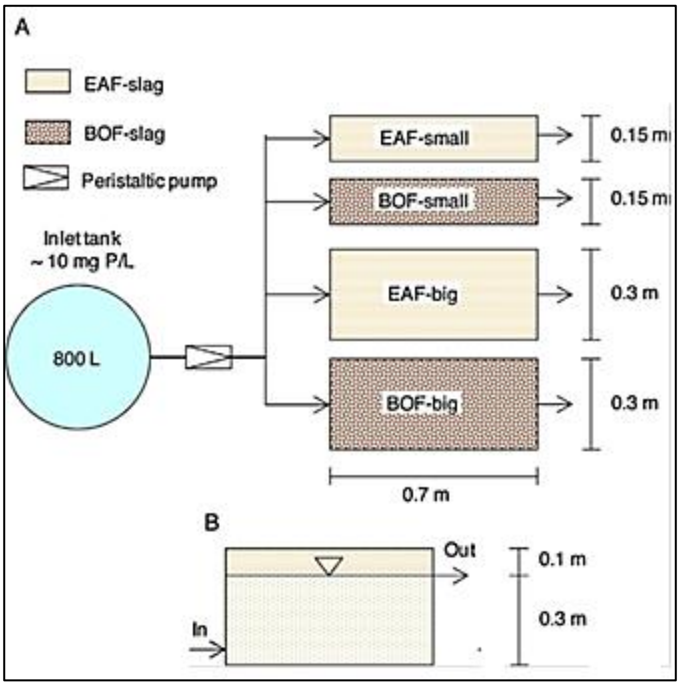

Figure 3.1 Experimental setup: (A) top view of the filters; (B) cross section view of the filters (modified figure reported in Barca et al., 2014).

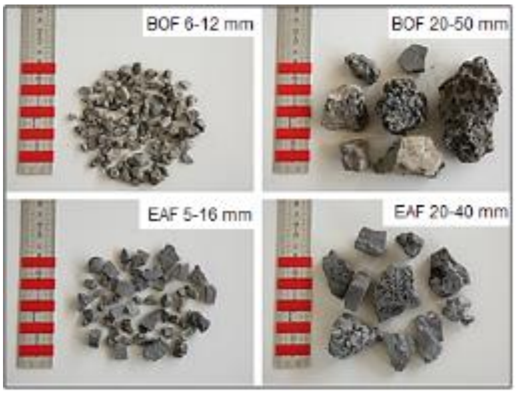

Figure 3.2 Slags used in column experiments.

The HSSF was adopted to limit the dissolution of atmospheric CO_2 in water, because it is known that CO_2 is an inhibitor of P precipitation (Valsami-Jones, 2001). The HRT of 24 h was chosen because previous studies showed good P removal performances of steel slag filters (Shilton et al., 2005; Drizo et al., 2006).The synthetic P solution used in this study was prepared without the addition of organic content and suspended solids. However, it was considered sufficiently representative, from the chemical point of view, of a real effluent, as constructed wetland effluents in France usually have very low levels of organic content and suspended solids (Molle et al., 2005).

Four peristaltic pumps (Masterflex® L/S Tubing Pumps) were used to feed every column, by taking the wastewater from a tank that every 3 days was filled. The experiments were performed under room temperature condition (approximately 20°C).

Treatment performances

Every week the inlet volume of the columns was checked, while phosphorous analyses were made every two weeks. Monthly also tracer test was carried out to evaluate hydrodynamics of the columns and their evolution over time.

For the analyses water samples (about 100 mL) were collected from the inlet tank and from the outlet of each column. pH, total phosphorous (TP) and phosphate (PO_4-P) concentrations were measured. pH was measured in fresh water samples using model C5010 conductivimeter (Consort bvba,Turnhout, Belgium). TP and PO_4-P analyses were performed according to the Ammonium Molybdate Spectrometric Method (EN ISO 6878, 2004). TP and PO_4-P contents were determined after acidification of the samples by adding some drops of 1M HCl to dissolve suspended Ca phosphate precipitates; to find the PO_4-P content a filtration of samples with 0.45 µm filters occurred before acidification. The content of phosphorous was detected after reading of the absorbance (880 nm) with the model U DR/4000 spectrophotometer (Hach Company, Loveland, USA).

Tracer tests and data modelling

Tracer tests were made using fluorescein as tracer substance, with a concentration of 4g/L by performing a pulse injection of a volume of 25 mL for small-size columns, and 50 mL for big-size columns. The samples were taken every 2 hours with a total number of samples of 36 after the pulse injection.

During a period of 3 years, a total number of seven tracer tests were performed for each pilot system (Table 3.1), of which 5 tracer tests were made before the commissioning period (week 0-week 153). The results of tracer tests gave a general overview of the evolution of hydrodynamics over time. From the tracer test, the RTD (residence time distribution) curve was calculated and compared to the theoretical retention time τ of 24.6h.

By comparing the theoretical (τ) and observed (ts) HRTs, certain unique hydraulic behavior was revealed:

• ts > τ: Inflow crosses the reed bed without reacting; this is an indicator of short-circuiting. Fluid follows a preferential path and the first peak is seen at an earlier stage of the curve than in the theoretical curve, which peaks at a later stage.

• ts < τ: Fluid stagnates in the reactor and does not participate in reactions. This phenomenon is due to the presence of dead or stagnant zones.

Then two conventional models have been employed to describe the non-ideal flow: the plug flow reactor with axial dispersion (=dispersion plug flow model (DPFM) and the tank in series model (STSM). In the DPF model, a dimensionless number is thus used, the Péclet number:

$$Pe = Ul/D$$

where: U is the flow speed (m s^{-1}), l the flow length (m), and D the diffusion coefficient (m^2 s^{-1}).

The DPFM applies in the case of flow close to plug flow and is based on the superimposition of a simple convective plug flow with an unpredictable dispersion model obeying Fick’s law (Levenspiel, 1972). In the STSM, the flow path in the non-ideal reactor is represented through a series of n equal-size ideal stirred tanks (STR) separated by waterfalls. If $n \rightarrow 1$, then there is a large dispersion, and if $n > 100$, flow is similar to a plug flow (Chazarenc et al., 2003). The models were tuned using the software excel (solver by minimizing the pearson correlation between the experimental curves and the theoretical model data).

Table 3.1 Tracer tests performed in the experimental plant, with number, date and number of progressive operational week.

Number	Date	N° of progressive week
1	August 2010	0
2	September 2011	53
3	July 2012	98
4	April 2013	136
5	August 2013	153
6	October 2013	165
7	November 2013	170

RESULTS

Tracer tests results

Because the given column system had a slight vertical slope from bottom to top, the main flow direction was not rectangular to the longitudinal cross-section. Thus, the columns cannot represented an ideal plug flow, but anyway the hydraulic behavior should be very similar.

The EAF columns showed in the first week the lowest traced recovered quantities (around to 76.5%), which were increased over time around almost 100% since the week 136 (Table 3.2). The observed HRT (ts) in the big EAF column was in general greater than the theoretical HRT. Before regular operation (week 0) and in week 136 the EAF big column showed a similar effect of shortcut flow (ts=23 h). In the other weeks tracer tests indicate massive channelling effects which get stronger over time. One explanation of this phenomenon can be that crystallisation of $CaCO_3$ and Ca-P-complexes blocks the shortcut flow paths. The hard edged chip shape of EAF-slag may lead to widely different loop way lengths, which occur as multi peaks in the later tracer curves.

The HRT of the small EAF column was greater before regular operation (41 h) and over time it was more similar to the theoretical HRT, although it was greater, in the range 28-32 h. Differently from the bigger one, the smaller grain showed mixed flow and recycling before system launch and changed to channelled flow after (Table 3.2).

The BOF columns showed different hydraulic behaviour according to the slag grain size. The traced recovered quantities in big BOF column were irregular over time, in general higher than 70%. The small BOF column showed more regular tracer test recover, with percentages approximately of 100% in 2013 (Table 3.2). The flow characteristic of big BOF column before system launch displayed a massive shortcut effect. On week 98 the observed HRT was really high (39 h) , with plug flow shape presenting a wide spread, indicating that the main flow paths were clogged and dead volumes were activated. Afterwards the observed HRT was more similar to the theoretical HRT, although different phenomena inside the reactor occurred, highlighted from a widely spread curve due to flow distribution for flow mixing or preferential flow paths.

The smaller BOF-slag showed on week 0 an unpredictable hydraulic behaviour as a mix of shortcut and channelled flow. This improved in the following weeks of regular operation, and after 53 weeks the hydraulic characteristic was closed to an ideal plug flow, with HRT in the range 23-25 h in general (Table 3.2) . The efficiency was better than for the bigger size, what might be explained due to the ratio between slag size and reactor volume.

Table 3.2 Pulse input tracer details of pilot scale columns systems (τ =24.6 h).

	EAF big		**EAF small**		**BOF big**		**BOF small**	
	Tracer recovered %	ts (h)	Tracer recovered %	ts (h)	Tracer recovered %	ts (h)	Tracer recovered %	ts (h)
week 0	76.0	23	77.1	41	82.2	26	66.0	32
week 53	82.6	34	83.3	31	82.3	30	83.3	25
week 98	87.2	31	91.9	30	77.2	39	88.4	25
week 136	96.0	23	100	28	100	30	100	23
week 153	90.9	26	100	32	86.1	28	100	25
week 165	96.4	33	99.1	29	78.3	30	98.2	25
week 170	99.5	29	100	26	100	27	100	27

Hydraulic performances

The modeling results showed for the EAF columns an increase in the *Pe* number and in the number of STR, indicating an improvement of the flow behavior over time (Table 3.3). Values of the theoretical HRT found with the STS model are very close to the observed HRT values, with HRT between 23 and 35 h for big EAF column and between 21 and 49 h for the small EAF reactor.

The big EAF column at the beginning (week 0) of the experiment till the week 53 and from the week 165 presented slow internal circulation. Similar happened for the small EAF column, but irregular behaviour was observe from week 136 to week 170 (Table 3.3). The lowest values found at the beginning and at the end of experiment probably indicate the presence of dead zones in the columns.

The modeling of the BOF columns displayed in general an increase in the *Pe* number and in the number of STR, indicating an improvement of the flow behavior over time (Table 3.4). The columns with smaller slags shows highest *Pe* and STR number , suggesting that it had a better behavior compared to the big size slags reactor. Values of the theoretical HRT found with the STS model are very close to the observed HRT values, with HRT between 26 and 48 h for big BOF column and between 17 and 47 h for the small BOF reactor.

Table 3.3 Modeling results of the big and small EAF columns.

	EAF big				EAF small			
	Pe	τDPF	number of STR	τSTS	Pe	τDPF	number of STR	τSTS
week 0	1.8	14	2	24	1.4	28	2	49
week 53	5.3	25	3	33	7.3	25	4	31
week 98	21.1	27	10	29	29.2	28	14	29
week 136	30.1	22	14	23	28.0	21	19	21
week 153	28.9	25	13	26	11.8	26	6	30
week 165	7.7	29	5	35	21.3	25	11	27
week 170	13.3	26	6	29	9.9	22	6	26

Table 3.4 Modeling results of the big and small BOF columns.

	BOF big				BOF small			
	Pe	τDPF	number of STR	τSTS	Pe	τDPF	number of STR	τSTS
week 0	1.1	13	1	38	1.0	17.0	1	47
week 53	2.4	19	2	30	2.4	17.0	2	27
week 98	1.4	26	2	48	6.1	18.1	4	23
week 136	5.5	20	3	27	7.2	13.8	4	17
week 153	5.9	22	4	27	7.8	17.0	4	21
week 165	5.4	21	4	26	8.4	19.1	5	23
week 170	4.9	20	3	27	10.2	21.1	6	24

P removal and pH monitoring results

Monitoring of pH showed stable conditions at inlet with values between 7.1 and 8.1 (7.7±0.2), lower in the weeks 134-171 (Figure 3.3). Outlet pH values were different according on the used slag, but in general all columns shower higher values at the beginning of the experiment and afterwards they presented outlet values with the same inlet trend . The lowest pH was detected at outlet of the big EAF column, with values between 7.5 and 10.4 (8.6±0.7). The small EAF columns showed a slightly higher pH compared to the previous reactor, with pH in the range 11.2-8.14 (9.5±0.9). The BOF columns showed the higher pH then EAF reactors, with outlet pH values of 12.3-9.2 (10.8±0.7) for the big size and 11-12.6 (11.6±0.4) for the small size (Figure 3.3).

The experimental results indicated that steel slag has a clear tendency to produce high pH in the effluents as a result of CaO dissolution during water filtration. CaO dissolution from slag is supposed the main parameter to cause the increase in pH (Chazarenc et al., 2008). The higher CaO content of BOF-slag should account for the higher outlet pH observed using BOF-slag. Also, small-size BOF-slag produced higher pH (and Ca^{2+} concentration, besides it was not detected in this work) than big-size BOF-slag, most likely because the smaller is the size, the greater is the specific surface available for CaO dissolution (Anjab, 2009). The pH of effluents decreased during the operation of the filters, suggesting that the rate of CaO dissolution from slag decreased over time.

The TP and PO_4-P at inlet were higher than 10 mg/L in until the week 45, afterwards the TP ranged between 10.2 and 8.5 mg/L (10.2±1 mg/L), and the PO_4-P between 10.1-8.9 mg/L (10.1±1 mg/L). The highest outlet phosphorous concentrations were detected in big EAF column, with maximum values of 8.0 mg/L of TP (2.5±2.1 mg/L on average) and 7.4 mg/L of soluble-P (mean 2.2±2.0 mg/L), followed by the small EAF column with maximum values for TP of 4.5 mg/L (average 0.4±0.6 mg/L) and soluble-P of 1.9 mg/L (0.3±0.4 mg/L, on average) (Figure 3.3). The BOF columns showed in general the lowest concentrations. The big BOF reactor showed ranges of 0.0-29.1 mg/L for TP (3.0±6.1 mg/L) and 0.0-7.8 mg/L (0.3±0.9 mg/L) for soluble-P. The small BOF reactor instead showed the lowest outlet concentrations in the ranges of 0.0-0.7 mg/L for TP(0.1±0.1 mg/L) and 0.0-0.3 mg/L (0.04±0.1 mg/L) for soluble-P. At increment of outlet TP concentration, there was the decrement of the pH, and vice versa. Also, a clear trend of decrease in the outlet PO_4-P concentrations was associated with an increase in outlet pH. Over time the columns showed to change their behaviors and consequently the P treatment. This is easily viewable from the outlet values with inconstant trend, in several periods very high and in other periods very low (Figure 3.3).

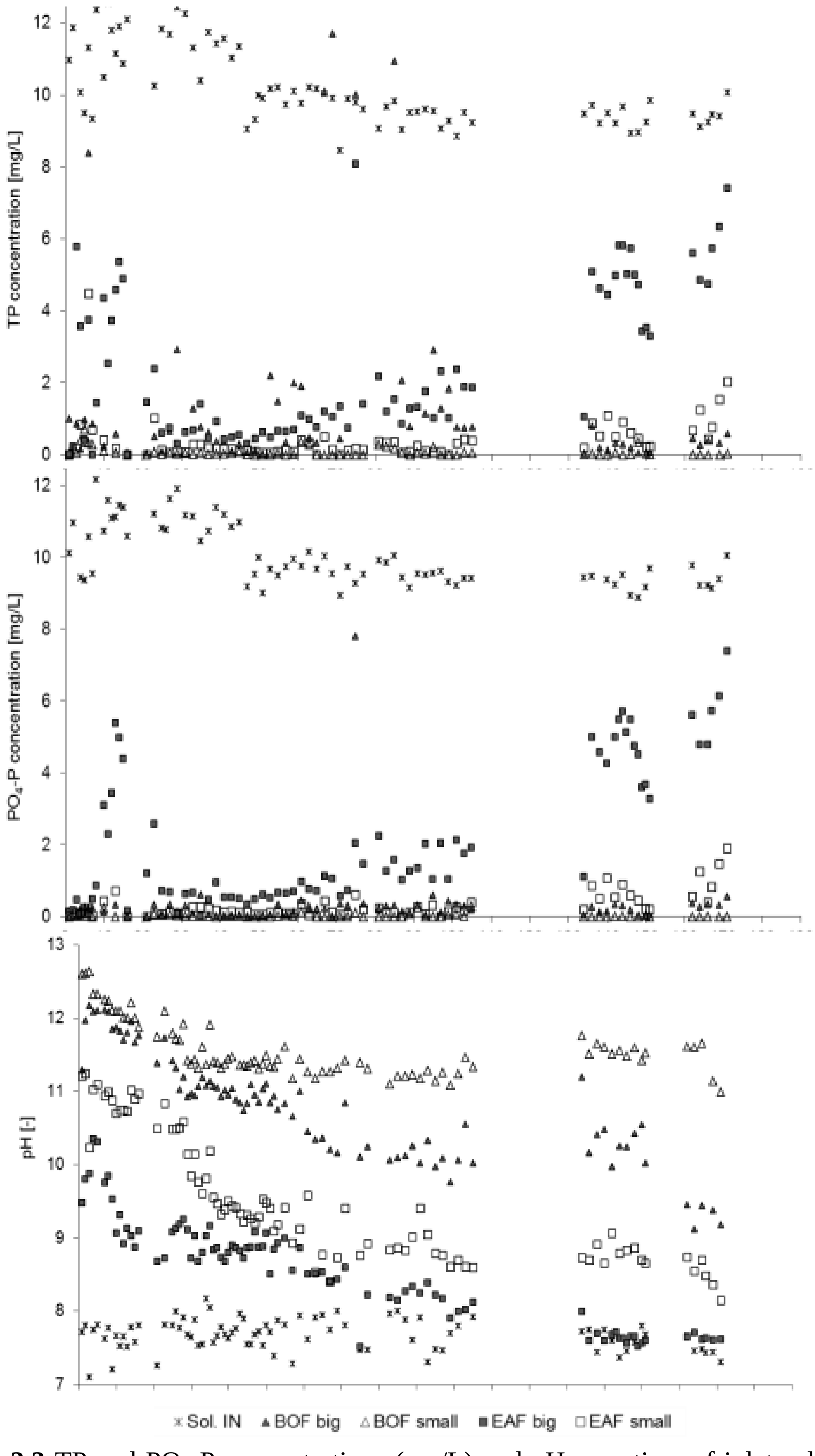

Figure 3.3 TP and PO_4-P concentrations (mg/L) and pH over time of inlet solution (Sol. IN) and at outlet of the different columns: BOF big, BOF small, EAF big, EAF small.

The P retention over time by columns was more stable for those filled with smallest granular size slags, in particular for the small EAF reactor (Figure 3.4). In both EAF and BOF columns filled with greatest granular size slags unstable removal efficiencies have been observed over time with increment of the P concentration at the outlet of the BOF big reactor. According to the pH outlet values, unstable removal efficiencies could be indicate a close end of CaO leachate. Because the inlet P was given mainly by P-salt dissolution there are two sources of differences between PO_4-P and TP. Small amounts of organic P coming from tap water can be neglected compared to the main concentration. As a second reason precipitated Ca-P-complexes can be transported by the water flow. Correlations or reasons could not be found until now, but sampling methods could be excluded.

Since CaO dissolution from slag followed by Ca phosphate precipitation was considered as one of the main mechanisms of PO_4-P removal using steel slag (Chazarenc et al., 2008), it is likely that Ca phosphate precipitates were removed from the solution by self-filtration and then accumulated into the filters as described by Claveau-Mallet et al. (2012).

For the filters filled with big-size slag, the average PO_4-P removal performances (79% for EAF-big and 97% for BOF-big) were higher than the average TP removal performances, especially for BOF-big (65%, vs. 76% of EAF-big). This may indicate that a fraction of the inlet PO_4-P that reacted with Ca to form P precipitates was not retained into the filters and was leached out. Moreover in the EAF columns from the week 134 till the end of the experiment there is a constant decrement of the TP removal (as PO_4-P removal) over time, especially for the EAF-big (Figure 3.4). Differently from the filters EAF-big and BOF-big, the filter filled with small-size slag (EAF-small, BOF-small) showed very high values for both average PO_4-P and TP removal performances (97% for both EAF-slag and >99% BOF-slag).This suggests that small-size slag was more efficient than big-size slag for the self-filtration of P precipitates. These results confirm that columns (both EAF slag and BOF-big) are changing their hydraulic over time, where channelling and dead volume with diffusion phenomena are increasingly evident, leading to the decrease of P removal. A previous work on this study (Barca et al., 2014), in which the system was investigated over a period of 52 weeks, in fact, reported higher removal performances compared to that reported in this research, with TP of 88% for EAF-big and 95% BOF-big and PO_4-P of 92% for EAF-big and 99% BOF-big. Besides, for both average PO_4-P and TP the removal performances were >98% for EAF-small and >99% for BOF-small. Barca et al. (2014) affirmed that TP retention levels increased with increasing duration of the experiments and/or inlet P concentrations. In this study, instead, the investigation for other 119 weeks showed that columns (except the BOF-small) are reducing over time the P removal performances, probably because the are saturating of P.

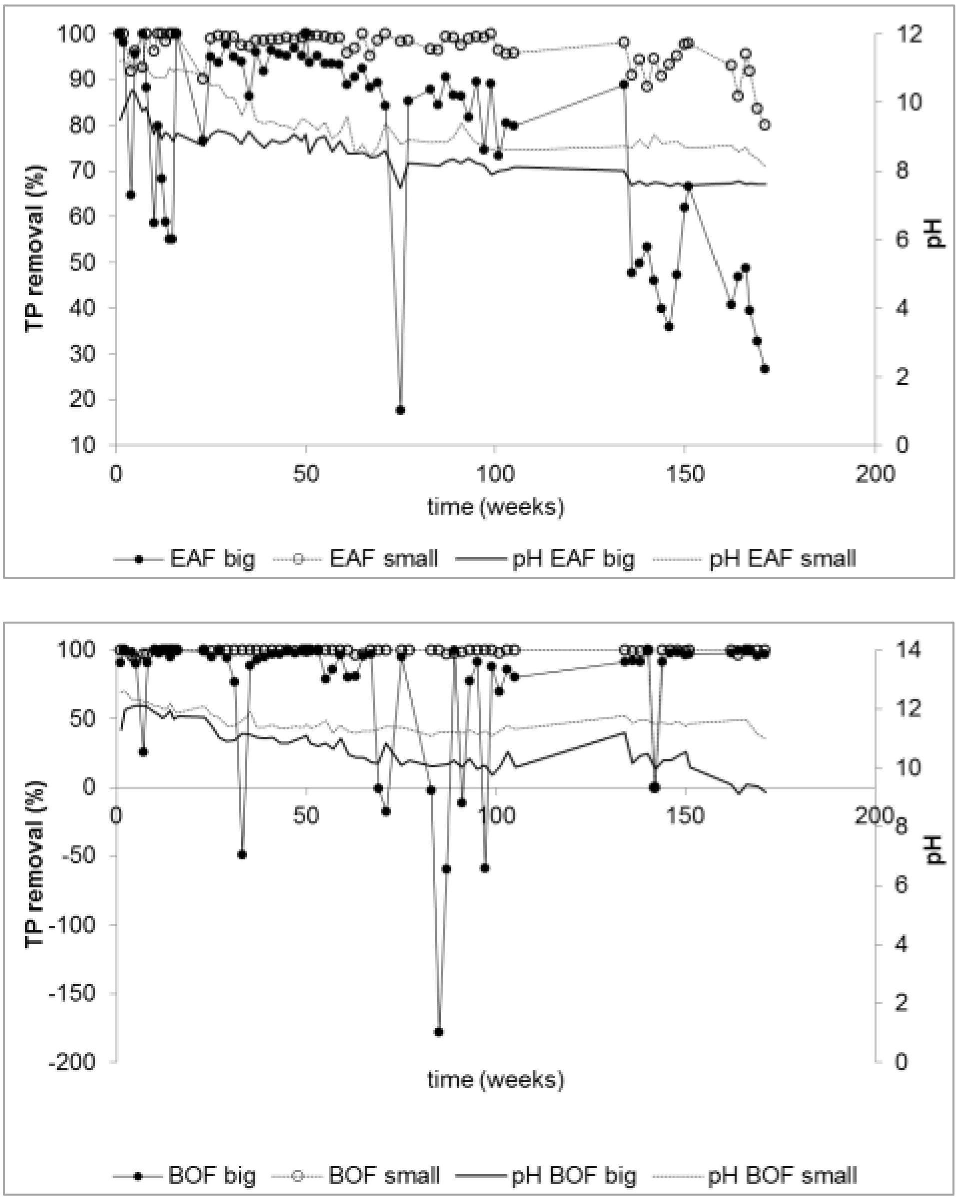

Figure 3.4 TP removal (%) and pH of the effluent of mesocosms.

CONCLUSIONS

For both EAF and BOF columns modeling with DPF model seemed to give inferior results than modeling with STS model, which presented R^2 approximately to 1, suggesting that columns studied had flow path more similar to non-ideal reactors.

The TP retention capacity of the filter material is usually considered as a parameter to estimate the longevity of an active filter system (Drizo et al., 2002; Cucarella and Renman, 2009). The system, studied over a period of 170 weeks, removed 76-97% (EAF-filter) and 65-100% % (BOF-filter) of the inlet TP, and about the 79% (EAF-filter) and 97% (BOF-filter) of the inlet soluble-P, with highest removal performances for the smallest grain size slags. P removal performances increased according to the increase in pH, suggesting that P retention was mostly linked to Ca-P-OH precipitation. The results indicated that P removal performance improved with increasing the content of CaO in slag and with decreasing the slag size, most probably because the specific surface available for CaO dissolution was increased. Also, small-size slag was more efficient than big-size slag for the self-filtration and accumulation of P precipitates into the filters. This study highlighted the hydraulic behavior of these columns over time, showing the decrement over time (especially in the last 7 months of the experiment) of the performance on P removal, more evident in the filters filled with EAF slags and BOF-big slags. The column with BOF-small slags, instead didn’t show particular changing over time. This likely depended on reactions of slag hydration, slag carbonation, and Ca carbonate precipitation during water filtration.

The investigation of the filters hydraulic using tracer test and applying hydraulic models has been useful to interpret their development over time. The results obtained from the hydraulic study were closely related to the performance on the P treatment. EAF-slags columns and BOF-big slags column are losing their efficiency probably for dead volume and diffusion phenomena. However also the media P saturation could be the reason of the reduction on P removal performances.

Chapter IV

INTEGRATED PILOT SYSTEM OF FILTERS AND A CASCADE CONSTRUCTED WETLAND TO TREAT PIGGERY WASTEWATER

THE CASE STUDY

This research started in 2012 with the financial support of the "SEES PIG" project – Solutions for Environmental and Economic Sustainability of PIG manure. Grant n° 2010-2220 filiera del suino- in which four partners were involved: Milano, Udine, Torino and Padova Universities. It ended in March, 2014.

An integrated pilot system (IPS) for treating piggery wastewater was set up in 2012 and monitored for two years (2012 and 2013). It was composed of a pretreatment system lo lower the high pollution load followed by a phytodepuration system to depurate the pretreated wastewater. The concept of the phytodepuration system, called "cascade constructed wetland (CCW)", was to reduce the surface area necessary for the treatment by taking advantage of verticality. The use of economical, reusable, easily available and disposable materials was a prerequisite for implementation of the pretreatment system.

The IPS was monitored for the first two years, and final analyses done in the last year. In particular the beginning of the first year (2012) was spent planning and creating the plant. Once set up, only the pretreatment system was studied the first months, with preliminary tests for filtering behaviour and plant design. The pretreatment and phytodepuration systems were then combined and studied till November 2012.

In the second year (2013) the IPS was modified to improve the plant engineering and find more efficient solutions for the treatment of piggery wastewater. In the first months some changes were made on plant engineering and the plant was then monitored till the end of the year.

In 2014 the IPS was stopped, and final analyses were conducted on media for both systems.

MATERIALS AND METHODS

Experimental set-up

The IPS was located in an open field in North-Eastern Italy, at the Experimental Farm "Lucio Toniolo" of Padova University at Legnaro (8 m a.s.l., Padova; N45° 20', E11° 58'), close to a stable where about 100 pigs were reared (Figure 4.1). The pigs were fed with two types of diet differing in high and low protein content (14% and 11%). The rearing cycle began in late February and ending in late September of each year.

The IPS occupied an area of 72 m² and was composed by a combination of an upstream pretreatment system and downstream phytodepuration system to treat the piggery wastewater coming from the stable. The pretreatment system consisted of six independent filters and was placed before the phytodepuration system with the purpose of retaining the solid particles and pollutants of the wastewater (Figure 4.2). The piggery wastewater, made up of faeces, urine, and cleaning water of the stable, was collected in a storage tank (ST) sited under the building. It was treated first by the pretreatment system (1) and then the filtered wastewater was sent to the phytodepuration system (2). Finally, the purified wastewater was collected in a cistern (final collection tank; FCT) which was discharged weekly.

Figure 4.1 Location of the experimental plant in the University's farm, with a view of the stable where the pigs were reared.

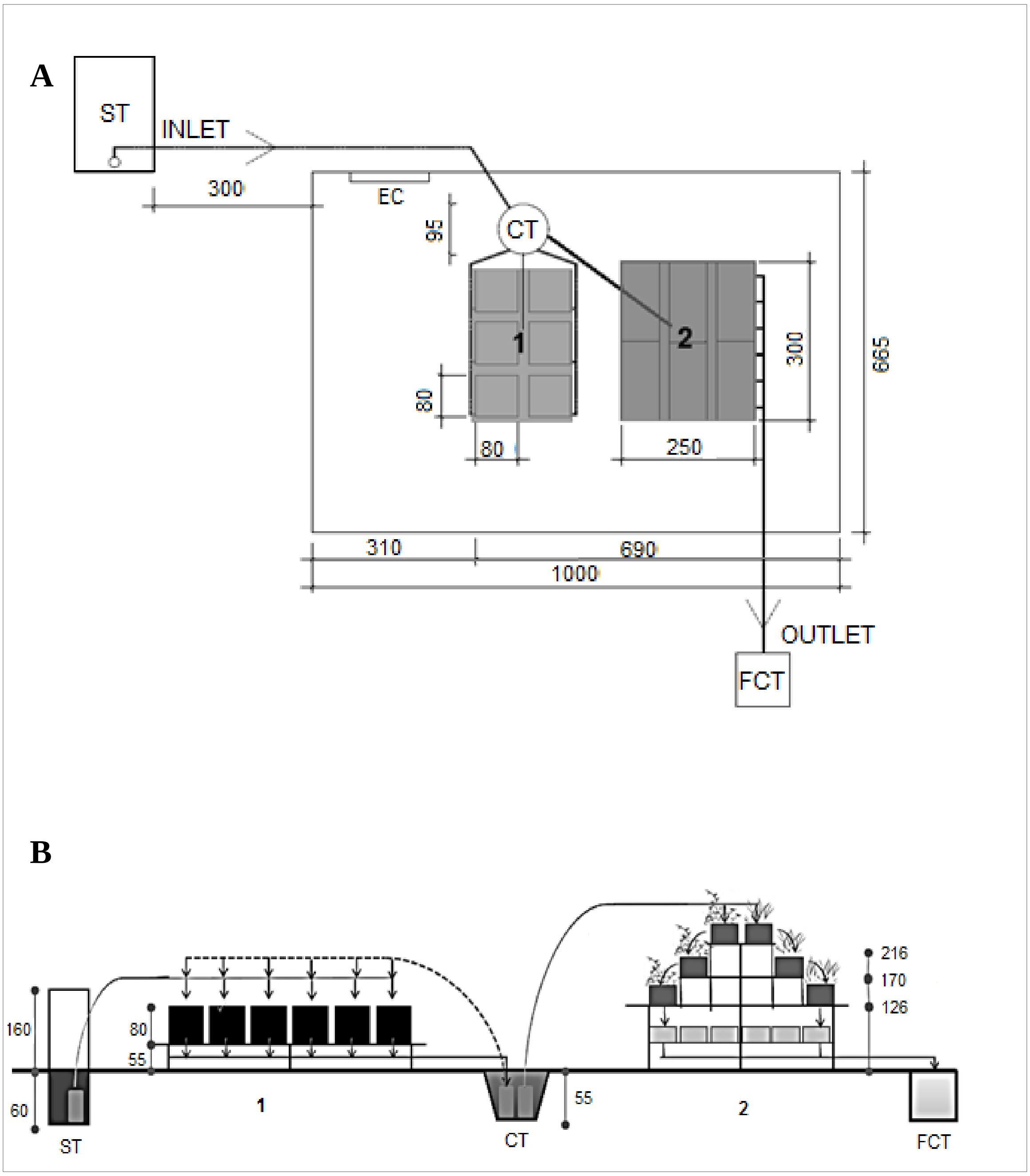

Figure 4.2 Experimental layout of the integrated pilot system (IPS); measurements are in centimetres. A: plant plan; B: plant side section. ST: piggery wastewater storage tank (INLET); CT: collection tank before and after the wastewater treatment by the pretreatment system; 1: pretreatment system; 2: phytodepuration system; FCT: final collection tank after the wastewater treatment by the phytodepuration system (OUTLET); EC: electrical cabinet.

Pretreatment system

The pretreatment system was composed of 6 independent filters located half a metre off the ground, in which the wastewater moved vertically. Filters had a 0.512 m^3 volume capacity (0.8 m x 0.8 m x height 0.8 m) and an iron structure. A perforated a stainless steel plate with a 1 cm holes diameter and a cover net of 3 mm mesh were placed at 5 cm from the bottom, to allow aeration and avoid clogging. These also to sustained the filter material placed above, up to 5 cm from the top (filtering volume 0.448 m^3). On the lower part of each filter there were two taps, one placed laterally for wastewater sampling, the other on the bottom for wastewater discharge (Figure 4.3).

The filters were filled with different substrates, with the aim of using economical, easily available, disposable and reusable materials. Substrates with excellent nitrogen removal properties (ammonia in particular) were considered for the treatment of piggery wastewater. The filter materials were selected with specific characteristics (such as particle size and type) to prevent clogging. The filters had different filling media depending on the experimental year.

The experimental layout was the same in both years. The pretreatment system was fed with the piggery wastewater coming from the stable through a peristaltic pump (Pedrollo®, TOP VORTEX, max load 100 L/min), covered by a perforated plastic tube (nominal diameter 300 mm; holes of 10 mm) and a thin mesh net (holes of 3 mm), which was placed inside the ST (Figure 4.4).

The wastewater moved vertically on the filtering substrate of each filter, to be collected in the CT, where another peristaltic pump recirculated the wastewater (Figure 4.5). The pumps were regulated by timers. Afterwards the filtered wastewater was sent to the phytodepuration system. The plant system and feeding system of the pretreatment system were different according to the experimental year.

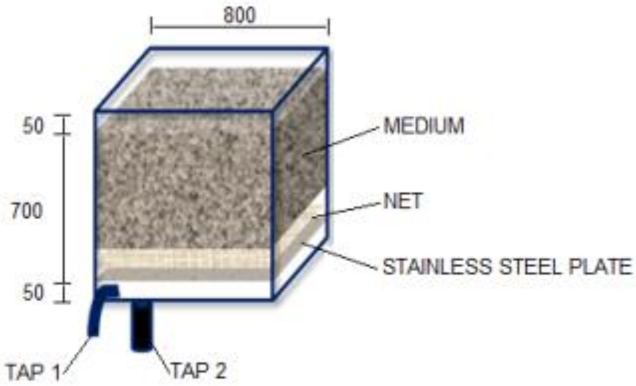

Figure 4.3 Construction detail of each filter; measurements are in millimetres.

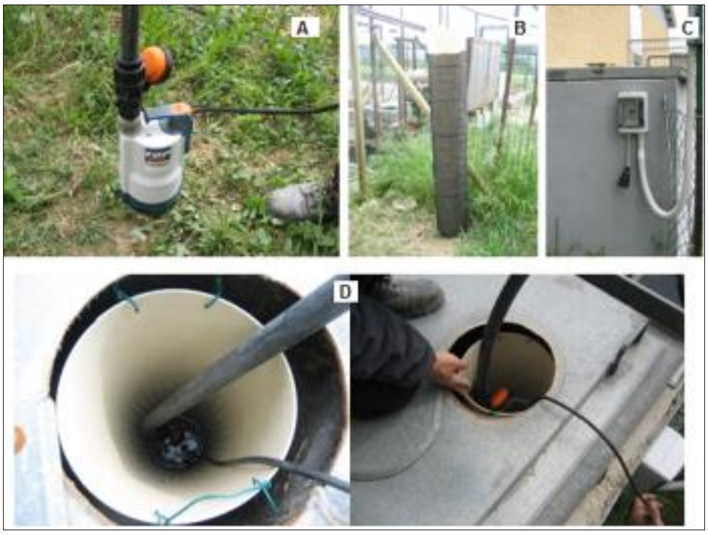

Figure 4.4 Peristaltic pump used for the wastewater distribution from the storage tank to the pretreatment system. A: pump; B. pump cover; C: timer; D: pump placement in the storage tank.

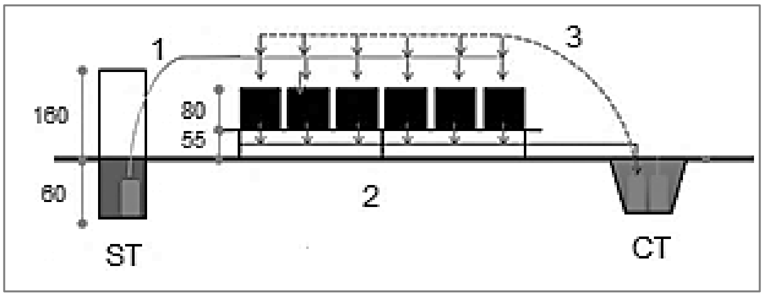

Figure 4.5 Pretreatment system operation; measurements are in centimetres. ST: storage tank of the piggery wastewater with pump; CT: collection tank of the filtered wastewater with pumps. Numbers indicate different wastewater steps: 1: feeding; 2: filtration; 3. recirculation.

May 2012-November 2012 (First year)

In the first experimental year the pretreatment system was not covered and the six filters were filled with different substrates, according also to previous studies on treating piggery wastewater. Among the materials used for the treatment of this type of wastewater, sand and zeolites are the most common (Nguyen and Tanner, 1998; Nikolaeva, 2002; Zheng et al., 2012; Reddy et al., 2013; Huang et al., 2014). However, also other materials are used for the depuration of swine wastewater, like semi-soft plastic media (Wei et al., 2010) or a mixture of peat moss and wood chips (Buelna et al., 2008).

The filter materials were chosen on the basis of studies reported in literature, but also for their cheapness, availability, disposal. For these reasons, other substrates never previously used for filtration purposes in swine wastewater treatment, but interesting for the features previously reported, were chosen as filtering media in this research.

The filters were filled with one material or with a mixture of two (Table 4.1). Filter n°1 had a stratification of three filtering media: pumice (particle size of 15-20 mm), medium/coarse gravel (12-20 mm) and fine gravel (4-8 mm). The filter was realized taking a cue from Nikolaeva et al. (2002). The pumice was placed on the top of the filter because of its feature to break apart over time, and to prevent clogging (Figure 4.6). The pumice had a low zeolite content (17.5% of chabazite) and was produced in Italy (Tessennano, VT). The pumice was composed mainly of SiO_2 (46.5%) and Al_2O_3 (15%), with a SiO_2/Al_2O_3 ratio 3:1 (Table 4.2); high silica content results in high abrasive characteristics. Pumice is characterised by its light appearance and porous structure. It has an average porosity of 90% (Kitis et al., 2004). Deposits of natural zeolites containing chabazite are widespread in the regions of central Italy (Toscana and Lazio) (de'Gennaro and Langella, 1996). The relative chabazite abundances, in terms of the equivalents of the different cations, are arranged according to the following series: $Ca^{++}>K^{+}>>Na^{+} \sim Mg^{++}$. On the basis of its structure, chabazite appears to be a rather "open" zeolite with three systems of interconnected channels, delimited by "access windows" formed by rings with six or eight tetrahedrons (Gottardi and Galli, 1985). The gravel used in the same filter was a river gravel and was bought from building material supplier in Padova. A total of 490 kg of filling medium was utilised, of which 223 Kg (total volume of 0.29 m^3) was pumice, 146 kg (about 0.10 m^3) medium/coarse gravel and 121 kg (about 0.06 m^3) fine gravel. The average porosity (ø) of the filter was 39% (Table 4.1).

Table 4.1 Filtering system composition and characteristics.

Filter number	Filling materials	Composition	Grain size/length/diameter range (mm)	Ø (%)	Total weight (kg)
1	MIX GRAVEL - PUMICE	Pumice 64% Gravel 36%	4/8- fine gravel*;12/20- medium-coarse gravel*;15/20- pumice	39	465
2	MIX SAND-GRAVEL	Sand 44% Gravel 56%	0/5-sand; 12/20- medium-coarse gravel*	33	688
3	GIANT REED	-	90- stems length; 20- stems diameter	62	97
4	PLASTIC TOPS	-	30/60	68	75
5	GRAVEL	-	4/8	37	677
6	BAMBOO	-	50- stems length ; 25- stems diameter	51	157

* Grain size classification according to the *Udden-Wentworth scale* (Wentworth, 1922)

Filter n°2 was composed by five alternating layers of medium/coarse gravel (3 layers) and sand (2 layers) (Figure 4.7). It was realized relying on Zheng et al. (2012). The gravel (particle size of 12-20 mm) was used on the top, in the middle and on the bottom of the filter to prevent clogging. The sand had a particle size of 0-5 mm and was placed in the central layers between gravel layers. In total the filling media weighed 688 kg, of which 395 kg (total volume of about 0.26 m^3) was gravel and 293 kg (about 0.19 m^3) sand. The average porosity (ø) of the filter was 33% (Table 4.1).

The other four filters were filled with one material. Two of these had organic material as filtering medium (Figure 4.8): giant reed (*Arundo donax* L.) stems were used in filter n°3 and bamboo (*Phyllostachys pubescens* (Pradelle) Mazel ex J.Houz.) stems in filter n°6.

Organic materials such as wood chips, wheat straw or sawdust, are often used for the treatment of wastewaters, in particular for NO_3 removal (Saliling et al., 2007; Schipper et al., 2010). Colin et al. (2007) and Camargo and Nour (2001) also experimented with bamboo for the reduction of COD, showing good results (40-91%). Bamboo is a fast-growing biomass source, with a wide range of species, making it suitable for growing in most temperate and tropical climates. The use of bamboo as an energy crop for Europe was reviewed and it was concluded that it can be grown under European climate conditions with good yields and is also useful as crop for phytoremediation on marginal contaminated lands (NL Agency Report, 2013). Relied on for thousands of years by rural households, bamboo is quickly becoming a popular feedstock for commercial products such as flooring, furniture, plywood, pulp, paper, linen, building materials and other products. Currently, 80% of bamboo products are produced China, particularly in 10 counties of China's southeast (NL Agency Report, 2013).

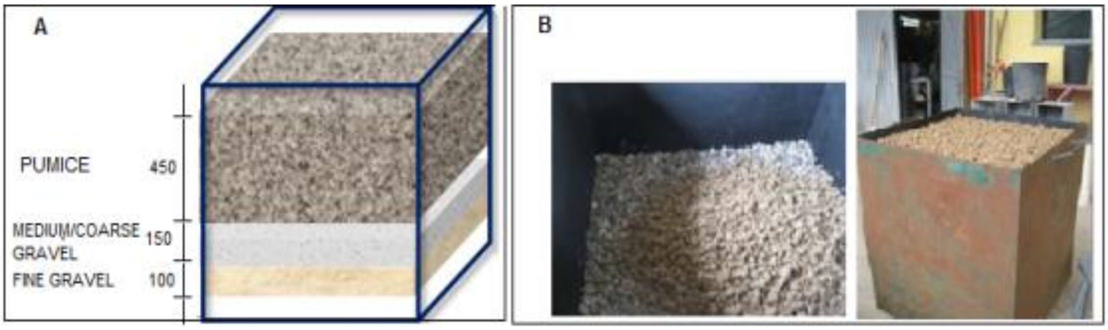

Figure 4.6 Representation of filter n°1. Arrangement of the filling materials (A; measurements are in mm) and construction (B).

Table 4.2 Chemical composition (wt,%) of the pumice used as filling medium: analysis of representative sample from the quarry faces.

SiO_2	Al_2O_3	K_2O	Fe_2O_3	CaO	Na_2O	TiO_2	MgO	SO_3	MnO	SrO	BaO	P_2O_5	pH
46.5%	15%	6%	3%	10%	0.6%	0.5%	2.3%	0.3%	0.1%	0.1%	0.05%	0.1%	7/8

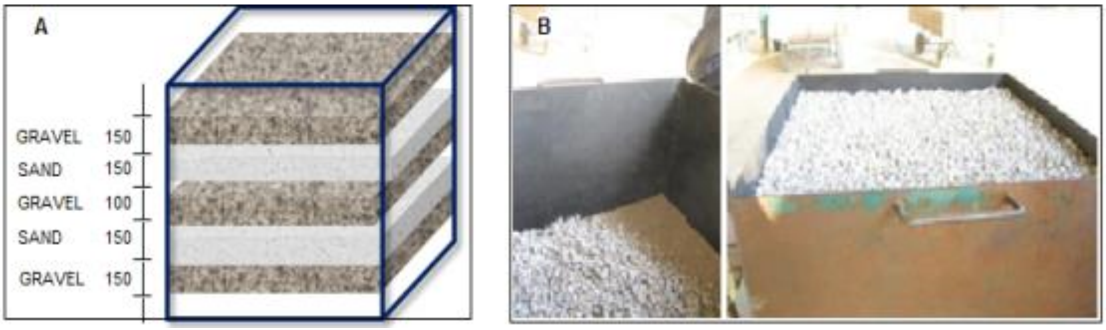

Figure 4.7 Representation of filter n°2. Arrangement of the filling materials (A; measurement are in mm) and construction (B).

Arundo donax (giant reed) was chosen for its easy availability in the natural environment. This availability and its low cost made it ideal to use in the filters. It is a perennial rhizomatous grass that grows in all the temperate areas of Europe and can easily be adapted to different climatic conditions (Pignatti, 1982; Polunin and Huxley, 1987). Its high growth rate makes it an invasive and aggressive species (Quinn and Holt, 2008; Cushman and Gaffney, 2010; Gordon et al., 2011) and it is one of the most promising biomass crops, due to its capacity to produce more biomass using less fertilizer and without pesticides than any other crop reported from Mediterranean to sub-tropical environments (Williams et al., 2009; Mirza et al., 2010). Like bamboo, the stems of giant reed are also used in agricultural buildings, but also for paper pulp production, biofuels and in building materials and musical instruments (Ververis et al., 2004; Abrantes et al., 2007; Nassiet al., 2010). Moreover this non-wood plant has applications in preserving and reconsolidating hydro-geological risk areas, contaminants removal and several traditional usages in agriculture (Papazoglou et al., 2005).

The bamboo canes were bought from a building material supplier in Padova; they had a diameter of 25 mm and were cut in 5 cm length pieces (Figure 4.8B). The filter contained 175 cane pieces, with a total weight of 157 Kg and average porosity of 51% (Table 4.1). Giant reed plants were picked manually on a riverbank in Padova. Stems had an average diameter of 2 cm; they were cleaned of leaves and cut in about 9 cm length pieces (Figure 4.8A). A total of 97 Kg of stems were used to fill the filter, with an average filter porosity of 62% (Table 4.1).

Several analyses were performed on gravel, pumice and organic media before starting to feed the system. X-Ray Powder Diffraction (XRPD) and Cation Exchange Capacity (CEC) tests were done for gravel and pumice materials, plus a kinetic test was performed for the latter. Hemicelluloses, cellulose and lignin contents in giant reed and bamboo media were analysed over the time. A general characterisation of the mineral contents in raw piggery wastewater was also carried out using ion chromatography and ICP OES analyses.

At the beginning of the experiment preliminary tests were performed on the pretreatment system to study the filling media features. A percolation time trial was carried out in March and April, in which a tap water volume of 10 L was poured manually on every filter, after media saturation. Depending on the medium, the percolation time was detected until 349 hours from the water entrance.

The filters were then fed with piggery wastewater. On June 28th the effect of a single load (33L/filter of load volume, poured manually) was tested for each substrate, measuring pH, electrical conductivity (EC), dissolved oxygen (DO), temperature (T), contents of total

Kjeldhal nitrogen (TKN), ammonium-nitrogen (NH_4-N) and phosphorus (TP) and wastewater volumes at inlet (IN) and outlet of every filter. Moreover, for the entire system, the effect of different recirculation (30 and 60 times) was studied on July 26th and 27th to understand the variances with different loads, measuring pH, EC and chemical parameters (total nitrogen (TN), NH_4-N, and TP) at inlet (IN) and at outlet (OUT).

From the end of August 2012 the pretreatment system started to work steadily with the piggery wastewater formed of urine, faeces, and cleaning water of the stable. The pump, located in the storage tank, sent the wastewater directly to the distribution system of the pretreatment system through an HDPE (High Density Polyethylene) 100 pipe (nominal diameter 50mm). The wastewater distribution system consisted of a manifold with 6 lines (regulated by manual control valves, connected to 6 bent HDPE pipes (nominal diameter 25 mm) ring-shaped (ring diameter 500 mm), which were perforated (holes of 4 mm), positioned over the filling medium in the filter (Figure 4.9).

The filtering system was fed from August 28th to November 9th with an intermittent daily load volume of 5 litres(L)/filter, with 5 re-circulations at intervals determined according to the filter with higher hydraulic retention time (about 3 hours), activated by a peristaltic pump (10 L min^{-1}) located in the collection tank (CT) (volume of 0.29 m^3) where the filtered wastewater was collected (Figure 4.10). There was another pump of the same type in the CT to send the wastewater to the phytodepuration system.

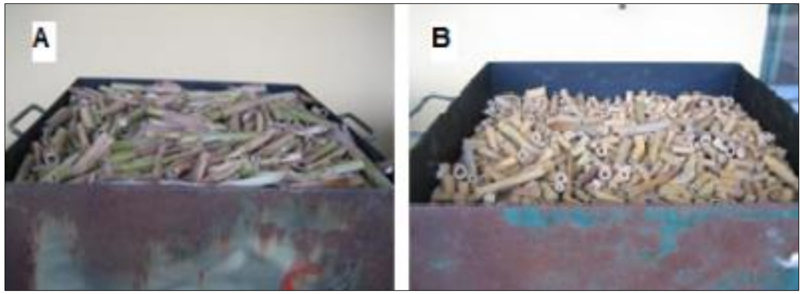

Figure 4.8 Plant materials used as filtering media: giant reed stems in filter n°3 (A) and bamboo stems in the filter n° 6 (B).

Figure 4.9 Wastewater distribution system of the pretreatment system used in 2012.

Figure 4.10 Collection tank (CT) with pumps used in 2012.

Upgrade 2013 (Second year)

In the second experimental year (2013), the pretreatment system was modified on the basis of the results of the previous year. The filling medium was changed in filter n°2 (mix of sand and gravel), as a result of clogging that occurred in October 2012, and replaced by the same type of pumice used in filter n°1 in 2012 (average filter porosity: 39%; total material weight: 323 kg). Moreover, giant reed (filter n°3) was added on the top of the filter used the previous year (total volume: 0.096 m^3; material weight: 9.11 kg) to have the same filtering volume as the other filters.

The system was protected by a permanent corrugated plastic cover for a better study of the filtering media (Figure 4.11A). The plant system was improved for a better wastewater distribution and a higher feeding load. The pump positioned in the ST sent the piggery wastewater to a CT with higher volume capacity (500 L) than in 2012, with a partial concrete step (height 10 cm) on the bottom to allow the total emptying of the tank. Three peristaltic pumps were placed inside the CT: one to feed the pretreatment system (and recirculation), one to feed the phytodepuration system, one to empty the remaining wastewater from the tank (Figure 4.11B). The distribution system was composed of a cylindrical plastic tank (120L volume capacity) with six pipes departing in a radial pattern (30 cm length, 1.6 cm diameter) at the bottom. Each pipe sent the wastewater into a plastic box (40 cm x 30 cm x 17 cm) in the bottom of which there was another pipe (one inch diameter) that joined the ring distribution system used in 2012 (Figure 4.11A).

The pretreatment system was fed from 3rd June to 18th August with an intermittent daily load volume of 10L/filter; thereafter up to mid-November it was fed daily with 15 L per filter to guarantee more wastewater to the CCW. As in the previous year, the filtered wastewater was recirculated 5 times per day.

Figure 4.11 Pretreatment system experimented in 2013. A: permanent corrugated plastic cover and wastewater distribution system; B: CT of the wastewater with pumps.

CCW

May-November 2012 (First year)

The phytodepuration system studied in this research derived from a previous experiment of the same research group of the University of Padova (Tamiazzo et al., 2014). It was placed in the experimental farm in January 2012, and subsequently modified for this project. The CCW consisted of a terraced system with two opposite sides to facilitate the monitoring operations. It occupied a total surface of 7.5 m^2 (m 2.50 x 3.00) and was composed of iron poles supporting wooden planks situated at three levels to sustain six phytodepuration lines: heights of 140 cm (lowest level; L3), 195 cm (mid-level; L2) and 250 cm (highest level; L1) above the ground. Each line was made of three plastic tanks positioned at the different heights to allow the flux of wastewater by gravity ("cascade" flow) (Figure 4.12).

Each tank had a total volume of 0.06 m^3 (0.50x0.40xh.0.29 m) and was filled with LECA® (light-weight expanded clay aggregates) produced in Italy by Laterlite, with 35-40% porosity range. The effective grain size was between 20 and 50 mm. LECA consists of small, lightweight, bloated particles of burnt clay and was chosen because it is a light medium (300-500 Kg m^{-3} depending on the grain size) that is very suitable for structures hanging on walls. The thousands of small, air-filled cavities give its strength and thermal insulation properties. It has higher porosity and specific surface area than other media. LECA is used in CWs as medium for plants (Albuquerque et al., 2010) and for the removal of recalcitrant pollutants (Dordio and Carvalho, 2013), pharmaceuticals (Dordio et al., 2010), ammonia and nitrate (Vilpas et al., 2005; van Deun and van Dyck, 2008), phosphorus and phosphate (Johansson, 1997; Drizo et al., 1999; Farahbakhshazad and Morrison, 2003) and many types of organic molecules (Dordio et al., 2007; Calheiros et al., 2008; Dordio et al., 2009). LECA is also used as filter material to study for example nitrification efficiency (Lekang and Kleppe, 2000), and for the removal of ammonium (Sharifnia et al., 2013) and heavy metals (Malakootian et al., 2009). A porous matrix, like LECA, provides a greater surface area for treatment contact and biofilm development.

Inside each tank a vertical plastic tube with an open bottom (diameter 100 mm) was installed close to a side where a tap was connected at 15.4 cm from the bottom. Based on the communicating vases principle, the entering wastewater forced the wastewater present inside the cell to pass through the substrate and then rise along the tube to exit and fall into the lower cell (Figure 4.13). The system functioned as a vertical flow system, with alternation of phases in which the cells were completely saturated or partially empty. Specifically, the feeding load was intermittent and, once it was done, the cells filled up (prevalence of anaerobic conditions). Between two feeding cycles the water level in the cells dropped due to water consumption by the plants, determining aerobic conditions inside the tank.

The CCW was intermittently fed with the wastewater on the top where the six upper tanks were located, and the wastewater moved by gravity into the two lower levels. Finally, a plastic container (volume 0.05 m^3), placed in the lower part of the structure, collected the effluent of every line through an LDPE (low density polyethylene) pipe (nominal diameter 16 mm) connected to the lowest tank. Each tank had 2 valves, one on the side for effluent sampling, the other on the outer part of the bottom for emptying, which was done through an LDPE pipe (nominal diameter 16 mm) ending in a plastic cistern (1 m^3 volume) that collected the depurated wastewater.

In 2012 the system was vegetated with plant species planted since 2010: *Typhoides arundinacea* (L.) Moench (syn. *Phalaris arundinacea* L.) var. picta (PHAAP), *Mentha aquatica* L. (MENAQ) and *Carex divisa* Hudson (CRXDW), one plant species per line with two replications (Figure 4.14). These are rhizomatous perennial plants that can live as emergent species in wetland habitats. They are suitable for use in a wall constructed wetland, because they have limited development of the aerial and root systems compared to other wetland plants. *T. arundinacea* var. picta (ribbon grass) belongs to the Poaceae family and, having white streaked leaves, is used as an ornamental plant (Dalla Fior, 1985). *T. arundinacea* (reed canarygrass) lives in tidal freshwater marshes and its shoot elongation increases as water depth increases (Cronk and Fennessy, 2001); the aerial part grows to 60-150 cm (Shilling and Kiniry, 2007). It is a fast-growing plant that does not need replanting or careful management and is a highly competitive species (Cronk and Fennessy, 2001; Kadlec and Wallace, 2009). *Mentha aquatica* (aquatic mint) is an aromatic plant of the Lamiaceae family that grows up to 90 cm with stems that bend by gravity. It is considered an amphibious plant because it presents two distinct types of leaves, submerged and emerged or submerged and floating. Leaves are scented and lilac flowers appear from June until October (Kumar et al., 2011; Borin, 2003). This plant species is widely used in CWs for the removal of heavy metals (e.g. Zurayk et al., 2002; Marchand et al., 2010). *Carex divisa* (divided sedge) is a typical species of wet permanent grassland of the Cyperaceae family, common on the French Atlantic coast and in Northern Italy up to 600 m a.s.l. (Bouzillé, 1992; Pignatti, 1997). It is able to spread laterally by rhizomes and the aerial part grows to 60-70 cm (Dalla Fior, 1985).

Figure 4.12 The phytodepuration system (CCW) experimented in 2012.

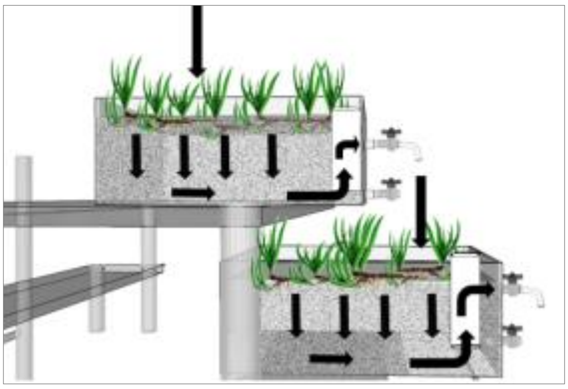

Figure 4.13 Representation of the wastewater flow (black arrows) inside each tank.

The phytodepuration system was fed by a peristaltic pump (Pedrollo®, TOP VORTEX) regulated by a timer (Figure 4.15B) and located in the CT. By means of an HDPE pipe (nominal diameter 25 mm), the pump was connected to a manifold at 6 lines of LDPE pipes (nominal diameter 16 mm) regulated by manual control valves, in which six flow meters were installed (Figure 4.15A).

Until the system implementation, plants were supplied weekly with tap water giving a sufficient quantity to ensure constant water presence inside each tank, although the volumes were not measured in this phase. Once set up, the CCW was differently fed with piggery wastewater during the experiment. At the beginning of the experiment (from 10th to 15th June) it was fed with filtered wastewater coming from the pretreatment system to test the survival of plants. The daily volume load was about 20 L of wastewater per line (34.2 L/m^2 per day).

This trial caused marked stress in plants and led to supplying water, which was done from 20th to 27th June, with a daily water volume load of about 15L/line (25.6 L/m^2). A manual water washing was then carried out in all tanks of the CCW (from 27th to 29th June) for a faster pollutant removal. Subsequently until the end of August the system was fed with tap water (10 L/line (17 L/m^2) per day). During this phase the aerial part of the plants was cut. Specifically, on July 3rd plants of PHAAP were shortened to 15 cm on average and MENAQ to between 8 (L3) and 26 (L1) cm, while on July 19th the whole aerial part of CRXDW plants was cut.

From August 29th to November 9th the phytodepuration system was fed with diluted wastewater coming from the pretreatment system. Every day after filtration the wastewater collected in the CT was diluted (dilution ratio of about 1:7) to achieve a salinity suitable for the plant species, EC of between 2 and 3 mS/cm. This range corresponds to normally used irrigation water values (Beltrán, 1999). The water load volume was regulated by a controller connected to a water tap next the stable. The theoretical daily load volume of the CCW was 60 L of wastewater (10 L/line (17 L/m^2) per day), with an average hydraulic retention time (HRT) of 4 days.

Figure 4.14 The plant species used to vegetate the CCW in 2012: before (A) and during (B) the experiment.

Figure 4.15 Manifold at six lines with flowmeters (A) and pump timer (B) of the CCW.

Upgrade 2013 (Second year)

In 2013 the plant species and the wastewater distribution system in the phytodepuration system were modified. To reduce wastewater dilution, salt resistant plants were chosen to vegetate the system. The plant species were chosen according to the results of a previous study by the same research group (Pavan et al., 2014), where halophytic plants were tested to treat digestate liquid fraction in a floating wetland treatment system.

Six plant species were selected to vegetate the CCW (Figure 4.16): *Puccinellia palustris* (Seen.) Hayek (PUCPA), *Halimione* (syn. *Atriplex*) *portulacoides* (L.) Aellen (HANPO), *Sarcocornia fruticosa* (L.) A.J. Scott (SALFR), *Cynodon dactylon* (L.) Pers. (CYNDA), *Artemisia coerulescens* L. (ARTCO), and *Phragmites australis* (syn. *P. communis*) (Cav.) Trin (PHRCO) as reference species for wetland treatment.

Puccinellia palustris (sea poa) belongs to the Poaceae family. It is a perennial plant with buds on the ground and aerial part that dries in winter. Stems are erect, slightly swollen at the base. The leaves can be glaucous, with 2-4 mm wide blade, a convoluted shape (diameter 1.0-1.5 mm) and acute ligule, 0.9-1 mm wide in the basal leaves, 1.5-3 mm wide in the upper leaves. Flowering is from June to September, and consists of a pyramidal panicle 10-20 cm, with a number of 7-11 floral spikelets, 10-12 mm long (Pignatti, 1997). In the salt marsh of the Venice lagoon *Puccinellia palustris* grows along the edges of creeks and channels, usually more elevated and it is more sensitive to soil elevation than other halophytes (Silvestri et al., 2003).

Halimione portulacoides (sea purslane) is a perennial, shrubby C3 halophyte belonging to the Chenopodiaceae family that is widespread on salt marshes around the coasts of Europe, North Africa and South-West Asia (Cambrollé et al., 2012). It has woody prostrate stems and glaucous leaves, opposite, from linear-lanceolate to lanceolate (7-14 x 30-60 mm). The small flowers are sessile and are gathered in panicles sometimes mixed with leaves. *H. portulacoides* has hair to remove excess salt accumulated in the leaves; the accumulation of dead hair and salt on the surface of the leaf increases the reflection of solar radiation by reducing the heat received and limiting evaporation (Pignatti, 1997). This plant is frequently the physiognomic dominant in well-drained and upper marshes, often fringing channels and pools that are flooded at full tide (Chapman, 1950). This species has been shown experimentally to maintain growth over a range of salinities, particularly under high nitrate availability (Jensen, 1985). It is also well known that, like other halophytes, it accumulates high salt concentrations in its leaves (Jensen, 1985; Freitas and Breckle, 1992), notwithstanding its ability to remove salt through epidermal bladders on both surfaces of its leaves (Freitas and Breckle, 1992). Several recent studies have explored the phytoremediation potential of this species (e.g. Duarte et al., 2007; Sousa et al., 2008; Almeida et al., 2009; Cambrollé et al., 2012).

Sarcocornia fruticosa (froggrass or glasswort or leadgrass) is a perennial plant that belongs to the Amaranthaceae family (subfamily of Salicornioideae). Plants are upright, without underground or creeping stems, 30 – 120 cm high; seeds with short, conical hairs. In the genus *Sarcocornia*, the leaves are highly reduced and the assimilatory surface is effectively composed of succulent branches. The photosynthetic part of the branch has an articulated structure, formed of succulent cylindrical internodes (Castroviejo, 1990). At both the proximal and distal ends of the branch, this succulent covering may atrophy, leaving hard, dry lignified sections of stem. In *S. fruticosa*, branching is decussate. Axillary buds on the main stem give rise to primary branches, which in turn give rise to secondary and tertiary branches. *S. fruticosa* is distributed throughout the coastal systems of south and west Europe (Ball 1993). The responses of this plant species to salinity are of particular interest, as it is one of the few that is found growing in a wide range of heterogeneous soil salt concentrations.

Cynodon dactylon (bermudagrass or dog's-tooth grass or Indian doab) is a perennial low-growing stoloniferous grass of the Poaceae family (Jacobs, 1993). Its distribution is very wide but it predominantly occurs in tropical and warm temperate regions throughout the world (Chaudhary, 1989). Although it is a favourite lawn grass, it makes an excellent hay and is considered as a potential fodder plant in Australia, Pakistan and Southeast Asia (Chaudhary, 1989; Jacobs, 1993). This species is known for its extremely variable habit and adaptation to a variety of habitat types. *C. dactylon* is frequently found growing on sandy or saline soils of open sites, including roadsides, on agricultural fields, along irrigation canals, in orchards, waste places and metal-contaminated wastelands, the latter particularly with high loads of Pb, Zn and Cu (Gleason and Cronquist, 1991; Shu et al., 2002; Río-Celestino et al., 2006; Wu et al., 2006). Natural populations of this plant possess a great magnitude of genetic variation for growth traits, including erect vs. prostrate stems, root penetration and tolerance of extreme soil temperatures, salinity and drought (Grattan et al., 2004; Wu et al., 2006). Some ecological and distribution patterns are correlated well with the ploidy levels of this grass (De Silva and Snaydon, 1995; Wu et al., 2006). *C. dactylon* has a good tolerance to salinity, but shows slow growth on salt affected soils. Moderate salinities may increase its yield, though it is capable of resisting relatively high salinities (Grattan et al., 2004; Grieve et al., 2004).

Artemisia coerulescens (Asteraceae family), commonly called maritime wormwood or sea wormwood, is a perennial herb widespread along the coasts of the Mediterranean region. Aerial parts of *A. coerulescens*, especially flowers, are used for culinary purposes and as a stomachic, digestive, antipyretic, and antihelmintic agent (Anoe et al., 1998). *A. coerulescens* is a plant with erect stems, woody only at the base. It has grey-ash leaves with little hair frizzy, linear-spatulate to obovate, 3-14 x 50-90 mm. The flower is a large pyramidal panicle; flower heads more or less erect, fusiform (1.5 x 5 mm); linear scales, streamlined, slightly hairy on the spine (Pignatti, 1997).

Phragmites australis (common reed) is widespread around the world between 10 and 70° latitude, and is the dominant plant of many European marshes (Hawke and José, 1996). It grows along the banks of rivers and lakes, and in wet meadows (Cronk and Fenessy, 2001). It is a tall perennial grass (of the Poaceae family) with large, pennant-like leaves that grows in both salt and freshwater marshes, in swamps and ditches, and along shorelines. *P. australis* grows up to 4 m or more in height and towers above most other emergent wetland vegetation (Cronk and Fenessy, 2001, Vymazal, 2013b). Its stalks bear a conspicuous inflorescence with bisexual wind-pollinated florets. Each flowering stalk typically produces 500 to 2000 wind-dispersed seeds. *P. australis* rapidly colonises areas opened by human development and marsh construction or restoration activities (Cronk and Fenessy, 2001). This plant species spreads vegetatively along stolons and rhizomes that grow out from the main stem, usually from the upland boundary toward the wetter parts of a marsh. The primary method of spreading is through rhizomes as seed viability is very low (Sainty and Jacobs, 2003; Wallace and Knight, 2006). The extensive rhizome system of this grass usually penetrates to depths of about 0.6–1.0 m (Vymazal, 2013b) . The stolons have been observed to grow at a rate of 10.8 cm day^{-1} and a new plant can arise at each stem node. *P. australis* tolerates a wide range of inundation and salinity levels and can grow in salinities as high as 30 ppm (Voss, 1972; Shay and Shay, 1986; Cook, 1996; Wijte and Gallagher, 1996a, b). Wherever this plant species grows, it is usually the dominant or co-dominant plant, displacing other plants because it grows and spreads rapidly, shades other plants, and accumulates a large amount of litter that covers and shades the substrate.

In April 2013, the halophytic plants coming from the Venice lagoon were obtained from a specialised supplier; CYNDA was collected in the area around the plant and PHRCO was taken from an experimental surface flow constructed wetland next the plant, studied for the treatment of agricultural drainage waters (Borin and Tocchetto, 2007; Passoni et al., 2009; Maucieri et al., 2014). Before vegetating the phytodepuration system, any loam adhering to the plant roots was removed, to avoid clogging inside the tank. To allow this operation without creating serious damage to the plants, the root system of the halophytes (that were in pots) was soaked in tap water, and subsequently planted with the aerial part. Plants of PHRCO were deprived of the aerial part, then rhizomes were washed, divided into sections (average length 18 cm) and planted with an angle of 45 °C from the profile of the medium with an overground end (Figure 4.17A). Plants of CYNDA were directly planted (2 cm deep) with the small aerial part after stolon sectioning (average length 15 cm) (Figure 4.17B). For all species the entire plant was weighed before planting.

Figure 4.16 Plant species used in the second year (2013) to vegetate the CCW: *Puccinellia palustris* (A), *Halimione portulacoides* (B), *Sarcocornia fruticosa* (C), *Artemisia coerulescens* (D), *Cynodon dactylon* (E), *Phragmites australis* (F).

Figure 4.17 Plants placement before planting in the tanks: *Phragmites australis* (A), *Cynodon dactylon* (B), *Puccinellia palustris* (C).

New filling medium was bought for the plant growth. Every tank was filled till 2 cm from the top (total volume 0.05 m^3) with the same substrate used in 2012 (LECA), with 35.7% porosity. Planting was done on May 6th. During the month there were problems of rooting took place, so there were various additions and replacements of the plants. The last planting was done on July 2nd, with a different number of plants per tank, according to the plant size (Table 4.2). The total fresh plant weight per tank was very different among the species, ranging from 1,014-1,140 g of ARTCO to 24-29 g of CYNDA (Table 4.3). After planting, the tanks were taken to the water regime, using tap water.

Table 4.3 Planting details of the CCW, with plant species used in 2013, plants or rhizomes number and total plant weight (g) per tank: 1: tank at the highest level; 2: mid-level tank; 3. tank at the lowest level.

Plant species	**Tank**	**N° of plants/rhizomes per tank**	**Total plant fresh weight per tank (g)**
Puccinellia palustris (PUCPA)	1	4	486
	2	5	554
	3	4	526
Halimione portulacoides (HANPO)	1	4	886
	2	3	884
	3	2	692
Sarcocornia fruticosa (SALFR)	1	2	616
	2	1	590
	3	3	694
Cynodon dactylon (CYNDA)	1	5	24
	2	5	26
	3	5	29
Artemisia coerulescens (ARTCO)	1	3	1,014
	2	3	1,140
	3	2	1,056
Phragmites australis (PHRCO)	1	5	36
	2	6	34
	3	4	38

The CCW was fed from May 29th to November 22nd with diluted piggery wastewater coming from the pretreatment system to achieve an EC of between 7 and 8 mS/cm (dilution ratio of about 1:2). This salinity value was chosen on the basis of results obtained by Pavan et al. (2014). Every line was fed with a daily wastewater volume of 8÷21 L·day^{-1} (14÷36 L/m^2 per day), in relation on the plant water needs during the season. Specifically, from August 19th to September 30th the system was fed doubling the wastewater load volume.

In 2013 the CCW system differed from the previous year to ensure a better wastewater distribution. A cylindrical plastic tank (60L volume capacity) with six tubes departing in a radial pattern (40÷50 cm length, 1.6 cm diameter) at the bottom was located in the highest part of the structure (raised by 30 cm) to feed the system. By gravity, each pipe carried the wastewater into the tank located at the highest level of the structure (Figure 4.18).

Figure 4.18 Wastewater distribution system of the CCW used in 2013.

Monitoring activity

First year

<u>Pretreatment system</u>

In 2012, the monitoring of the pretreatment system started on August 28th and ended on November 9th. It regarded the study of the entire system and each filter. For the entire system, the monitoring was performed at the inlet (IN) and outlet (OUT) concerning:

- wastewater volumes (once per week),
- on-site chemical-physical analyses (once per week) and
- chemical contents (irregularly, for a total of 6 monitoring cycles).

The total solids (TS) and volatile solids (VS) contents of the wastewater were also analysed occasionally in the system.

The single filters were studied occasionally (1 monitoring cycle in October), in which a wastewater load volume of 5L/filter was poured manually on every filter and recirculated manually 3 times; for the first 3 loads the recirculation interval was 3 h, while for the last it was 13.5 h. Also in this case, the volumes and the physical and chemical parameters of the wastewater were checked at the inlet (IN) and outlet of each filter. The chemical contents were analyzed immediately after sampling in a chemical laboratory. The inlet and outlet volumes were measured manually using sized buckets.

CCW

The monitoring activity of the CCW started in mid-June (2012) when it was fed with filtered piggery wastewater. Afterwards there were several unforeseen changes (water feeding, water washing, cutting of the aerial part of the plants), which were required in order not to jeopardise plants survival. In this period the monitoring regarded:

- volumes and physical and chemical analyses of piggery wastewater at inlet (IN) and outlet of every phytodepuration line;
- plant growth and development;
- volumes at IN and outlet when plants were fed with water;
- physical parameters of the wastewater during water washing stage.

From August 29th to November 9th the phytodepuration system was fed steadily with diluted piggery wastewater and it was monitored measuring:

- wastewater IN and outlet volumes of every phytodepuration line (once per week);
- wastewater on-site analyses at the IN and the outlet of every phytodepuration line (once per week);
- wastewater chemical contents at the IN and outlet of every phytodepuration line (irregularly, 4 monitoring cycles in the months of September, October and November);
- plant growth and development.

The plant growth and development was checked for all tanks situated at the three levels (first level (L1), the highest level; second level (L2), mid-level; third level (L3), lowest level) of each phytodepuration line from April 2012, when the CCW was fed weekly with tap water giving a sufficient quantity to ensure constant water presence inside each tank. For volumes, the inlet was detected through flow meters and the outlet manually measuring the volume in the CT of every line. The growth was recorded measuring height aboveground for PHAAP and CRXDW and shoots length for MENAQ of 5 sample plants per tank. Their development in terms of plant phenological stage was also detected, using the general Biologische Bundesanstalt, Bundessortenamt and Chemical industry (BBCH) scale, a system designed to uniformly encode phenological stages of development observable in both monocot and dicot species. Its structure encloses all the existing scales; it can also be used for all those species for which there are currently no specific scales available. The BBCH scale, which is based on the scale of Zadoks et al.

(1974), is divided into primary and secondary stages of development (Table 4.4). Each stage is indicated by a code consisting of two digits.

Table 4.4 BBCH scale stages.

Stage	Description
0	Germination / sprouting / bud development
1	Leaf development (main shoot)
2	Formation of side shoots / tillering
3	Stem elongation or rosette growth / shoot development (main shoot)
4	Development of harvestable vegetative plant parts or vegetatively propagated organs/booting (main shoot)
5	Inflorescence emergence (main shoot) / heading
6	Flowering (main shoot)
7	Development of fruit
8	Ripening or maturity of fruit and seed
9	Senescence, beginning of dormancy

Second year

Pretreatment system

In 2013, the pretreatment system was monitored from May 20th to November 22nd, regarding for the IN and outlet:

- wastewater volumes of the filtering system (once per week);
- wastewater on-site analyses of the filtering system (twice per week);
- wastewater chemical contents of the filtering system (once every 2 weeks);
- occasionally also the wastewater physical and chemical analyses for every filter (r).

The single filters were studied regularly (4 monitoring cycles: one in July, one in August, one in September and the last in November), in which a wastewater load volume of 10L/filter was poured manually on every filter and recirculated manually 4 times; for the first 3 loads the recirculation interval was 3 h, while for the 4th it was 13.5 h; the last outlet was instead detected 3 h after the 4th.

CCW

For the constructed wetland, the 2013 monitoring started in May, but the effective starting date for the results is 2nd July. It ended on November 22nd. The measurements were made for each phytodepuration line and regarded:

- inlet and outlet volumes (twice per week);
- on-site analyses of the IN and outlet (twice per week);
- chemical contents of the IN and outlet (once every 2 weeks).

Analyses for filtering media characterisation

X-Ray Powder Diffraction (XRPD)

The X-Ray Powder Diffraction (XRPD) was done for gravel and pumice materials in collaboration with the Geosciences of the University of Padova, with the same procedure reported in Chapter II. The XRPD is a fast and economical technique, that allows the various components of a solid sample to be quantified, and also to obtain information on the crystalline structure and the size of the crystallites.

Cation exchange capacity (CEC) and kinetic tests

The cation exchange capacity (CEC) test for ammonium was done for gravel and pumice substrates, using ammonium solution (Gualtieri et al., 1999), with the same procedure described in Chapter II.

To evaluate the ammonium adsorption over time, a kinetic test was performed for pumice material. The procedure was the same as for CEC test but adsorbed ammonia-nitrogen was determined more often: after 10 and 20 minutes, and after 1, 2, 3, 4, 24 hours. In this way the exchange velocity of the pumice was detected.

Wastewater analyses

On-site parameters

The parameters of the piggery wastewater detected at the inlet and outlet of the pretreatment system and the CCW analysed on-site were: electrical conductivity (EC, mS/cm), pH, dissolved oxygen (DO, mg/L and %) and temperature (T, °C), using a Hach Lange HQD 40d multi-parameter with interchangeable probes according to standards methods (APHA, 1998). Before testing, each probe was carefully calibrated according to the manufacturer's procedures. For the pretreatment system and the single filter studies, the probes were placed directly inside the container where the wastewater was before and after treatment. For the CCW, the probes were positioned on the CT where there was the wastewater to be treated, and on the container of every line that collected the phytodepurated wastewater. In both systems turbidity (NTU) of the wastewater was also measured before and after treatment, using a portable HI83414 (HANNA Instruments) turbidimeter.

Total (TS) and volatile (VS) solids

Total solids (TS) and volatile solids (VS) analyses were performed using the method described in Chapter II. For the TS and VS determinations the wastewater was sampled at the inlet and outlet of both systems and stored at -20 °C until analyses, when it was defrosted. In 2012, a total of 35 samples (14 samples pretreatment system and 21samples CCW) were analysed for TS content and 17 samples for VS content (9 samples pretreatment system and 8 samples CCW). In the second year, a total of 72 samples were analysed for TS content (34 samples pretreatment system and 38 samples CCW) and 31 samples for VS content (8 samples pretreatment system and 23 samples CCW).

Chemical contents

Nitrogen and phosphorus forms, COD and BOD_5

Chemical contents of the piggery wastewater were analysed immediately after sampling for both systems before and after treatment as concentrations (mg/L) in a chemical laboratory of the University.

For the first preliminary test of the pretreatment system conducted in 2012 (single load trial per filter), chemical analyses differed from the subsequent ones. Ammonia-nitrogen (NH_4-N) and Total Kjeldahl Nitrogen (TKN) were determined using a Kjeltec 2300 Analyser (Foss, Denmark) according to the macro-Kjeldahl method (APHA, 1998). Total phosphorus (TP) was determined using the Valderrama method (Valderrama, 1981).

For the other tests and monitoring activity, total nitrogen (TN), nitrate-nitrogen (NO_3-N), ammonia-nitrogen (NH_4-N), total phosphorus (TP), soluble phosphorus (PO_4-P) and chemical oxygen demand (COD) were determined photometrically using Hach-Lange DR-2800 spectrophotometer and adequate cuvette test kits (cuvette-tests LCK 014, 338, 340, 348, 350, 514) (Hach-Lange, 1989), according to DIN (1985). The 5-days biological oxygen demand (BOD_5) was also measured through the respirometric method with the Oxitop® system (WTW). Before the chemical analysis adequate sample dilutions were made with a stock supply of deionised water.

Ions

The general characterisation of the raw piggery wastewater before the treatment by the IPS consisted of ions analyses. This was performed in both 2012 and 2013 (one sample per year, before the starting up of the plant), through ion chromatography (IC) and heavy metal content analyses. Samples of defrosted wastewater were analysed for ions, after storage at -20 °C.

The IC was conducted with the same procedure described in Chapter II, determining the concentrations of Cl, Br, SO_4, Li, Na, K, Mg, Ca. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Ciros Vision EOP, SPECTRO Analytical

Instruments GmbH, Kleve, Germany) was used as reference method to determine the content of heavy metals (As, B, Cd, Co, Cr, Cu, Mo, Ni, Pb, Se, Zn) in the wastewater.

Analyses over time on filtering media

Changes in the media bed height of the filters filled with giant reed and bamboo media were measured before (2012), during (2013) and after (2014) the experimentation. Chemical composition of these media was also measured to analyze hemicelluloses, cellulose and lignin changes over time. They were determined using FAO methods (2011) for Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and lignin (ADL) analyses.

Giant reed and bamboo, in fact, are natural fibres (ligno-cellulosic fibres) that consist mainly of cellulose, hemicelluloses and lignin. Natural fibres derived from plants consist mainly of cellulose fibrils embedded in a lignin matrix. Each fibre has a complex layered structure that contains a primary cell wall and three secondary cell walls (Figure 4.19). The thick middle layer of the secondary cell walls determines the mechanical properties of fibre. It consists of a series of helically wound cellular micro fibrils formed from long chain cellulose molecules. Each cell wall is made up of three main components: cellulose, hemicelluloses and lignin. Lignin-hemicelluloses acts as matrix while micro fibrils (made up of cellulose molecules) acts as fibres (John and Thomas, 2008; Dittenber et al., 2012). Other components include pectins, oil and waxes (John and Thomas, 2008; Wong et al., 2010). Lumen is present in natural fibre, making it a hollow structure unlike synthetic fibres (Liu et al., 2012). These parameters can give an estimation of the degradability of these media. In plants, cellulose is found in the form of slender rod-like crystalline microfibrils, aligned along the length of the fibre. Cellulose is resistant to hydrolysis, strong alkali and oxidising agents, but is degradable to some extent when exposed to chemical and solution treatments. Hemicelluloses are lower molecular weight polysaccharides that function as a cementing matrix between cellulose micro fibrils, forming the main structural component of the fibre cell. They are hydrophilic and can be easily hydrolysed by dilute acids and bases. Lignin is a complex hydrocarbon polymer that gives rigidity to the plant and assists in the transportation of water. It is hydrophobic, resists acid hydrolysis and most microorganisms attacks, is soluble in hot alkali, readily oxidised, and easily condensable with phenol (Azwa et al., 2013).

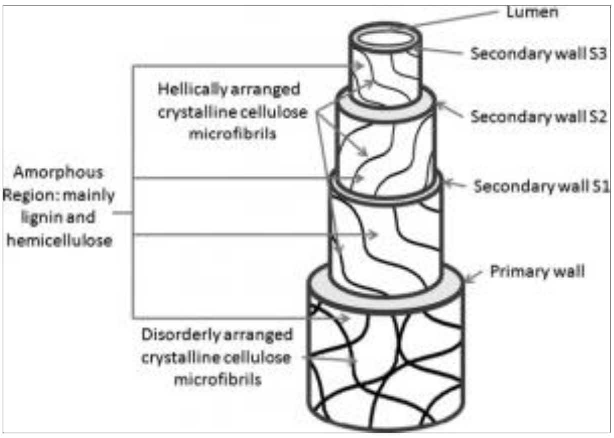

Figure 4.19 Structure of biofibre (Azwa et al., 2013).

Vegetation production and nutrient uptake measurements

At the end of each monitoring activity the aerial part of the plants grown in the CCW was harvested and analysed for chemical content. The harvesting was done in all tanks in November 2012 (10th) and 2013 (25th) by cutting the stems to calculate the biomass production of the plant species, subdivided by L1 (higher level tanks), L2 (mid-level tanks), L3 (lower level tanks). The total fresh weight of the collected aboveground biomass was weighed on site. Afterwards, samples were dried at 65 °C in a forced draught oven for 36 hours, mechanically powered, and analyzed to determine total Kjeldahl nitrogen (N) and phosphorus (P) contents, using the FAO official method (FAO, 2011). A portion of the sample (10 g) was dried at high temperature (130 °C) for 24 h to measure the residual moisture content (%) and calculate the dry mass production (DM, g/m^2) of the aboveground part.

Final surveys

Pretreatment system

The final surveys were performed in May and July 2014 and regarded the final state of the filter media tested in 2013 to treat the piggery wastewater and their sampling to analyse chemical contents. The substrates were weighed and dried to examine the moisture content. Samples were also analysed for carbon (C), nitrogen (N), sulphur (S) and organic matter contents; in addition a desorption test for ammonium was conducted on the pumice substrate.

<u>Sampling of the filling media and measurements</u>

For the sampling every filter was emptied of the filling medium. For the filters with one filling medium the samples were taken in the two central depths situated at 0.28m and 0.52 m from the top (Figure 4.20), subdividing the medium in three layers (1- above; 2- intermediate; 3- below). For the multiple materials filter the sampling was done in the middle of every layer to the depth of 0.28 m (pumice layer), 0.58 m (medium/coarse gravel layer), 0.70 m (fine gravel layer) from the top. Eight samples were gathered for every depth next to the ring tube for the wastewater distribution (Figure 4.20). This was done because the medium had a darker colouring around the tube ring, a sign of greater adsorption of wastewater and the presence of organic matter in that area (Figure 4.21). The weight of the samples ranged between 77 and 686 g.

During the filters empting (Figure 4.22), observations were made about insects presence and adsorption area. For each layer the substrate was weighed on-site and subsequently part of it was placed in an oven at 105 °C for 48h to determine the moisture content (not for the tops medium). For the analyses, all media samples were dried at 65 °C for 24h.

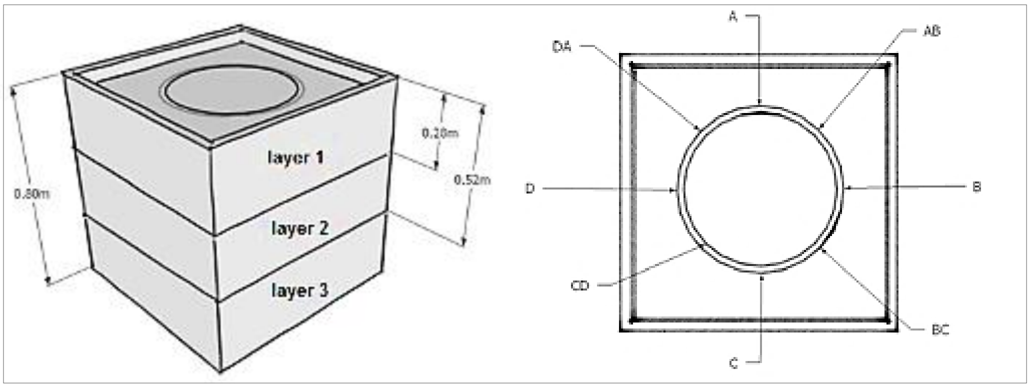

Figure 4.20 Schematic representation of the sampling points of the medium, at various depths (0.28 m and 0.52 m for the filters with one medium) and at different zones for every depth.

Figure 4.21 View of the top of some filters: A- filter with pumice and gravel; B- filter with gravel; C- filter with pumice.

Figure 4.22 Empting of the filters with separation of the substrates by depth level.

C, N, S and organic matter analyses

For the analyses of C, N, S contents, bamboo and giant reed substrates were mechanically pulverised, while pumice was manually milled. They were then sieved to <50 µm (Figure 4.23). C, N and S contents were measured with a CNS automatic analyzer (Elementar vario MACRO CNS, Elementar Analysen systeme GmbH, Hanau, Germany). The operating principle is based on the Dumas method (1831), which provides a complete and instantaneous oxidation (flash combustion) of the sample with conversion of all organic and inorganic substances into gaseous products.

For the tops and gravel materials it was not possible to do this analysis as it was impossible to crush them. For this reason the C, N, S analysis was done on the organic matter of these media. Their organic matter quantity was measured removing it with a solution of Hydrogen Peroxide (H_2O_2) solution 40 m/v. In this way the organic matter was oxidised. H_2O_2 is used for the treatment of soil to remove organic matter prior to particle size analysis (Gee and Bauder, 1986) and also before characterisation of soil mineralogy (Feller et al., 1992). Two samples of each medium were analysed, except for tops in which 2 samples per depth (total 4) were tested. Before the treatment they were weighed. For the treatment the medium was put inside a beaker (1L volume), and then completely covered by the solution. The beaker was then placed, under a hood, on an electromagnetic stirrer with heating in which the temperature was steadily increased (till 280 °C). The process was continued until the end of oxidation. Subsequently the medium was washed with tap water and dried in an oven at 65 °C for tops and 105 °C for gravel, for 48 h or until constant weight (Figure 4.24). Finally the sample was weighed and the organic matter content was calculated from the difference between initial and final weight.

Ammonium desorption test for pumice material

The ammonia desorption tests were based on regeneration tests of NH_4 used for saturated zeolite. The desorption of ammonium ions from pumice samples was done with a similar procedure to that experimented by Šiljeg et al. (2010). Some studies (Lahav and Green, 1998; Guo et al., 2013) demonstrated the increment of ammonium desorption (then complete regeneration) with increase of sodium or sodium chloride concentration.

The samples were treated with a 2mol/L NaCl solution at liquid-to-solid ratio 100:1 (10 g of zeolite in 1L of solution in a plastic bottle). The mixtures were stirred at 120 rpm on the industrial magnetic stirrer at room temperature (23-25 °C) for 48 hours (Figure 4.25). After stirring the bottles were carefully decanted and the supernatant (50 mL) was sampled for ammonium analyses. The initial and final pH of the solution were detected with a Hach Lange HQD 40d multi-parameter with interchangeable probes according to standards methods (APHA, 1998).

Figure 4.23 Dried, pulverised and sieved samples of bamboo (1), giant reed (2) and pumice (3) materials prepared for the analyses of C, N and S.

Figure 4.24 Removal of organic matter from the tops. The photos show the several phases from before (left) to after (right) the treatment.

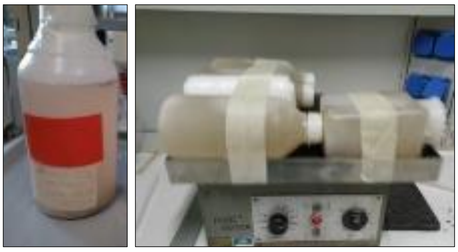

Figure 4.25 Pumice samples before and during stirring for the ammonium desorption test.

CCW

The final surveys for the phytodepuration system were performed in correspondence with those of the pretreatment system, in which the plant growth substrate was sampled and analysed for chemical contents (C, N and S). The tanks were emptied of wastewater and the samples were taken the following day. The sampling of the LECA was made in the central part of each tank, to a depth between 5-10 cm below the overflow level (Figure 4.26). Eighteen samples with a fresh weight between 181 and 329 g were taken and dried at 65 °C for 48 hours. The samples were then deprived of plant parts, manually milled, sieved to <50 µm, and finally subjected to the C, N, S analyses, with a CNS automatic analyzer (Elementar vario MACRO CNS, Elementar Analysen systeme GmbH, Hanau, Germany).

Figure 4.26 LECA sampling.

Meteorological data

Microclimatic data for both 2012 and 2013 were gathered from an automatic weather station close to the experimental plant (ARPAV, Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto). They regarded daily measurements of: air temperature (min, mean, max (°C), at 2 m; °C), wind speed (at 10 m; m/s) and direction, rainfall (mm), global radiation (MJ/m^2), relative humidity (min and max at 2 m; %) and soil temperature (°C).

Data elaboration

Data were elaborated using Microsoft Excel 2010. The statistical analyses were conducted for both pretreatment system and CCW when there were more than two data, using STATISTICA 8.0 software (StatSoft Inc. 2007). The data series of the parameters did not follow normal distribution even after transformation. Statistical analyses were thus conducted with the Kruskal-Wallis non parametric test; different letters were used to indicate significant differences (at $p < 0.05$ by Kruskal–Wallis test).

For the pretreatment system the statistical analysis was performed to determine differences among IN and OUT and among the filters (only 2013) in physical parameters and chemical concentrations; it was also applied among the filters (then the media) for chemical removals and mass abatements. For the CCW the statistical analysis was executed to find differences among the IN and the plant species used in the same year on physical parameters and chemical concentrations, and among the plant species for chemical removals and mass abatements. For the phytodepuration system the statistical analysis also compared the aerial biomass production in the three levels. In addition it compared the C, N, S contents of LECA between the three levels, regardless of the species.

The depurative performances of the pretreatment system and the CCW were studied as:

1. on-site parameters reduction (R,%) (for turbidity, EC, TS and VS) calculated on second quartile (Q2; median) values as: $R = [(V_{IN} – V_{OUT})/V_{IN}] \times 100$, where V_{IN} is inlet value and V_{OUT} is outlet value;
2. chemical concentration reduction (A,%), calculated on second quartile (Q2; median) concentration values as: $A = [(Cin – C_{OUT})/C_{IN}] \times 100$, where C_{IN} is inlet concentration (mg/L) and C_{OUT} is outlet concentration (mg/L);
3. mass abatement (MA, %) for all chemical parameters, calculated as: $MA = [(C_{IN} *V_{IN})–(C_{OUT} *V_{OUT})/(C_{IN} *V_{IN})]\times 100$, where V_{IN} is the wastewater inlet volume (L/m^3 for filters; L/m^2 for plants) and V_{OUT} is the wastewater outlet volume (L/m^3 for filters; L/m^2 for plants);
4. quantity of chemical elements removed (g/m^3/d for filters; g/m^2/d for plants).

The water consumption of plants was calculated for both experimental years by subtracting the V_{OUT} (L/m^2) from the V_{IN} (L/m^2; wastewater and rain) of every phytodepuration line, obtaining the actual evapotranspiration (ET, L/m^2). Evapotranspiration is the sum of evaporation and plant transpiration from land surface to the atmosphere. Evapotranspiration from the plant depends on various abiotic factors, mainly temperature, solar radiation, wind, and relative humidity, as well as on the plant’s physiological properties and surface area of the leaves (Doneen, 1971). ET data were compared with the reference evapotranspiration (ET_0), calculated using the FAO Penman-Monteith equation (Aellen et al., 1998), which is usually valid for monotypic or poorly diverse vegetation cover. It refers to a lawn area, 8-15 cm high, uniform and completely shading the ground, not subjected to water stress (Borin, 1999). Lastly, the monthly plant coefficient (*Kp*) was calculated as the ratio ET/ET_0 for both seasons (June-November 2012 and June-November 2013).

RESULTS

First year

Environmental conditions

In general in the first experimental year of 2012 the air temperature increased from January to August (Figure 4.27), with average monthly values of 1.8 °C in January, 2.3 °C in February, 11.5 °C in March, 12.9 °C in April, 18.1 °C in May, 23.3 °C in June, 25.4 °C in July, 25.4 °C in August, 19.9 °C in September, 14.8 °C in October, and 10.8 °C in November (till 9th November). The minimum air temperature detected during the experimentation was detected on 9th November (2.3 °C), while the maximum was recorded on 22nd August (37.4 °C).

Rainfall was more frequent during spring and autumn 2012 (Figure 4.27). Specifically, the total monthly rainfall was 77.4 mm in April, 87.4 mm in May (of which 45.4 mm fell on 21st May), 30.4 mm in June (during the first 13 days), 0.4 mm in July, 40.6 mm in August (in just four days), 95.2 mm in September, 136.6 mm in October (mostly on the 26th and 27th) and 21.6 mm in November (till 9th November).

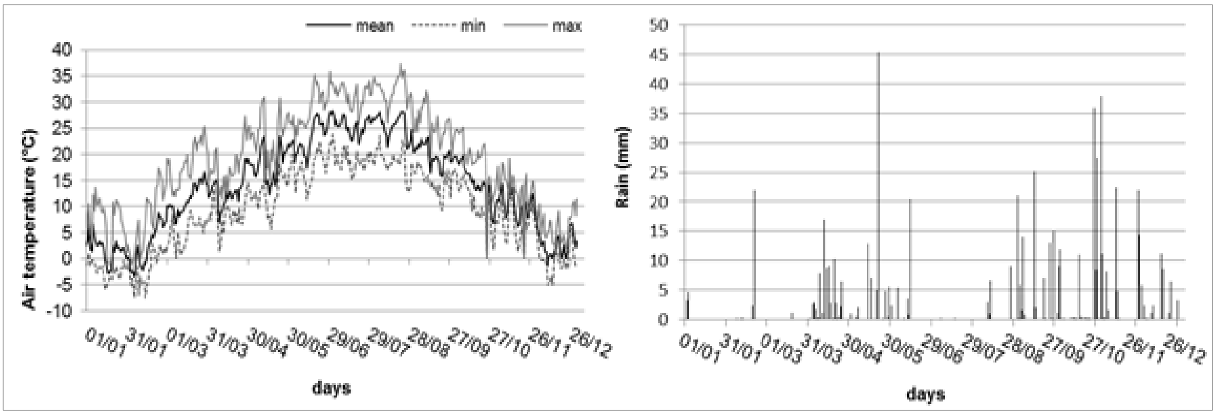

Figure 4.27 Air temperature (°C) and rainfall (mm) during the experimental year 2012.

General characterisation of the wastewater

The ion composition of the piggery wastewater revealed high contents of K and ions Cl and Na, indicators of high salinity of the wastewater (Table 4.5). Among metals, a high value of Zn was detected that by far exceeds the Italian limits (Italian Legislative Decree No. 152/2006) for the discharge into surface water and sewerage. However, Zn like Cu represent the metals prevalent in swine slurries due to their presence in pig feeds; these concentrations are extremely wide-ranging and dependent on the age of the pigs and the quantities of Zn or Cu supplements added to the diet (Moral et al., 2008). Contaminants such as heavy metals are present in livestock diets at background concentrations. They may be added to certain feeds as supplementary trace elements for health and welfare reasons, or as growth promoters (Petersen et al., 2007). Copper is added to growing pig diets as a cost-effective method of enhancing performance, and is thought to act as an anti-bacterial agent in the gut. Zinc is also used in weaner pig diets for the control of post weaning scours. For all livestock, the majority of heavy metals consumed in feed is excreted in the faeces or urine, and will thus be present in manure that is subsequently applied to land or excreted during grazing. A survey of manures collected from commercial farms in England and Wales in the mid-1990s (Nicholson et al., 1999) found the highest concentrations of Zn and Cu in pig slurry and laying hen manure, reflecting higher levels of dietary supplementation in these livestock types. Dach and Starmans (2006) confirmed that electroremediation, by which an electric current is passed through a liquid manure and metal ions are precipitated on an electrode, can decrease metal concentrations. However, at present the technology is unproven at the farm-scale and is unlikely to be cost-effective.

Table 4.5 Ions concentration (mg/L) of the piggery wastewater. "*n.f.*" acronym indicates that the value was not found.

Ion	Concentration (mg/L)	Ion	Concentration (mg/L)
As	<0.01	Cl	1,399
B	0.38	Br	*n.f.*
Cd	< 0.001	SO_4	254
Co	<0.002	Li	*n.f.*
Cr	0.04	Na	761
Cu	0.51	K	2,749
Mo	0.03	Mg	96
Ni	0.08	Ca	207
Pb	<0.01		
Se	<0.01		
Zn	2.86		

Pretreatment system

Features of the filtering media

The gravel powder diffraction spectrum obtained by the XRPD revealed its composition (Figure 4.28A): 51.5% Dolomite, 50.1% Magnesium Iron Aluminum silicate hydrate, 17.2% Potassium Aluminium Silicate hydroxide, 13.1% Calcium Carbonate, 8.1% Albite calcian and 5.1% Silicon oxide. Diffraction spectrum of pumice substrate (Figure 4.28B) shows a greater content of Sodium Potassium Aluminium silicate (47.5%), and smaller contents of Sanidine (22.2%), Chabazite (17.2%), Phlogopite 2M1 (12.1%) and Silicon oxide (1%). On this basis, the pumice was a material with a low percentage of zeolite (chabazite), which is the mineral with peculiar characteristics of ammonium adsorption.

Results of the kinetic test revealed that with the increasing of contact time between ammonium solution and pumice substrate, the ammonium adsorption increases. The initial NH_4Cl solution had a NH_4 concentration of 303.7 mg/L and pH 6.3. The NH_4 concentration in the solution decreases increasing the contact time (Figure 4.29A), as result of the increasing adsorption over time by the pumice (Figure 4.29B). After 10 minutes the substrate adsorbed 68% of the ammonium present in the solution (40.6 mg NH_4 adsorbed per gram of pumice), reaching 81.5% (49.6 mg NH_4 adsorbed per gram of pumice) after 24 hours. The pH during the kinetic test ranged between 5.7 (first hours) and 6.9 (last value detected after 24h). This test revealed that the exchange kinetic is very fast (0.99 mg min^{-1} adsorbed in the first four hours) and that a short contact time is sufficient to adsorb the larger quantity (more than 50%) of the ammonium in the solution. However a longer contact time could probably have given a greater ammonium adsorption, till medium saturation.

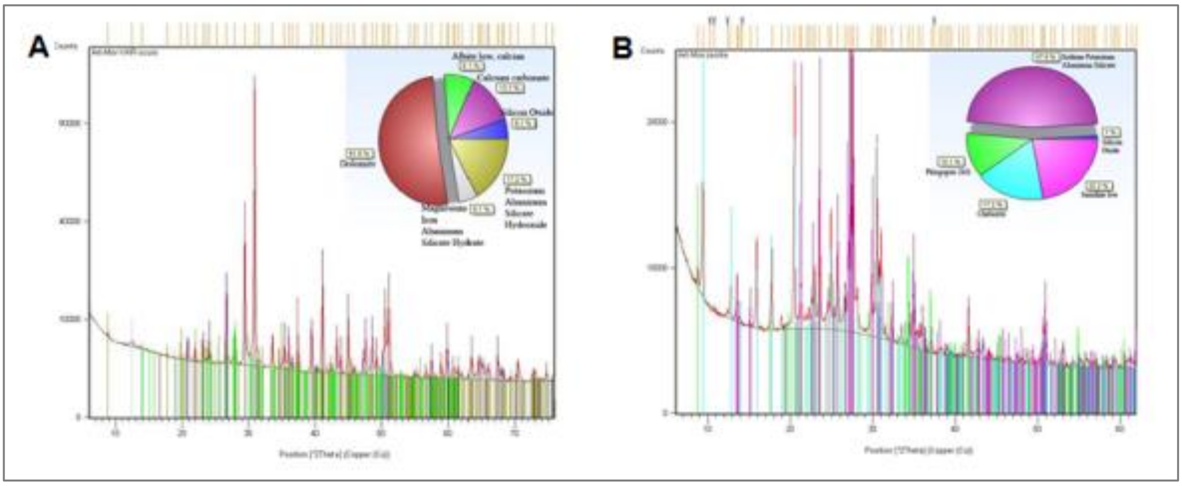

Figure 4.28 Gravel (A) and pumice (B) powder diffraction spectrums and quantitative phases ratio.

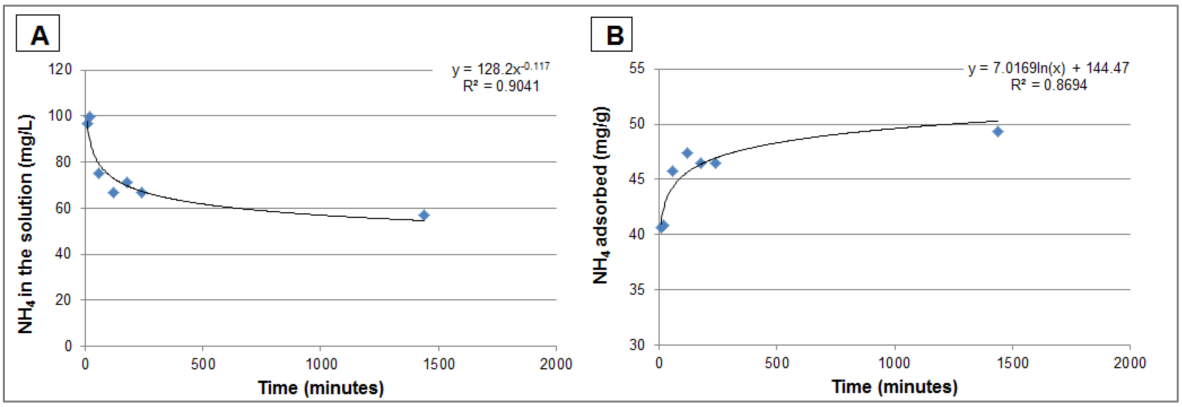

Fig. 4.29 Kinetic results on pumice substrate. A: ammonium concentration (mg/L) in the solution over time; B: ammonium content adsorbed (mg/g) over time.

High CEC values and selectivity for ammonium removal are generally known for zeolite minerals (Passaglia et al., 1999; Reddy et al., 2013). Pumice material showed a great ion-exchange capacity compared to the gravel substrate (Table 4.6): 274 meq/100g (pumice) vs. 1.5 meq/100g (gravel). From these results, pumice was shown to have higher degree of NH_4 exchange than zeolites (heulandite-clinoptilolite and phillipsite; Passaglia and Laurora, 2013). A similar value to that obtained from this study on pumice has been reported in the literature (2.5 meq/g; Lahav and Green, 1998). Mumpton (1999) reported that natural zeolites have CECs from 2 to 4 meq, about twice the CEC of bentonite clay, and a CEC of 3.84 meq/g for chabazite. Exchange capacity of zeolitic materials may depend on several factors. Many literature studies experimented higher ammonium exchange capacity of zeolites decreasing grain size (Ames, 1960; Hlavay et al., 1982;

Passaglia and Laurora, 2013). Moreover attached biofilm on zeolites may interfere with ion-exchange, although Lahav and Green (1998) reported that biofilm had no effect on the ion exchange characteristics of chabazite. These authors also reported that in chabazitic material an equivalent Na^+ is sufficient to exchange an equivalent NH_4^+. The high CEC value for ammonium on pumice is probably due to the smaller grain size and experiment set-up. This substrate was milled and then sieved at 2 mm before testing, so the CEC test was done on grain size up to 2 mm. The smaller the particle size distribution (PSD) is, the bigger the specific surface area will be and consequently the CEC value increases.

The CEC test revealed that pumice could adsorb 4924.8 mg of ammonium (Table 4.6). However this high value cannot be assigned to the material used to fill the filters, because it presented a greater PSD. Besides in the laboratory experiment the substrate was also washed with a sodium solution, creating an ideal condition for cationic exchange.

Table 4.6 CEC test results on gravel and pumice materials: NH_4 concentration in solution (mg/L), NH_4 adsorption on the substrate surface (mg and mg/g), CEC value (meq/100g) and solution pH.

	NH_4 concentration (mg/L)	**NH_4 adsorption (mg)**	**NH_4 adsorption (mg/g)**	**CEC (meq/100 g)**	**pH**
NH_4Cl solution	235.8	-	-	-	6.3
GRAVEL	296.7	7.0	0.3	1.5	8.4
PUMICE	56.9	246.8	49.4	273.6	6.9

<u>Medium performance</u>

1. Preliminary results

-Percolation time

In most filters the effluent discharge started almost immediately after application at the top, except the filter with the sand and gravel mixture, where the wastewater outflow started after 3 hours and 30 minutes. During this trial, the filters retained a good part of the entered water volume, also after 349 hours from the inlet (Figure 4.30). In particular, the filter filled with the mixture of sand and gravel showed only 35% outlet water volume, compared to the inlet, which remained stable after 123.5 hours. Filter filled with tops showed 60% of the water outflowed after 169 hours, value obtained by the filter with gravel after 73 hours, 25 hours by the mixture with pumice and gravel filter, 23 hours by the giant reed filter, 4 hours by the filter with bamboo. Gravel was the material that showed the highest water volume outflow, with 8.8 L (88% of the inlet volume) after 147 hours. These results suggested that the filters with lower porosity (but also with lower grain size) retain more water and the filtration is slower. Besides porous materials, such as pumice, could adsorb water and decrease the percolation time, increasing the hydraulic retention time.

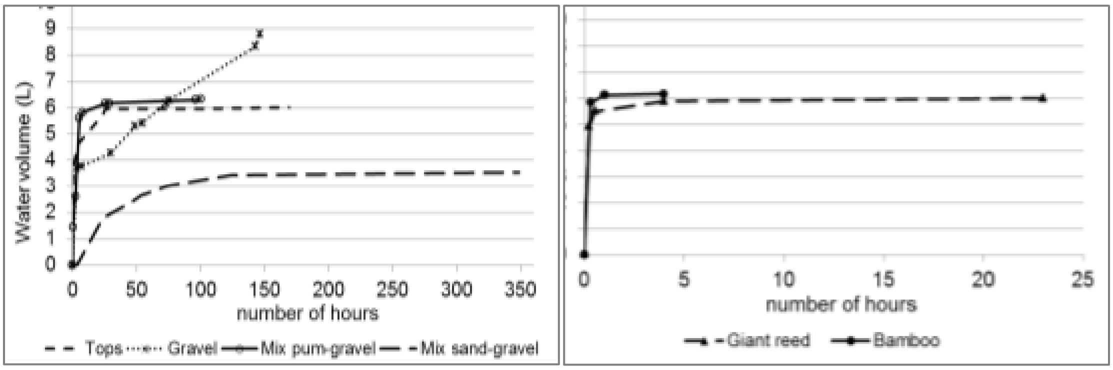

Figure 4.30 Water volume (L) at the outlet of each filter detected during the percolation test.

-Effect a single load

The results of the single piggery wastewater transition showed different responses in the physical and chemical parameters analyzed depending on the filter (Table 4.7). The pH value of 7.89 of the inlet wastewater (IN) had increased after filtration in most filters, reaching 8.2 at the outlet in the filters with tops and giant reed. The EC, which was higher than 15 mS/cm at inlet, decreased after filtration, especially with the filling media of gravel and pumice (outlet EC 13.8 mS/cm, R= 9%), except in the giant reed and bamboo filters (15.2 mS/cm at outlet), in which it remained similar. These last, with tops, presented outlet DO concentrations equal to the IN (0.07 mg/L), which instead increased after filtration by the other materials, especially in gravel filters (0.21-0.22 mg/L at outlet) (Table 4.7).

The TKN concentration (more than 1,170 mg/L at IN) decreased after filtration, particularly by the filter filled with sand and gravel (A= 36%), apart by the bamboo filter, which presented 1200 mg/L at the outlet (A=-2%) (Table 4.7). All filters reduced the ammonia-

N content, higher than 1,000 mg/L at IN; the best results on abatement were given by the filters filled with gravel, showing outlet concentrations between 329 mg/L (A=68%; only gravel) and 390 mg/L (A=62%; mixture of gravel and pumice). The TP content was a little higher than 5 mg/L at IN and was lowered after filtration by all filters with abatements in the range 52% (gravel)-7.4% (bamboo), except by the plastic tops medium, which presented an outlet value higher than 8 mg/L (A=-54%) (Table 4.7). This higher TP concentration at the outlet is probably due to the release of P compounds still present in the tops coming from detergents, even if they had been previously washed with water. Some studies in fact reported that detergents contain petroleum hydrocarbons, heavy metals, phosphorus, nitrogen, ammonia, total suspended solids, and surfactants (Scott and Malcolm, 2000; Bakacs et al., 2013).

Table 4.7 Values of physical and chemical parameters of the wastewater detected at inlet (IN) and outlet of every filter.

	pH	**EC** mS/cm	**DO** mg/L	**T** °C	**TKN** mg/L	**NH_4-N** mg/L	**TP** mg/L
IN	7.89	15.13	0.07	26.8	1,177	1,014	5.4
GRAVEL AND PUMICE	8	13.8	0.21	26.7	916	390	3.0
SAND AND GRAVEL	7.86	14.76	0.22	27.1	755	650	3.3
GIANT REED	8.2	15.22	0.08	25.4	1,088	573	4.9
TOPS	8.16	14.86	0.07	24.4	968	574	8.3
GRAVEL	7.95	14.32	0.10	26.2	827	329	2.6
BAMBOO	8.05	15.2	0.07	25.0	1200	664	5.0

From the single load trial test, the filters showed the highest chemical removal for NH_4-N, ranging between 31 and 56 g/m^3 (Table 4.8). Specifically, similar removals were obtained by the filters filled with bamboo and the mixture of sand and gravel (31-32 g/m^3 and MA 42-43%), for the giant reed and tops filters (43-16 g/m^3 and MA 57-61%), for the filters with gravel and the gravel-pumice mixture (about 55 g/m^3 and MA 74%). TKN removal by all media was similar (37-45 g/m^3 per filter), apart from bamboo, which gave the lowest removal quantity (8 g/m^3) and mass abatement (MA=9%). For the TP the removal and the mass abatement was higher for gravel filters (0.25 g/m^3 and 62% of MA), lower for the others (0.07-0.18 g/m^3; MA 18-46%), while tops presented higher TP outlet content.

Table 4.8 Chemical removal quantity (g/m^3) of every filter, with mass abatement (MA, %).

	TKN		NH_4- N		TP	
	removal (g/m^3)	MA (%)	removal (g/m^3)	MA (%)	removal (g/m^3)	MA (%)
GRAVEL AND PUMICE	40.3	46	54.9	74	0.25	62
SAND AND GRAVEL	37.5	43	32.3	43	0.18	46
GIANT REED	32.1	37	45.9	61	0.15	38
TOPS	33.1	38	42.9	57	-0.06	-16
GRAVEL	38.7	45	55.6	74	0.25	62
BAMBOO	8.2	9	31.3	42	0.07	18

2. Effect of recirculation

All the wastewater physical and chemical parameters were affected by the recirculation. The first three loads were carried out in sequence at a distance of three hours, while the 4th load was performed after 13 and a half h after the 3rd because of a technical impossibility. This led to different responses for almost all the parameters analysed, common to all filters. The piggery wastewater volume decreased steadily till the 3rd outlet, while it augmented with the last recirculation, probably due to the greater time for the wastewater release (Figure 4.31). Higher water retention was achieved by the filter filled with the pumice-gravel mixture, which showed a volume of about 3L at the 3rd outlet (40% less than the inlet volume). On the contrary the filters with tops, giant reed and gravel substrates gave the lower water retention, reducing the inlet volume by 20% at the 3rd outlet, and releasing about 6 L with the last recirculation (tops and giant reed).

The electrical conductivity of the piggery wastewater was around 9 mS/cm, and with the recirculation it was lowered in all filters, regularly and over time by the gravel material (Figure 4.31) to less than 7 mS/cm in the last outlets. The mixed filter and the tops filter showed no recirculation effect, maintaining EC outlet values close to 8 mS/cm, while giant reed and bamboo had similar behaviour till the 3rd outlet, afterwards the former increased the outlet value to 8.5 mS/cm while the latter dropped to 7.2 mS/cm. The behaviour of the giant reed in the last recirculation can be related to the higher outlet volume that caused the release of Na^+ and Cl^- ions (which didn't happen in the bamboo).

The inlet wastewater pH of 7.62 was augmented after filtration (Figure 4.31), in particular in the filters filled with tops and bamboo (outlet values higher than 8.2). The recirculation seems to have raised the pH value, except for the filter with pumice and gravel that showed an outlet pH around 7.8 during the first three loads, lowering to 7.6 at the 4th outlet.

Wastewater temperature at the inlet was approximately 19 °C, which was maintained between 18.4 and 20.1 °C by all filters until the 1st and the 2nd outlets, while it progressively decreased till 15 °C at the last outlet (Figure 4.31). These results are due to the higher air temperature during afternoon hours when the first two recirculations were performed.

The DO of the inlet piggery wastewater was very low (0.2 mg/L) and was raised with filtration (Figure 4.31). In general, the recirculation increased the DO content, particularly in the filter with pumice and gravel, which showed a final DO outlet concentration of 8.5 mg/L (90% of DO). The other filters reduced the DO content at the last outlet, probably due to the long permanence of the effluent in the collection bucket. The bamboo medium showed the lowest DO content at the outlet, ranging between 2 and 5 mg/L (20-50% of DO in the solution).

Filtration and recirculation also affected the piggery wastewater turbidity, which was 1,081 NTU at the inlet (Figure 4.31). For almost all filters the 2nd and 4th loads lowered this parameter the most, whereas the pumice-gravel mixture showed a regular downward trend over time, with values descending from 1,028 to 181 NTU. Turbidity was particularly lowered by the giant reed, tops, and the mixture with reductions of 93%, 75% and 83% at the 4th outlet.

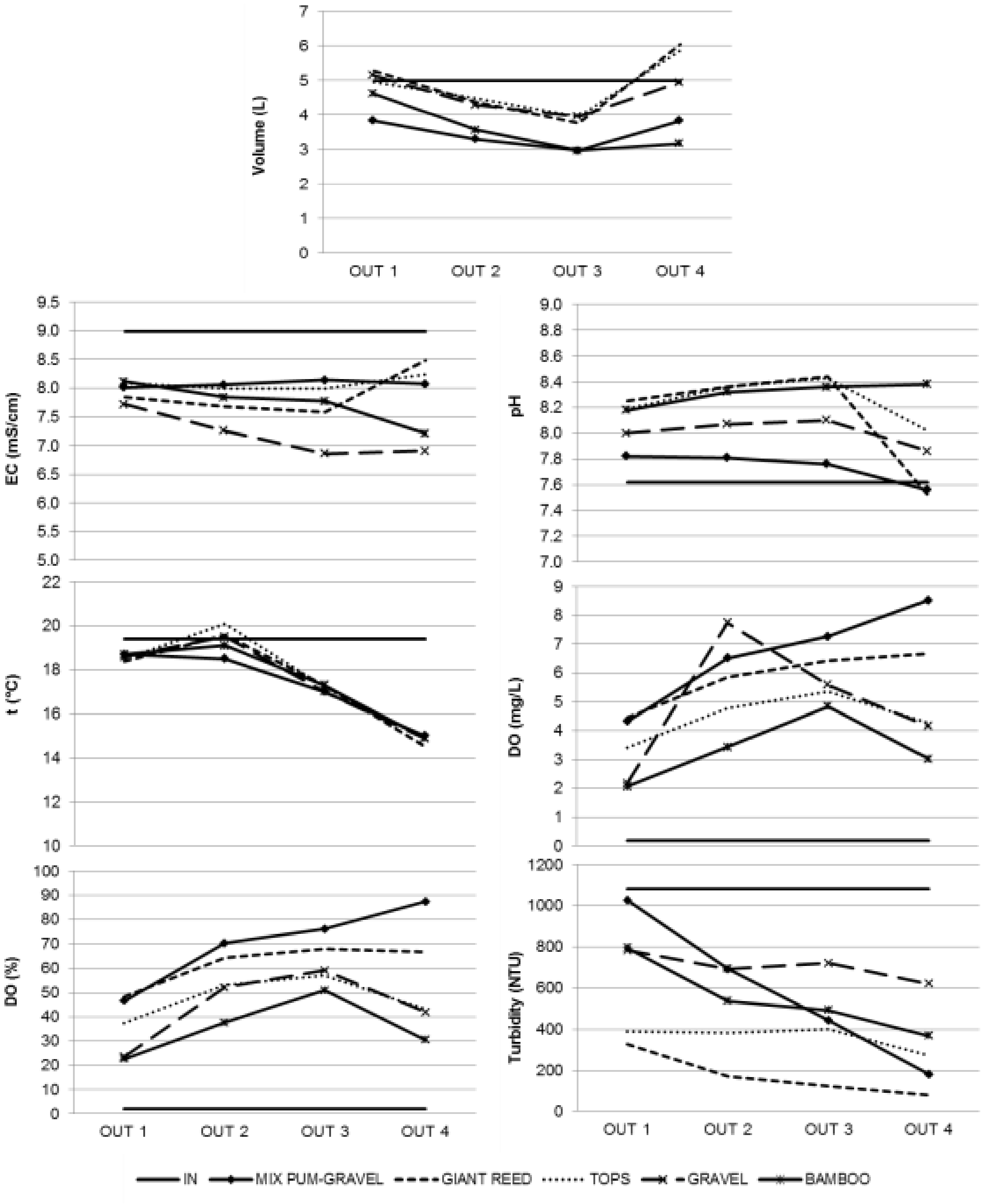

Figure 4.31 Effect of recirculation on volume and on-site parameters.

The recirculation of the piggery wastewater also affected the chemical content at the outlet. Inlet concentrations were 456 mg TN/L, 221 mg NH_4-N/L and 43.7 mg NO_3-N/L (Figure 4.32A). The recirculation gave similar behaviour among the filters for NH_4-N and NO_3-N, while TN showed different responses. The TN concentration detected at outlets was in general lower than the IN for all filters, apart from the one filled with pumice and gravel, which progressively increased the TN content from 520 to 820 mg/L with recirculation, as a result of the increment of nitrate-N from 378 to 756 mg/L (Figure 4.32). In the other filters, up and down TN values were obtained at the outlet; bamboo showed the lowest TN outlet contents ranging from 252 (OUT 2) to 384 (OUT 3) mg/L. Recirculation led to the formation of NO_3-N, which steadily increased from the OUT 1 to OUT 3; vice versa the NH_4-N was increasingly lowered through recirculation (also at the last outlet), in particular in the filters filled with giant reed and the pumice-gravel mixture, finally reaching a 98% reduction.

Aeration on the filter systems allowed by the intermittent recirculation resulted in the reduction of NH_4-N concentration by oxidation and its transformation (together with organic nitrogen) in the nitric form. Recirculation is usually adopted for water treatment in aerobic biological filters; many benefits have been obtained using recirculation, such as equalising filter load, enhancing treatment efficiency and reducing influent odour (Wei et al., 2010).

Each filter received about 5000 g/m^3 of total-N, of which 50% was ammonium-N and 10% nitrate-N (Figure 4.32B). For NO_3-N and NH_4-N, the quantity trends were similar to the concentration trends, while the TN differed. Regarding the TN outlet quantities, in fact, also the filter filled with pumice and gravel showed lower content compared to the inlet. Apart from the last outlet, in which high nitrate-N quantities had formed, this filter, like bamboo, tops and giant reed reduced the TN quantity increasing recirculation (especially with the second load), showing on average a mass abatement from 13 (OUT 1) to 31% (OUT 3). The gravel filter instead showed up and down values for TN outlet quantity. The nitrate-N quantity reached at the last outlet ranged from 1,125 g/m^3 (bamboo, MA= -131%) to 6,462 g/m^3 (pumice-gravel mixture; MA= -1,225%), while the ammonium-N was between 35 g/m^3 (pumice-gravel mixture; MA= 99%) and 816 g/m^3 (tops; MA= 67%).

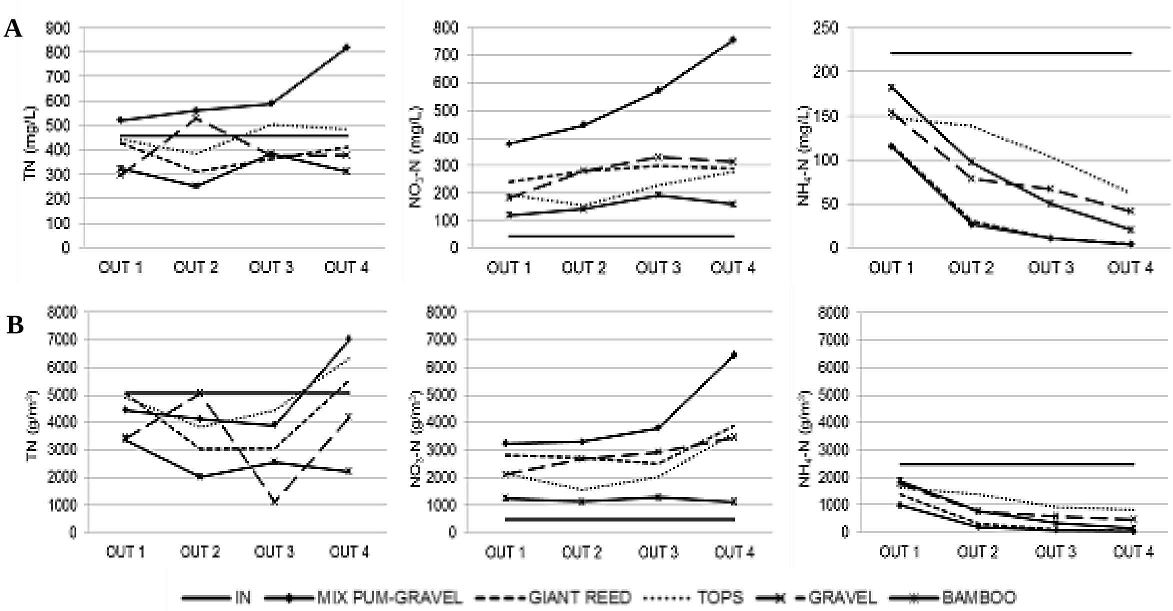

Figure 4.32 Concentration (mg/L; A) and quantity (g/m^3; B) of nitrogen forms at inlet and at the different outlets for every filter (mg/L).

The total phosphorus in the wastewater at inlet was close to 25 mg/L, of which 20 mg/L was in the soluble form (Figure 4.33A). With the recirculation all filters, except the bamboo, reduced these parameters with similar behaviour. The bamboo filter, in fact, presented higher TP outlet concentration compared to the inlet, reaching more than 30 mg/L at the 2nd outlet. Besides, the same filter presented a different behaviour for the PO_4-P, with outlet values always higher than the IN, but reducing it in the last three outlets (to around 24 mg/L). The lower phosphorus concentration was found in the pumice-gravel mixture filter, which reached 16 mg TP/L (A=35%) and 13.4 mg PO_4-P/L (A=35%) at the 3rd outlet. All filters reduced the phosphorus quantity increasing the recirculation, except at the last outlet (Figure 4.33B). The lower TP outlet values, reached at the 3rd outlet, were around 215 g/m^3 for tops and bamboo (MA=23%, on average), 179 g/m^3 for giant reed and gravel filters (MA=35% for both), and 107 g/m^3 for the pumice-gravel filter (MA=61%). For the latter, the same mass abatement was achieved for the soluble-P, while the other filters showed higher mass abatements, between 30% (tops and bamboo) and 38% (giant reed and gravel).

The inlet piggery wastewater had contents of approximately 2,100 mg COD/L and 500 mg BOD_5/L (Figure 4.34A). During recirculation the bamboo and tops filters showed similar COD outlet concentration to the IN, while the other filters revealed gradually lower outlet concentrations increasing the recirculation; this occurred especially in the filters with gravel medium, in which the concentration was reduced by between 25 (only gravel) and 48% (gravel+pumice) at the last outlet. The BOD_5 concentration was reduced with recirculation, with final outlet values between 300 mg/L (bamboo; A=40%) and 60mg/L (pumice-gravel; A=88%). The similar behaviour of the filters occurred also for the outlet COD and BOD_5 quantities, indicating that they had similar outlet wastewater volumes (Figure 4.34B). At the third outlet the filters showed the lower COD quantity, between 8,130 g/m^3 (pumice-gravel; MA=66%) and 19,408 g/m^3 (tops; MA=18%). The BOD_5 removal reached at the OUT 4 was between 2,270 g/m^3 (MA=91%) for the pumice-gravel filter and 1,325 g/m^3 for the tops (MA=53%).

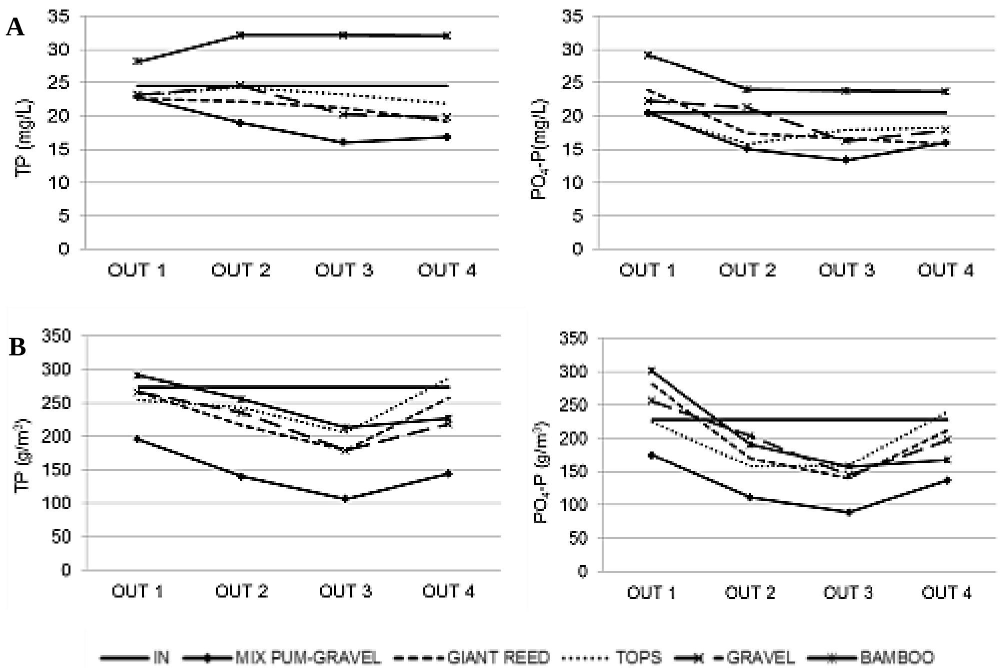

Figure 4.33 Concentration (mg/L; A) and quantity (g/m^3; B) of phosphorus forms at inlet and at the different outlets for every filter (mg/L).

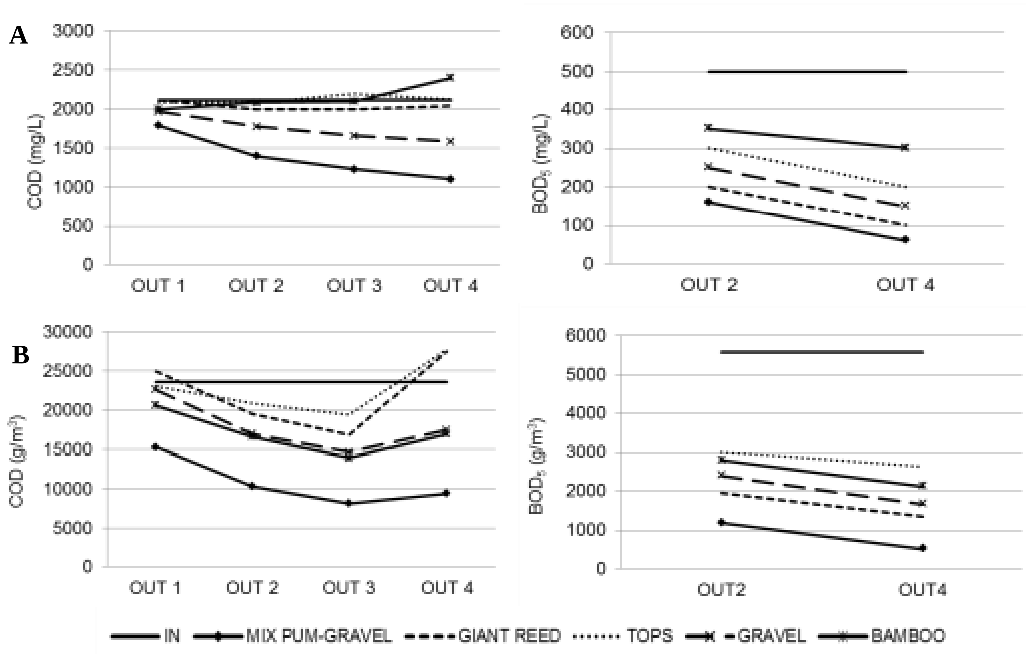

Figure 4.34 Concentration (mg/L; A) and quantity (g/m^3; B) of COD and BOD_5 at inlet and at the different outlets for every filter (mg/L).

<u>System performance</u>

1. Preliminary results on recirculation

The effect of different recirculation considering the entire system showed no important differences among the number of loads (Table 4.9). In both trials the pH rose to 9.5-9.9 and the EC decreased particularly with 30 recirculations (R=38% vs. 20% with 60 recirculations). The TN was reduced in both tests with similar abatement (44-42%), and the same occurred for the TP (A=32-33%). The ammonium-N increased after filtration, especially with fewer recirculations.

This trial didn't lead to a clear understanding of the difference among recirculations. Probably the high wastewater volume load (200 L/filter) and the very close recirculation over time (one every 15 minutes) caused adsorption and release phenomena, also due to the previous tests.

Table 4.9 Wastewater physical and chemical parameters detected at inlet (IN) and outlet (OUT) of the pretreatment system after 30 and 60 recirculations, with abatement (A, %).

		pH	EC mS/cm	TN mg/L	NH_4-N mg/L	TP mg/L
30	IN	7.73	14.71	1080	126	32.5
	OUT	9.92	12.16	604	242	22.1
	A %	-	-	44	-92	32
60	IN	7.96	13.84	1110	114	32.7
	OUT	9.54	11.04	646	207	21.8
	A %	-	-	42	-82	33

2.Wastewater treatment performances over time

-*On site parameters*

The physical-chemical composition of the piggery wastewater at inflow changed over the experimental period (Figure 4.35). The inlet EC was higher in the first week (13.37 mS/cm), it then remained more stable till the end of the experiment ranging between 6.39 and 9.24 mS/cm, with median value of 9.1 mS/cm (Figure 4.35). From September 6th 2012 the filtered wastewater (OUT) presented lower EC than the IN, less than 7.5 mS/cm, till the end of the experiment. Filtration significantly reduced the wastewater EC (outlet median 7.4 mS/cm), resulting in 19% of reduction by the filtering treatment. The pH of the IN wastewater ranged between 7.66 and 9.46 (median 8.5), with higher values in the first two weeks (August 28th - September 12th). After the filtration the pH of the wastewater remained similar to the inlet, in the range 7.88-9.71 (median 8.7), decreasing over time as the IN trend (Figure 4.35). The wastewater temperature before filtration oscillated between 25.4 °C (August 29th) and 19.0 °C (September 18th), with median value of 23.5 °C (Figure 4.35). The filters treatment significantly lowered the wastewater temperature, which ranged from 23.3 °C to 15.5 °C (median 19 °C). The wastewater had a low DO content, with concentration between 0.15 and 0.63 mg/L (2.9-7.3%) and median value of 0.2 mg/L (Figure 4.35). The DO concentration was significantly higher after the treatment by the filtration system (median 1.5 mg/L), although the outlet had up and down values, probably due also to the rainfall during September, in particular continuously from August 31st to September 5th, September 12th, and the end of the month, which created jumps in the dissolved oxygen content in the wastewater.

Turbidity at the inlet oscillated between 3,718 NTU and 1,145 NTU (median 1,516 NTU), higher in the first week and decreasing over time (Figure 4.36). The outlet turbidity was slightly lower, ranging between 3,844 and 190 NTU (median 752 NTU), with a similar trend to the IN.

Regarding the solids content, the wastewater contained 0.62% of TS and 0.27% of VS as median values. With the filtration treatment the outlet TS was 0.65% (R=-3.7%), while the VS was lowered to 0.25% (R=8.7%).

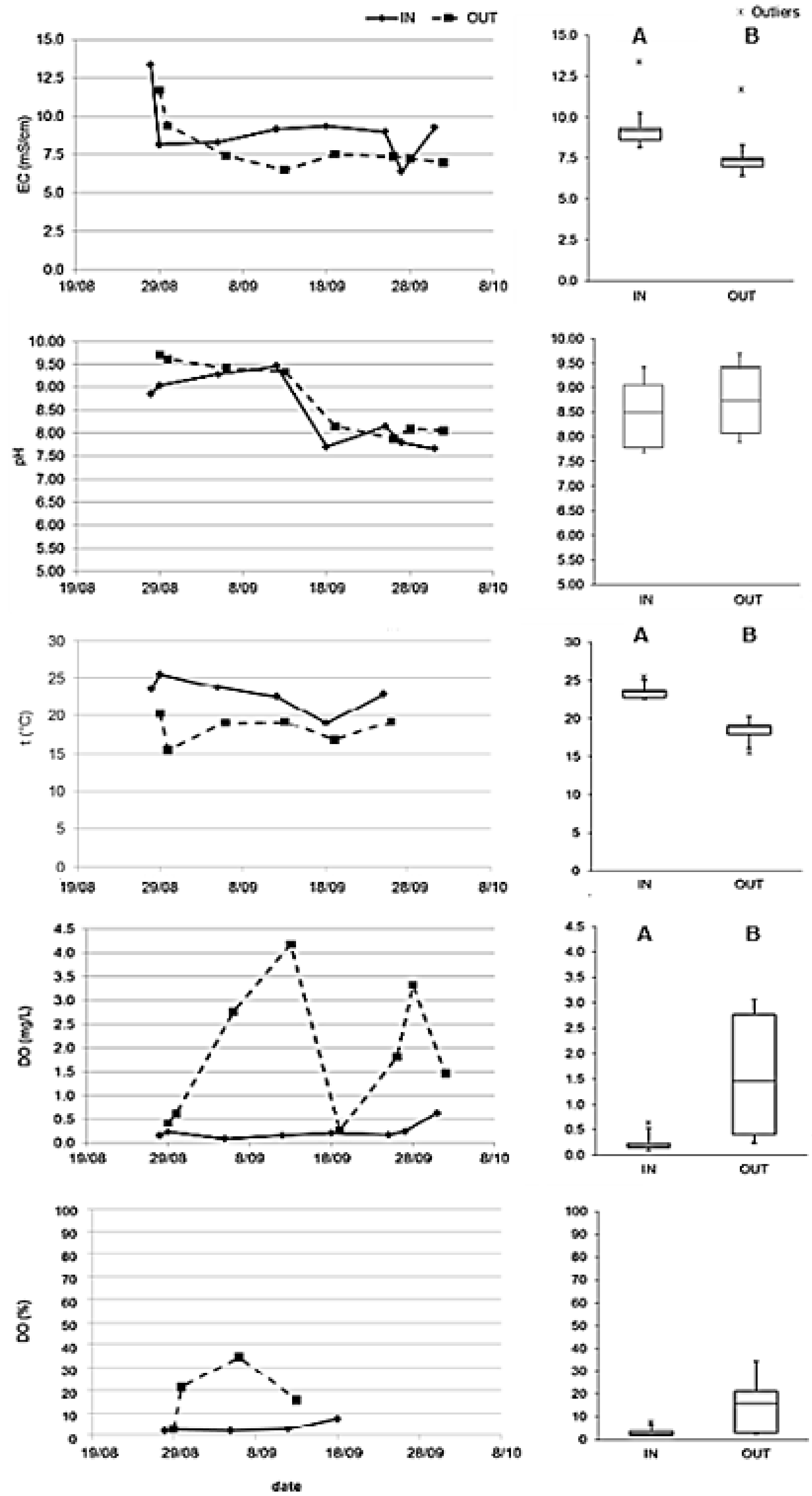

Figure 4.35 On-site parameters of the piggery wastewater detected in 2012 at inlet (IN) and outlet (OUT) of the pretreatment system. Trends over time (at left) and box-plot diagrams (at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

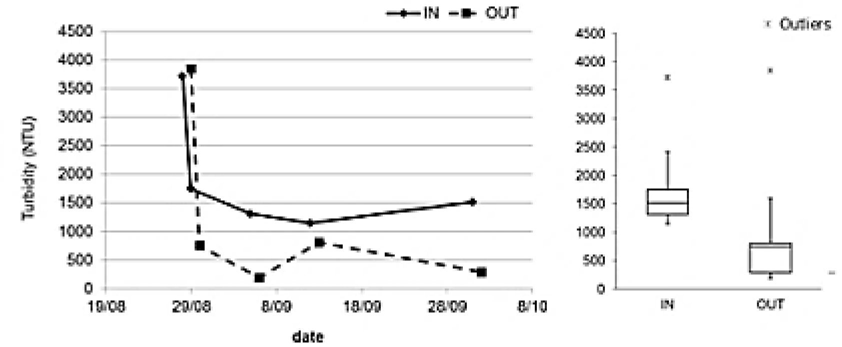

Figure 4.36 Turbidity (NTU) detected at inlet (IN) and outlet (OUT) of the pretreatment system in 2012. Trends over time (at left) and box-plot diagram(at right).

-Chemical contents

The piggery wastewater chemical concentrations varied over time, in general with higher values in the first experimental week. The inlet total nitrogen (TN) concentration decreased constantly over time from 1,030 mg/L to 523 mg/L (median 580 mg/L) (Figure 4.37). The TN concentration of the filtered wastewater was lower than 800 mg/L throughout the period, showing a similar trend to the IN, ranging between 765 mg/L and 357 mg/L (median 436 mg/L). The ammonia nitrogen (NH_4-N) contained in the inlet wastewater varied from 122 mg/L to 239 mg/L (median 208 mg/L), presenting steady values in the last experimental month (range of 60-90 mg/L) (Figure 4.37). Although the outlet concentration was lower than the IN (67-233 mg/L; median 87 mg/L), the pretreatment system didn't significantly reduce this parameter. The nitric nitrogen (NO_3-N) at the IN increased over time, from 23.3 mg/L to 63.4 mg/L (median 25 mg/L) (Figure 4.37), and after the filtration the wastewater NO_3-N concentration had large fluctuations from 21 mg/L to 271 mg/L (median 64 mg/L). These high values of wastewater nitric-N at the outlet are probably due to rainfall on the days prior to the monitoring (specifically the September 12th and October 2nd, when 25mm and 12mm fell), which caused oxygenation in the filters, creating NO_3-N generation. During the experiment the pretreatment system received a TN quantity between 11.5 (August 28th) to 5.8 (October 1st) g/m^3/d (median 6.5 g/m^3/d); the outlet showed a TN in the range 3.0-8.7 g/m^3/d (median 4.9 g/m^3/d), exceeding the IN quantity in the 4th monitoring cycle (September 13th) due to nitrate-N generation (Figure 4.38). The inlet NH_4-N quantity oscillated from 1.5 to 2.6 g/m^3/d (median 2.3 g/m^3/d), while the outlet was between 1.9 and 0.7 g/m^3/d (median 1.4 g/m^3/d) (Figure 4.38). The inlet NO_3-N content remained stable over the period (0.3-0.7 g/m^3/d), while at the outlet it reached 4.8 g/m^3/d on 13th September (median 0.5 g/m^3/d) (Figure 4.38). The pretreatment system showed a median TN removal of 2.5 g/m^3/d, of which 0.9 g/m^3/d was in the ammonium form, while the nitrate nitrogen increased (Table 4.10). The median mass abatements were 40% for NH_4-N and 34% for TN.

The total phosphorus (TP) concentration in the inlet wastewater had higher values at the beginning of monitoring, which decreased over time; it ranged between 21.7 mg/L and 54.5 mg/L, with a median value of 28.3 mg/L (Figure 4.39). After filtration the TP concentration oscillated between 21.2 and 27.3 mg/L (median 24.5 mg/L). Similar trends were obtained for soluble phosphorus (PO_4-P), which represented 80% of the TP. This parameter ranged from 45.6 to 18.1 mg/L at the inlet (IN median 22.5 mg/L), and from 15.9 to 21.7 mg/L at the outlet (OUT median 21 mg/L) (Figure 4.39). For both parameters the filtering system proved to significantly decrease their content at the outlet, with concentration abatements of 13% for TP and 7% for PO_4-P. The TP quantity entering the system was in the range 0.2-0.6 g/m^3/d (median 0.32 g/m^3/d), while the outlet median corresponded to 0.23 g/m^3/d (Figure 4.40). Similar values were found for the PO_4-P, which showed not significant differences between the IN and OUT quantities. The pretreatment system removed 0.06 g TP/m^3/d (MA=26%), of which 92% was removed in the form of soluble P (MA=28%) (Table 4.10).

Like the previous chemical parameters, the chemical oxygen demand (COD) of the wastewater at the inlet was particularly high in the first experimental week (6,831 mg/L), it then remained stable at about 2,100 mg/L (2,242 mg/L as median) (Figure 4.41). During the entire experimental period, the outlet COD concentration was steady and lower than the IN (1,532-2,086 mg/L), and significantly reduced by the system (median 1,867 mg/L) by 17%. The 5-days biological oxygen demand (BOD_5) at the inlet ranged from 1,200 to 700 mg/L (median 650 mg/L), and like the COD, it was significantly reduced by the filtration to a median value of 200 mg/L (A=69%). The daily median COD quantity received by the filters system was 25 g/m^3/d, ranging from 76 (August 28th) to 21 (October 2nd) g/m^3/d, significantly lowered to less than 18 g/m^3/d (median) (Figure 4.42), showing a removal quantity of 8 g/m^3/d and 35% of MA (Table 4.8). The BOD_5 daily quantity also significantly dropped from 7.3 g/m^3/d to 2.2 g/m^3/d (medians, Figure 4.42), with a daily removal of 5 g/m^3/d and MA of 71% (Table 4.10).

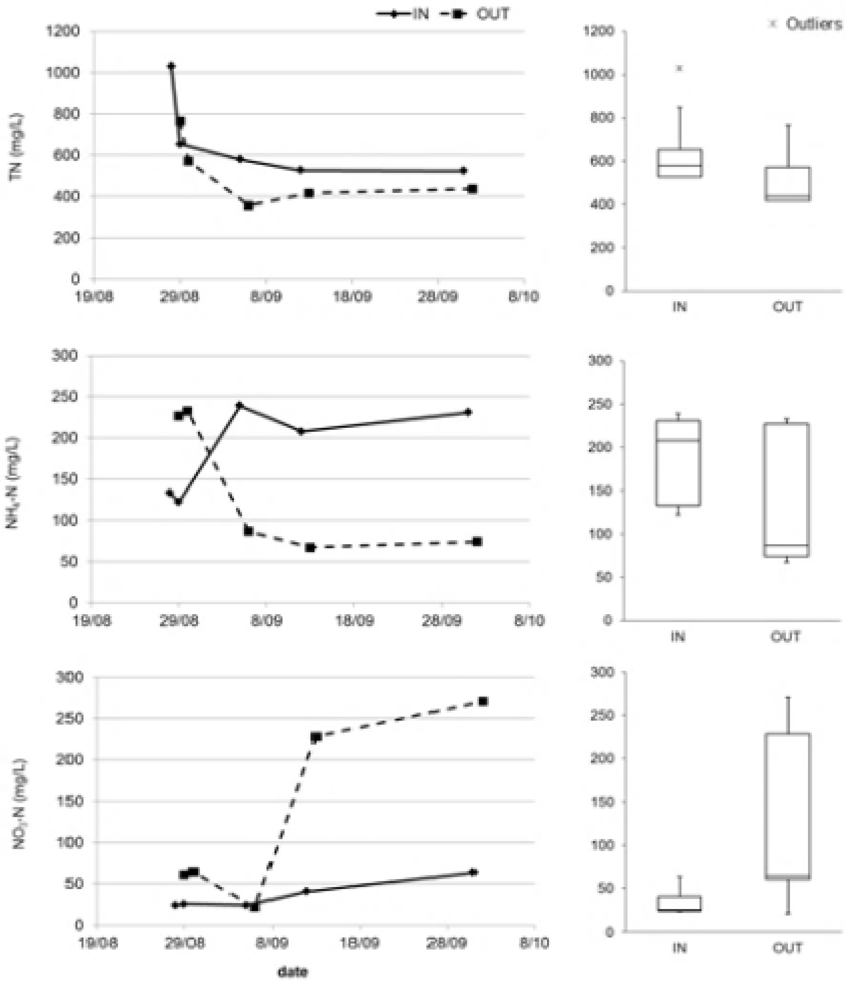

Figure 4.37 Concentrations (mg/L) of nitrogen forms at inlet (IN) and outlet (OUT) of the pretreatment system. Trends over time (at left) and box-plot diagrams (at right).

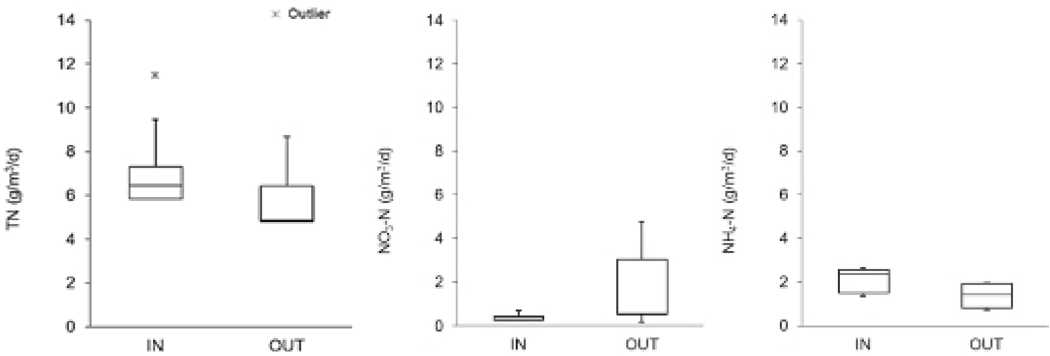

Figure 4.38 Box-plot diagrams of the nitrogen quantities (g/m^3/d) at inlet (IN) and outlet (OUT) of the pretreatment system.

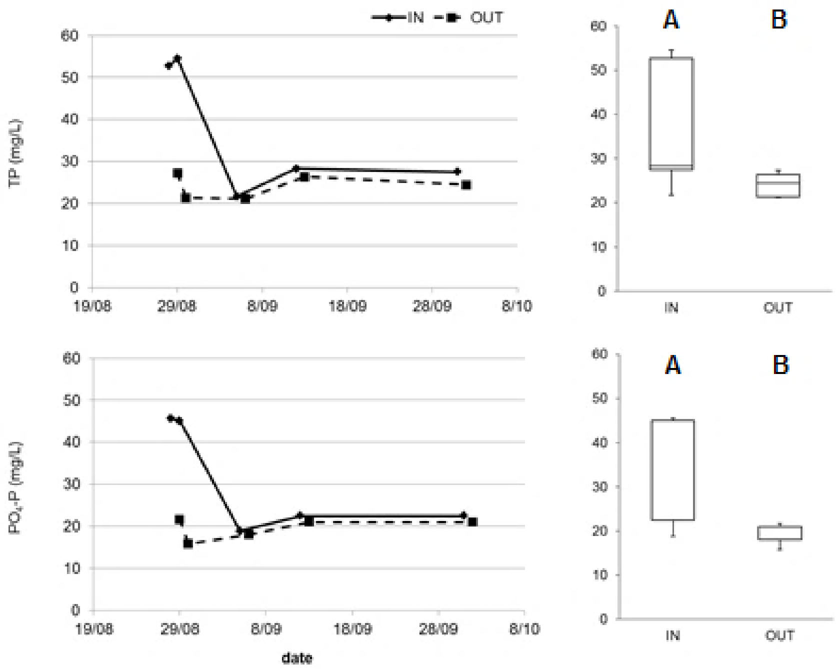

Figure 4.39 Concentrations (mg/L) of phosphorus forms at inlet (IN) and outlet (OUT) of the pretreatment system. Trends over time (at left) and box-plot diagrams (at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

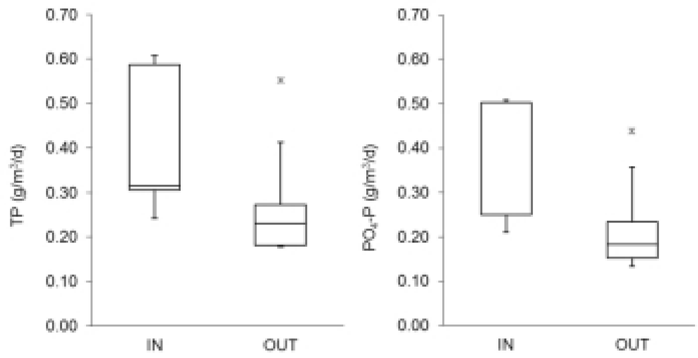

Figure 4.40 Box-plot diagrams of the phosphorus quantities ($g/m^3/d$) at inlet (IN) and outlet (OUT) of the pretreatment system.

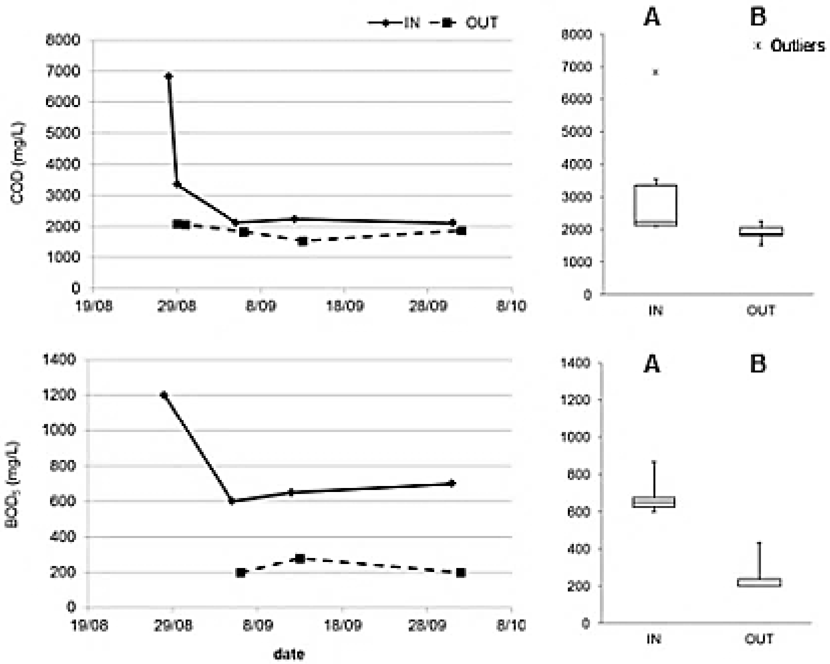

Fig. 4.41 Concentrations (mg/L) of COD and BOD_5 at inlet (IN) and outlet (OUT) of the pretreatment system. Trends over time (at left) and box-plot diagrams (at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

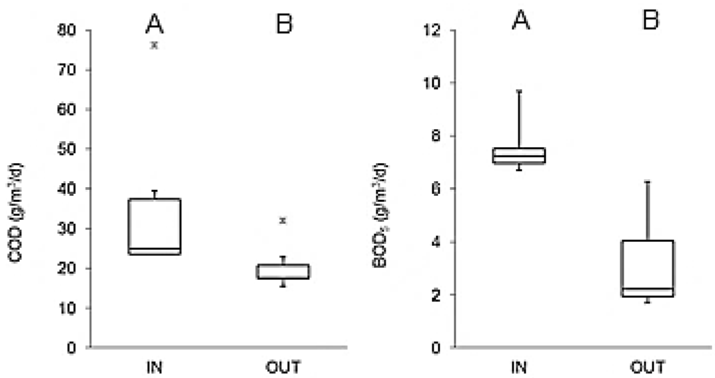

Figure 4.42 Box-plot diagrams of the COD and BOD_5 quantities ($g/m^3/d$) at inlet (IN) and outlet (OUT) of the pretreatment system. Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

Table 4.10 Median values of removal ($g/m^3/d$) and mass abatement (MA, %) of the chemical compounds in the pretreatment system.

	Removal ($g/m^3/d$)	**MA (%)**
TN	2.5	34
NO_3-N	-0.3	-96
NH_4-N	0.9	40
TP	0.06	26
PO_4-P	0.06	28
COD	8.2	35
BOD_5	5.0	71

Observations on filter clogged

During the experimentation the filter N°2, filled with sand and gravel, presented small outlet wastewater volume, and on October 8th the filter was excluded from the pretreatment system. Afterwards it was manually emptied, removing the filling material in small layers from the top to the bottom, to investigate where the clogging occurred. The wastewater stagnation was seen at the top of first sand layer (about 20 cm from the top of the filter). All the underlying material was soaked in wastewater, and once having removed the clogged sand layer, the wastewater started to exit the filter.

Although the filter was filled with sand and alternating layers of coarse gravel, clogging anyway occurred after about 40 days from the feeding with the piggery wastewater. Probably the pores of sand particles occlusion derived from the organic matter and biofilm formation. Healy et al. (2011) revealed that the biofilm formation in intermittently-loaded sand, glass and soil polishing filters occurs mainly in the uppermost 0.12 m-deep filter layer. Clogging didn't occur in Zheng et al. (2012), where the filter composition was similar and in which a biological sand filter system was applied as a partial nitritation treatment of anaerobically digested effluent of swine wastewater and studied for 45 days. The filter used in this study was based on biofilms establishing on sand grains, accommodating microorganisms that are responsible for biotransformation, biodegradation, mineralisation and nutrient assimilation processes involved in wastewater purification. Compared with the investigation of Zheng et al. (2012), the inlet piggery wastewater studied in the present work showed higher TN concentration, similar NH_4-N content, and almost 2-3 times more COD concentration, suggesting that probably this wastewater contained more organic compounds that blocked on the layer of sand. Leverenz et al. (2009) revealed that clogging involves several mechanisms, such as reduction of pore space by TSS and bacterial growth on entrapped or dissolved solids. The presence of a clogging layer may be characterised in terms of physico-chemical parameters, such as organic matter and nutrients, or physical parameters, such as water retention capacity (Rodgers et al., 2004).This circumstance prevents its use for this experiment.

CCW

Plant growth and stage

The plants used in 2012 to vegetate the phytodepuration system were fed with filtered piggery wastewater only from 10^{th} to 15^{th} June. In the previous period, they were constantly fed tap water at a sufficient quantity to ensure constant water presence inside each tank. Throughout these months (April, May, and half of June) plants grew constantly (Figure 4.43). In the first monitoring week the average height of *Typhoides arundinacea* (PHAAP) was similar for the plants in the second and third levels (29±1.4 and 26±0.7 cm), and higher in the first level (39±1.4 cm; Figure 4.43). In this phase they grown constantly reaching a mean height of 56±0.7 cm in L1 (0.3 cm per day), 49±2.1 cm in L2 (0.4 cm per day) and 46±5 cm in L3 (0.4 cm per day). At the beginning of April these plants were at the shoot development stage, and by mid-June they were flourishing (Figures 4.44 and 4.45A). *Carex divisa* (CRXDW) growth during this period was similar, which from an initial height of 21± 1.4 cm (L3)-26±0.0 cm(L1), reached similar heights at the end of May (49 cm), growing on average about 0.5 cm per day. Differently from PHAAP, in April this species was flowering and in mid-June it produced seeds (Figures 4.44 and 4.45A). In agreement with this result, Esmaeili et al. (2009) reported that for this species flowering and fruiting occurs from May until July. In the same months also *Mentha aquatica* (MENAQ), which initially had a mean shoot length between 3.8 (L2 and L3) and 7 (L1) cm, grew constantly reaching 24.5 cm in L1 and L2 and 20.5 cm in L3 (Figure 4.43), with a daily growth between 0.39 (L2) and 0.32(L3) cm. Contrarily to CRXDW, which showed an advanced phenological stage, the MENAQ plants were still forming leaves and side shoots during this period (Figures 4.44 and 4.45A).

During the short period of feeding with raw piggery wastewater plants suffered a strong stress, manifested by a yellowing followed by drying ("burning" effect) of the aerial part. Compared to other species the shoots of MENAQ seemed to grow (Figure 4.43). In the subsequent phases of water feeding and water washing, till the cutting of the aerial part of plants (on July 3^{rd} for PHAAP and MENAQ, July 19^{th} for CRXDW), growth was not detected. During the last period of water feeding (from the second week of July to August 28^{th}), the plants recouped growth (Figure 4.45B), with greater differences among the levels in the CRXDW plants. This species reaching a height of between 22 and 30 cm at the end of August, with a daily increment of 0.54 cm in L3, 0.63cm in L1 and 0.73 cm in L2. The PHAAP showed similar mean plant height among L1, L2 and L3, with values of 35-40 cm (Figure 4.43), corresponding to a daily growth of 0.33, 0.39, 0.37 cm from the higher to the lower level. Also MENAQ gave similar shoot length results, with average growth of 0.34, 0.39 and 0.42 cm per day in L1, L2 and L3, respectively. During this period, plants of MENAQ were flowering in all levels (from mid-August), which continued till mid-October 2012. In this study, flowering of this species occurred later than reported in Borin (2003) (June); probably the use of raw piggery wastewater and the plants cutting influenced and delayed its development.

All plants showed growth during the diluted piggery wastewater feeding in all levels (Figures 4.43 and 4.45C). Specifically, PHAAP showed continuous elongation in the second and third levels while plant growth in L1 seemed to slow till the end of the experiment. The final average height reached on November 9th was between 40 and 60 cm, similar in L2 and L3, 54±5.9 cm and 56±5.2 cm, lower in L1 with 44±4.4 cm. The daily increment of PHAAP plants was 0.29 cm in the lower level, 0.22 in the mid-level, 0.12 in the higher level. MENAQ revealed a continuous growth in particular in the mid-level, and the same average shoot length in November in the first two levels, with 77±8.5 cm (L1) and 76±7.6 cm (L2). The plants in L3 showed less data differences between repetitions but smaller growth (51±0.1 cm). The daily elongation of this species was 0.51 cm in L2, 0.44 cm in L1 and 0.26 cm in L3, respectively. The plants of CRXDW had more similar growth among levels, reaching in November mean values of 32±4 cm, 36± 0.6 cm and 28±2.7 cm in L1, L2, L3 (Figure 4.43), with the same daily increment of 0.08 cm in all levels.

Concerning the plant development considering the entire period, the three species gave similar daily growth results in the first experimental stage during spring 2012. In the second period, during summer, in which water feeding and water washing were applied, plants of CRXDW showed higher daily increment compared to the other species. In the last period with the diluted piggery wastewater feeding, CRXDW growth slowed, which continued regularly in the other species, particularly in MENAQ.

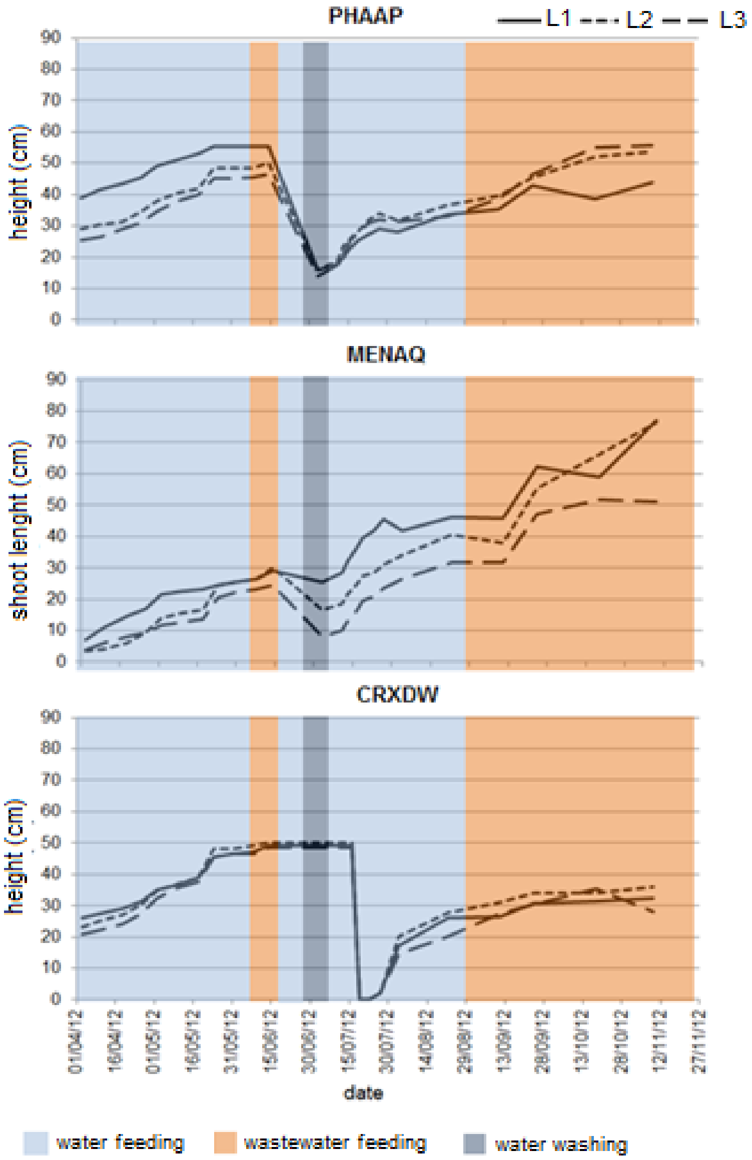

Figure 4.43 Average plant height or shoot length (cm) trends over time of every plant species in the three levels of the CCW (L1: highest level. L2: mid-level. L3: lowest level).

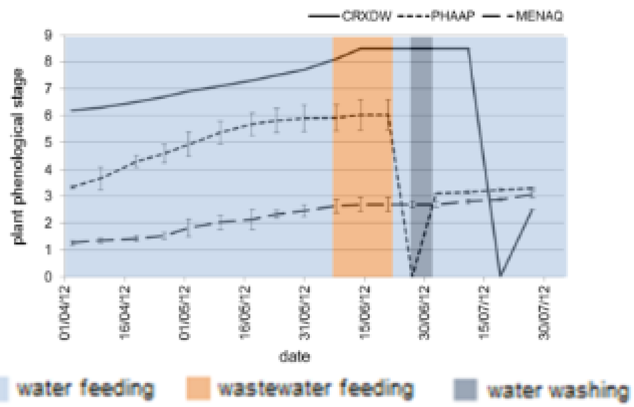

Figure 4.44 Phenological development stage of the three plant species detected over time in 2012.

Morphological differences in the aerial parts and phenological development of the three plant species gave different growth results. PHAAP and CRXDW, although they present different phenology, have similar morphology, as they able to spread laterally by rhizomes to form permanent grassland. MENAQ instead, develops later and forms long horizontal runners above ground and relatively rigid erect stems.

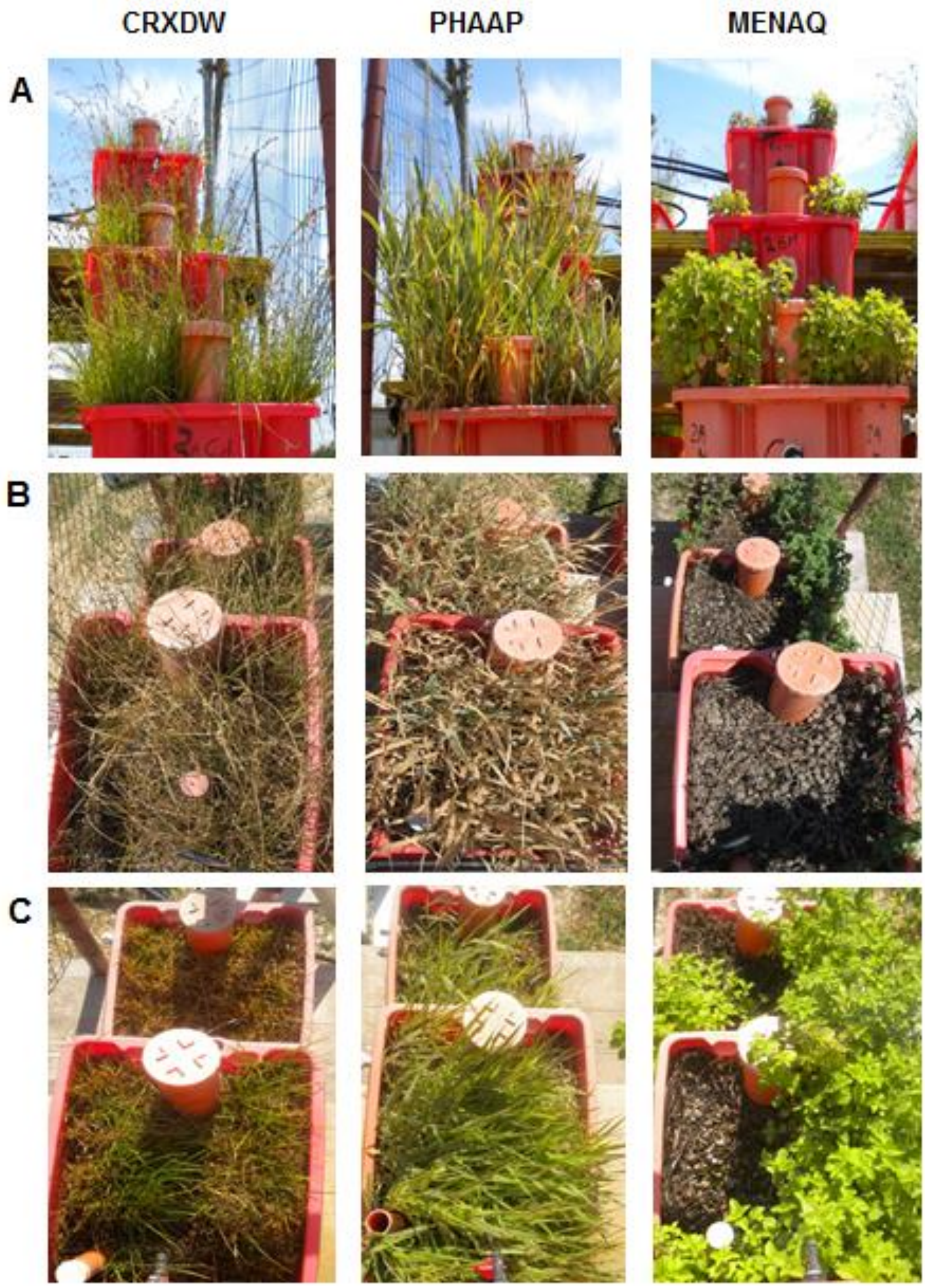

Figure 4.45 Plants in the different monitoring periods of 2012: May 24 (A; first period with water feeding), June 27 (B; period following raw wastewater feeding in which water washing occurred), July 30 (C; third period with wastewater feeding).

Wastewater characteristics in the initial periods

The raw piggery wastewater applied to the CCW (IN) had alkaline pH (more than 9), salt content around 9 mS/cm as EC, and low DO content (0.14 mg/L) (Table 4.10). The TKN was almost 440 mg/L, of which 67% was in the ammonium form, TP was slightly more than 3.7 mg/L, and PO_4-P was lower than 1 mg/L (Table 4.11). At the outlet of the phytodepuration system the pH was around to 9.7, EC was lower, in the range of 7.8 (CRXDW)- 8.4 (PHAAP) mS/cm, as was DO. Regarding chemical compounds, TKN concentration increased after the treatment, especially in PHAAP (561 mg/L) and also NH_4-N (particularly in MENAQ, 485 mg/L at outlet). The TP concentration increased at the outlet of the PHAAP and MENAQ treatments, while soluble-P was lowered to 0.28 mg/L (PHAAP) (Table 4.11).

Table 4.11 Mean values of the raw piggery wastewater physical and chemical parameters detected at inlet (IN) and outlet of every treatment. The "*n.f.*" acronym indicates that data were not found.

	pH	**EC**	**DO**	**TKN**	**NH_4-N**	**TP**	**PO_4-P**
		mS/cm	mg/L	mg/L	mg/L	mg/L	mg/L
IN	9.32	9.25	0.14	441	297	3.73	0.94
CRXDW	9.68	7.76	*n.f.*	535	301	2.93	0.63
PHAAP	9.67	8.41	0.08	561	347	4.06	0.28
MENAQ	9.69	8.34	0.05	449	485	4.03	0.55

Regarding the chemical quantities, the system received on average between 64 and 129 g TKN/m^2/d and 43-87 g NH_4-N/m^2/d, higher in the lines vegetated with MENAQ (Table 4.12). The outlet contents for these elements were in the range of 59-71 g/m^2/d for TKN and 37-65 g/m^2/d for ammonium-N, with higher removals for MENAQ, around 70 g TKN /m^2/d and 22 g NH_4-N /m^2/d. The total phosphorus applied to the CCW was between 0.54-1.0 g/m^2/d and soluble-P in the range 0.14-0.28 g/m^2/d (Table 4.12). CRXDW lines reduced the TP quantity at the inlet (till 0.35 g/m^2/d), vice versa they presented the higher PO_4-P quantity at the outlet. The daily removal was between 0.19 and 0.48 g/m^2 for TP and from 0.06 to 0.21 g/m^2 of PO_4-P, higher for both parameters in the MENAQ treatments.

The application of raw piggery wastewater to the phytodepuration system led to stress in the plants, deducible from the aerial part (as previously reported). Various factors could have caused this, such as the high values of pH and EC, but also the chemical content of NH_4-N and probably of organic compounds which were not studied in this experimental stage. Although wetland plants are able to live in a very wide range of environments, their survival is compromised when they are not used to living under certain conditions. Besides the several contaminants in the raw piggery wastewater, the stress could have been caused by the fact that they had been fed for a long time with just water. Nevertheless during the feeding with raw wastewater the treatments showed chemical concentration reduction and chemical removals; these results can be related to the influence of the LECA medium, which probably adsorbed the chemicals, although the depuration effect of MENAQ is not to be excluded, since it gave the best results for all parameters.

During the plants water washing phase (three washes in three days), EC values at the outlet constantly decreased in all tanks. On the first day EC at the outlet was between 1,53 mS/cm (PHAAP) and 1,84 mS/cm (CRXDW), the pH in the range 7.5 (PHAAP)-7.9(MENAQ and CRXDW) and DO concentration between 2.81(PHAAP) and 3.23 (MENAQ). With washing the EC reached 1.20-1.35 mS/cm, the pH remained similar (7.5-7.8) and DO augmented till 3.38-3.83 mg/L.

Table 4.12 Average daily chemical element quantity ($g/m^2/d$) at inlet (IN) and outlet (OUT) of every treatment (CRXDW, PHAAP, MENAQ).

	IN			**OUT**		
	CRXDW	PHAAP	MENAQ	CRXDW	PHAAP	MENAQ
TKN	64	72	129	65	71	59
NH_4-N	43	49	87	37	44	65
TP	0.54	0.61	1.0	0.35	0.51	0.52
PO_4-P	0.14	0.15	0.28	0.08	0.04	0.07

<u>Water consumption</u>

The water quantity entering the phytodepuration system during the entire experimental period was more than 2,500 L/m^2 (Figure 4.46). The MENAQ lines received higher average inlet volumes than other lines, reaching at the end of the experimentation 2,733 L/m^2 of water and piggery wastewater, or rather 17.5 $L/m^2/d$. The CRXDW treatment received the lower inlet quantity over the period, with an average value of 2,115 L/m^2 on 8th November (13.5 L/m^2/d); the PHAAP lines were instead fed with an average daily volume of 14.3 L/m^2 (total volume 2,242 L/m^2). The cumulated rainfall during the experiment has also to be considered. A total 319 L/m^2 of rain fell (Figure 4.46), which corresponded to 12% of the inlet volume in MENAQ lines, and to 14% and 15% of the inlet volumes of PHAAP and CRXDW treatments.

The outlet water volumes were higher in the CRXDW lines, which showed an average value of 1,706 L/m^2, versus 1,580 L/m^2 in MENAQ and 1,536 L/m^2 in PHAAP.The mean daily outlet volume was 11 L/m^2/d for the sedge lines, 10 L/m^2/d for the mint and 9.8 L/m^2/d for the reed treatments (Figure 4.46).

Regarding plant evapotranspiration (ET), MENAQ showed higher values throughout the period, with an average cumulated ET of 1,473 L/m^2 in the last monitoring cycle, to be more precise a daily ET of 9 L/m^2/d. The lower ET was instead given by the CRXDW lines, with a final value of 774 L/m^2 (5 L/m^2/d). The PHAAP lines had intermediate results, with average ET of 1,025 L/m^2 on November 8th, or rather 7 L/m^2/d (Figure 4.46). Considering the period during which the system was fed with diluted piggery wastewater, MENAQ treatments had the higher water consumption, with 9.3 L/m^2/d, followed by PHAAP with 7.2 L/m^2/d and CRXDW lines with 3.8 L/m^2/d.

Studies on *Phragmites australis* grown in the Veneto region (Borin et al., 2011) showed ET values in the range 0.7-5.0, lower than the mint ET values obtained in this study. Higher ET values for *P. australis* were observed in warmer climates, with up to 57 mm/d (El Hamouri et al., 2007; Headley et al., 2012), while lower in colder countries (0.2-6.3mm/d; Herbst and Kappen, 1999; Fermor et al., 2001).

The cumulated ET$_0$ trend increased constantly over time with a marked difference from ET, especially in MENAQ and PHAAP, reaching a final value of 706 L/m^2 (4.5 L/m^2/d) in November (Figure 4.46).

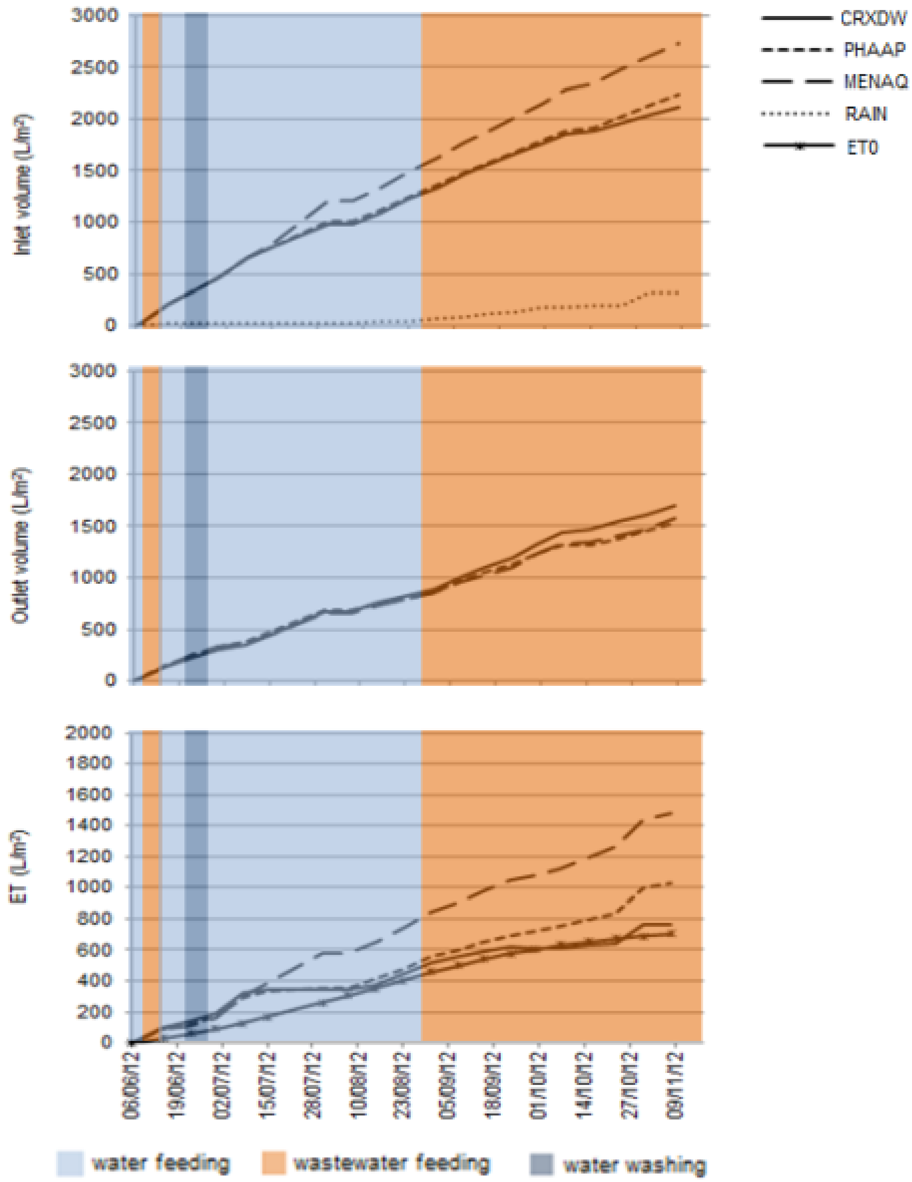

Figure 4.46 Cumulated trends of average inlet volume (above), outlet volume (middle) and actual evapotranspiration (ET; below), in L/m^2, of each plant species. Inlet volume graph also shows the cumulated rain (L/m^2); ET graph also shows potential evapotranspiration (ET_0, L/m^2).

The plant coefficient (*Kp*) has been calculated for from the months of June, July, August, September, while the coefficients for October and November have been computed as one, since the experiment ended on November 8th (Table 4.13). The *Kp* differed depending on the plant species, with in general higher values for MENAQ and lower for CRXDW. In June the plants showed similar *Kp* values (1.8-2.1); in the following three months the *Kp* of PHAAP and CRXDW lowered, remaining at 1.1 for the former, and from 0.8 to 0.6 for the latter, while MENAQ had *Kp* between 2.4 in July and 1.7 in September. In the last months (October-November) the *Kp* was higher for all plants with values of 1.3, 3.0 and 3.9 for CRXDW, PHAAP and MENAQ, respectively.

It is known that *Kp* values are related to plant growth and physiology, becoming greater in the summer months, as reported by various studies on wetland plants (e.g. Borin et al., 2011; Anda et al., 2014). In this study, the *Kp* increment in the summer season was not detected (especially for CRXDW and PHAAP lines), undoubtedly because the plants had stress problems due to the raw piggery wastewater feeding and being cut, jeopardizing the phenological stage and consequently also the water consumption. In a previous research (Tamiazzo et al., 2014) these plant species showed a *Kp* increase from May to August, with an average monthly increment of 1.04, 1.02, and 0.69 for PHAAP, MENAQ and CRXDW. Nevertheless these results confirmed that wetland vegetation has greater water demand than other plants (*Kp*>1), leading to higher evapotranspiration rates as reported by Mueller et al. (2005). The daily plant coefficient found in *Phragmites australis* can range between 0.2-8.5, depending on the environment (Walkovszky, 1973; Fermor et al., 2001; Boldirsar 2007; Zhou and Zhou, 2009; Borin et al., 2011). In the Veneto region the *Kp* of this plant was found to be between 4.7 (June) and 8.4 (August) (Borin et al., 2011).

Mint lines showed the greatest ET; this may be due to its water requirements, with the increment of shoots elongation and water consumption under increasing water availability (Lensenn et al., 2000). Vice versa, the sedge had the lowest ET, mainly from July to September when ET_0 was higher, as a result of lower plant development. Reed canarygrass showed intermediate water consumption, probably for the problems previously reported, even if this species is more able to tolerate drought than many other grasses (Vose, 1959; Klimešová, 1995; Henry and Amoros, 1996; Odland and del Moral, 2002).

Table 4.13 Monthly plant coefficient (*Kp*) of each plant species in 2012.

	June	**July**	**August**	**September**	**October-November**	**average**
PHAAP	1.9	1.1	1.1	1.1	3.0	1.6
MENAQ	1.8	2.4	1.3	1.7	3.9	2.2
CRXDW	2.1	0.8	0.9	0.6	1.3	1.2

Wastewater treatment performances

-*On-site parameters*

During the feeding of the phytodepuration system with diluted piggery wastewater coming from the pretreatment system, the values of the parameters measured on-site showed several changing over time (Figure 4.47). pH at the inlet (IN) was higher in the first ten days (9.4-8.1), while from September 14th it ranged between 7.5 and 8. The average outlet pH had similar trends among the three different treatments, in general higher at the beginning (around to 9.2) and decreasing over time till about 7.5, exceeding the IN pH until October 18th. The treatments with outlet median in the range 7.9-8.1 didn’t show changes in pH compared to the inlet (Figure 4.47).

The inlet piggery wastewater had up and down trends of EC till the beginning of October, ranging from 1.6 to 3.1 mS/cm, while it decreased in the last two cycles (Figure 4.47). The average outlet EC trends were similar among the lines, and contrasting the IN trend, with an EC increase from the beginning to October 18th from 0.6 mS/cm to more than 2.5 mS/cm (MENAQ almost 3.5 mS/cm on average), afterwards they decreased. These behaviours in the first weeks, common to all treatments, were probably due to the presence of water in the tanks, previously used to feed the system, which presented a low EC, and that gone out with the passing of time. For this reason, high data dispersion was detected and no significant changes happened in the EC.

The wastewater inlet temperature was higher on the first monitoring day (almost 25 °C) and from September 19th to October 2nd, between 20.3 and 22 °C, afterwards it decreased till 14.7 °C (Figure 4.47). With the phytodepuration treatment average temperature at the outlet was very similar among the lines, in general lower than the IN, and ranging between 15.8 (September 13th) and 8.4 °C (October 30th). Lines showed similar dispersion, and considering all data the IN temperature (median 21.2 °C) was significantly reduced by the PHAAP treatment (median 16.1 °C).

The dissolved oxygen contained in the inlet wastewater ranged from 0.17 mg/L (2%) on September 27th to 7.4 mg/L (77%) on October 18th, with a discontinuous trend between the two dates (Figure 4.47). Considering the average DO values, the outlet concentration trends were similar among the lines, with ups and downs in the range 5.6-8.8 mg/L; also the outlet DO percentage had similar trends (in general average values between 50 and 90%) , but on September 28th CRXDW treatment showed a low value (37%) at the outlet. The phytodepuration of the PHAAP and CRXDW lines proved to significantly increase the DO concentration, from 6.4 mg/L to 7.8 mg/L (medians).

The diluted piggery wastewater showed a turbidity between 35 and 108 NTU (median 65 NTU), higher at the beginning of October (Figure 4.48). The CCW reduced the turbidity, with lower average data at outlet and with similar trends for CRXDW and PHAAP (mean outlet values 3.5-4.1 NTU), while MENAQ slightly increased over time from 3.5 to 11.6 NTU. The CRXDW treatment presented a significant reduction (R= 95%) in the turbidity. *C. divisa* is a rhizomatous grass which has tufts of leaves that create a dense vegetation cover, retaining solid particles; this could be the reason for its turbidity reduction.

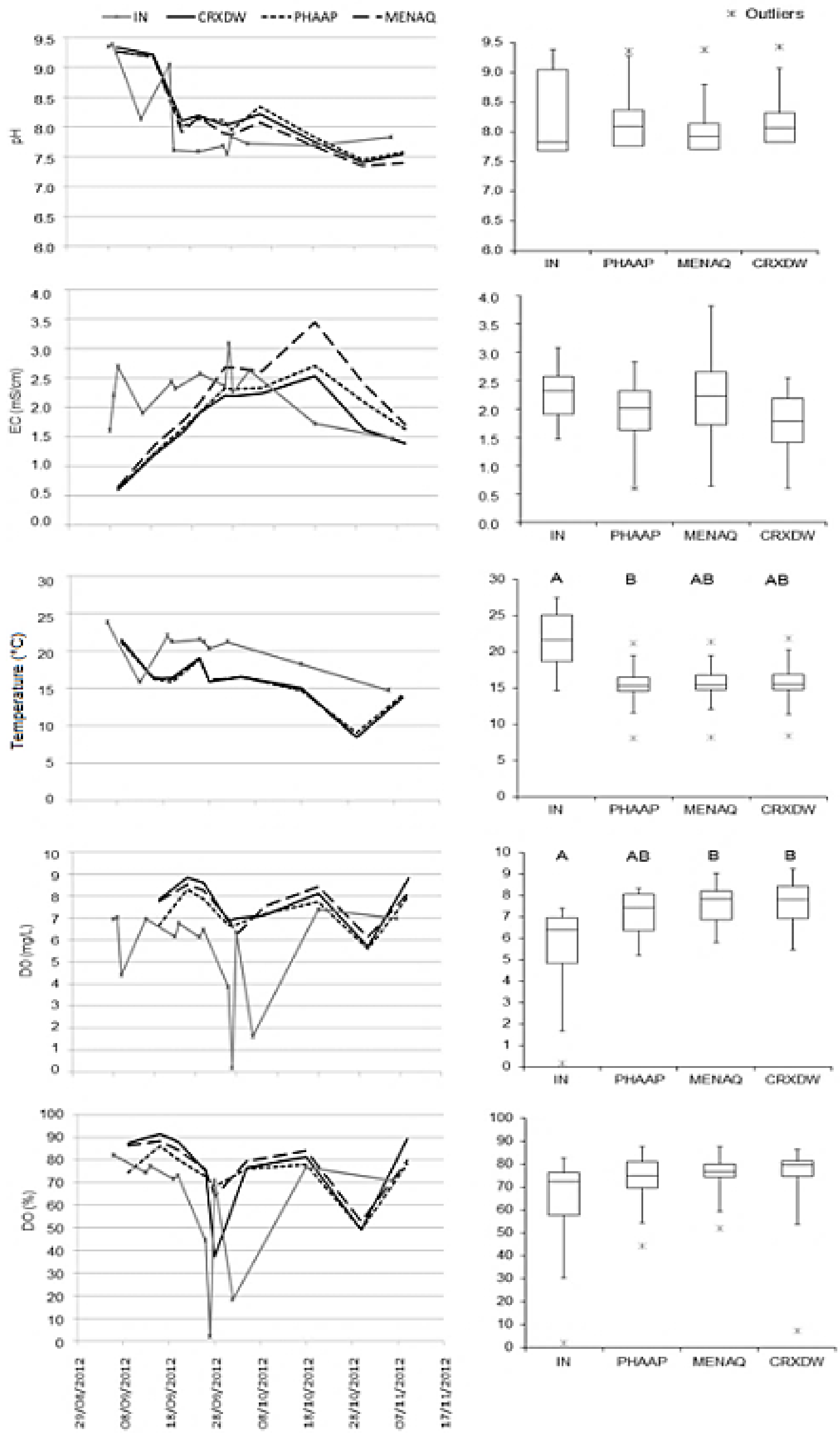

Figure 4.47 On-site parameters of the piggery wastewater detected at inlet (IN) and outlet of every phytodepuration line. Trends over time (at left) and box-plot diagrams (at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

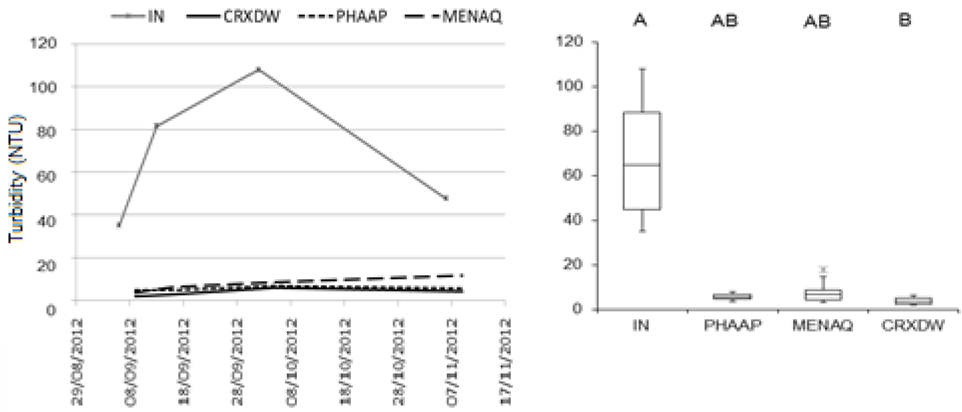

Figure 4.48 Turbidity (NTU) detected at inlet (IN) and outlet of every phytodepuration line. Trends over time (at left) and box-plot diagram (at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

-TS and VS

The analysis on total and volatile solids showed a median inlet content of 0.17%, with high data dispersion (TS of 0.15% as first quartile and 0.5% as third quartile), and lower data dispersion at the outlet of the phytodepuration system, with similar medians between treatments (TS 0.07-0.08%) (Figure 4.49). Volatile solids at the inlet was around to 0.2% (median), while at the outlet the TS was in the range 0.005-0.03%. For both analyses, although outlet contents were lower than the IN, not significant differences were found.

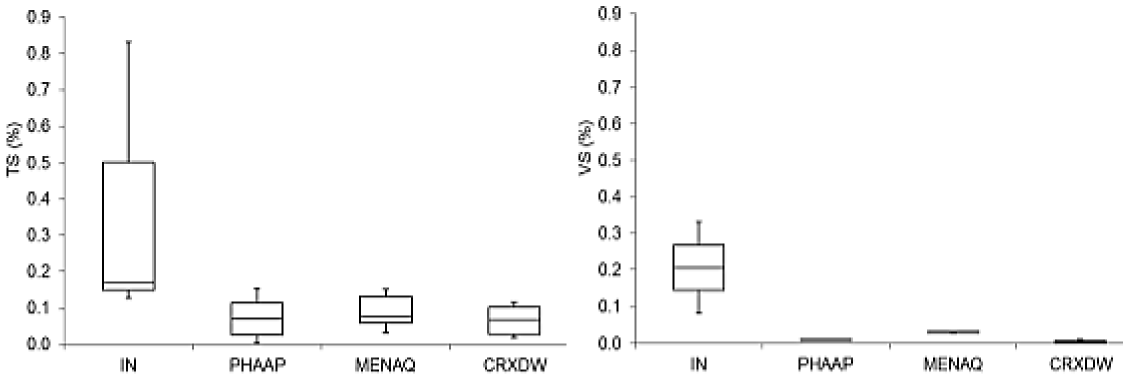

Figure 4.49 Box-plot of total solids (TS) and volatile solids (VS) contents (%) detected at in inlet (IN) and outlet of every phytodepuration line.

-Chemical parameters

The diluted piggery wastewater applied to the CCW in 2012 had a TN concentration between 75 and 131 mg/L, which increased from the first to the third monitoring cycles, to decrease in the last (Figure 4.50A). The same occurred for the NO_3-N and NH_4-N concentrations at inlet (Figure 4.50A); the IN nitric-N content ranged from 24 to 42 mg/L, while the IN ammonium-N from 9 to 22 mg/L, on average. The reason for higher values in the 2nd and 3rd cycles is due to the rainfall events on the previous days (specifically September 12th and October 2nd, when 25 and 12 mm fell), with filtered wastewater presenting higher TN and NO_3-N contents, as previously reported. After the phytodepuration treatment, all nitrogen concentrations were reduced, to less than 80 mg TN/L, 60 mg NO_3-N/L and 0.6 mg NH_4-N/L, with similar trends among the CRXDW and MENAQ lines for TN and NO_3-N. For these parameters, instead, MENAQ raised the concentrations from the first to the last cycle. The lines vegetated with PHAAP significantly reduced the TN concentration from 97mg/L (IN median) to 19.4 mg/L (OUT median; A=80%), and also the NO_3-N with 93% of abatement (medians of 54 mg/L at IN and 3.9 at OUT) (Table 4.14). The NH_4-N was instead significantly decreased by the other two treatments, with median outlet values of 0.22-0.26 mg/L for MENAQ and CRXDW (A=98-99%).

During this experiment, each line of the CCW received different a chemical quantity at the inlet (depending on inlet volume), with daily values ranging from 0.8 to 2.5 g/m^2 of TN, 0.4-1.4 g/m^2 of NO_3-N and 0.1-0.4 g/m^2 of NH_4-N per line. For all nitrogen forms, in general, the removal quantities increased with the entering quantities (Figure 4.50B), except for CRXDW, which showed different removal trends, decreasing over time. The mass abatement decreased from September (which was more than 90% for TN, 80% forNO_3-N and almost 100% for NH_4-N) to November with final values of 30-70% for TN, 15-55% forNO_3-N and almost 98-99% for NH_4-N. For TN the median removal quantity of the CCW was between 0.31 and 0.50 $g/m^2/d$, with MA in the range 65-90% (Table 4.15). Among nitrogen forms, ammonium-N was the element most reduced with median MA of 99% and median removal of 0.08-0.09 $g/m^2/d$. The nitrate-N removal was 52-91% with a median of 0.09-0.23 $g/m^2/d$.

The TP concentration of the inlet wastewater ranged between 4.8 and 9.3 mg/L, mainly present as soluble form (4 and 7.9 mg/L) (Figure 4.51A). Both TP and PO_4-P showed IN higher values in cycles 2 and 3 due to the more concentrated filtered wastewater. For both parameters, the average outlet concentrations remained stable over time and similar for PHAAP and MENAQ lines (ranges of 0.6-0.4mg/L for TP and 0.3-0.15 mg/L for PO_4-P), which significantly reduced both chemicals with an abatement of 91-92% for TP and 93-96% for PO_4-P (Table 4.13). The outlet concentration of tanks vegetated with CRXDW instead rose till 3.6 mg TP/L (median 1.5 mg/L) and 2.3 mg PO_4-P/L (median 1.1 mg/L), with significantly higher outlet concentration than MENAQ lines (Table 4.14).

The daily quantity entering the system was between 0.07 and 0.18 g TP /m^2 and 0.06-0.15 g PO_4-P/m^2. Also for these parameters (as nitrogen forms) the chemical removal of the lines vegetated with MENAQ and PHAAP (differently from CXDW) was higher with greater inlet quantity (Figure 4.51B). The mass abatement steadily decreased from September to November only for the CRXDW lines from 95% (TP and PO_4-P) to 42% for TP and 58% for PO_4-P. The system removed a TP quantity between 0.03 and 0.04 g/m^2/d, with MA of 85-97% (Table 4.15). The removal of soluble-P was similar, but in this case MENAQ showed a significantly higher MA (99%) compared to CRXDW (MA= 85%).

The chemical oxygen demand in the piggery wastewater presented a concentration between 451 and 688 mg/L (median 508 mg/L), with a similar trend to the previously compounds and higher values in the 2nd and 3rd cycles (Figure 4.52A). Different from this were the outlet COD concentration trends of the average values, which were similar between the treatments, lower in the 3rd cycle (171 mg/L) when the IN concentration was higher. The biological oxygen demand at the IN increased from 40 to 300 mg/L over time (median 80 mg/L), while it remained steady at the outlet (between 10 and 17.5 mg/L, on average), with similar trends among the treatments. Considering median values, MENAQ and CRXDW significantly reduced the BOD_5 wastewater concentration by 12.5-15% (Table 4.14).

The daily quantity entering in the system was between 4.2 and 13.2 g COD/m^2 and 0.6-5.7 g BOD_5/m^2. Also for these parameters the chemical removal was higher with greater inlet quantity (Figure 4.52B). As for nitrogen, the mass abatement decreased from September (that was more than 90%) to November with final values of 24% for MENAQ, 13% for PHAAP, and negative MA for CRXDW (-6%). The BOD_5 mass abatement remained steady over time for all treatments. For COD, the system removed between 1.98 and 2.48 g /m^2/d (median), showing a median mass abatement of 75-87%, while the BOD_5 presented lower daily removal quantity (0.31-0.42 g /m^2/d) and higher MA (90-96%) (Table 4.15).

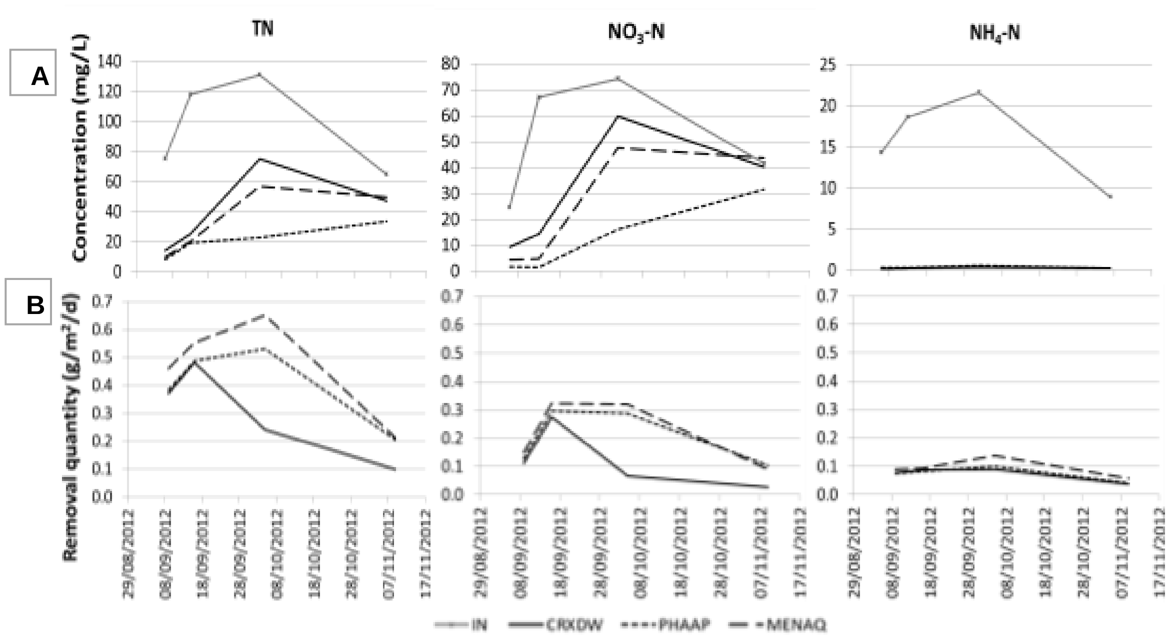

Figure 4.50 Trends of the average concentrations (mg/L) at inlet and outlet of every treatment (A) and of the average removal quantities (g/m^2/d) per treatment (B) for total nitrogen (TN), nitrate-nitrogen (NO_3-N) and ammonium nitrogen (NH_4-N).

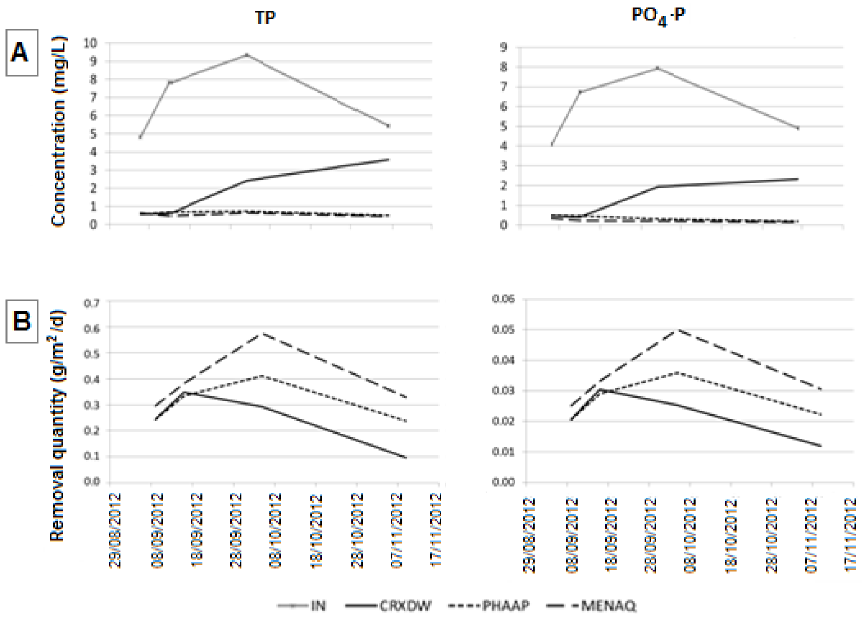

Fig 4.51 Trends of the average concentrations (mg/L) at inlet and outlet of every treatment (A) and of the average removal quantities ($g/m^2/d$) per treatment (B) for total phosphorus (TP) and soluble phosphorus (PO_4-P).

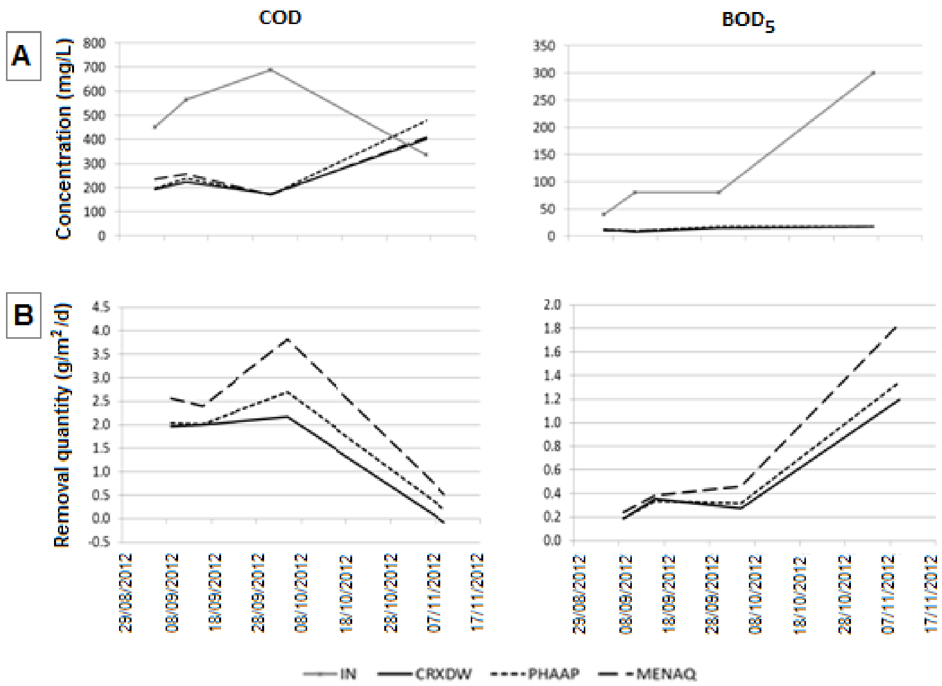

Figure 4.52 Trends of the average concentrations (mg/L) at inlet and outlet of every treatment (A) and of the average removal quantities ($g/m^2/d$) per treatment (B) for the chemical oxygen demand (COD) and biological oxygen demand (BOD_5).

Table 4.14 Median concentrations (mg/L) of the chemical parameters at inlet (IN) and outlet (OUT) of every treatment, with abatement (A, %). Different letters indicate statistical differences among plant species (Kruskal-Wallis test $p<0.05$).

	IN	PHAAP		MENAQ		CRXDW	
	(mg/L)	OUT (mg/L)	A (%)	OUT (mg/L)	A (%)	OUT (mg/L)	A (%)
TN	96.7 a	19.4 b	80	32.6 ab	66	38.3 ab	60
NO_3-N	54.4 a	3.92 b	93	21.3 ab	61	26.6 ab	51
NH_4-N	16.5 a	0.31 ab	98	0.22 b	99	0.26 b	98
TP	6.63 a	0.60 b	91	0.54 b	92	1.46 ab	78
PO_4-P	5.81 a	0.39 bc	93	0.22 c	96	1.11 ab	81
COD	508	219	57	245	52	201	60
BOD_5	80.0 a	15.0 ab	81	12.5 b	84	15.0 b	81

Table 4.15 Median values of daily chemical removal ($g/m^2/d$) and mass abatement (MA, %) of every treatment. Different letters indicate statistical differences among plant species (Kruskal-Wallis test $p<0.05$).

	PHAAP		MENAQ		CRXDW	
	removal ($g/m^2/d$)	MA (%)	removal ($g/m^2/d$)	MA (%)	removal ($g/m^2/d$)	MA (%)
TN	0.43	90	0.50	86	0.31	65
NO_3-N	0.21	91	0.23	82	0.09	52
NH_4-N	0.08	99	0.09	99	0.08	99
TP	0.03	95	0.04	97	0.03	85
PO_4-P	0.03	97 ab	0.03	99 a	0.02	85 b
COD	2.02	82	2.48	87	1.98	75
BOD_5	0.33	92	0.42	96	0.31	90

Aerial biomass production and nutrient uptake

The aboveground biomass production of the plants used in 2012 differed depending on species and not significant differences were found among levels (Table 4.16). The average fresh weight, depending on the level, ranged from 1,738 to 7,015 g/m^2 for PHAAP, from 5,656 to 11,533 g/m^2 for MENAQ, and from 1,908 to 1,600 g/m^2 for CRXDW. Considering all levels, MENAQ plants had the higher average fresh weight (9,049 g/m^2), followed by PHAAP plants (4,499 g/m^2), and lastly by CRXDW plants (1,757 g/m^2). Average dry biomass percentage was higher for sedge plants (Table 4.16), between 21 and 23% and lower for the others, 14-18% for mint and 13-18% for reed canarygrass. The highest average dry weight was detected in MENAQ plants (818-1,863 g/m^2, 1,464 g/m^2), while the lowest dry weight was found in CRXDW plants with a range of 338-438 g/m^2 (396 g/m^2 on average). Plants of PHAAP showed a dry weight between

307 and 935 g/m^2, 692 g/m^2 on average. The higher standard deviation for the dry weight among repetitions was found in mint plants, especially in the L1 and L3. Beyond this, *Mentha aquatica* gave the higher dry matter production, similar to the results reported in Tamiazzo et al. (2014) in which it was tested for the treatment of surfactant-polluted water, and where it showed lower dry weight than *Typhoides arundinacea* var.picta plants. *T. arundinacea*, in fact, is a fast growing and high yielding species used as a bioenergy crop, especially in Europe and the US. Anderson (1995) reported yields of 1.6-12.2 t DM ha^{-1} yr^{-1}; Vymazal and Kröpfelová (2008), with production data collected from different sources, concluded that maximum aboveground biomass in natural stands varies from 440 to 2,304 g DM m^{-2}, depending mostly on the trophic status of the stand. This species showed highest growth and yield in wet soils and organic soils (Lewandowski et al., 2003; Vymazal and Kröpfelová, 2005). Nutrient enrichment also increases its production, as reported by Maurer and Zedler (2002). The *Typhoides* used in this work has different characteristics because it is an ornamental cultivar. Nevertheless its productivity falls within the species range and agrees with the results of Salvato and Borin (2010) and Borin and Salvato (2012) who used the cv. picta in an experiment with a nitrogen-rich wastewater, revealing that dry matter production increased over time, from 307 g m^{-2} in the1st year to 4,824 g m^{-2} in the 3rd year. Moreover in Tamiazzo et al. (2014), its average dry matter production was between 900 and 2,200 g/m^2.

Regarding the aerial biomass, production of *Carex divisa* plants obtained in this study agrees with Vymazal and Kröpfelová (2008) who reported a maximum aboveground standing crop range of 300-3000 g m^{-2} of dry mass for short emergent plants, while it was lower than that in Bernard et al. (1988), who reported that aboveground dry mass from standing crops in *Carex* wetlands is in the range of approximately 500–1050 g m^{-2}. The same *Carex* species tested in Tamiazzo et al. (2014) gave dry matter production values almost triple those found in this study.

N content in the aerial part was higher in PHAAP plants (2.9-3.1%, 3.0% on average), between 1.9-3.2% in MENAQ and 2.6-5.9% in CRXDW (Table 4.16). The *T. arundinacea* values are higher than those reported in Borin and Salvato (2012), in which it presented on average 1.7% of N in the aerial part, and in Vymazal 2010, who found a N aboveground concentration between 1.33 and 2.26%. Other studies on constructed wetlands, instead, agreed with the N concentration found in this work for *T. arundinacea*.

Bernard and Lauve (1995) reported N concentrations between 2.2 and 4.6% in a system treating landfill leachate in the United States (New York). Behrends et al. (1994) reported values between 2.2 and 3.3% in experimental constructed wetlands in Alabama, and Hurry and Bellinger (1990) found nitrogen concentrations up to 4.5% in a treatment wetland in England. In enrichment experiments, Dubois (1994) reported a range of 1.6–3.6%. For *M. aquatica* the aboveground N concentration was higher than the value reported in De Stefani (2012), which corresponded to 1.01%, on average.

The higher N uptake was given by mint (29-56 g/m^2), followed by reed (8.9-29 g/m^2) and lastly by sedge (10-11 g/m^2). In agreement with this study, Vymazal (2010) reported aboveground N uptake for *T. arundinacea* between 21.6 and 29.2 g N/m^2 (average 27.4 g N/m^2).

The P content in the aerial part was similar among plant species, 0.3% on average. The P uptake was greater for MENAQ plants (2.0-8.6 g/m^2) and lower for PHAAP (0.7-3.0 g/m^2) and CRXDW (1.1-1.2 g/m^2) (Table 4.16). The aboveground P concentration of *T. arundinacea* resulted as higher than the values reported in Vymazal (2010), who found values in the range 0.15-0.27%. Higher P aboveground concentration in this plant was found in Vymazal (1995), who reported P concentrations up to 0.48% in two systems in the Czech Republic and in Hurry and Bellinger (1990), who gave values between 0.14 and 1% in England. Regarding the aboveground P uptake, lower values for *T. arundinacea* were found in this study compared to that reported in Vymazal (2010) (3.63g P/m^2).

Table 4.16 Average (±st.dev.) fresh and dry weights (g/m^2), dry biomass (%), and nutrient (N and P; % and g/m^2) stored in the aerial part of the plants, subdivided by levels (L1- highest level; L2- mid-level; L3- lowest level) and with average value.

Plant	**Level**	**Fresh weight**	**Dry weight**	**Dry biomass**	**N**		**P**	
		g/m^2	g/m^2	%	%	g/m^2	%	g/m^2
PHAAP	L1	1,738±370	307±0.1	18±3.9	2.9±0.7	8.9 ± 2.1	0.2± 0.07	0.7± 0.2
	L2	4,744±703	836±129	18±0.1	2.9±0.3	24± 5.8	0.2± 0.08	2.0±0.9
	L3	7,015±508	935±233	13±4.3	3.1±0.1	29± 6.1	0.3± 0.01	3.0±0.7
	average	4,499±2,405	692±325	16±3.4	3.0±0.3	21 ± 10	0.3± 0.1	1.9± 1.2
MENAQ	L1	9,959±7,949	1,713±1,231	18±2.0	1.9±0.3	35± 29.4	0.2±0.09	3.7±3.8
	L2	5,656±544	818±157	14±1.4	2.3± 0.1	19± 4.0	0.2± 0.09	2.0±3.3
	L3	11,533±1,066	1,863±1,055	16±7.7	3.2± 0.6	56± 21.8	0.5± 0.15	8.6±2.5
	average	9,049±4,508	1,464±887	16±4.0	2.5± 0.7	36 ± 23	0.3± 0.2	4.8± 3.7
CRXDW	L1	1,908±798	438±162	23±1.2	2.6 ±0.1	11± 3.9	0.2±0.03	1.1±0.5
	L2	1,764±160	412±25	23±0.7	2.8± 0.0	11± 0.6	0.3± 0.03	1.1±0.2
	L3	1,600±29	338±23	21±1.0	2.9± 0.0	10±0.7	0.4± 0.08	1.2±0.2
	average	1,757±389	396±87	22±1.4	2.8± 0.2	11 ± 2	0.3± 0.1	1.1± 0.3

Second year

Environmental conditions

In the second experimental year of 2013, the air temperature showed in general an increase until the beginning of August and a constant downward trend till the end of the year (Figure 4.53). Considering the experimental period (May 20th -November 22nd), the minimum air temperature oscillated between 3.7 and 23.7°C, the mean air temperature between 7.6 and 29°C, and the maximum air temperature between 10.8 and 35.2 °C, respectively. Specifically, the highest value was detected on August 5th and the lowest value on December 15th. The mean monthly air temperature was: May 16.3°C; June 21.7°C ; July 25.0°C; August 23.5°C; September 19.4°C; October 15.1°C; November (till 22nd November) 11.9°C.

The rainfall was particularly more frequent in May and in autumn 2012 (Figure 4.53). Specifically, the total monthly rainfall was of 127 mm in May (of which 32.6 mm fallen to May 30th), 16.6 mm in June, 39.6 mm in July, 75.6 mm in August (of which 28.8 mm fallen to 27th August), 49.2 mm in September, 106.2 mm in October (higher to the 26th and 27th days) and 75.6 mm in November (till 22nd November).

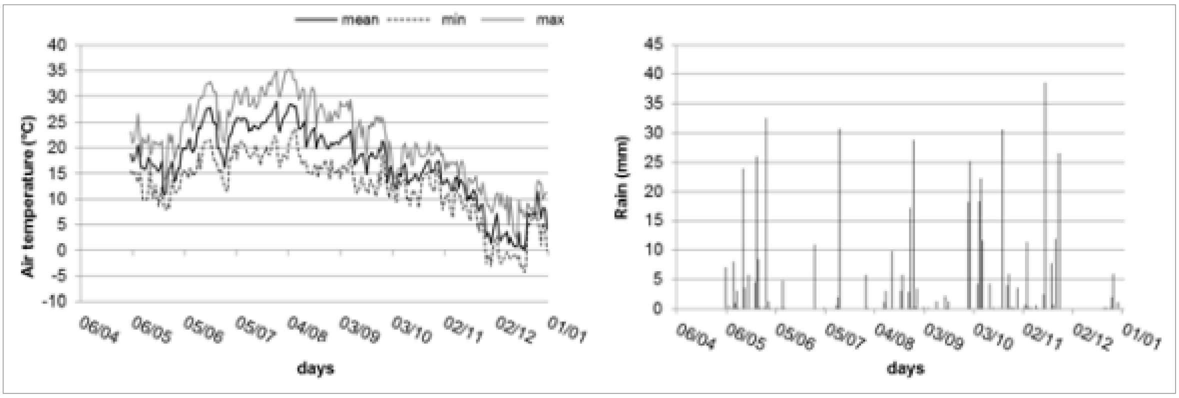

Figure 4.53 Air temperature (°C) and rainfall (mm) during the experimental year 2013.

The ion composition of the piggery wastewater revealed also in the second year high contents of K and ions Cl and Na, indicators of high salinity of the wastewater (Table 4.17). Specifically, all these parameters presented higher values compared to 2012, especially Cl with more than 1,700 mg/L and K exceeding 5,500 mg/L. The soluble salts in pig slurries are mainly excreted in the urine, and specific hazards are associated with Na and Cl ions. Li-Xian et al. (2007) studied the salinity of three manures (chicken, pig and pigeon manures), finding that they was mainly composed of sulfate and chloride of potassium and sodium, which are commonly added to animal diets as sodium sulfate, sodium chloride, potassium chloride and/or sodium bicarbonate (Dong et al., 2001).

The ion composition of the animal slurry is therefore related to the animal feeding and can change over time. Higher K and lower Ca and Mg in manures could be ascribed to the excessive K provided in most diets since metabolism of K in animal is poorly understood (Goff, 2006) and Ca and Mg metabolism (Eliam-Cisse et al., 1993; Hill et al., 2001; Apple et al., 2002), resulting in more precise additions into diets. Previous studies have also pointed out that the efficacy of potassium salts exceeds sodium compounds in enhancing bird performance during heat stress (Deyhim and Teeter, 1991; Liu and Yang, 2000), and this may be the reason for a higher K over Na in manures. All this evidence implies that a large quantity of salt added to feed is excreted as manure.

Table 4.17 Average (±st.dev.) ions concentration (mg/L) of the piggery wastewater. "*n.f.*" acronym indicates that the value was not found.

Ion	Concentration (mg/L)
Cl	1,729±980
Br	*n.f.*
SO_4	18.2
Li	*n.f.*
Na	948±380
K	5,508±429
Mg	210±28
Ca	511±110

Pretreatment system

Medium performance

1. Effect of recirculation

All the wastewater physical and chemical parameters showed to be affected by the recirculation. The first three loads were carried out in sequence at a distance of three hours, while the 4th load was performed after 13 h and a half from 3rd for technical impossibility; the 5th outlet was detected after 3 h from the 4th . This led to different responses for almost all the parameters analyzed, common for all filters, especially between the 4th and the 5th outlets. The piggery wastewater volume decreased steadily till the 3rd outlet, while it augmented with the third recirculation (OUT 4), probably due to the greater time for the wastewater release (Figure 4.54). Lower water retention was achieved by the filter filled with gravel, which showed about a volume of 8L in the 3rd and 5th outlets; considering the last value, the volume was reduced by 17%. On the contrary the filters with bamboo substrate gave the higher water retention, reducing the inlet volume for the 42% considering the OUT 5. The others filters showed a volume reduction in the range 31 (mix pumice and gravel medium) - 37% (giant reed and tops media).

The mean electrical conductivity of the piggery wastewater was 12.8 mS/cm, and with the recirculation it was lowered in all filters at less than 12 mS/cm (Figure 4.54). In particular for all the filters, the first load had the major effect to lowering the EC. The giant reed, bamboo and pumice filters showed to reduce the outlet values with recirculation, while for the others the values remained similar. The filter filled with the pumice showed the low EC at outlet, decreasing from 10.1 (OUT 1) to 9.1 mS/cm (OUT 5), slightly lower to the bamboo medium, while tops showed the high EC values at outlet, in the range 11.7 (OUT 1)-11.2 (OUT3) mS/cm.

The mean pH of the inlet wastewater was around to 8.0, and differences among filters were observed (Figure 4.54). Giant reed, bamboo and tops showed an increment of the pH with filtration, in particular tops which increased the outlet values with recirculation from 8.27 (OUT 1) to 8.66 (OUT 4). Vice versa, the others three filters showed to reduce the outlet pH, with same trend for the pumice filters, which with recirculation steadily decreased till to 7.48 (gravel) and 7.21 (gravel+pumice and pumice filters). Bilardi et al. (2013) reported that the pH decrease of pumice material could be attributed to acidic sites at the pumice surface.

The average wastewater temperature at inlet was approximately of 24°C, which was increased for all filters in the 2nd and 3rd outlets, reaching in the OUT 2 mean values of 27.4 °C in tops, 26.1°C in gravel and bamboo, and 24-25°C in the other filters (Figure 4.54). Inversely, the lowest wastewater temperature was detected in the 4th outlet with similar values among filters (about 19.3 °C). These results derived from to the fact that the air temperature peak occurred during afternoon hours in which the first two recirculations were performed, while the third recirculation happened in late-afternoon, remaining the entire night outside.

The inlet piggery wastewater presented on average a DO concentration of 0.64 mg/L (2.6%) and was risen with filtration (Figure 4.54). In general, the recirculation showed to increase the DO content, particularly heightened by the filters filled with the pumice medium, which showed a final DO outlet concentration of 7.4 mg/L (80% of DO) in pumice filter and 6.8 mg/L (71% of DO) in the mixed filter. A particular the DO increment happened between the 4th and the 5th outlets, from 38% to 58% of DO content on average (Figure 4.54). The tops medium showed the lower content of DO at outlet, ranging between 1.4 and 4.6 mg/L (14-41% of DO in the solution).

As the other parameters, filtration and recirculation showed to effecting the piggery wastewater turbidity, which was of 2,052 NTU at inlet, on average (Figure 4.54). All filters showed to reduce this parameter especially with the first load (R=56-60%, OUT 1) and presented similar trends, with decreasing values from OUT 1 to OUT 5. The giant reed filters (followed by the pumice filter) showed the lowest outlet values, which ranged from 1,153 to 248 NTU, while the tops and the mixed filters gave the highest outlet values, ranging from 1,230 to 664 NTU, respectively.

The total solids (TS) content of the wastewater was 0.44%, on average, and on the contrary of the turbidity, it increased with filtration (till maximum 0.9%), remaining similar with the recirculation, except in the last outlet (OUT 5), in which it increased till more than 3.0% in the tops and giant reed filters (Figure 4.54). These are results contrasting with the turbidity detected at the outlet, that suggest sampling error as it only one sample was analyzed.

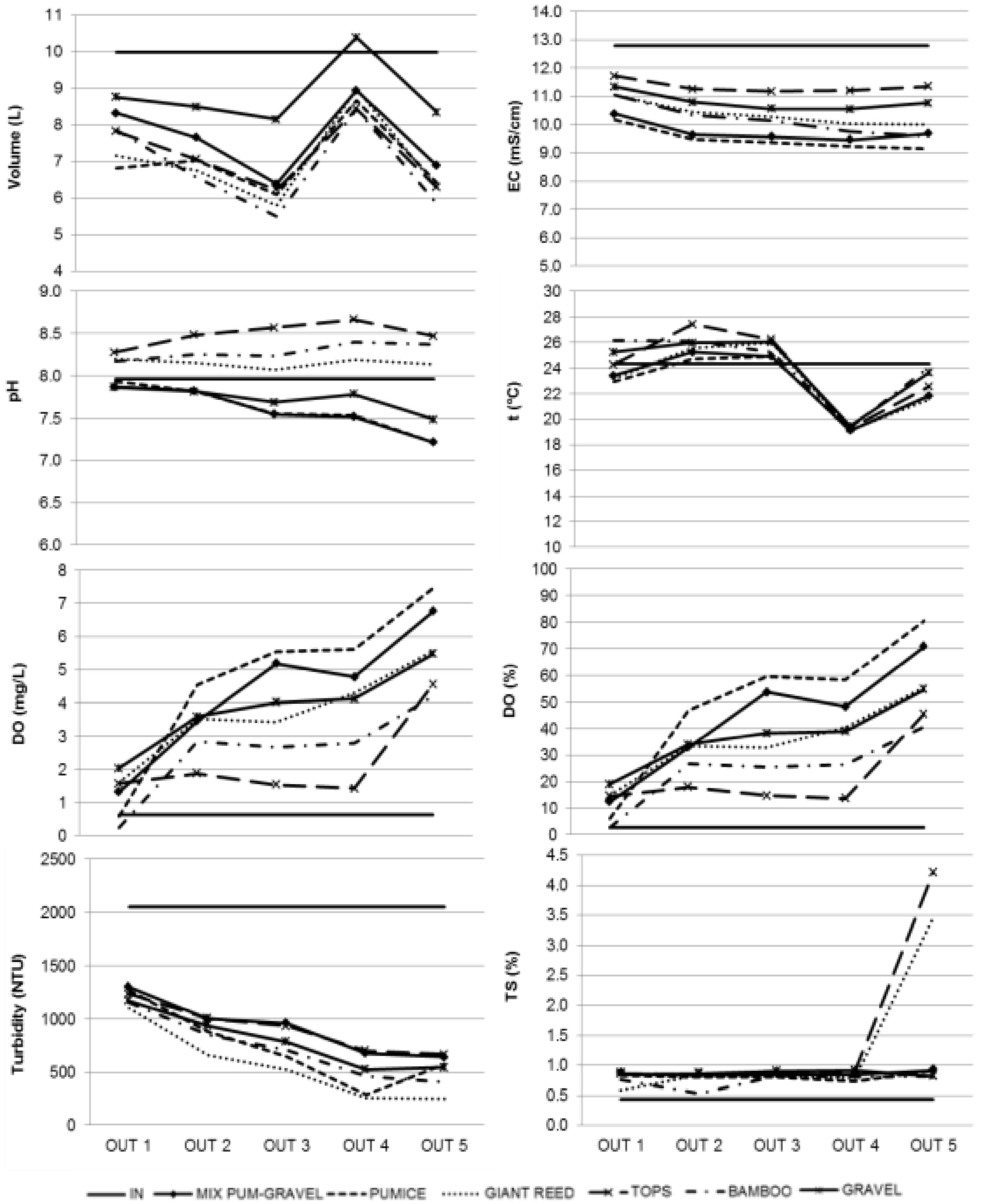

Figure 4.54 Effect of recirculation on volume and on on-site parameters.

Concerning the nitrogen compounds, the recirculation gave similar results of the previous year, especially for NO_3-N and NH_4-N concentrations (Figure 4.55A). The mean inlet wastewater TN concentration of 1,096 mg/L was reduced after filtration, in all filters and especially with the first outlet (R=17-30% in OUT 1). The outlet values of TN concentration revealed that for the filters filled with pumice they remained similar with recirculation, while with the bamboo, giant reed and gravel media the concentration decreases from OUT 1 to OUT 5 (Figure 4.55A); the tops filter showed instead up and down trend. The bamboo, followed by giant reed, showed the lowest mean TN concentrations at outlets, ranging from 763 (OUT 1; R=30%) to 573 mg/L (OUT 5; R=48%). Regarding the nitrate-nitrogen concentration, it was increased at the outlet of all filters, with upward trend rising the recirculation, especially in the gravel filter and filters with pumice medium (Figure 4.55A). Considering these filters, the increment rate with recirculation for NO_3-N outlet concentration was +521% between IN and OUT 1, +26% between OUT 1 and OUT 2, +13% between OUT 2 and OUT 3, +9% between OUT 3 and OUT 4, and +9% between OUT 4 and OUT 5. In general, the bamboo (followed by the tops medium) gave the lowest NO_3-N concentration increment, while the filter with pumice and gravel showed the highest outlet concentration values. The increment of the nitrate-N derived by the oxidation of the ammonium-N, which in fact was reduced with filtration, especially with the first transition, and all filters (except the gravel in OUT 4) showed a progressively outlet NH_4-N concentration reduction with increasing recirculation (R=59%, OUT 1; R=79%, OUT 5, on average) (Figure 4.55A). The filters with pumice medium gave same trend and the similar lowest values at the outlet for NH_4-N concentration, which decreased from 223 mg/L in OUT 1 to 67 mg/L in OUT 5.

Regarding the influence of the recirculation on nitrogen mass removal (MA), results showed that similar behavior was detected among the filters, especially for TN and NO_3-N (Figure 4.55B). All filters, except pumice medium, increased the TN mass abatement till the 3rd outlet (+ 10-15% on average), while in the 4th outlet they lowered the MA, for the increment of the nitrate-nitrogen occurred during the night. The bamboo gave the highest TN mass abatement (42-70%), followed by giant reed (39-64%). The gravel showed instead the lowest TN MA, in the range 12-33%. As the NO_3-N concentration, also the NO_3-N quantity increased with recirculation, showing not positive results on MA (Figure 4.55B). The bamboo and tops filters showed the lowest NO_3-N increment at outlets with no recirculation effects, while the other filters revealed a constant NO_3-N quantity generation from OUT 1 to OUT 5, especially the gravel filter. Contradictory to these, there are the results on NH_4-N mass abatement (Figure 4.55B), which increased in all filters from the OUT 1 to the OUT 3, similar to TN MA trends, and afterwards it was reduced in the OUT 4, to increase in the OUT 5. Gravel and tops displayed the lowest NH_4-N MA (about 55-80%, considering the first three outlet), while filters with pumice gave best results on NH_4-N mass abatement, with about 85-94% considering the first three outlet and achieving almost the 100% with the last recirculation.

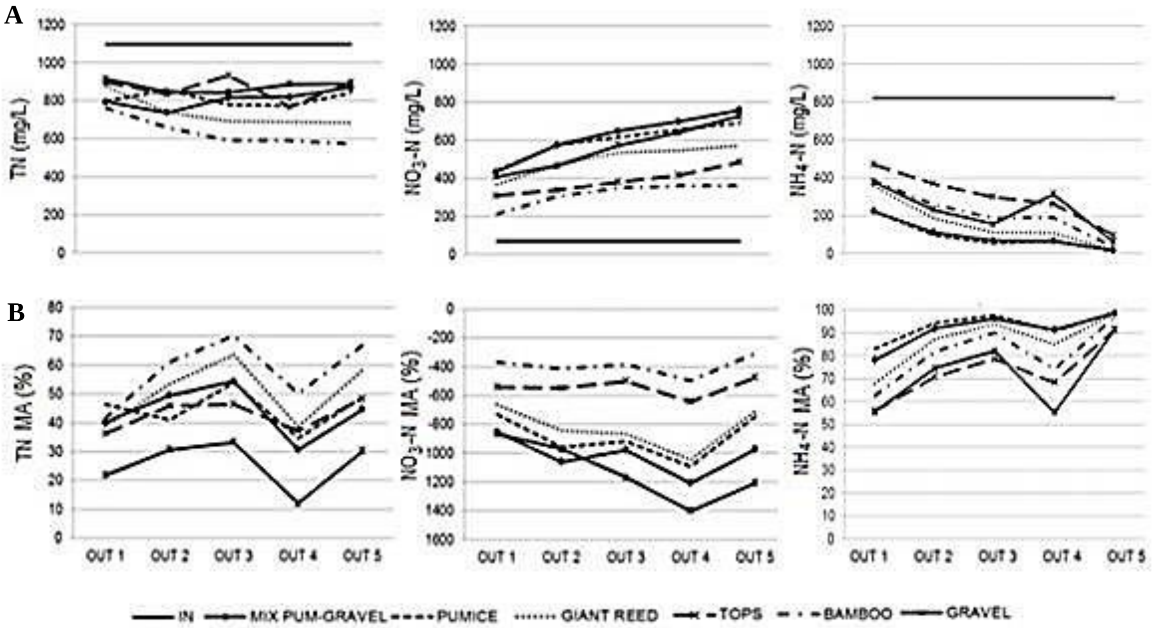

Figure 4.55 Concentration (mg/L; A) at inlet and at the different outlets and mass abatement (MA,%; B) of every filter of nitrogen forms (mg/L).

The recirculation had effect also for the phosphorous contents. In general the greatest effect was shown for the TP concentration (Figure 4.56A), dropped with filtration, and which diminished increasing recirculation (especially from OUT 1 to OUT 4), while the PO_4-P concentration at the outlet remained similar in all filters. Filters filled with pumice material (particularly the filter with only pumice) showed the greater TP and PO_4-P reductions, while gravel and bamboo filters gave the lowest reductions of both parameters (Figure 4.56A). Regarding the mass abatement, instead, similar results were obtained among filters (except gravel), which lowered the TP and PO_4-P quantity from OUT 1 to OUT 3, increasing the MA, that ranged from 28 to 62% for total-P and from 27 to 62% for soluble-P, with the best results for the filter filled with pumice (Figure 4.56B). Gravel, instead, gave the lowest MA results, and as the other filters, it showed an increasing mass abatement from the first to the third outlet just for TP (MA=28-41%), while it gave the higher PO_4-P MA with the first recirculation (OUT 2; MA=42%).

As the TN, NH_4-N and P forms, also the COD concentration was reduced with filtration, and especially with recirculation (Figure 4.57), in all filters. Considering mean values, it was reducted by 11% with the 1st recirculation, by 15% with the 2nd, by 10% with the 3rd recircualtion, while the outlet COD concentration remained similar with the 4th recirculation. The lower outlet concentration was found in pumice filter (3,035 (OUT 1) - 1,494 (OUT 4) mg/L), while the higher was gave by the filter with tops (3,582 (OUT 1)- 3,006 (OUT 4) mg/L). Concernig the mass abatement of this parameter (Figure 4.57), it is evident its increment from the 1st to the 3rd outlet, on average from 41-56% to 55-77%, while in OUT 4 it slightly decreased, as TN, NH_4-N and PO_4-P mass abatements.

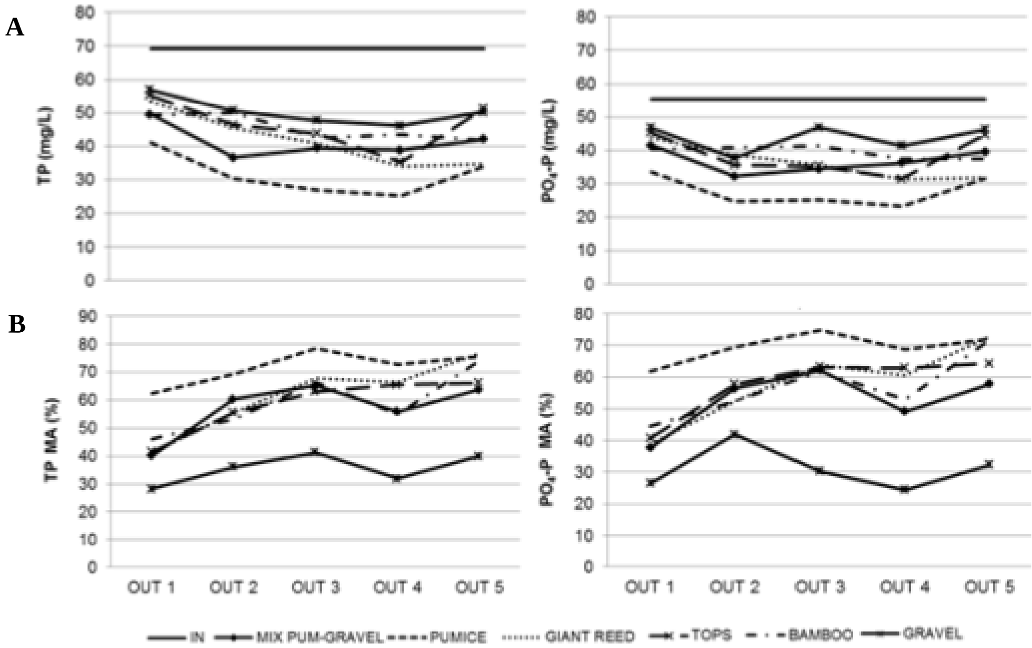

Figure 4.56 Concentration (mg/L; A) at inlet and at the different outlets and mass abatement (MA,%; B) of every filter of phosphorous forms (mg/L).

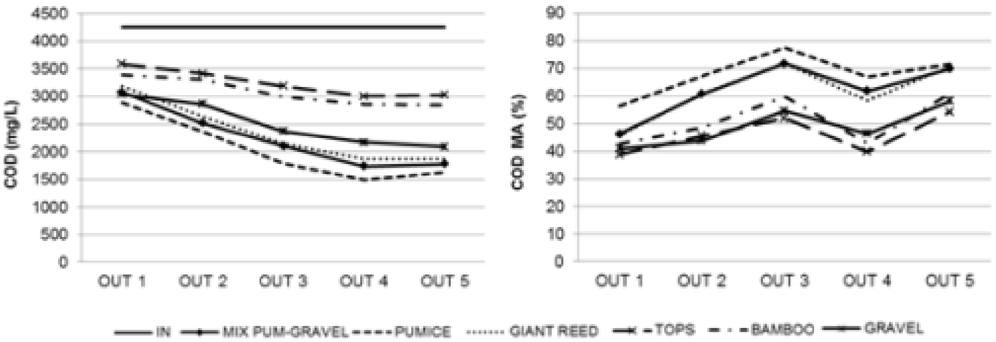

Figure 4.57 Concentration (mg/L) at inlet and at the different outlets and mass abatement (MA,%) of every filter of COD (mg/L).

The results of the recirculation effects on physical and chemical parameters has been altered from the third recirculation, in which, as previously reported, the outlet wastewater was analysed after a far greater number of hours (10 h and half more than the previous outlets) for technical impossibility, which caused an higher outlet wastewater volume (also exceeding the inlet volume in the gravel filter) and a greater wastewater permanence in the collection bucket that could have influenced the various parameters analysed, in particular on wastewater dissolved oxygen and temperature. Consequently the increment of the wastewater outlet volume led to a higher outlet chemical quantity, than a mass abatement decrease of the chemical compounds analysed compared to the previous cycle (OUT 3).

2. Effects over time

The effects over time of the filters were elaborated considering the average values of the inlet piggery wastewater and of the outlet wastewater derived from the last recirculation (OUT 5).

-*Wastewater volume*

During the study of the single filters, the piggery wastewater volume at inlet was of 10 L/filter (22.3 L/m^3). Over time the volume at the outlet of all filters increased (Figure 4.58), achieving in the last cycle similar values among the filters. Specifically, at the beginning the bamboo, pumice and tops filters presented a volume reduction between 65 and 70%, with higher values compared to the mixed filter (50%), giant reed (50%) and gravel (37%), meaning that these filters had higher water adsorption (or retention in the case of tops medium) than the others. Over time the reduction of the wastewater outlet volume dropped, meaning that there was an higher outlet volume, mainly in November, in which was detected between 9 and 9.5 L of outlet volume (volume reduction 5-10%). These results derived from the fact that in July the air temperature was higher of the following months and it has dropped over time, causing greater water evaporation on the substrates. Besides the tops filter was more exposed to the sun, compared to the other filters, and this could have caused a greater water evaporation, highlighted by the highest volume reduction value in September.

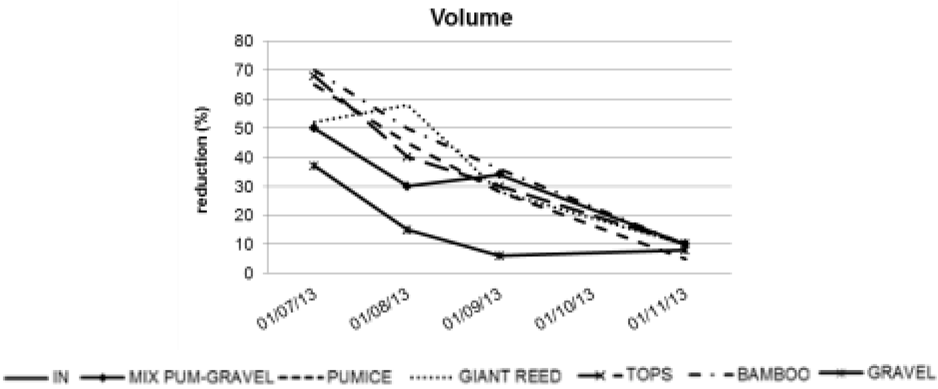

Figure 4.58 Reduction (%) of the outlet volume compared to the inlet volume of the wastewater.

- *On-site parameters*

Regarding the on-site parameters, EC, pH and turbidity were detected over time, while the DO was measured only in the first and in the last monitoring cycles, since the probe was not working.

The pH of the piggery wastewater at inlet remained steady to 7.8 in the first three cycles, while it increased to 8.5 in November (Figure 4.59) (IN median 7.83). The filters filled the with bamboo, gravel and pumice and the mixed filter had similar outlet pH trends of the IN, while tops and giant reed increased over time the outlet pH values. The outlet pH of tops, giant reed, and bamboo media ranged from 7.7 to 9.0, while it was between 7.0 and 8.0 in the other filters. Considering median values, however, no differences were found among the factors (Table 4.18).

The EC of the inlet piggery wastewater remained between 12 and 13 mS/cm, except in September, which was 13.6 mS/cm (Figure 4.59). All filters showed EC outlet values lower than IN and similar trends of the IN, except the giant reed medium, which maintained the outlet EC approximately to 10 mS/cm. The median EC at inlet was significantly lowered by 29% by the filter filled with pumice medium (median outlet EC of 9.05 mS/cm) (Table 4.18), probably due to the adsorption mechanisms of this material, than to the ion exchange for Na and Cl in this case.

The wastewater temperature at IN oscillated between 38.8°C (August) and 13.2 °C (November) (median 23°C; Table 4.18). The filtration showed an increase of the wastewater temperature at outlet only in the month of July, afterwards it was lower than the IN (Figure 4.59). The filters showed differences in the outlet temperature at the beginning of the experiment (July and August), while subsequently they showed similar values, showing 12°C as wastewater temperature at outlet in November.

The DO at inlet was between 0.31 and 0.97 mg/L (3.6-1.6%) (median values of 0.6 mg/L and 2.6%) (Table 4.18), and with filtration it increased till 4.2-7.5 mg/L (40.8-80.5%), without significant differences among the several factors studied.

The turbidity at inlet ranged between 1,110 NTU (November) to 2,260 NTU (September) (median 2,220 NTU). From the results, the filtration showed to reduce the turbidity by 67-89% (medians, Table 4.18), with different trends among the filters over time (Figure 4.59). Giant reed showed similar turbidity reduction over time, between 85 and 95%, while gravel, tops and the mixed filters had up and down trends, achieving in the last cycle a similar turbidity reduction (around to 75%). For the pumice filter instead, the reduction of the turbidity decreased over time, from 94 to 34%, probably for the feature of this material to disintegrate over time. Pumice is in fact a fragile matter and it may crumble during the filtration compared to other inert materials (as sand or gravel), but no deformation was observed for this material (Farizoglu et al., 2003). However greatest turbidity removal efficiency of pumice substrate was observed by Farizoglu et al. (2003) in comparison to a sand bed under the same experimental conditions. A pumice bed has a greater porosity, so it has a greater capacity for the accumulation of particulate matter. The results obtained in these study disagree with what reported in Farizoglu et al. (2003) ; the turbidity reduction over time of the pumice can be explained as the effect of time on the use of a high organic wastewater as the piggery wastewater, during which it accumulated ever more particulate matter till the pores obstruction, decreasing the turbidity reduction, in addition to its crumble over time.

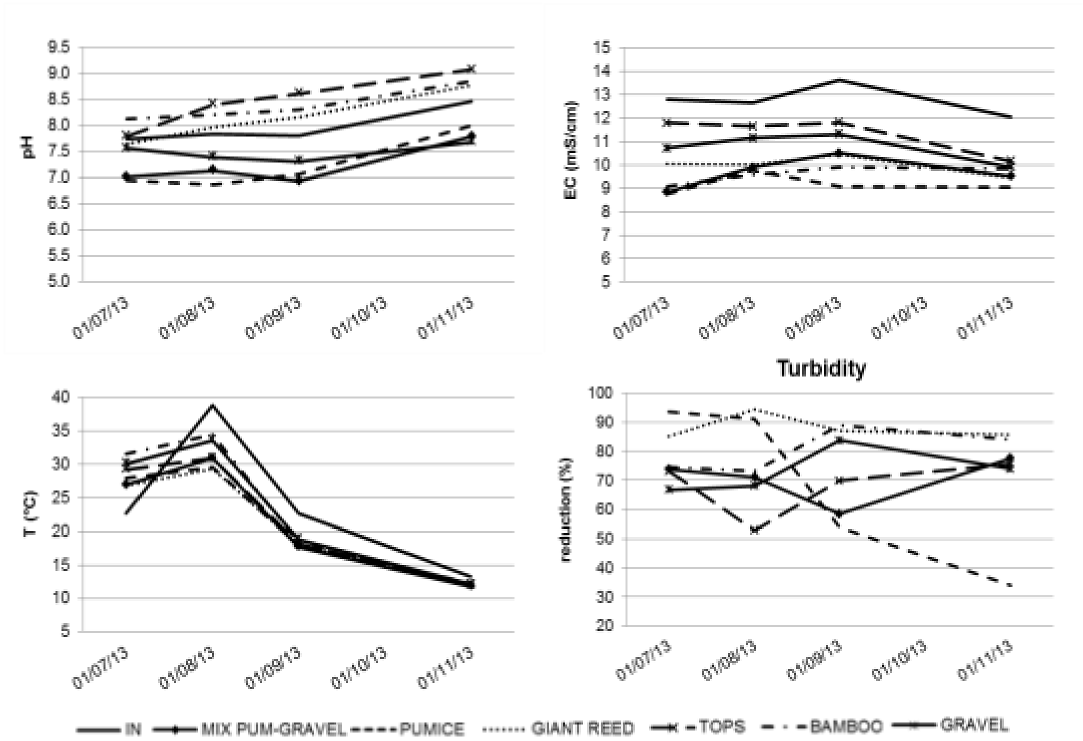

Figure 4.59 Trends over time of pH, temperature (T, °C) and EC (mS/cm) at the inlet and at the outlet of every filter, and turbidity reduction (%).

Table 4.18 Median values of the wastewater on-site parameters at inlet (IN) and outlet of every filter. Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

	EC		**pH**	**DO**		**DO**	**T**	**Turbidity**	
	mS/cm	Variation %	-	mg/L	Variation %	%	°C	NTU	Reduction %
IN	12.73 a	-	7.83	0.6	-	2.6	23	2,220	-
MIX PUM-GRAVEL	9.71 ab	24	7.07	6.8	-956	70.8	22	608	73
PUMICE	9.05 b	29	7.01	7.5	-1064	80.5	23	452	80
GIANT REED	10.04 ab	21	8.06	5.6	-767	55.7	22	254	89
TOPS	11.72 ab	8	8.51	4.6	-612	45.6	24	742	67
GRAVEL	10.93 ab	14	7.48	5.5	-755	54.8	24	521	77
BAMBOO	9.71 ab	24	8.25	4.2	-559	40.8	25	402	82

- *Chemical contents*

The piggery wastewater chemical composition changed over time, presenting higher values of TN and BOD_5 at the beginning of the experiment. The TN concentration at inlet decreased from 1,260 mg/L (July) to 965 mg/L (November) (median 1,088 mg/L) and with filtration it was lowered, with outlet TN concentrations that remained steady over time

(Figure 4.60). In particular the inlet TN concentration was significantly reduced by the filters filled with bamboo (outlet TN of 569 mg/L) and giant reed (outlet TN of 676 mg/L) media (A= 48-38%). The nitrate-N at the inlet ranged from 21 and 128 mg/L (median 64 mg/L) , which was augmented with filtration, between 300 and 900 mg/L, with different trends over time among filters. Specifically it significantly increased in the filters with the mix pumice-gravel and with the gravel, with outlet concentrations of 826 mg/L (mix) and 784 mg/L (gravel) (Figure 4.60). The NH_4-N content at inlet represented the 76% of the total nitrogen with median NH_4-N concentration of 810 mg/L, which ranged between 960 and 700 mg/L (Figure 4.60). For this chemical element the data obtained in the last monitoring cycle were not considered for inconsistency of the values compared to previous tests. The filtration reduced the outlet NH_4-N concentration till less than 150 mg/L, with significant results on ammonium-N abatement by the filter filled with pumice medium (A=99% ; median outlet NH_4-N concentration of 11 mg/L).

Regarding the nitrogen quantity, every filter received between 28 and 22 $g/m^3/d$ of TN (median 23 $g/m^3/d$) and 21-15 $g/m^2/d$ of NH_4-N (median 19 $g/m^3/d$); the NO_3-N content was instead in the range 0.5-2.7 $g/m^2/d$ (median 1.4 $g/m^3/d$) (Figure 4.60). In general at the outlet of the filters there is an increment of the TN quantity, especially from July to September, consequent to the NO_3-N formation, less visible in the bamboo filter. During these months the TN mass abatement was increasingly reduced, remaining stable till November. With filtration, the median TN removal ranged between 7 and 11 $g/m^3/d$, with daily TN mass abatement in the range 31- 69% (Table 4.19). The ammonium-N quantity at the outlet of the filters was lower than 2.5 $g/m^3/d$, with different trends among filters, and mass abatement more constant for the filters filled with pumice medium (Figure 4.60). The NH_4-N removal was similar among the filters, between 15 and 17 $g/m^3/d$, with daily mass abatement in the range 90-99% (Table 4.19). As effect of the ammonium-N reduction, there was the NO_3-N increment at the outlet of the filters with quantity between 2.5 and 17 $g/m^3/d$, which lowered or remained steady in the last monitoring cycle (Figure 4.60). The NO_3-N was not removed with filtration, but significant differences considering the removal (negative removal; Table 4.19) was found between the filters filled with tops and bamboo (from -3 to -5 $g/m^3/d$) and the filters with the mix pumice-gravel and gravel (from -9 to -11 $g/m^3/d$).

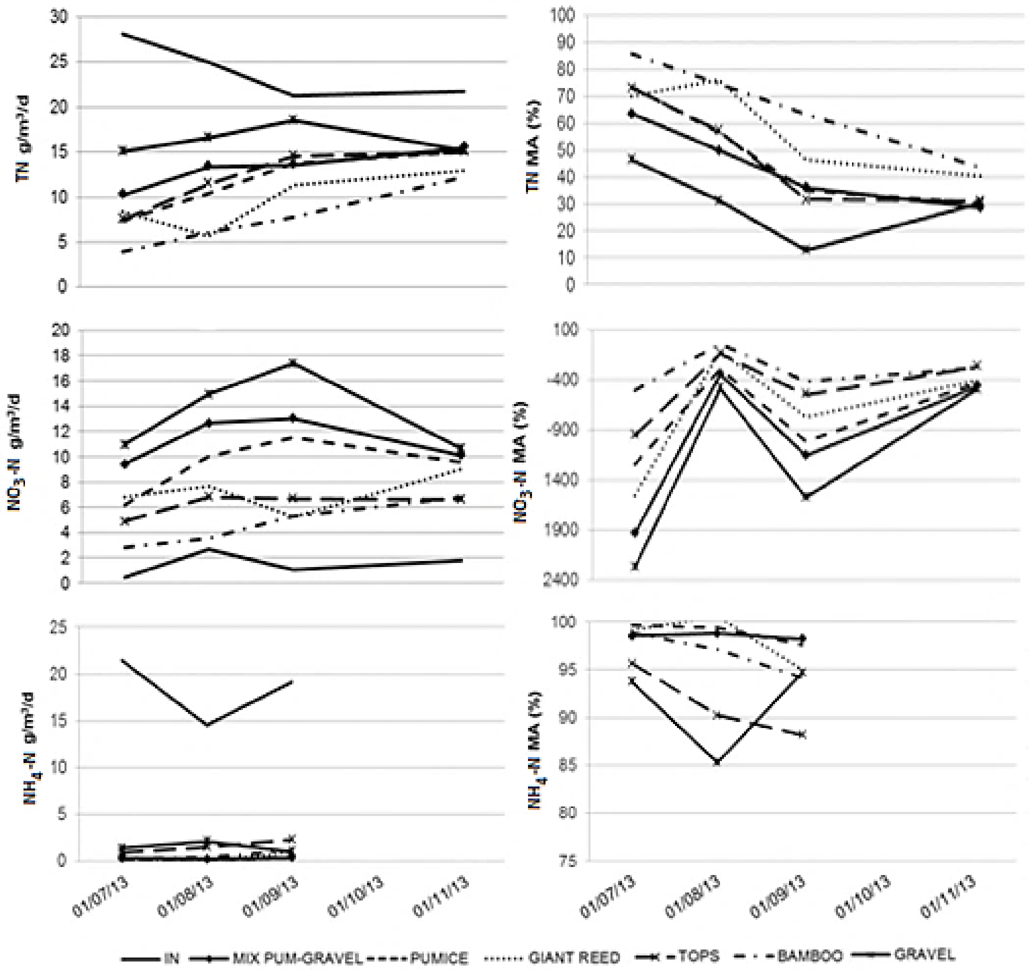

Figure 4.60 Trends of the nitrogen quantities (g/m^3/d) at inlet (IN) and at outlet of every filter, and filters mass abatements (MA, %).

The phosphorous analyses were performed just in the first two monitoring cycles. The piggery wastewater at inlet presented median concentrations of 69 mg TP /L and 55 mg PO_4-P/L (Figure 4.61). Both parameters were lowered after filtration, with median outlet values of 34-50 mg/L for TP (A=27-50%), and 32-46 mg/L for PO_4-P (A=17-43%). Every filter received between 1.7 and 1.3 g/m^3/d of TP (median 1.5 g/m^3/d) and 1.3-1.1 g/m^3/d of PO_4-P (median 1.2 g/m^3/d) (Figure 4.61). Regarding the outlet quantities, the same filter showed similar trends for both parameters. In general at the outlet there are opposite trends compared to the IN in all filters, except in those with giant reed medium, which showed in fact an increment of the mass abatement from the first to the second cycle (Figure 4.61). In general the pumice, bamboo and giant reed filters showed the highest P mass abatements. Onar et al. (1996) reported an adsorption capacity of a pumice powder sample of 0.95 mg PO_4 g^{-1} pumice powder. Yang et al. (2004) showed that the modified pumice exhibited substantially higher P-removal ability than natural pumice. The increment of the mass abatement observed only for the giant reed medium can be due to the P holding due to its decomposition, increasing the adsorbing surface and supporting the microbial biomass responsible of decomposition and nutrient removal. Different authors confirmed that also for substrates able to remove phosphorous (as sand medium) it is not possible to obtain a sufficient high capacity to bind phosphorus for a prolonged period (Arias et al., 2001; Del Bubba et al., 2003; Arias and Brix, 2005). A suitable calcite material was identified, but the full-scale tests showed that removal initially was good, but after a few months, the filters were saturated with phosphorus and there were problems with clogging. It has not subsequently been possible to solve the inherent problems with this material, and chemical precipitation with an aluminium compound is often the solution to solve this problem (Brix and Arias, 2005). The median outlet values oscillated from 0.35 to 0.85 $g/m^3/d$ for TP, and from 0.32-0.78 $g/m^3/d$. The median removal gave by every filter was between 0.6 and 1.1 $g/m^3/d$ for TP (40-77% of median daily MA) and 0.4-0.9 $g/m^3/d$ for soluble-P (32-73% of median daily MA) (Table 4.19).

The COD content of the piggery wastewater ranged from 4,000 and 4,500 mg/L (median IN Cod concentration 4,226 mg/L), higher in the first and in the last monitoring cycles (Figure 4.62). In general the filters had trends of COD outlet concentrations similar to the IN, except the tops filter which didn't show important changes on COD outlet concentration over time (2,820-3,210 mg/L). In particular this element was significantly abated by 65-70% by the filters filled with the mix gravel-pumice (median outlet COD concentration of 1,499 mg/L) and the pumice media (median outlet COD concentration of 1,249 mg/L). The BOD_5 of the piggery wastewater was higher in the 2^{nd} cycle, with 1,050 mg/L, afterwards it decreased till 500 mg/L (median 925 mg/L). Filtration showed to diminish also this parameter, and significant low BOD_5 outlet concentration was found in the filters filled with the mix gravel-pumice (100 mg/L at outlet) and the pumice medium (50 mg/L at outlet) (A=89-95%). During the experiment, every filter received between 83 and 104 $g/m^3/d$ of COD and 20-11 $g/m^3/d$ of BOD (Figure 4.62). In general the filters increased the outlet COD quantity over time with mass abatement lowering from July to November. For this parameter the median removal showed by filters was between 45 and 67 $g/m^3/d$, with median mass abatement in the range 51-80% (Table 4.19). The BOD_5 at outlet was in general lower than 5 $g/m^3/d$, remaining steady over time in all filters (Figure 4.62). The BOD_5 mass abatement, instead, was reduced over time by the filters filled with giant reed and the mix pumice-gravel, on the contrary of the pumice and bamboo filters, while gravel and tops had up and down trends. The BOD_5 was removed with a median daily quantity of 17-19 g/m^3/d and a median daily abatement of 82-98% (Table 4.19).

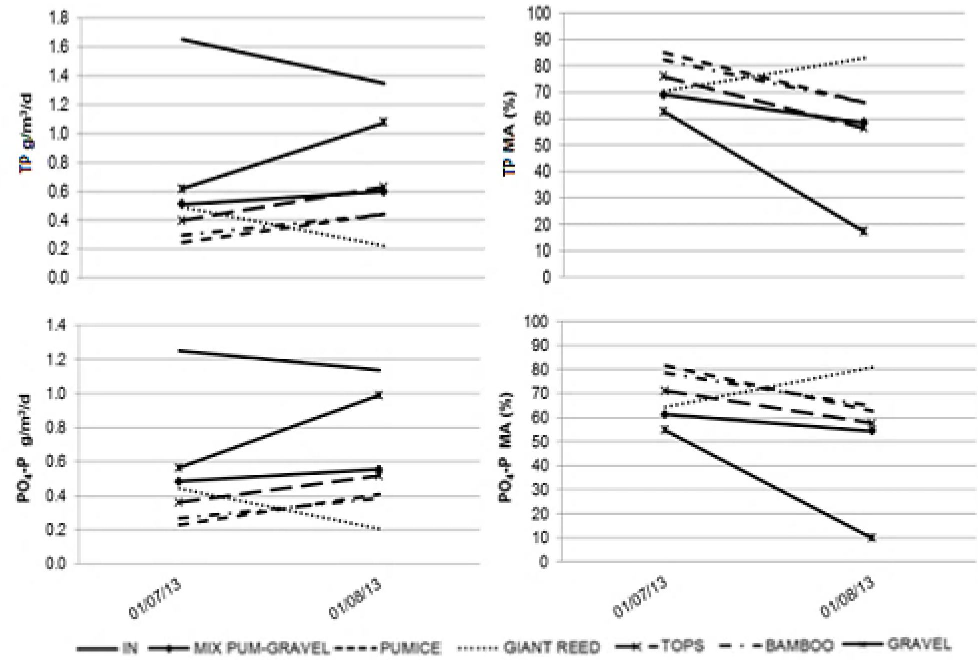

Figure 4.61 Trends of the phosphorous quantities (g/m^3/d) at inlet (IN) and at outlet of every filter, and filters mass abatements (MA, %).

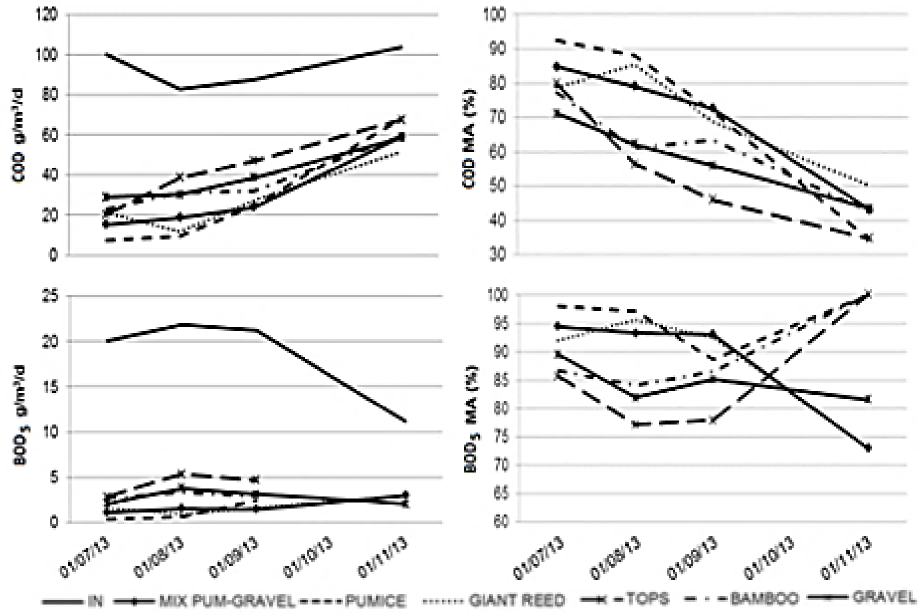

Figure 4.62 Trends of the COD and BOD_5 quantities (g/m^3/d) at inlet (IN) and at outlet of every filter, and filters mass abatements (MA, %).

Table 4.19 Medians values of filters removal (g/m^3/d) and mass abatement (MA, %).

	TN		**NO_3-N**		**NH_4-N**		**TP**		**PO_4-P**		**COD**		**BOD_5**	
	removal (g/m^3/d)	MA (%)	removal (g/m^3/d)	MA (%)	removal (g/m^3/d)	MA (%)	removal (g/m^3/d)	MA (%)	removal (g/m^3/d)	MA (%)	removal (g/m^3/d)	MA (%)	removal (g/m^3/d)	MA (%)
MIX PUM-GRAVEL	11	43	-9 b	-808	17	99	1.0	64	0.7	58	67	76	19	93
PUMICE	11	46	-8 ab	-720	16	99	1.1	75	0.9	72	67	80	19	98
GIANT REED	14	58	-7 ab	-588	16	99	1.1	77	0.8	73	64	74	19	92
TOPS	11	44	-5 a	-405	15	90	1.0	66	0.8	64	45	51	17	82
GRAVEL	7	31	-11 b	-1035	15	94	0.6	40	0.4	32	49	59	18	84
BAMBOO	16	69	-3 a	-346	16	97	1.1	74	0.8	72	52	62	18	87

System performance

-On-site parameters

The piggery wastewater entering to the pretreatment system showed in general constant values over time for the on-site parameters.

The IN electrical conductivity was higher compared to the previous year and ranged between 13.7 and 12.0 mS/cm, highest from mid-September to mid-October, with lowest value of 9.7 mS/cm detected in the last monitoring cycle (November 14th) (Figure 4.63).

The EC at the outlet of the system presented different trend compared to the IN, especially irregular till mid-June and from the end of October. The system showed to reduce significantly the EC content from 12.6 mS/cm to 10.1 mS/cm, considering medians (R=20%) (Figure 4.63).

The wastewater presented a pH at inlet oscillating from 7.6 to 8.5 that increased over time, lower compared to 2012. The filtration system showed an irregular trend for the pH values detected at the outlet, greater of the IN pH from the beginning of the experimentation till the end of July (Figure 4.63). Statistical differences were found between the median values of the IN pH (7.9) and the OUT pH (7.8).

The wastewater temperature of the inlet (Figure 4.63) increased in general from May to the end of August, to drop until the end of the experiment, with a range of 13.9-31.2°C. The outlet temperature was lower and had similar trend than IN, ranging from 12.0 and 25.8°C. Considering medians (IN, 21.1°C; OUT 18.5°C), it was significantly lowered by the pretreatment system (Figure 4.63).

The DO content of the inlet wastewater was in general around to 0.1 mg/L, higher the on July 31st (2.1 mg/L) and especially on November 14th (5.8 mg/L). The percentage of DO was instead near to 2.4 %, with values of 17% and 55% on July 31st and November 14th, respectively (Figure 4.63). The filtration system showed a significant increment of this parameter from 0.2 to 1.1 mg/L (2.4-12.4% in the case of the DO percentage), although up and down trends at the OUT were observed.

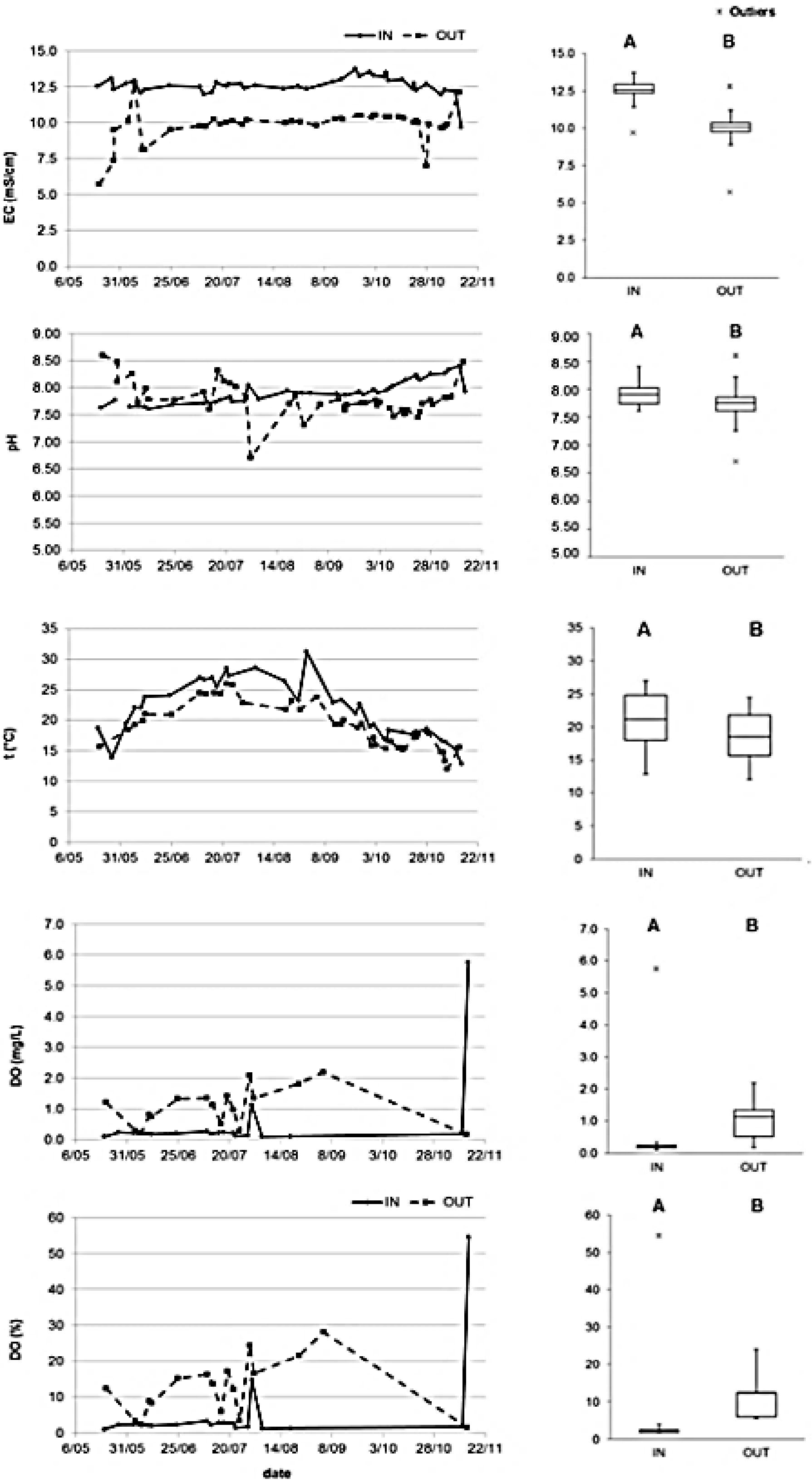

Figure 4.63 On-site parameters of the piggery wastewater detected in 2013 at inlet (IN) and outlet (OUT) of the pretreatment system. Trends over time (at left) and box-plot diagrams (at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

The turbidity of the inlet wastewater ranged from 3,890 to 1,715 NTU (median 2,367 NTU), with up and down trend till the end of June and decreasing over time until the end of September (Figure 4.64), higher of about 800 NTU (considering medians) respect the previous year. The turbidity of the filtered wastewater (OUT) remained more steady over time, ranging from 1,386 and 672 NTU (median 1,239 NTU), significantly reduced by the pretreatment system (R=48%) (Figure 4.64).

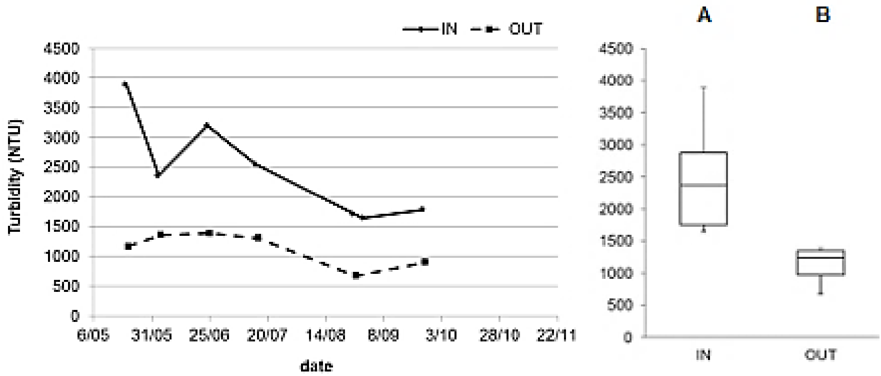

Figure 4.64 Turbidity (NTU) detected at inlet (IN) and outlet (OUT) of the pretreatment system in 2013. Trends over time (at left) and box-plot diagram(at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

-TS and VS contents

The contents of total solids and volatile solids of the inlet piggery wastewater remained steady over time, with percentages between 0.68 and 0.77% (median 0.77%) for TS and in the range 0.26-0.27% (median 0.26%) for VS (Figure 4.65). Similar values were detected after filtration, and not significant differences among the IN and the OUT contents for both parameters were found.

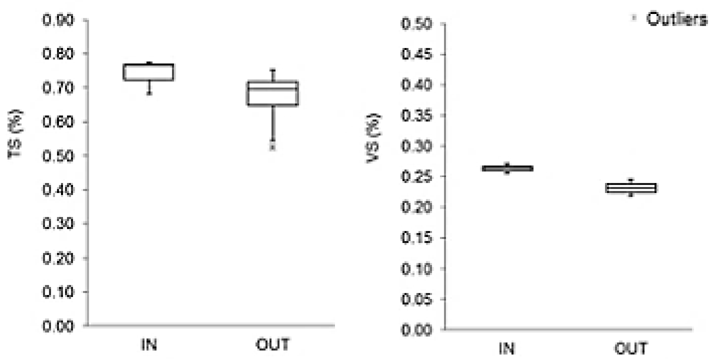

Figure 4.65 Box-plot of the total solids (TS) and volatile solids (VS) contents (%) detected at inlet (IN) and outlet (OUT) of the pretreatment system in 2013.

-*Chemical contents*

The piggery wastewater tested in 2013 for this experimental research presented higher chemical contents compared the previous year.

The total nitrogen concentration of the wastewater at inlet had up and down trend over time, oscillating between 775 and 1,210 mg/L (median 1,001 mg/L) (Figure 4.66). The outlet TN concentration remained in general lower of the IN, except on June 4th in which it exceeded the IN concentration, showing an outlet median value of 726 mg/L. For this parameter, the system showed significant differences among the IN and OUT TN concentrations, with an abatement of 28%.

The ammonium nitrogen content found in the piggery wastewater represented the 72% of the TN concentrations, considering median values. The IN NH_4-N concentration was lower at the beginning of the experiment (till the beginning of June), in the range 175-133 mg/L, while it remained stable at 720-800 mg/L until the end of September (median 724 mg/L) (Figure 4.66). The wastewater detected at the outlet of the pretreatment system had lower data dispersion for the NH_4-N concentration, with values between 305 and 80 mg/L (median 102 mg/L), lower in the last three monitoring cycles. Also this parameter, as TN, was significantly reduced with the filtration treatment, by 86%.

The nitrate-nitrogen concentration of the IN piggery wastewater was very low in the first two monitoring cycles (about 20 mg/L), afterwards it remained around to 99 mg/L (median 97 mg/L). The outlet NO_3-N concentration was higher than IN and presented higher data dispersion, with irregular trend over time (Figure 4.66). As the effect of the ammonium-nitrogen reduction for oxidation phenomena, the pretreatment system increased significantly the concentration of NO_3-N at the outlet, reaching a median value of 386 mg/L.

Regarding the nitrogen quantities, the pretreatment system received progressively from 16 to 35 $g/m^3/d$ of TN (median 25 $g/m^3/d$) and from 2.8 to 27 $g/m^3/d$ of NH_4-N (18 $g/m^3/d$ as median). The nitrate-nitrogen quantity at inlet had similar trend to the IN concentration, with values in the range 1.0-3.1 $g/m^3/d$ (median 2.8 $g/m^3/d$). At the outlet of the system the quantity of TN oscillated between 5.6 and 25 $g/m^3/d$ (21 $g/m^3/d$), with high data dispersion similar to the IN (Figure 4.67). The median daily TN removal obtained with filtration corresponded to 10.6 $g/m^3/d$, while the median MA was 87% (Table 4.20). For the NH_4-N, if at the inlet there was an high data dispersion, at the outlet the NH_4-N quantity detected remained similar over time, with value in the range 2.0-5.7 $g/m^3/d$ (outlet median of 3.3 $g/m^3/d$), which was significantly reduced from the system with a median daily removal of 16 $g/m^3/d$ (median MA=87%) (Table 4.20). The NO_3-N quantity at the outlet showed similar irregular trend of the outlet NO_3-N concentration, and oscillated between 1.0 and 20 $g/m^3/d$ (median 12.7 $g/m^3/d$), significantly higher than the IN quantity. The median daily nitrate-nitrogen increment was 9.7 $g/m^3/d$ (Table 4.20).

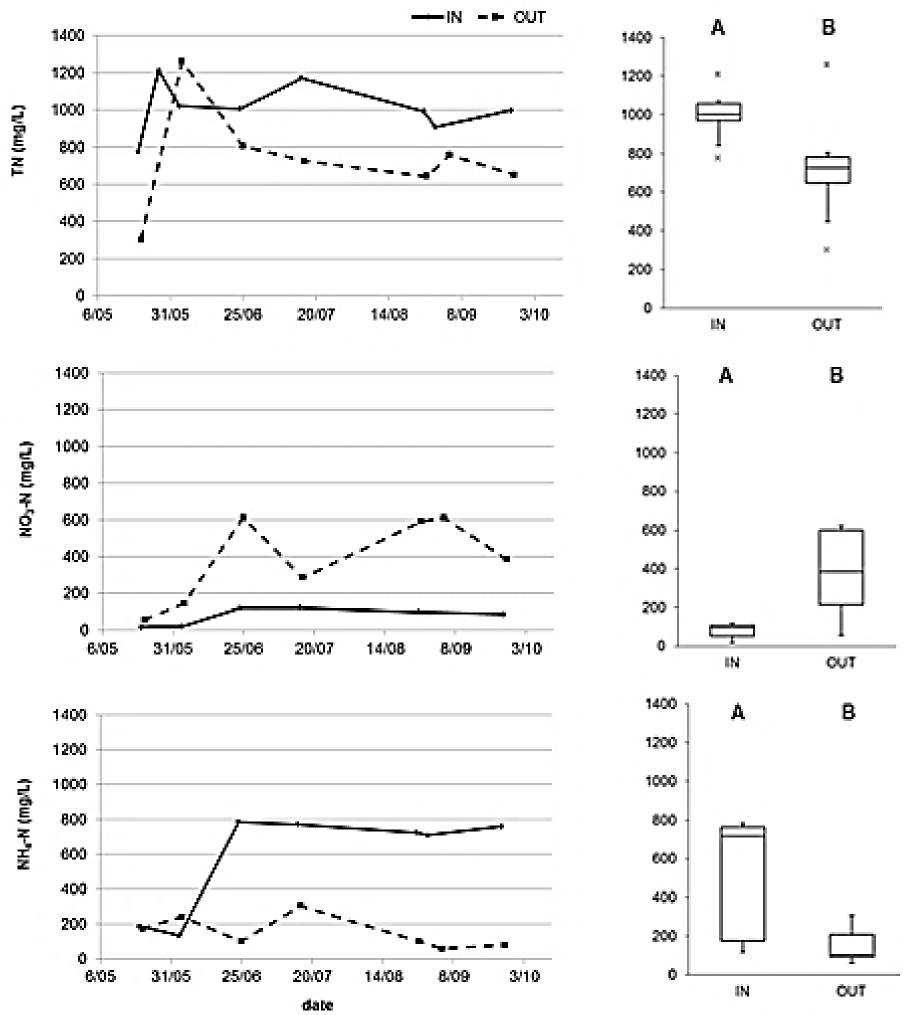

Figure 4.66 Concentrations (mg/L) of nitrogen forms at inlet (IN) and outlet (OUT) of the pretreatment system. Trends over time (at left) and box-plot diagrams (at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

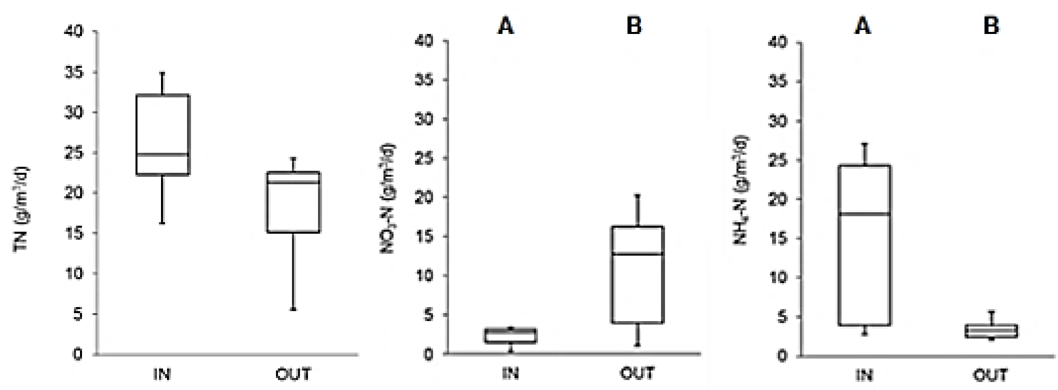

Figure 4.67 Box-plot diagrams of the nitrogen quantities (g/m^3/d) at inlet (IN) and outlet (OUT) of the pretreatment system. Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

The piggery wastewater presented up and down trends for phosphorous concentration, lower at the beginning and at the end of the experiment, higher from the end of June to the end of August. At the IN the TP concentration oscillated between 36 and 102 mg/L (median 57 mg/L) and the PO_4-P was in the range 30-99 mg/L (median 49 mg/L) (Figure 4.68). The filtration showed outlet concentrations of 24-64 mg TP/L and 13-48 mg PO_4-P/L, with similar trend to the IN for TP, which was instead different for the soluble form showing an increment over time (Figure 4.68). Same trends of the IN and the OUT concentration for both TP and PO_4-P were observed for the quantities. During the experiment the system received from 0.8 to 2.4 g TP/m^3/d (median 1.7 g/m^3/d) and between 0.6 and 2.3 g PO_4-P/m^3/d (median 1.5 g/m^3/d) (Figure 4.69). The median quantities detected at the outlet were instead 0.9 g/m^3/d of TP and 0.6 g/m^3/d of PO_4-P. For TP, the filtration showed median removal of 0.5 g/m^3/d and a median daily mass abatement of 37%, while for PO_4-P the median removal was 0.3 g/m^3/d with a daily median mass abatement of 46% (Table 4.20).

The chemical oxygen demand concentration, as the biological oxygen demand, of the piggery wastewater at the inlet of the system decreased over time (Figure 4.70). It fluctuated between 5,242 (May 20th) and 2,270 (August 30th), with median of 4,455 mg/L. The outlet COD concentration had up and down trend until mid-July, afterward it decreased till 1,690 mg/L (median 2,250 mg/L), significantly reduced by 41% (Figure 4.70). The BOD_5 of the inlet wastewater ranged from 900 to 600 mg/L (median 800 mg/L), significantly reduced over time (A= 53%) with the filtration with outlet values between 600 and 200 mg/L (median 375 mg/L) (Figure 4.70). The entering daily COD quantity to the pretreatment system oscillated from 94 to 138 g/m^3/d (median 105 g/m^3/d), particularly higher on August 26th and September 25th (about 138 g/m^3), while the outlet COD was between 50 and 73 g/m^3/d (median 61 g/m^3/d) (Figure 4.71). The BOD_5 at the inlet ranged from 19 to 24 g/m^3/d (median 20 g/m^3/d) and at the outlet it was between 11 and 7 g/m^3/d (median 9 g/m^3/d). Both parameters were significantly reduced by the system, which removed almost 48 g/m^{3}of COD per day and 11 g/m^3 of BOD_5 per day (medians). The daily median mass abatement was 47% for the COD and 55% for the BOD_5 (Table 4.20).

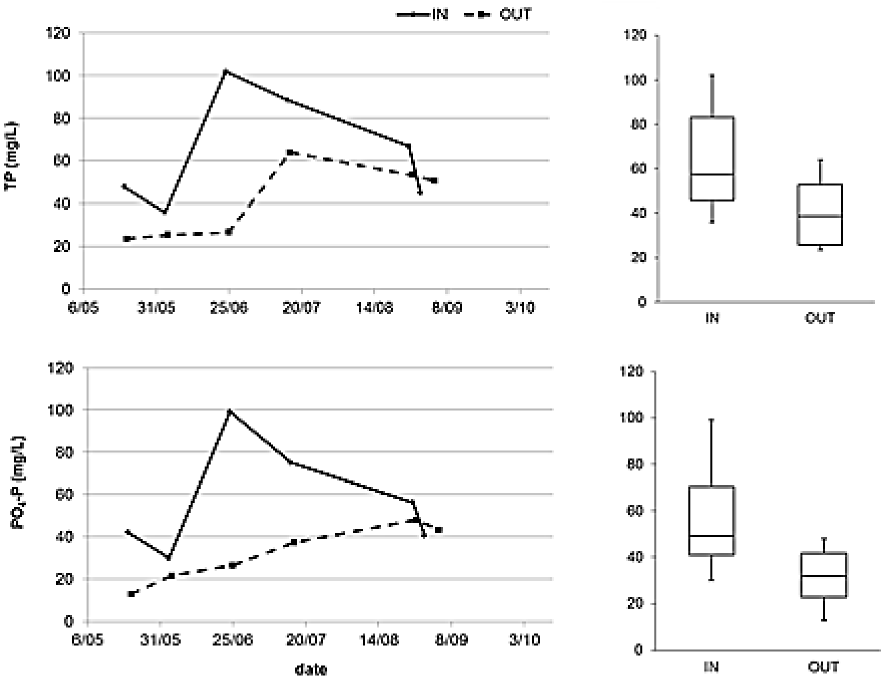

Figure 4.68 Concentrations (mg/L) of phosphorous forms at inlet (IN) and outlet (OUT) of the pretreatment system. Trends over time (at left) and box-plot diagrams (at right).

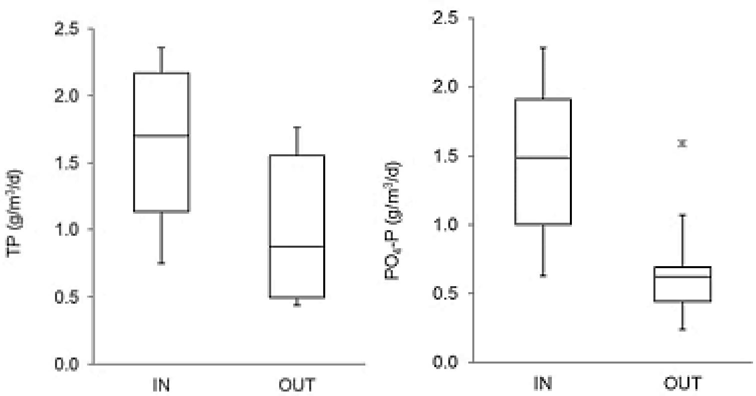

Figure 4.69 Box-plot diagrams of the phosphorous quantities (g/m^3/d) at inlet (IN) and outlet (OUT) of the pretreatment system.

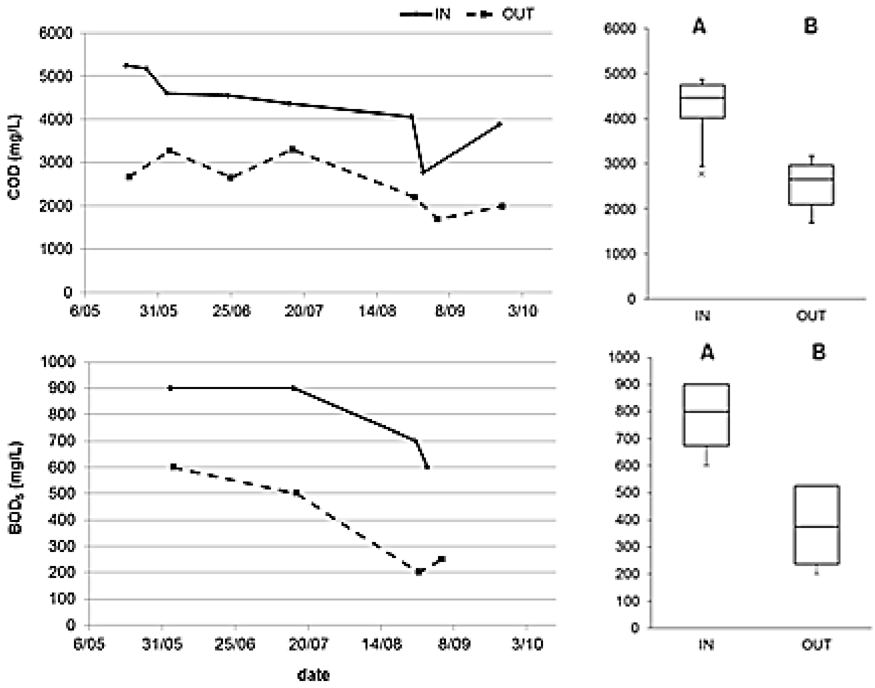

Figure 4.70 Concentrations (mg/L) of COD and BOD_5 at inlet (IN) and outlet (OUT) of the pretreatment system. Trends over time (at left) and box-plot diagrams (at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

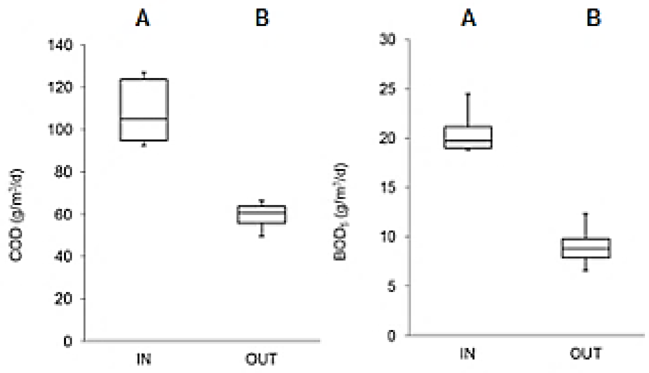

Figure 4.71 Box-plot diagrams of the COD and BOD_5 quantities ($g/m^3/d$) at inlet (IN) and outlet (OUT) of the pretreatment system. Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

Table 4.20 Median values of removal ($g/m^3/d$) and mass abatement (MA, %) of the chemical compounds of the pretreatment system.

	Removal ($g/m^3/d$)	MA (%)
TN	10.6	37
NO_3-N	-9.7	-359
NH_4-N	16.0	87
TP	0.5	37
PO_4-P	0.3	46
COD	49.7	47
BOD_5	10.9	55

CCW

Plants establishment

All plant species used in 2013 to vegetate the phytodepuration system showed difficulty in the taking root, as various replacements due to their mortality took place in the month of June and at the beginning of July.

Over time, the plants of *Phragmites australis* (PHRCO), *Cynodon dactylon* (CYNDA), *Artemisia coerulescens* (ARTCO), *Halimione portulacoides* (HANPO) and *Sarcocornia fruticosa* (SALFR) taken root and survived till the end of the experiment, growing the aerial part. *Puccinellia palustris* (PUCPA), instead, didn't take root, even after several replacements and additions. For this reason this phytodepuration line was renamed as "PUCPA/BLANK", as the dead plants were left in the tanks. In Pavan et al. (2014), contrary to this work, *P. palustris* gave the higher development and survival in treating the digestate liquid fraction (DLF) in a floating wetland treatment system, although its transplantation occurred in summer. In Pavan et al. (2014) , the DLF was characterized by anoxic conditions in which the median redox potential of almost two years experiment was -159 mV. According with results obtained in Pavan et al. (2014), Lang et al. (2010) confirmed that *P. palustris* (as other halophytes, such as *J. maritimus* and *P. australis*) is characteristic of histic soils, and this affirms its not adaptability to oxic conditions.

The taken root plants grown and lived throughout the experimental period (than they survived) (Figure 4.72). The plant taking root/survival was calculated only for PUCPA, HANPO, SALFR and ARTCO plants, counting the plants number for tank (Table 4.21). This was not possible for CYNDA and PHRCO, which are rhizomatous and stoloniferous plants. As previously reported, PUCPA didn't taken root; vice versa, ARTCO showed the higher plant survival (88%, on average), followed by HANPO (75%, on average) and finally by SALFR (50%, on average) (Table 4.21). In Lang et al. (2010) *H. portulacoides* and *S. fruticosa* were identified as indicators for oxic soils, implying that they occur mainly at oxic sites. The high oxygen demand of *H. portulacoides* is well documented in literature (Chapman, 1950; Jensen, 1985). Another reason for the preference of that plant species for well-drained and aerated soils is that nitrate, which forms only under oxic conditions, seems to be *H. portulacoides*'s major form of N nutrition (Jensen, 1985). In addition, Sanchez et al. (1998) found that *H. portulacoides* occurs only at sites with a redox potential >200 mV, where the oxidation of Fe and Mn (Brümmer, 1974) guaranties subtoxic levels of these elements. The lower taking root of *S. fruticosa* compared to the other can be due to the its feature to avoid water-logged and flooded soils (Alvarez-Rogel et al., 2006; Davy et al., 2006).

Table 4.21 Number of planted and taken root/survided plants, with plant taking root/survival (%), divided for level: L1, tanks of the higher level; L2, tanks of the mid-level; L2, tanks of the lower level.

Plant species	Level	N° of plants/tank	Taken root/survived plants (N°)	Plant taking root/survival (%)
	L1	4	0	0
PUCPA	L2	5	0	0
	L3	4	0	0
	L1	4	2	50
HANPO	L2	3	2	67
	L3	2	2	100
	L1	2	1	50
SALFR	L2	1	1	100
	L3	3	1	33
	L1	3	3	100
ARTCO	L2	3	2	67
	L3	2	2	100

Figure 4.72 Plants used in 2013 in July and in November: PUCPA/BLANK (1), HANPO (2), SALFR (3), PHRCO (4), ARTCO (5), CYNDA (6).

Water consumption

The water quantity entering to the phytodepuration system during the entire experimental period of 2013 was higher than 2,500 L/m^2 (Figure 4.73). The HANPO line received highest inlet volumes respect the others lines, reaching at the end of the experimentation 3,056 L/m^2 of wastewater, or rather 21.5 L/m^2/d on average. The SALFR treatment received over period the lowest inlet quantity, with average value of 2,488 L/m^2 on 22th November (17.5 L/m^2/d); the others plants received about a total inlet volume of 2,700-3,000 L/m^2 with an average daily volume of 19-21 L/m^2. To these values, also the cumulated raifall occurred during the experiment has to be considered. Totally 346 L/m^2 of rain have fallen (Figure 4.73), which corresponded to the 11-14% of the inlet volume.

The cumulated outlet wastewater volume was higher in the PUCPA/BLANK treatment, that showed a final value of 2,741 L/m^2, versus 1,811 L/m^2 of the CYNDA treatment which had the lower cumulated outlet volume.The other lines showed intermediate cumulated outlet volume. The mean daily outlet volume was 13 L/m^2/d for the CYNDA line, 16 L/m^2/d for the ARTCO line, 19 L/m^2/d for the PUCPA/BLANK and 17-18 L/m^2/d for the other treatments (Figure 4.73).

Regarding the plant evapotranspiration, CYNDA showed the highest values throughout the period, showing in November a cumulated ET value of 1,302 L/m^2, to be more precise a daily ET of 9.2 L/m^2/d (Figure 4.73). ARTCO and HANPO treatments showed similar ET values, with 852-729 L/m^2 as cumulated final values and daily ET of 5-6 L/m^2 . PHRCO had intemediate ET results, with a final cumulated value of 614 L/m^2 (daily ET of 4.3 L/m^2). The lowest ET was instead gave by the SALFR and PUCPA/BLANK lines, showing final cumulated ET of 382-346 L/m^2 (about 2.5 L/m^2/d). PHRCO, SALFR and PUCPA/BLANK gave lower ET values than the cumulated ET_0 (Figure 4.73). The ET_0 trend, in fact, increased constantly over the time reaching a final value of 723 L/m^2 (5 L/m^2/d) in November. The highest evapotranspiration was recorded in the month of July for the SALFR, CYNDA and PHRCO treatments , while for PUCPA/BLANK, HANPO, and ARTCO lines in the month of August.

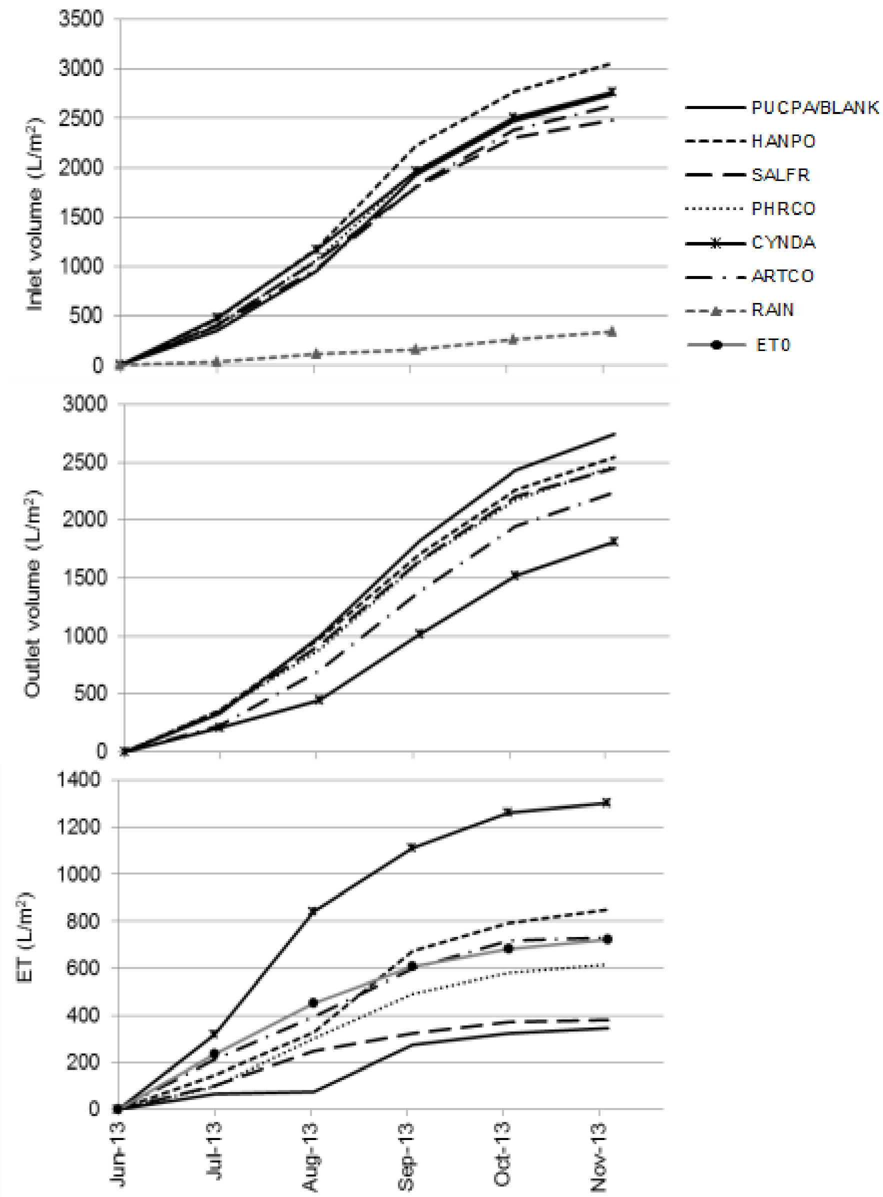

Figure 4.73 Cumulated trends of inlet volume (above), outlet volume (middle) and actual evapotranspiration (ET; below), explained in L/m^2, of every plant species. Inlet volume graph shows also the cumulated rain (L/m^2); ET graph shows also the potential evapotranspiration (ET_0, L/m^2).

The plant coefficient (*Kp*) of halophytic plants was in general lower than the plant species used in 2012, except CYNDA which showed the highest plant coefficient (Table 4.22). The PUCPA/BLANK, HANPO, PHRCO and ARTCO lines showed an increment of the *Kp* from July to September, afterwards it decreased till November. CYNDA gave highest *Kp* in August (2.4), and also SALFR which presented similar *Kp* also in October (0.7). The average coefficient ranged between 0.5 (SALFR) and 1.7 (CYNDA).

The calculated values of *Kp* are similar (except for *C. dactylon*) compared to the typical plant coefficient for agricultural crops, which in most cases are in the range 0.9–1.2 (Allen et al., 1998). The concept of the increment of the plant coefficient in the summer season as confirmed by many authors (e.g. Borin et al., 2011; Anda et al., 2014) was observed only in *C. dactylon*, while the other plant species showed highest *Kp* in September or October, probably due to the transplanting occurred in July which led to the delay of the plant development and therefore of the water consumption. CYNDA gave similar *Kp* of wetland plants, which is usually greater than 1 (Kadlec and Wallace, 2009), even if this species is able to live and survive to drought conditions (Wu et al., 2006).

Table 4.22 Monthly plant coefficient (*Kp*) of each plant species in 2013.

	July	August	September	October	November	average
HANPO	0.6	0.8	2.3	1.5	1.5	1.3
SALFR	0.4	0.7	0.5	0.7	0.2	0.5
PHRCO	0.4	0.9	1.3	1.1	0.9	0.9
CYNDA	1.4	2.4	1.8	1.9	1.0	1.7
ARTCO	0.9	0.8	1.4	1.5	0.3	1.0

Wastewater treatment performances

-On-site parameters

The diluted piggery wastewater coming from the pretreatment system used to feed the phytodepuration system in 2013 presented a pH in the range of 7-8.3, particularly low the September 6th (Figure 4.74), with median value of 7.9. The treatment by plants seems not have affected this parameters, which remained similar to the IN with median outlet values 8.0-8.1. The electrical conductivity of the IN wastewater remained steady over time, in the range 5.9-8.5 mS/cm (median 6.9 mS/cm) (Figure 4.74). Similar outlet EC trends than the IN were observed in PUCPA/BLANK, HANPO, SALFR and PHRCO treatments, while ARTCO and especially CYNDA lines showed higher data dispersion, due to higher values on July 19th and August 9th, in which CYNDA exceeded 20 mS/cm and ARTCO showed 12 mS/cm (Figure 4.74). These EC outlet values are related to the higher water consumption of these plants (especially *C. dactylon*) in the days before, which caused the presence of a small wastewater volume in the tank, concentrating the salts present in the wastewater. When the feeding was augmented (double IN volume), the wastewater started to exit from the tanks, presenting high values which decreased over time, achieving similar values of the other lines. Not differences were found for the EC at the IN and at outlet of the system.

The wastewater temperature of the inlet wastewater ranged between 31.4 (July 19th) and 13.5 °C (November 22nd), with a downward trend over time (median 24.2°C). The treatments showed similar values, and same decreasing trends, without changing of the IN temperature (Figure 4.74).

The dissolved oxygen of the inlet wastewater increased constantly over time from 1.0 to 7.9 mg/L (median 3.4 mg/L), as the percentage (from 12.6 to 76.2%; 40.2% as median value) (Figure 4.74).The increase can be related to the filtration system, which in general increased over time the DO at the outlet, and also to the dilution which occurred before the CCW feeding. Opposite trends were instead observed for the outlet DO contents (both concentration and percentage), which decreased over time, and considering medians, no differences were found for this parameter among the factors. These results can be related to the fact that the DO analysis was carried out just three times (July 10th and 19th and November 22th) for the probe that was not working; these data are than few to give a correct interpretation, and in particular data are missing in the period in which plants grown, and in which probably the DO at the outlet increased for the plant development.

The turbidity of the inlet wastewater was especially higher at the beginning (more than 900 NTU on July 16th), afterwards it was around to 250-360 NTU (median 360 NTU) (Figure 4.75). The depuration system showed a decrement of this parameter with outlet values stable over time, and significant reduction by the lines vegetated with PUCPA/BLANK, SALFR, PHRCO and CYNDA (Figure 4.75) (medians in the range 63-69 NTU; R= 82% on average).

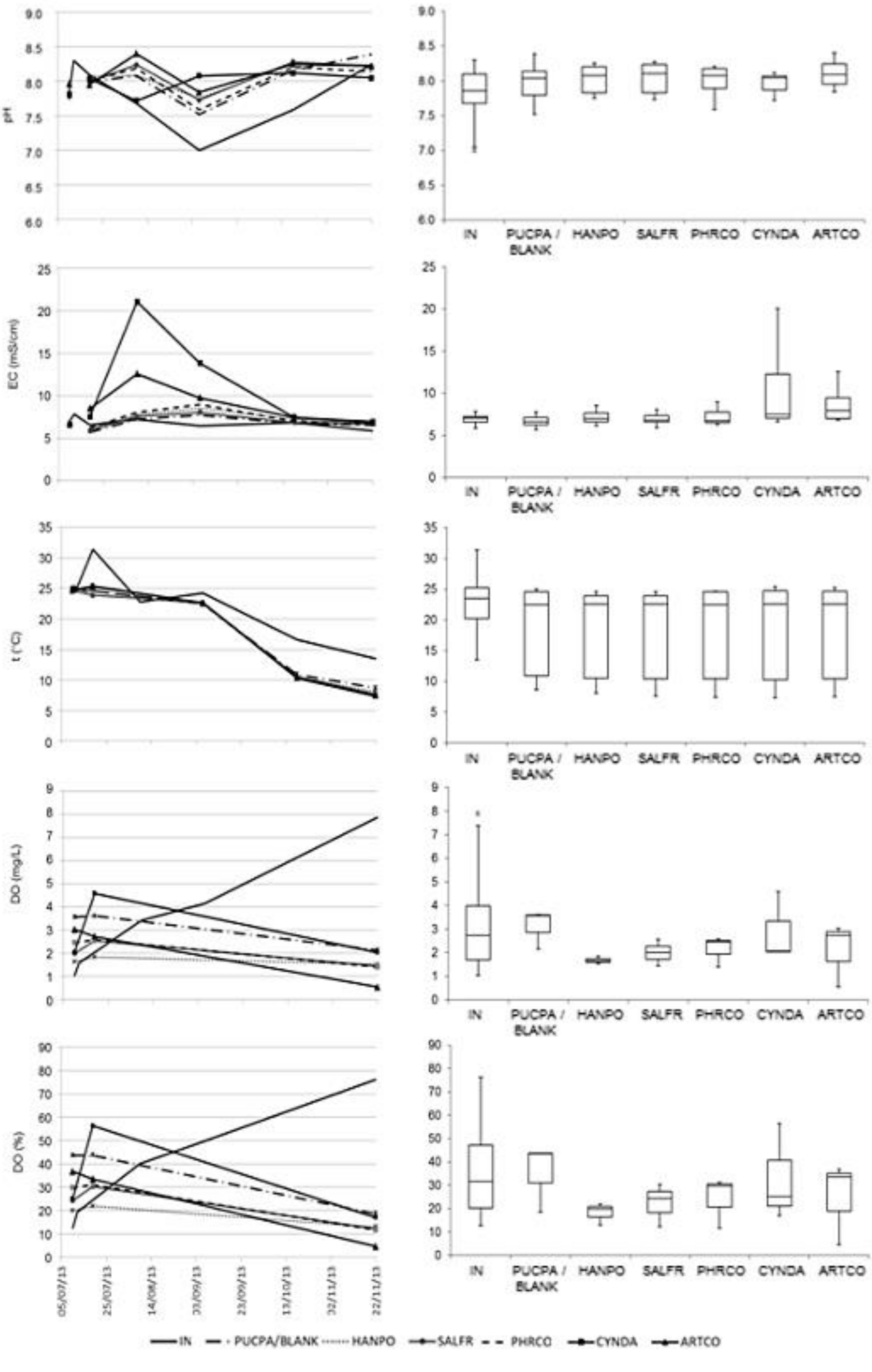

Figure 4.74 On-site parameters of the piggery wastewater detected at inlet (IN) and outlet of every phytodepuration line. Trends over time (at left) and box-plot diagrams (at right).

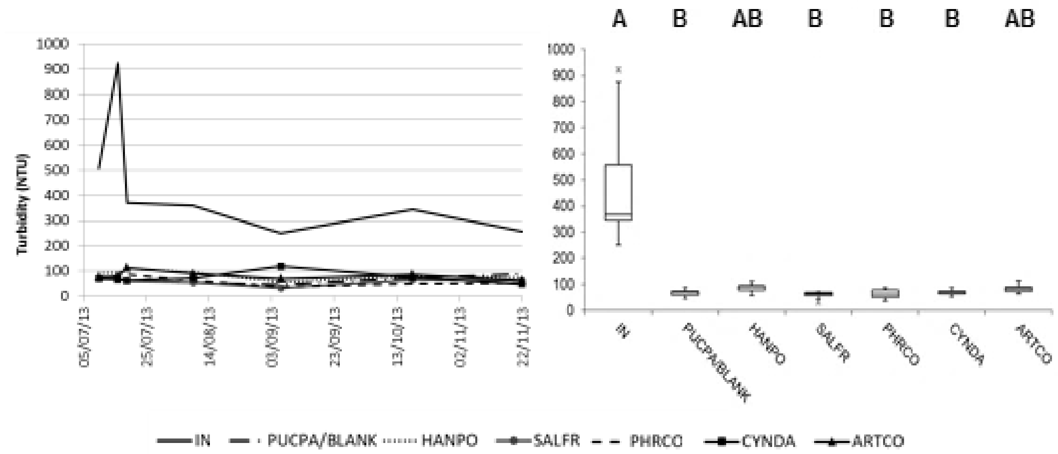

Figure 4.75 Turbidity (NTU) detected at inlet (IN) and outlet of every phytodepuration line. Trends over time (at left) and box-plot diagram(at right). Different letters indicate significant differences ($p < 0.05$) by Kruskal–Wallis test.

-*TS and VS*

The contents of the total solids and volatile solids of the wastewater at inlet remained steady over time, ranging between 0.38 and 0.56% for TS (median 0.48%) and 0.17-0.19% for VS (median 0.19%) (Figure 4.76). The phytodepuration treatment gave similar outlet values of the IN for both parameters. In particular ARTCO and especially CYNDA showed higher data dispersion for the TS contents at outlet. These results are related to the fact that the TS were analyzed in some samples of August, in which these plants had higher wastewater consumption and the presence of a small wastewater volume in the tank, that led an accumulation of the solids inside the tanks. For the VS content, also PUCPA and SALFR showed higher data dispersion (Figure 4.76).

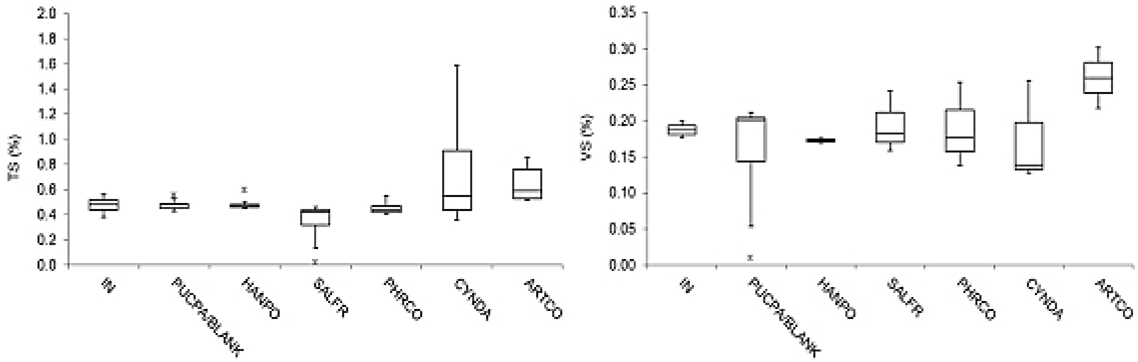

Figure 4.76 Box-plot of total solids (TS) and volatile solids(VS) contents (%) detected at in inlet (IN) and outlet of every phytodepuration line.

-Chemical contents

The diluted piggery wastewater applied to the CCW in 2013 was characterized by a TN concentration between 354 and 550 mg/L (median mg/L), steady over time (Figure 4.77A). The IN nitric-N concentration represented about the 62% of the TN, and ranged from 214 and 409 mg/L (median 282 mg/L), while the IN ammonium-N was between 57 and 194 mg/L (median 57 mg/L), particularly high on July 16th (Figure 4.77A). The system showed outlet values higher than the IN for the NO_3-N concentration, in the range 373-475 mg/L (medians), with higher data dispersion for the CYNDA and ARTCO treatments. The NO_3-N increment led the increment of the TN concentration, which was between 438 and 565 mg/L considering medians (Table 4.23). The NH_4-N concentration was reduced with the phytodepuration system, although it increased over time in all treatments (Figure 4.77A). The PUCPA/BLANK, SALFR, PHRCO, and CYNDA lines showed a significant reduction of this parameters with outlet medians in the range 3.0-7.0 mg/L . The median mass abatement ranged instead between 69 and 95% (Table 4.23).

During the experiment, every line of the CCW received different chemical quantity at inlet (depending on the inlet volume), with daily values ranging from 5.1 to 12.5 g/m^2 of TN, 2.6-10.9 g/m^2 of NO_3-N and 0.7-3.4 g/m^2 of NH_4-N per line. The highest nitrogen inlet quantities were observed in the PUCPA, HANPO, PHRCO and ARTCO treatments, while the CYNDA and SALFR lines had lowest inlet nitrogen quantities. For all nitrogen forms, in general, the removal quantities increased with the entering quantities (Figure 4.77B), less observable in the PUCPA/BLANK, HANPO and ARTCO lines for the TN and NO_3-N from the beginning of August to September. The mass abatement for the TN and NO_3-N was in general higher till the beginning of September, afterwards it decreased; for the NH_4-N the mass abatement was almost 100% for all lines in July and decreased steadily over time till less than 20% in November. For TN the median removal quantity of the CCW was between -0.1 and 1.04 g/m^2/d, with median MA in the range -3-42% (Table 4.24). Among nitrogen forms, the ammonium-N was element mostly reduced with median MA of 68-97% and median daily removal of 0.19-0.33 g/m^2/d . The nitrate-N was removed by -27-7%, considering median MA, with median removal of -0.35-0.09 g/m^2/d (Table 4.24). The negative mass abatement of TN and NO_3-N are related to the outlet concentrations often higher than the inlet concentrations, especially till October. In

November the outlet concentrations were lower but higher outlet volume of the inlet were detected, probably due to the low ET and rainfall phenomena.

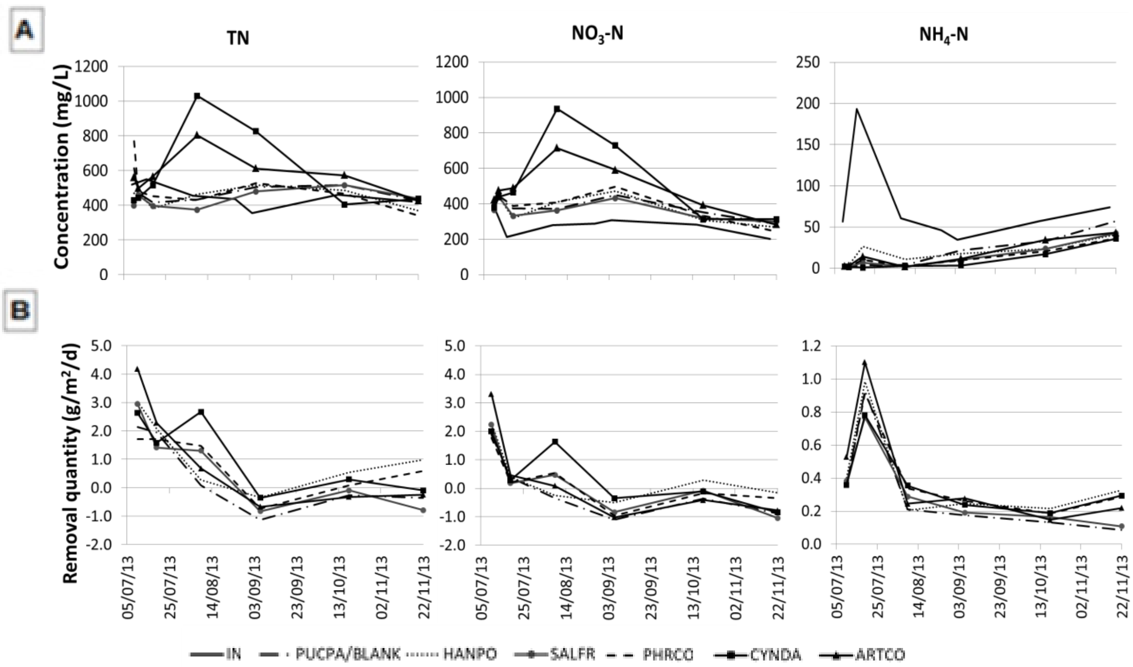

Figure 4.77 Trends of the average concentrations (mg/L) at inlet and outlet of every treatment (A) and of the average removal quantities (g/m^2/d) for treatment (B) for the TN, NO_3-N and NH_4-N.

The phosphorous concentration of the wastewater at the inlet of the CCW was higher in at the beginning and decreased over time (Figure 4.78A). The TP decreased from 30.4 to 19.1 mg/L, the PO_4-P (median 27.6 mg/L) from 27.2 to 17.6 mg/L (median 23.6 mg/L) (Table 4.23). For both parameters, the treatments showed similar behavior for the outlet concentration, which was in general lower than the IN concentration but it augmented over time (contrarily to the IN) exceeding the IN concentration in November (Figure 4.78A). A similar behavior was observed in the CRXDW lines tested in the CCW in 2012. As the NH_4-N concentration, both the TP and the PO_4-P concentrations were significantly reduced by the PUCPA/BLANK, SALFR, PHRCO, and CYNDA lines, which gave outlet values in the range 13.3-15 mg/L of TP and 10.9-10.1 mg/L of PO_4-P (Table 4.23). Not significant differences were found for the mass abatement among treatments, which was between 39 and 52% for TP and 39 and 54% for the P-soluble (Table 4.23).

The daily quantity entering in the system was between 0.31 and 0.98 g TP /m^2 and 0.28-0.79 g PO_4-P/m^2. The chemical removal of these parameters was higher with greater inlet quantity (Figure 4.78B). All lines showed outlet phosphorous quantities than exceeded the IN in November, due to the increment of the concentrations and also for the higher outlet volumes for the low ET and rainfall phenomena. For all lines, than, the higher phosphorous mass abatement was detected at the beginning of the experiment (higher than 80% for both parameters), which decreased over time till November, becoming negative. The system removed a TP quantity between 0.09 and 0.13 g/m^2/d, with median MA of 49-82% (Table 4.24). The median daily PO_4-P removal was in the range 0.08-0.11 g/m^2/d, and the median PO_4-P MA between 48 and 84% (Table 4.24).

The COD of the wastewater at the inlet had up and down trend, ranging from 2,135 to 606 mg/L (median 998 mg/L), higher on July 16th and November 19th. Regarding the outlet COD concentration, all lines, except the CYNDA, showed similar trends, in general with lower values compared to the inlet, a part in November, when they exceeded the IN (Figure 4.79A). The CYNDA line gave higher outlet values than the IN in the monitoring cycles of August and September. The median COD concentration of the outlet wastewater was between 739 and 881 mg/L, and it was abated by the CCW by the 12-26% (Table 4.23). The BOD_5 at the inlet was in general lower than 150 mg/L, even if it achieved almost 400 mg/L on July 16th (median 120 mg/L) (Figure 4.79A). The outlet BOD_5 was similar among the lines, with median values of 40-55 mg/L. (Table 4.23); the mass abatement of this parameter presented by the phytodepuration treatment was between 58 and 67%.

The daily quantity entering in the system was between 8.4 and 43.3 g COD /m^2 and 0.8-7.0 g BOD_5/m^2, lower for CYNDA and SALFR lines, as the previous chemical parameters. For the BOD_5 the chemical removal was higher with greater inlet quantity (Figure 4.79B), while for COD this happened only for PHRCO and HANPO. All treatments showed similar behavior on the mass abatement, which dropped for the BOD_5 from almost 100% of July to become progressively negative in November. The same occurred for the COD, but with more steps: in July the MA was higher than 80%, afterwards it decreased to 16-43% in October, and it became negative in November. For COD, the system removed between 1.53 and 2.68 g /m^2/d (median), showing a median mass abatement of 37-56%, while the BOD_5 presented lower daily removal quantity (0.48-0.71 g /m^2/d) and higher MA (74-91%) (Table 4.24).

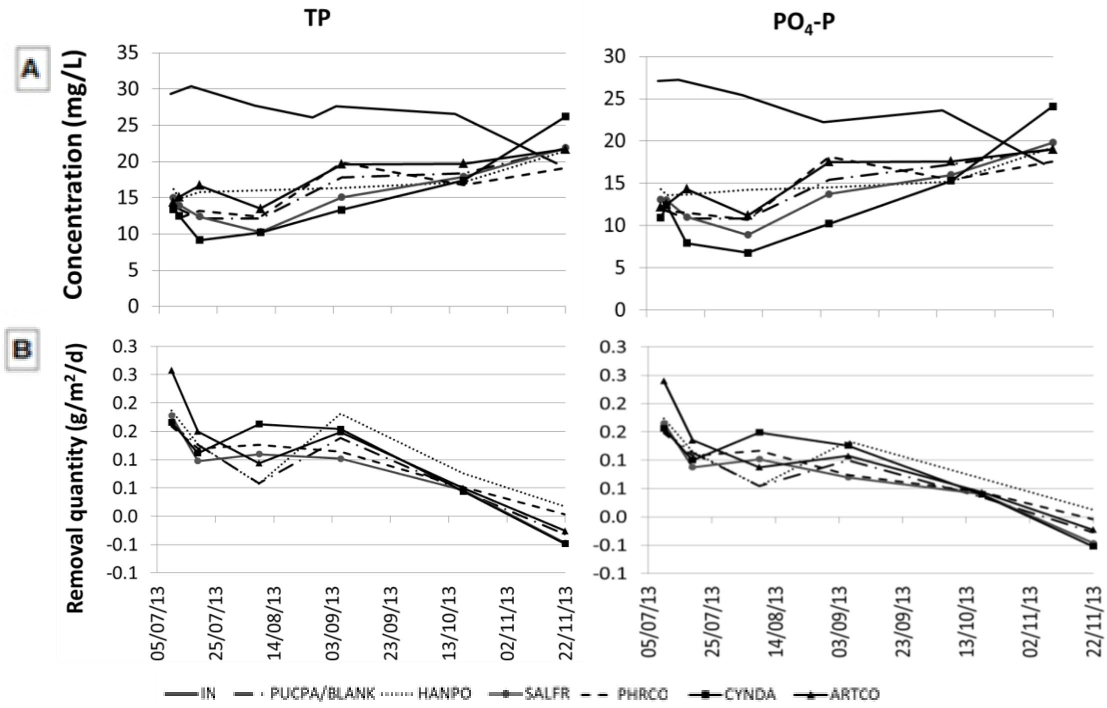

Figure 4.78 Trends of the average concentrations (mg/L) at inlet and outlet of every treatment (A) and of the average removal quantities (g/m^2/d) for treatment (B) for the total phosphorous (TP) and soluble phosphorous (PO_4-P).

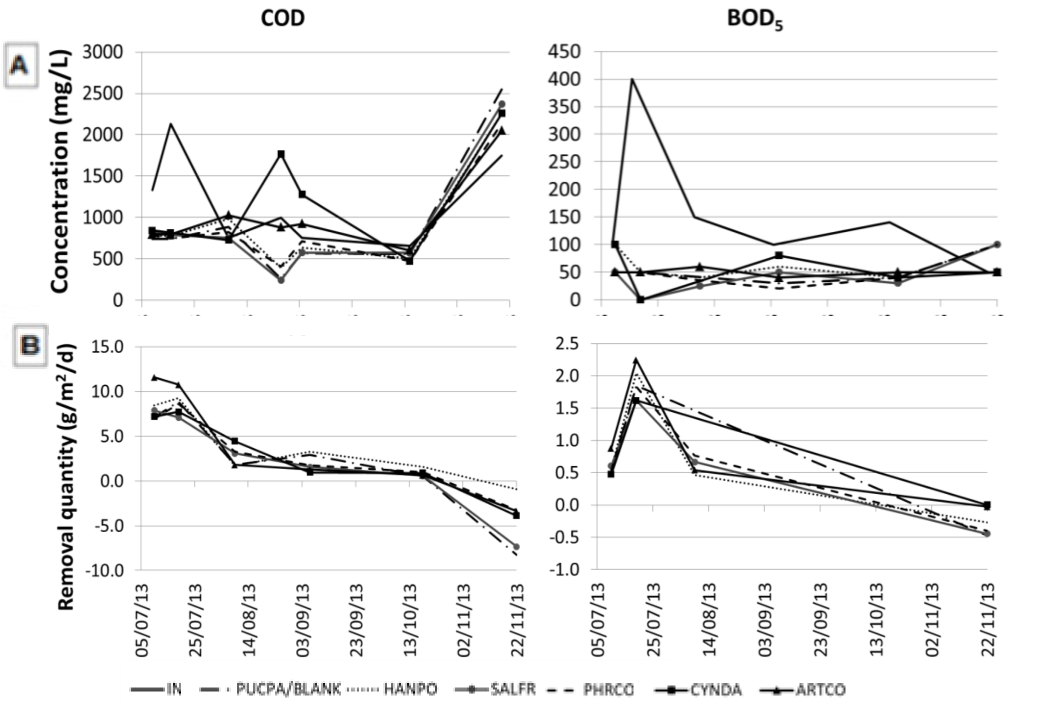

Figure 4.79 Trends of the average concentrations (mg/L) at inlet and outlet of every treatment (A) and of the average removal quantities (g/m^2/d) for treatment (B) for the chemical oxygen demand (COD) and biological oxygen demand (BOD_5).

Table 4.23 Median concentrations (mg/L) of the chemical parameters at inlet (IN) and outlet (OUT) of every treatment, with abatement (A, %). Different letters indicate statistical differences among plant species (Kruskal-Wallis test p<0.05).

	IN (mg/L)	PUCPA/BLANK		HANPO		SALFR		PHRCO		CYNDA		ARTCO	
		OUT (mg/L)	A (%)	OUT (mg/L)	A (%)	OUT (mg/L)	A (%)	OUT (mg/L)	A (%)	OUT (mg/L)	A (%)	OUT (mg/L)	A (%)
TN	455	471	-3	469	-3	438	4	465	-2	442	3	565	-24
NO_3-N	282	375	-33	410	-45	363	-29	402	-43	438	-55	475	-68
NH_4-N	57 a	3.9 b	93	18 ab	69	7 b	88	10 b	83	3 b	95	12 ab	80
TP	28 a	14 b	50	16 ab	41	15 b	46	14 b	49	13 b	52	17 ab	39
PO_4-P	24 a	12 b	49	14 ab	39	13 b	44	12 b	49	11 b	54	14 ab	39
COD	998	739	26	777	22	754	24	749	25	845	15	881	12
BOD_5	120	50	58	55	54	40	67	45	63	50	58	50	58

Table 4.24 Median values of daily chemical removal ($g/m^2/d$) and mass abatement (MA, %) of every treatment.

	PUCPA/BLANK		HANPO		SALFR		PHRCO		CYNDA		ARTCO	
	removal ($g/m^2/d$)	MA (%)	removal ($g/m^2/d$)	MA (%)	removal ($g/m^2/d$)	MA (%)	removal ($g/m^2/d$)	MA (%)	removal ($g/m^2/d$)	MA (%)	removal ($g/m^2/d$)	MA (%)
TN	-0.10	-3	0.76	25	0.60	27	1.04	35	0.94	42	0.22	14
NO_3-N	-0.35	-27	0.07	5	0.05	7	0.02	4	0.09	12	-0.16	-9
NH_4-N	0.19	68	0.29	77	0.24	85	0.31	87	0.33	97	0.26	88
TP	0.09	49	0.10	54	0.10	63	0.12	61	0.13	82	0.12	67
PO_4-P	0.08	48	0.09	53	0.08	60	0.09	59	0.11	84	0.10	65
COD	2.36	49	2.48	50	2.31	54	2.48	53	2.68	56	1.53	37
BOD_5	0.54	85	0.52	78	0.64	90	0.65	86	0.48	74	0.71	91

Aerial biomass production and nutrient uptake

Over time all plants which taken root in July showed to grow till the end of the experiment, without showing stress signs. *H. portulacoides* was in flowering from the end of August to mid-September; this happened also for *A. coerulescens* (from the beginning of September) and also for *S. fruticosa* from mid-September to mid-October. However some changes were observed in some plants. *A. coerulesces* was attacked by eriophyid mites from the beginning of September to the end of the experimentation, which live in different plants. Acaricidal treatments have not been made, to not alter the system and because the plants were not so much compromised. *P. australis* plants showed instead yellowing of the aerial part from August to the end of the experimentation. This circumstance could be due to the high piggery wastewater salinity, because it was picked up from a constructed wetland used for treatment of agricultural drainage waters, in which the EC detected in the previous years (measured in a 1:2 (soil:water) suspension) was 0.18-0.22 mS/cm (Passoni et al., 2009).

The aboveground biomass production of the plant species used in 2013 was different depending on the type and not significant differences were found among levels (Table 4.25). The average fresh weight ranged from 550 g/m^2 of PHRCO to 7,265 g/m^2 of CYNDA, with higher standard deviation for SALFR and CYNDA. Ventura and Sagi (2013) revealed an annual fresh biomass of *S. fruticosa* grown under field conditions on sand dune soil irrigated with moderate salinity (10 mS/cm) of 20-28 Kg/m^2, depending on the genotype.

Average dry biomass percentage was higher for CYNDA (46%) (Table 4.25), and lower for the others, 33% for PHRCO, 36 % for ARTCO, and 27-28% for HANPO and SALFR. The average dry weight was highest in CYNDA plants (3,294 g/m^2 on average) , while the lower dry weight was found in PHRCO plants, with 172 g/m^2 on average. Grattan et al. (2004) reported values of dry weight shoot biomass production rates of *C. dactylon* between 50 Kg/ha per day dry weight with EC of 15 mS/cm and 56 Kg/ha per day with EC of 25 mS/cm. Low dry aboveground production was observed in this research, compared to that reported by Matos et al. (2009) in which *C.dactylon* (Tifton 85) used for the treatment of swine wastewater produced around 28.8 t ha^{-1} in monoculture per cutting and a total yield of 86 t ha^{-1} of dry matter .

The aboveground dry biomass of *P. australis* was very low compared to those reported in Kadlec and Wallace (2009), between 942 and 10,800 g/m^2. This can be explained for the fact that in this study it was planted as rhizomes in the CCW in July, and it was grown for just 3 months, in a not ideal period for the plant growth. Similar dry aboveground production of *S. fruticosa* was found in Pavan et al. (2014), in which it ranged between 400 and almost 700 g/m^2.

N content in the aerial part was highest in PHRCO and ARTCO plants (3.5% and 3.2% on average), mean in HANPO and SALFR (2.6-2.7%) and lower in CYNDA plants (2.3% on average) (Table 4.25).

This results are lower of that reported in Pavan et al. (2014), in which *C. dactylon* and *H. portulacoides* showed aboveground N content from 3% to more than 4%.

However the highest N uptake was gave by *C. dactylon* (mean 77 g/m^2), followed by HANPO and ARTCO (31-32 g/m^2 on average), while *P. australis* gave the lower N uptake (6.2 g/m^2). In this study the halophytic plants showed N uptake values far higher than those obtained by Pavan et al. (2014), which were less than 25 g/m^2. The N uptake of *C.dactylon* obtained in this study, instead, was lower of what reported by Matos et al. (2009), with value of 2,043 kg ha^{-1} in three cuttings.

The P content on the aerial part was similar among plant species, 0.2-0.3% on average. Also the P uptake was greater for *C. dactylon* plants (8.5 g/m^2 on average) and lower for *P. australis* (0.6 g/m^2) (Table 4.25). Also for the P uptake the halophytic plants showed values far higher than those obtained by Pavan et al. (2014).

In general, the nutrients uptake of the plant species is related to the ability to increase the plant biomass, and rhizomatous or stoloniferous plant species, as *C. dactylon* or *P. australis*, are favored compared to the halophytic plants used in this study. The halophytes, in fact, had a different vegetative system in which each plant originates from seed. C.dactylon would likely increased biomass production if grown in a larger area.

Table 4.25 Fresh and dry weights (g/m^2), dry biomass (%), and nutrient (N and P; % and g/m^2) stored in the aerial part of the plants, subdivided by levels (L1-the highest level; L2-the mind-level; L3-the lowest level) and with average value (±st.dev.).

Plant	Level	Fresh weight	Dry weight	Dry biomass	N		P	
		g/m^2	g/m^2	%	%	g/m^2	%	g/m^2
HANPO	L1	4,513	1,211	27	2.7	32	0.2	2.4
	L2	5,795	1,481	26	2.6	38	0.2	2.8
	L3	2,800	810	29	2.8	23	0.2	1.5
	average	4,369±1,502	1,167±337	27±2	2.7±0.1	31±8	0.2±0.01	2.3±0.7
SALFR	L1	472	172	36	2.3	4.0	0.1	0.2
	L2	4,718	1,131	24	2.9	32	0.2	2.2
	L3	2,297	536	23	2.5	13	0.1	0.7
	average	2,496±2,130	613±484	28±7	2.6±0.3	17±15	0.2±0.05	1.1±1.1
PHRCO	L1	277	99	36	3.1	3.0	0.3	0.3
	L2	851	240	28	3.9	9.4	0.4	1.0
	L3	523	177	34	3.6	6.3	0.4	0.6
	average	550±288	172±71	33±4	3.5±0.4	6.2±3	0.3±0.08	0.6±0.4
CYNDA	L1	10,523	4,504	43	2.6	118	0.3	15.1
	L2	6,236	2,969	48	2.2	64	0.2	6.0
	L3	5,036	2,408	48	2.1	50	0.2	4.3
	average	7,265±2,884	3,294±1,085	46±3	2.3±0.3	77±36	0.2±0.09	8.5±5.8
ARTCO	L1	3,046	1,096	36	2.9	32	0.3	3.0
	L2	2,533	894	35	3.4	30	0.2	2.1
	L3	2,728	1,001	37	3.3	33	0.2	2.2
	average	2,769±259	997±101	36±0.7	3.2±0.2	32±1.6	0.2±0.03	2.4±0.5

Final observations

Pretreatment system

1. Media state

In the final surveys of May 2014 in which the filters were manually emptied, the moisture content was detected and observation on media state were performed.

The filter filled with giant reed showed a decrement of the bed height of 5 cm (total volume of 0.032 m^3) compared to the initial mass, while bamboo didn't show changing on bed height.

Concerning the filters filled with one material, the moisture content was higher with increasing the depth (Table 4.26), except for bamboo that showed similar values. The giant reed medium showed the highest moisture content (64% on average), followed by the bamboo (54%), pumice (31%) and gravel (0.9%) materials. The giant reed material observed at the bottom of the filter presented in fact putrefactive phenomena. Concerning the mixed filter, instead, the moisture content was grater in the first layer of pumice medium (29%), and lower in the second layer (coarse gravel, 0.4%) and in the third layer (fine gravel, 2.4%). The pumice is a porous material which has high water adsorption capacity, as shown in the previous results. Also organic media as giant reed and bamboo can retain water, for their porous surface, although the giant reed can absorb more water than bamboo for the higher content of hemicelluloses (Azwa et al., 2013), as illustrated successively. The moisture absorption is related to the composition and internal structure of the fibers. Moisture content in the fibers also influences the degree of crystallinity, crystalline orientation, tensile strength, swelling behavior and porosity of the fibers (Sukumaran et al., 2001). However, it is worth noting that the high moisture absorption suggests ease of the microbial attack (biodegradation).

During the filters emptying spiders were observed especially with the increase of depth. Besides in the bamboo filter mud nests of a wasp, the *Sceliphron spirifex* (Sphecidae family), were found both close the bamboo canes and in the walls of the filter. Probably this insect, which feeds on spiders, used the filter as refuge (Figure 4.80A). Moreover in the giant reed medium saprophytic fungi were observed close the canes (Figure 4.80B), probably for the humidity conditions in the filter, symptom of the decomposition of this material. Fungi are an important class of decomposers due to their abundance and ability to decompose rather recalcitrant organic material. In fact, fungi are able to secrete enzymes that are capable of breaking down virtually all classes of plant compounds. Thus, fungi can decompose substrates such as fresh plant litter and some structural materials (e.g., lignin, chitin, and keratin) that are initially almost inaccessible to other decomposers (Wang and D'Orico, 2008). Obviously the decomposition of giant reed medium, as any other organic material, is also due to bacteria. Bacteria are another major group of decomposers. The bacteria include both aerobic and anaerobic organisms, which distinguishes them from the exclusively aerobic fungi. Recent studies have shown that bacteria are able to degrade cellulose/hemicellulose, lignin, and even intact fiber walls. Due to their small size and large surface to volume ratio, bacteria are able to rapidly absorb soluble substrates and to reproduce quickly in substrate-rich conditions. In substrate-rich environments such as the rhizosphere or dead animal carcasses, bacteria tend to undergo population 'explosions' and thereby become the dominant decomposers. These populations collapse as the freely available resources are consumed. Bacteria decomposition seems to be more common in situations where fungi are under stress. Bacteria have also been found to degrade substrates resistant to fungal decay (Wang and D'Orico, 2008).

Table 4.26 Average (±st.dev.) moisture content (%) detected at the different layers of the filters in the final surveys (2014).

Filter	Layer	Moisture content (%)	
MIX PUM-GRAVEL	pumice		29±0.7
	coarse gravel		0.4±0.04
	fine gravel		2.4±0.8
		average	11
PUMICE	0-28cm		28±6
	52-70 cm		33±3
		average	31
GIANT REED	0-28cm		52±15
	52-70 cm		75±2
		average	64
TOPS	-		-
GRAVEL	0-28cm		0.6±0.3
	52-70 cm		1.3±0.3
		average	0.9
BAMBOO	0-28cm		55±4
	52-70 cm		54±3
		average	54

A

B

Figure 4.80 State of the bamboo (A) and giant reed (B) filters observed during the final surveys (2014).

<u>2. Evolution of organic media</u>

The chemical composition of the two organic media used for the treatment of the piggery wastewater changes over time (Table 4.27). Considering the never used media (year 2012), the giant reed contained more hemicelluloses than the bamboo (27% vs. 17%) and lower content of lignin (12% vs. 18%). A higher hemicelluloses content indicates more moisture absorption and then easier biodegradation (microbial attack), while the higher lignin shows higher material rigidity (Azwa et al., 2013). Similar values of these parameters for bamboo was found in Azwa et al. (2013). In wood, the total concentration of hemicellulose s usually ranges from 20 to 30% (Berg and McClaugherty, 2008). In a natural fibre, the transport of water can be facilitated by three mechanisms which are by diffusion inside the matrix, by imperfections within the matrix (microspace, pores or cracks) or by capillarity along the fibre/matrix interface (Beg and Pickering, 2008; Assarar et al., 2011). When natural fibre is exposed to moisture, water penetrates and attaches onto hydrophilic groups of fibre, establishing intermolecular hydrogen bonding with fibres and reduces interfacial adhesion of fibre–matrix. Degradation process occurs when swelling of cellulose fibres develop stress at interface regions leading to microcracking mechanism in the matrix around swollen fibres and this promotes capillarity and transport via microcracks (Azwa et al., 2013). After long period, biological activities such as fungi growth degrade natural fibres (Chen et al., 2009). In both substrates, it is evident as over time the hemicelluloses decreased, till 16.4% in bamboo and 20.8% in giant reed, and the lignin content increased (till 19.2% in bamboo and 18.7% in giant reed), in a period of almost 2 years (Table 4.27). The lignin content increment can be seen as the material degradation (Barassa et al., 1992; Manfredi et al., 2006). For lignocellulosic fibres, ageing or degradation occurs due to ultraviolet radiation absorption by lignin, formation of quinoid structures, Norrish reactions, and reactions of photo yellowing that occur in lignin (Beg and Pickering, 2008). Generally, cellulose is responsible for strength of fibres, hemicelluloses for thermal, biological and moisture degradation, while lignin for UV degradation and char formation. Besides, weathering causes degradation of natural fibers through photo-radiation, thermal degradation, photo-oxidation and hydrolysis. These processes result in changes in their chemical, physical and mechanical properties (Azwa et al., 2013).

Table 4.27 Chemical composition (wt% on dry basis) of the two natural fibers detected over time and subdivided by depth (28 and 52 cm) in 2014, with average value.

			Hemicelluloses %wt	**Cellulose** %wt	**Lignin** %wt
GIANT REED	Never used (2012)		26.5	49.2	11.8
	End 2012		23.6	49.4	13.6
	2014	28 cm	20.7	34.2	18.1
		52 cm	21.0	36.3	19.3
		average	20.8	35.2	18.7
BAMBOO	Never used (2012)		16.6	51.0	18.0
	End 2012		16.6	54.6	19.8
	2014	28 cm	17.0	51.7	19.2
		52 cm	15.7	48.2	19.1
		average	16.4	49.9	19.2

3. C, N, S and organic matter contents

The analysis on carbon, nitrogen and sulfur contents on pumice, giant reed and bamboo substrates showed different results between the pumice and the organic materials (Table 4.28). Regarding the pumice, the never used material showed chemical contents around to 0.1% of C and S, and 0.04% of N. The pumice media used for the wastewater filtration, instead, presented an increment of the C (almost 7 times more than the never used pumice) and N (6 times more than the never used pumice) contents, while the S decreased (Table 4.28). It is known that it is a porous material, presenting higher water retention and higher chemical sorption compared to other not-porous substrate as gravel or sand (Farizoglu et al., 2003) , as shown in this study. Over time, this material adsorbed but also desorbed chemical compounds, as shown for other porous materials (like zeolites). Pollutants removed by the adsorption process may subsequently desorb (Chang et al., 2010). Moreover some studies (Woodcock et al., 1999; Eun et al., 2000; Caro et al., 2000; Mueller et al., 2008) reported the feature of porous materials, as zeolitic materials and lightweight aggregates, to expand the volume according with the temperature (thermal expansion), leading to a modification of the chemical composition. The expansion is affected by the Si and Al content, as indicated in Woodcock et al. (1999), in which the more siliceous zeolites had much greater expansion.

In the organic substrates high C quantity was found. The never used materials showed C, N, S contents of 43.9%, 0.41%, 0.19% in giant reed and 46.4%, 0.27%, 0.10% in bamboo, respectively (Table 4.28). Considering the never used media, the C:N ratio was 108 in giant reed and 169 in bamboo. Saliling et al. (2007) reported initial C:N ratio of 394 for wood chips and 135 for wheat straw. A lower C:N ratio indicates faster decomposition (Enriquez et al., 1993; Saliling et al., 2007). Woody plants have high C contents, as cellulose, hemicellulose and lignin are carbon rich polymeric materials used to produce physical support structures to plants, and are extremely low in N (Berg and McClaugherty , 2008). According with these results, Aber et al. (1990) found N concentration of 0.09% for red maple and 0.04% for white pine.

Regarding the chemical content after the piggery wastewater treatment, bamboo had a low increment of the N and S contents, while giant reed presented values of 1.75% of N and 0.45% of S (Table 4.28). Enriquez et al. (1993) affirmed that higher is the N content, higher is the decomposition rate, as N promotes the growth of decomposers microorganisms. For both materials the C was instead similar to the never used materials. Considering these values the C:N ratio was 24 for giant reed and 88 for bamboo. In general the bamboo material presented higher C:N ratio than the giant reed. Furthermore over time for both media the C:N ratio decreased, by 78% for the giant reed and 48% for the bamboo. Low C/N ratios favor biodegradation (Saliling et al., 2007).

During the degradation of these organic media, the N becomes bound in microbial biomass, and thus less available, as other nutrients. Moreover mineralization of C,N,P and S and degradation of proteins occur, thus converting a fraction of the assimilated nutrients into more available forms. With the mineralization there is the formation of inorganic N, P, S, in excess of microbial needs (Stevenson and Cole, 1999). Assimilation by synthesis of microbial cells and other organisms (immobilization) can also take place. During decay by microorganisms, much of the C is released to the atmosphere as CO_2, but a significant portion remains behind as soil organic matter and microbial components. Part of the native humus is mineralized concurrently (Stevenson, et al 1999). Chemical mechanisms are also related to the chemical content modification during degradation. For example, the formation of phenolic groups and quinones during this process led to the fixation of NH_3. Thus phenolic groups and quinone and also carbohydrates may react with NH_3, producing fixed N. The fixation process involves ammonia and therefore the reaction is faster at higher pH values(Berg and McClaugherty, 2008).

The higher content of S detected in giant reed could be due to the medium adsorption of S present in the wastewater, but also the mineralization process should be considered.

Table 4.28 C, N, S contents (%) detected in pumice, giant reed and bamboo substrates, in two moment of the experiment: in 2012, before the treatment (never used), and in 2014, after the treatment (at the two depths of the filter, with average value).

		C %	N %	S %
PUMICE	never used	0.09	0.04	0.12
	28 cm	0.69	0.24	0.06
	52 cm	0.52	0.23	0.04
	average	0.60	0.24	0.05
GIANT REED	never used	43.90	0.41	0.19
	28 cm	41.37	1.95	0.49
	52 cm	43.10	1.55	0.40
	average	42.24	1.75	0.45
BAMBOO	never used	46.38	0.27	0.10
	28 cm	46.48	0.54	0.13
	52 cm	46.25	0.54	0.12
	average	46.37	0.54	0.12

Regarding the organic matter quantity detected in tops and gravel, results showed highest content in the tops medium, (almost 35 g/Kg), followed by fine gravel (29 g/Kg) and coarse gravel (12 g/Kg) (Table 4.29). Both tops and gravel are not porous materials, and the reason that tops showed highest organic matter content is due to the grooves on their surface and to the “cup effect” given to their arrangement inside the filter. Regarding gravel, the particular size played an important role for the organic matter holding.

Table 4.29 Organic matter content (g/Kg) detected in the tops and gravel substrates.

	Organic matter (g/Kg)
TOPS	34.90
FINE GRAVEL	29.79
COARSE GRAVEL	12.26

4. Ammonium desorption of pumice

In the ammonium desorption test, the pumice material never used presented on average 0.14 g/Kg of NH_4. The contents detected in the pumice used for the treatment of the piggery wastewater were 0.83 g NH_4/Kg in the filter 1, 0.90 g NH_4/Kg in the filter 2 at 28cm of depth and 0.73 g NH_4/Kg in the filter 2 at 51 cm of depth (0.81 on average in the filter 2). To interpret these results, a balance in the NH_4 quantity was done (Table 4.30), considering the inlet quantity of the two experimental years and the medium final dry weight (detected in the final survey; drying at 65°C). Assuming that all the filter volume was soaked, 437 g NH_4/m^3 the pumice of the filter 1 (8.3% of the NH_4 entered) and 499 g NH_4/m^3 would be desorbed by the pumice of the filter 2 desorbed (11% of the NH_4 entered). In general these values suggest that beyond the feature of this material to have high exchange capacity of NH_4 (as previously verified), a lot of variable must be considered. Among these there are: 1) the presence of other cations in the wastewater (such as Mg^{2+}, Ca^{2+}, K^+) which compete with NH_4^+ for the ion-exchange; 2) the higher grain size of the pumice used in the filter compared to those tested in the CEC test, having lower NH_4 adsorption capacity for the lower specific surface area (Ames, 1960; Hlavay et al., 1982); 3) the organic matter of the wastewater which could result in the decrease in permeability of filtration media for surface clogging. Wang and Peng (2010) reported that the presence of cations such as $Ca^{2+,}$ Mg^{2+} and Na^+ in the feed solution reduced the clinoptilolite adsorption capacity to about 11.68 mgNH_4^+/g, by about 24%.

Table 4.30 Balance of the NH_4 desorbed by the pumice material used in the filters 1 and 2. Total NH_4 quantity received by the filters(g/m^3) and NH_4 desorbed (g/m^3, and % of desorption compared to the inlet).

	INLET NH_4 (g/m^3)		**NH_4 desorbed (g/m^3)**	**NH_4 desorbed (%)**
	2012	**2013**		
F1	232	5,007	437	8.3
F2	-	4,554	499	11.0

CCW

The analyses on LECA revealed that this natural material , never used (N.U.) for the treatment, contained almost 0.25% of S, 0.03% of N and 0.3% of C (Figure 4.81). After the use as media for plant growth in the CCW, the contents of C and N increased, while S decreased. The diminution of S content can be related to the feature of this porous material, as zeolitic materials, to expand the volume with the temperature, leading to a modification of the chemical composition, as previously reported, but also adsorption/desorption processes must be considered. In general, the lowest C content was found in the PUCPA/BLANK line, in the range of 1.35-0.44% , while the highest was found in SALFR line (0.55-1.59%) (Figure 4.81). The N was more similar among the treatments, with highest values for LECA of the SALFR line and contents between 0.05-0.14% (Figure 4.81). The S was highest in the LECA filling the PUCPA/BLANK line (0.12-0.17%) and lower in the other lines, between 0.09-0.14% (Figure 4.79). Although there is a general decrease for all contents of LECA from the L1 to the L3, not differences were found among the levels.

Significant differences were found among levels for the C, N and S, considering all treatments (Figure 4.82). The C content of the LECA in the L1 (median 1.17%) was significantly greater than the C in the L3 (median 0.53%). The N in L1 (median 0.12%) was significantly higher than L3 (median 0.06%). Finally, also the S content with 0.13% as median in L1, was significantly higher than the third level (median 0.10%).

These results confirmed that the tanks situated to the highest level (L1) received more nutrients than the tanks situated to the lower levels, in which the nutrients quantity increasingly decreased from the L1 to the L3. The progressive reduction of the nutrients content from the L1 to the L3 is due to the chemical, biological and physical processes that occur in constructed wetlands.

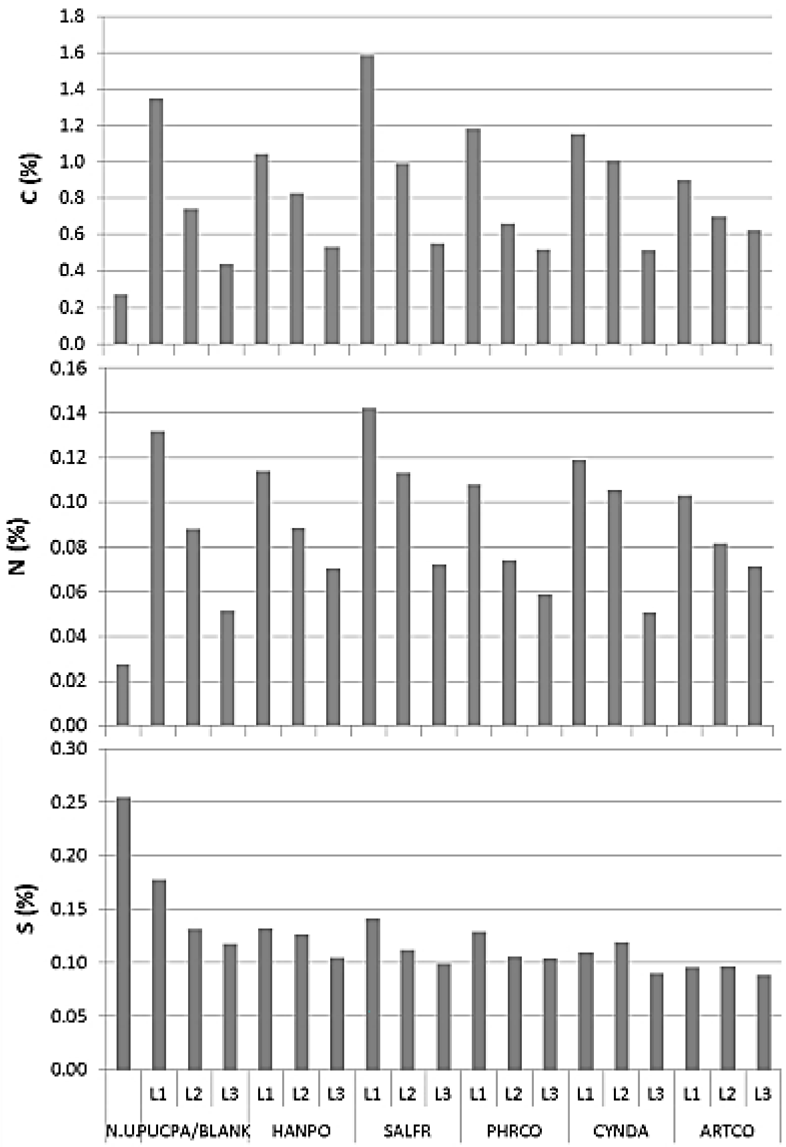

Figure 4.81 Contents (%) of C, N and S found in the LECA used as plant growth medium, subdivided for treatment and level. “ N.U.” is the LECA never used (clean).

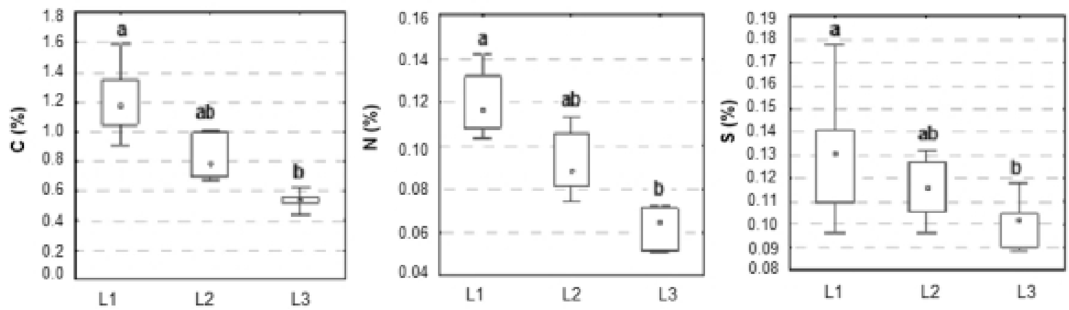

Figure 4.82 Contents (%)of C, N and S found in the LECA subdivided by level. Different letters indicate statistical differences among plant species (Kruskal-Wallis test p<0.05).

Overall considerations

Piggery wastewater

The characteristics of liquid pig manure vary highly depending on the amount of water used to clean the stable and the kind of pits used to collect the slurries (Sánchez and González, 2005), animal feeding habits, zone climatology (González-Fernández et al., 2008), as well as the number of animals on the farm, their health state, feed composition (Suzuki et al., 2010), means used for cleaning, washing and disinfection, and the sort of drugs used for animal treatment and prevention from diseases.

In this study, the piggery wastewater tested for two years in this research presented alkaline characteristics, with pH ranging between 7.6 and 9.5, with mean value of 8.0. The salinity was in the range 6.4-13.4 mS/cm, higher in the second year in which it was around to 12.5 mS/cm. To enhance the feed flavors and maintain cation–anion balance in the diet, salts are commonly added to animal feed (Dong et al., 2001; Goff, 2006). Salinity is defined as a kind of nonpoint pollutions like nutrient pollution by N and P, sediment, pesticides and pathogens (Shortle et al., 2001). Omeira et al. (2006) compared pH and electrical conductivity from different chicken types and production systems, and the results alerted farmers to possible salinity by application of litter with high EC values. Feeding of livestock influences nutrient flows and pollution sources at farm level in many different ways. Nutrients found in the manure, or in compounds emitted to the air and water, originate from the fraction of the feed which is not retained by the animals (Li-Xian et al., 2007). This indicates that manipulation of the diet could be an efficient way to control the amount of manure produced and its composition. The high electrical conductivity of slurries, and consequently the high concentration of soluble salts, suggests that addition of these wastes to soils without a percolating water regime would cause salinization. The turbidity of the piggery wastewater in this study oscillated between 1,110 NTU and 3,890 NTU.

In the second year the piggery wastewater presented higher chemical concentrations, with TN in the range 456-1,210 mg/L, that remained around to 1,050 mg/L in 2013, represented by the 76% by the ammonium-N in the second year, in the range of 210 mg/L in the first year - 800 mg/L in the second year (till also 960 mg/L). High ammonium concentration normally found in the piggery wastewater is the principal contaminant causing eutrophication of water body. It can be also higher than 3,000 mg/L (Im and Gil, 2011). The NO_3-N was instead in the range 20-128 mg/L. The TP ranged from 22 to102 mg/L, formed by about 90% of soluble-P, between 22-99 mg/L. The COD of the piggery wastewater oscillated from 2,100 to 5,000 mg/L (till 6,831 mg/L in 2012). Finally the BOD_5 was in the range 500-1,200 mg/L.

Filters performances

In this research it is clear the effect for physical and chemical parameters of the wastewater recirculation in filters. Generally, increasing recirculation the progressive reduction of the electrical conductivity and the turbidity and the rise of dissolved oxygen occurred. The turbidity was lowered till 93% in 2012 and 89% in 2013. For chemical compounds, the recirculation augmentation resulted in the ammonium nitrogen abatement, causing an increment of the nitrate nitrogen; nevertheless the total nitrogen content was reduced. Besides also the phosphorous and the chemical oxygen demand were progressively decreased at the recirculation increase. In general, the recirculation did not show to have effect on TN in the first experimental year, while in 2013 the TN concentration was reduced till 48% and the TN quantity was abated until 70%. The recirculation led to a progressive reduction of the ammonium nitrogen with maximum concentration abatement of 98% in 2012 and 79% in 2013, and a NH_4-N quantity lowered till 99-99.5% in both years. However the recirculation increased the NO_3-N concentration and quantity. Nitrification is in fact a microbiologically mediated process that occurs under aerobic conditions, resulting in the formation of nitrate (Chang et al., 2010). Gold et al. (1992) encountered poor denitrification performance of a sand filter when the nitrified effluent was recirculated through a recirculation tank with an a ratio of 4:1–5:1. TN removal increased from 8.4% (on a single pass through the sand filter) to 20% for recirculation. Again a low BOD_5:NO_3–N of approximately 1:1 in the recirculation tank limited denitrification. Optimal conditions for maximum denitrification occur at a COD: TN ratio in the range of 4:1–5:1 for organic sludge (Henze et al., 1997; Van Buuren et al., 1999). The total phosphorous and the soluble phosphorous were progressively lowered with recirculation in both years, with an maximum concentration reduction of 35% in 2012 and 62% in 2013. The bamboo filter did not showed effect on phosphorous reduction in the first year. The phosphorous quantity (both TP and PO_4-P) was reduced till 61% in 2012 and 80% in 2013. The effect of the oxygen due to recirculation resulted also in the abatement of the COD by oxidizing the organic content of the raw wastewater. Increasing recirculation also the COD was progressively dropped with mass abatement between 66% of 2012 and 80% of 2013; besides also the BOD_5 content was considerably lowered, by 91%. Wei et al. (2010) reported that when the recirculation rate is 1, 4 and 8, the COD reduction is 74.1%, 83.3% and 85.8%, respectively. They reported that the COD removal improves significantly at recirculation rate increasing from 1 to 4. When the recirculation rate is above 4, COD reduction enhances slightly with recirculation rate increasing, which indicates the remainder organic matter in the recirculation water is difficult for degradation by increasing recirculation rate and COD removal of effluent is not easy for further improving (Lai et al., 2008). The increasing of recirculation rate is not only diluting the influent but also prolonging the retention time and contacting time with microorganism for wastewater. According with Wei et al. (2010), the NH_3-N reduction increases slightly with recirculation rate enhancing. When the recirculation rate changes from 1 to 4, NH_3-N removal increases relatively quickly. For the recirculation rate changing from 4 to 8, the changes of NH_3-N reduction is small, which reflects NH_3-N reduction has few relations with the change of recirculation rate. The dissolved oxygen carried in recirculation water is benefit to nitrification, but when the recirculation rate is above 4, the accelerating impacts of dissolved oxygen carried in recirculation water on nitrifying bacteria are to limit (Wei et al., 2010). Brix and Arias (2005) documented the importance of recirculation for the treatment performance also in constructed wetlands. In this regard, the main goal of effluent recirculation in CWs is to enhance aerobic microbial activity through the intense interactions between pollutants and microorganisms, which are close to the plant roots and onto the substrate surface, without significant alterations in the system operation (Zhao et al., 2004). Recirculation in CWs is suggested as an easily applicable and effective method in the vertical-flow systems with high hydraulic conductivity values (Laber et al., 1997; Brix and Arias, 2005). However, in full-scale operating facilities, the recirculation of the effluent must be carefully evaluated as it may increase operation costs given additional energy consumption for pumping.

The study of the single filters over time for the treatment of the piggery wastewater revealed the different features of these low-cost substrates used as filtration media. Considering the results of the 2nd year, in general the filters manifested a decrease over time of the mass abatement for the total nitrogen, ammonium nitrogen, and the chemical oxygen demand. The nitrification was higher at the beginning of the experiment, afterwards it was reduced over time in all filters, with similar final value. Nitrification is a very limiting process in animal wastewater treatment, but a necessary condition to be able to remove large amounts of nitrogen using biological nitrification/denitrification systems (Loehr et al., 1973). With wastes rich in carbonaceous materials, as piggery wastewater, the nitrifying bacteria compete poorly with heterotrophic microorganisms. Nitrifiers need oxygen, lower organic carbon, a surface area, and a growth phase before sufficient numbers are present for effective nitrification. Nitrite produced by the ammonium-oxidizing autotrophs is rapidly oxidized to nitrate by *Nitrobacter* species (Hovanec and Delong 1996). All nitrifiers are obligate aerobes and hence a restricted nitrification under waterlogged or aquatic environments can be observed. In addition, these microorganisms, especially *Nitrobacter*, are fairly sensitive to acidic pH . Temperature and pH are some of the most influential variables affecting the nitrification rate and growth of nitrifying bacteria. Jones and Hood (1980) reported that the optimal temperature for nitrification occurred at approximately 30 °C . They informed that ammonium oxidation was significantly reduced at temperature below 30°C. Cho et al. (2014) experimented an optimal temperatures for a specific nitrification rate between 32.8°C and 34.1°C. In this study the wastewater temperature at the outlet ranged was approximately 30°C in the months of July and August (till 35°C in August), afterwards it decreased around to 18°C in September and 12°C in October. The nitrification decreased over time in particular in the filter with tops, gravel, pumice and the mix of pumice and gravel, with maximum inlet NO_3-N concentration increment of 3,786% (or 38 times more) in July and 617% (or 6 times more) in November. Giant reed and bamboo, instead, showed similar concentrations, especially from September to November. According with the previous studies, the effect on temperature in this research was observed in nitrification, that decreased at lower temperature. Regarding optimum pH for nitrification, there is a wide range in reported optimal pH (between 7.5 and 9.0). Some studies (e.g. Jones and Hood, 1980; Shammas, 1986) showed that as the pH decreases, the nitrification rate also decreases. Anthonisen et al. (1976) and Jones and Hood (1980) admitted that the optimum pH for nitrification is between 7.5 and 8.5, similar to those of the piggery wastewater treated in this study. Szögi et al.(2004) affirmed that the use of large populations of nitrifying bacteria entrapped in polymer resins offered much faster nitrification rates and avoided the problem of NH_3 volatilisation losses.

Pumice medium gave highest performances in electrical conductivity reduction and chemical contents in terms of NH_4-N, COD and BOD_5. The filter filled with only pumice gave the greater chemical reductions, with concentrations lowered by 99% for NH_4-N, 70% for COD and 95% of BOD_5. The traditional methods of removing ammonia nitrogen involve stripping, chemical precipitation, ion exchange, electric-chemical oxidation and biologic method (Zhang, 2001; Feng et al., 2004; Gao et al., 2008). Pumice material is a light, porous volcanic rock that is formed during explosive eruptions and resembles a sponge because it consists of a network of gas bubbles fixed amidst fragile volcanic glass and minerals (Bush et al., 2001). Pumice is actually a kind of glass and not a mixture of minerals (Juang et al., 2000; Lacour et al., 2001), and has a skeleton structure that allows ions and molecules to reside and move within the overall framework. The structure contains open channels that allow water and ions to travel into and out of the crystal structure. Pumice is used chiefly as an abrasive and is included in many scouring preparations. The sorption ability of this material is certainly the peculiarity that led to the highest abatement of ammonium and organic forms, although no have not been reported bibliographical studies of pumice for these chemical elements. However several other studies confirmed the sorption ability of this material. The adsorption, absorption, ion exchange, and precipitation processes are actually intertwined with the overall physicochemical process when removing nutrient via sorption (Chang et al., 2010). Pumice is used as heavy metals adsorbent (e.g. Lacour et al., 2001; Catalfamo et al., 2006; Yavuz et al., 2008) and as a biofilm support material in water and wastewater treatment because of its high porosity and large surface area (Di Lorenzo et al. 2005; Nabizadeh et al., 2008). The natural and modified pumice were explored to be a better adsorbent for organic and inorganic water pollutants in the recent years (Kitis et al., 2007; Ahmadian et al., 2012). Besides pumice has been found to be effective for the removal of phosphorous (Onar et al., 1996; Njau et al., 2003). The lack of information on the capacity of this material to remove ammonium and organic forms suggests that probably pumice is not used for the treatment of these compounds, maybe for its properties, besides it low-cost, and that certainly the presence of zeolite (chabazite), in our case, could have influences (increased) its chemical removal.

On the other hand, pumice showed a decrease over time on turbidity reduction, compared to the other materials. Farizoglu et al. (2003) reported that pumice used as filter bed gives higher turbidity reduction compared to sand, as it presents bigger particles which are retained in the filter bed, and smaller particles are retained inside the pores of the pumice. Consequently, in a pumice bed clogging progresses more slowly, and the volume of the bed is used more efficiently than in a sand bed. As smaller particulate material is deposited inside the pores of pumice grains, the filtration bed can be used more efficiently. Sand, as gravel, instead is not a porous material and particulate material can only be retained in the spaces between the grains in the filter bed, so smaller particulate matter can more easily drain through the sand media and escape in the effluent water. In our study, the turbidity reduction which was not reduced over time, is probably due to the high organic content in the piggery wastewater. However, as previously confirmed, pumice presents high potentialities also for the reduction of this parameter, that could be obtained through backwashing, which effectively cleans the pumice, as demonstrated by Farizoglu et al. (2003).

The nitrification process was particularly observed in the filters filled with gravel, in which the NO_3-N concentration were significantly augmented at the outlet (until +1,290%; almost of 13 times more), as the NO_3-N quantity (till 11 $g/m^3/d$). Szögi et al. (2004) which used a trickling filter 1.5 m × 0.6 m height, filled with marl gravel (pore space 57%), for the treatment of piggery wastewater to enhance nitrification, showed nitrification efficiency of 57% at total N loading rates of 249 g m^{-3} day^{-1}. They reported that an acclimation period of 6 weeks was needed to develop a functional nitrifying biofilm on the surface of the media, indicated by stabilisation of the nitrification activity. In this study, as gravel medium showed the higher nitrification, on the other hand it gave the lowest performances on reduction of the others chemical parameters.

The lowest nitrification occurred instead in the filters filled with tops, bamboo and giant reed, in particular in the first two, which presented a significant lower nitrification than the gravel filter. In these materials, in fact, higher outlet pH compared to the other filters were observed over time, possibly for the poor nitrification process. The process of nitrification, effectively, lead to lowering of pH of the medium due to release of H^+ (Kumar et al., 2015). Probably also for the lowest nitrification, the organic materials used for the filtration in this research, gave significant abatement of total nitrogen concentration, not showed by the other filters, with 38% for giant reed and 48% for bamboo. Besides these materials had natural changes over time, with degradation and decomposition phenomena, which led to chemical and physical modifications on their structure. They showed the highest water retention, compared to the other materials, suggesting that they decreased the porosity over time. Saliling et at. (2007) observed the decline of porosity in Kaldnes, wood chips, and wheat straw over a period of 140 days, from 82 to 37% in Kaldnes, 59 to 23% in wood chips and from 61 to 36% in wheat straw.

Considering the whole two-years period where the filters were tested for the treatment of the piggery wastewater, which corresponded to 244 days, the incoming total nitrogen quantity was between 4,585 and 6,046 g/m^3 per filter (Table 4.31). Every filter exhibited a total nitrogen removal quantity between 790 and 3,129 g/m^3 (4.6-18.2 g/m^3 per day), which corresponded to the 17-56% of the total N removal, with highest value for the bamboo filter and lowest value for the pumice filter. Similar values were obtained from the tops, gravel and mix pumice-gravel filters, with 23-25% of N removal. On the bases of the N contents analysed in the materials or in the organic matter in the case of tops and gravel substrates, an estimation on the N stored in the medium was performed. The estimation was done considering the 50% of the total filter volume, because during the final surveys only a part of the volume of the filter was seen to be soaked of the wastewater. Specifically the filters filled of pumice, the mix of pumice and gravel, gravel presented a soaked area in particular close to the wastewater distribution ring (in the surrounding 10 cm area), for the entire depth. The bamboo and tops materials had irregular soaked area, while the giant reed medium was soaked for all filter volume, especially increasing the depth. On the bases of these observations, in which it is inappropriate to establish for sure the filter volume, an estimation based on 50% of the volume has been proposed for this study (Table 4.31). From this computation, the estimated N stored in the medium was highest in pumice (13%), and lower in tops (8.6%) and in the mix pumice-gravel (7.2%) and the gravel (5.2%) media. For the giant reed and bamboo filters the estimated N stored in the medium was lower compared to the other filter: 1.4% for giant reed and 0.1% for bamboo. This estimation, although it is not exactly precise, may nevertheless clear up the behaviour of the different materials used in this study. The pumice material showed the best treatment performances on NH_4-N removal, meaning that this material can adsorb this N form, an probably the highest N storage is represented by this element. Also the fine gravel, with an intermediate estimated N storage, presented N abatement during the experiment, therefore for its physical properties together the microbial activity, it was able to retain and remove N. The organic media gave the highest N removal in this research, and the low values obtained from the estimation of N stored in they, are probably due to the complex processes of the microbial activity occurred inside the filter, which could have consumed high N quantity.

Table 4.31 Balance of the total nitrogen for the different filters, considering the whole two-years period.

	Total N at inlet g/m^3	**Total N at outlet** g/m^3	**Total N removal** g/m^3	**Total N removal** %	**Estimated N stored in medium*** %
MIX PUM-GRAVEL	5,509	3,927	1,146	23	7.2
PUMICE**	4,585	3,794	790	17	13.0
GIANT REED	5,406	3,088	1,882	38	1.4
TOPS	5,885	4,076	1,373	25	8.6
GRAVEL	5,754	4,015	1,303	25	5.2
BAMBOO	6,046	2,482	3,129	56	0.1

* *Estimation based on 50% of the total filter volume*
** *Tested only in 2013*

The nutrient removal revealed by the filters used in this research was a combination of chemical and physical (e.g. ion exchange, adsorption) processes of the materials, but it was accomplished also through biological process. The filter medium acts as a growth chamber for the microorganisms that are in the filters. Biological filtration has proved one of the most successful methods of rapid purification of used water by aerobic microbial oxidation. It is now the longest established secondary treatment method still widespread used (Bruce and Hawkes, 1983). Normally, the optimum pH is 6.0-8.0 for microorganism growth. The value of pH exceeding this range will result in negative effect on microorganism activity. Yu et al. (2008) reported the optimum pH range for COD reduction is 7.1-8.0. They affirmed that the NH_3-N reduction increases gradually with pH enhancing. and when pH is 10, NH_3-N reduction is highest (95.2%). Hill et al. (2002) informed that the performance of an aerobic biofilter treatment system was significantly affected by seasonal temperature changes in the reduction of enteric microbes in flushed swine waste. In addition to pH, also temperature is one of the important factors for microorganism metabolizing. In some temperature range, metabolism activities of most microorganism reinforce with temperature rising or weaken with temperature declining. The suitable temperature range of aerobic microorganism is 10-35°C. Normally, temperature below 10°C induces negative effect on biological treatment (Wu et al., 2003). Moreover, the medium and filter typology (e.g. filter depth, wastewater load, wastewater type) play an important role for microorganism growth. For example, Wang et al. (2011) observed high activity levels of the immobilised microorganisms on the surface of the fixed zeolite, indicating that the fixed zeolite is a stable and suitable carrier for microbes and that most microorganisms grew on the surface of the fixed zeolite, compared to the sunken zeolite in the bioreactor, mobile and unstable. The distribution of microbes in the liquid phase and on the surface of the support materials were about 5% and 95% respectively. However the microorganisms bond on the surface of the media can reduce the surface area available for nutrients adsorption preventing their removal (Chang et al., 2010).

Pretreatment system performances

Concerning the two years-study of the pretreatment system, interesting results on the piggery wastewater treatment for both physical and chemical parameters were obtained over time.

The electrical conductivity was lowered significantly by the filtration system in both years, with similar reduction (R=19-20%). The pH was instead significantly changed only in the second year, in which also the turbidity was significantly reduced by 48%.

Regarding the chemical parameters, the system tested in 2012 didn’t show significant concentration reduction for the nitrogen (total-N and ammonium-N, which were instead observed in the second year. In the second year, instead, both nitrogen forms were significantly reduced by the system. The organic compounds (COD and BOD_5) were significantly decreased by the filtration in both years, with greatest reduction in 2012 for the biological oxygen demand (A=69%) and in 2013 for the chemical oxygen demand (A=41%). The phosphorous concentrations, in terms of total-P and soluble-P, were instead significantly reduced only the first year.

The observation of the pretreatment system behaviour in these two years was important to detect its performance on of piggery wastewater over time. Different results were obtained in this period. The reason of diverse results can be due to its changing over time, which led to the diversification of the microbial activity, or to the natural modifications of the organic compounds (bamboo and giant reed) which caused physical and chemical changes. However the replacement of a filter occurred in 2013, using different medium: in the first year there was the filter filled with sand and gravel, replaced in 2013 from pumice medium. Therefore also this modification must be considered. Effectively, the system in the first year didn't show significant TN and NH_4-N reductions, which were observed instead in 2013, and the reason of this is probably due to the presence of the filter with pumice in the second year. On the other hand, phosphorous was significantly removed in the first year, besides the shorter working period of the system, and this could be due to the presence of the filter filled with sand and gravel for the first monitoring month. As several authors reported (e.g. Billore et al., 1999), in fact, there are different processes by which phosphates compounds may be removed from wastewater: adsorption, ionic exchange, and absorption. A great variety of different types of materials for P retention have been described, and among these sand filters have proved to be excellent material (Rustige et al., 2003). Because P is removed via sorption and precipitation processes, Ca, Fe and Al content is important in efficient P removal (Vohla et al., 2011).

Regarding the chemical quantities, in the second year the system received greater contents of nitrogen, phosphorous and organic compounds, due to both the higher concentrations of all elements and the higher wastewater volume, compared to the previous year. The highest chemical removal occurred in fact in 2013, especially for BOD_5 (+46%) and total nitrogen (+24%). The mass abatement of the chemical compounds (not considering nitrate-N), which is a significant parameter of the real removal efficiency, was higher in the second year for all chemical parameters, except for the BOD_5. In particular the mass abatement of TN was similar between years (34% of 2012 vs. 37% of 2013), while for NH_4-N the mass abatement was doubled in the second year. However highest nitrate-N generation was observed in 2013.

From these results it follows that the filtering system has been able to reduce the chemical compounds present in the swine wastewater, for a combination of media adsorption, ionic exchange, biological activity processes; besides, it is important to consider that also environmental factors played an important role on chemical removals of the filters.

Phytodepuration performances

The phytodepuration system tested in this two years- research gave an overview for the use of different plant species never previously used in general for the treatment of piggery wastewater. The different conditions on the type of effluent and on the state of the plants that have occurred during this period do not allow an effective comparison between the plants used in the two experimental years. However, several considerations may be given, especially in the case of systems like those tested in this work, where few bibliographic information is available. Effectively, in the first year the plant was fed with a more diluted piggery wastewater, presenting lower electrical conductivity, chemical contents and turbidity compared to the 2^{nd} year. Moreover in 2012 the plants were rooted since 2010, while in 2013 a new phytodepuration system was carried out, using new medium and plants not already rooted.

In both years the cascade constructed wetland showed no change the pH and the electrical conductivity. The dissolved oxygen was significantly augmented by the depuration only in the first year, after the treatment of *M. aquatica* and *C. divisa*. However the turbidity was significantly reduced in both years, in 2012 by the *C. divisa* plants (R=96%), in 2013 by the *P.palustris*/blank, *S.fruticosa, P.australis* and *C.dactylon* treatments (R=82%, on average). Regarding the dissolved oxygen, wetland plants present several physiological or morphological adaptions and different responses to flooding and waterlogging (Kadlec and Wallace, 2008). As other wetland plants, *M. aquatica* supplies its below-ground root system with oxygen by means of aerenchymatous gas-phase transport of either photosynthetically derived or atmospheric oxygen from shoot to root (Pedersen and Sand-Jensen, 1997), which is a more efficient means than through a hypertrophied stem base (Armstrong et al., 1994). The transport of oxygen to roots from lenticles and/or leaves is often evidenced by the oxidation of rhizospheres (Kadlec and Wallace, 2008). Biddlestone et al. (1991) stated that *Phragmites australis* has the ability to pass oxygen, from its leaves through stems and rhizomes and out from its fine hair roots into the root zone or rhizosphere. Different studies reported that the oxygen flux from roots for *P. australis* can be between 1 and 12 $g/m^2\ day^{-1}$ (Lawson, 1985, Armstrong et al.,1990; Gries et al., 1990). Furthermore, wetland plants growing in anoxic conditions can modify their root structure, creating fewer small roots and more large roots, presumably as a defense against the large oxygen supplies demanded by the small roots (Sorrell et al., 2000).

Regarding the chemical compounds, the phytodepuration system was able to reduce in particular the NH_4-N, with the highest reduction in 2012 by all plants (A=99%), while in 2013 the maximum reduction obtained corresponded to 95%. In particular in the first year the ammonium-N concentration remained stable over time, while the following year it increasingly augmented from July to November.

In CWs treatment, ammonium ion is generally adsorbed as an exchangeable ion on clays, and chemisorbed by humic substances, or fixed within the clay lattice. It appears that these reactions may occur simultaneously. The rate and extent of these reactions are reported to be influenced by several factors, such as nature and amount of clays, alternate submergence and drying, nature and amount of soil organic matter, period of submergence and presence of vegetation (Savant and DeDatta, 1982). However also ammonium uptake by plants occurred in CWs (Kadlec and Wallace, 2008). In this study, ammonia nitrogen loss through volatilization would probably be negligible since it generally requires a pH of 9.3 (Jing and Lin, 2004). Ammonium removal by the CCW observed in this research was probably mainly due to the medium sorption, especially observed in 2013. Light expanded clay aggregate (LECA) presents non-bonding electrons of oxygen in SiO_2 which can constitute the complex with ammonium ion in the solution. Therefore, the abundant values of SiO_2 in LECA structure is the most important factor of ammonium ion adsorption from aqueous solution (Albuquerque et al., 2008). Sharifnia et al. (2012) reported that the maximum monolayer coverage capacity of LECA for ammonium adsorption was obtained as 0.255 mg/g, at 25°C. The ammonium adsorption capacity decreases at lower pH values; in low pH values the competition between H^+ and NH_4 occurred, for occupation of the active sites on sorbent surface causes, decreasing than the ammonium ion adsorption capacity. The optimum ranges of pH for ammonium adsorption were reported to be 5–8 and 5–7 by other researchers (Yusof et al., 2010; Liu et al., 2010; Ji et al., 2007).

The phytodepuration system showed the reduction of the nitrate-N concentration only in the first year (by *T.arundinacea* lines in particular), while in 2013 the opposite situation occurred, in which the nitrate-N content exceeded the inlet concentration. Kadlec and Wallace (2008) reported that nitrate uptake by wetland plants is presumed to be less favoured than ammonium uptake. However in nitrate rich waters (as the piggery wastewater of this study), nitrate may become a more important source of nutrient nitrogen. Aquatic macrophytes utilize enzymes (nitrate reductase and nitrite reductase) to convert oxidized nitrogen to useable forms. The production of these enzymes decreases when ammonium nitrogen is present (Melzer and Exler, 1982). Ye and Li (2009) revealed that the nitrification process only occurs under aerobic conditions, at DO concentration at least ≥ 1.5 mg/L. The increase of the nitrate-N in the second year occurred probably for the high oxygenation inside the vegetated tanks. This could be derived from the new plant realization, occurred in July, where new LECA medium was used to fill tanks, and where small and not already rooted plants were planted. The higher porosity and specific surface area than other media of LECA is widely known (Albuquerque et al., 2010). Environmental factors known to influence denitrification rates include the absence of O_2, redox potential, soil moisture, temperature, pH value, presence of denitrifiers, soil type, organic matter, nitrate concentration and the presence of overlying water (Focht and Verstraete, 1977; Vymazal, 1995). Paul and Clark (1996) reported that the optimum pH range lies between pH 6 and 8. Opposed to the results obtained in the present research, many studies have found that high nitrate concentration enhances denitrification rate in wetlands (e.g. Hanson et al., 1994; Sartoris et al., 2000). Higher potential denitrification was detected in stands of emergent macrophytes than in open water (Hernandez and Mitsch, 2007). Denitrification in TWs was estimated to account for as much as 90% of overall N removal (Xue et al., 1999; Lin et al., 2002b). The plants used in the second year, which were not wetland plants (except *P.australis*), probably didn't use nitrate nitrogen for their growth or they were not able for its reduction. However Calheiros et al. (2012) reported that nitrate removal of *S. fruticosa* grown in a medium of Filtralite® and washed sand, for the treatment of tannery wastewater, between 55 and 95% ; however the mean inlet NO_3 was of 23 mg/L, lower compared to the present research. During this experimentation, the nitrate-N reduction achieved by the wetland plant in 2012 was reduced over time. Many individual wetland processes, such as microbially mediated reactions, are affected by temperature, and maybe this is the reason of the nitrate-N reduction over time. Processes regulating organic matter decomposition and all nitrogen cycling reactions (mineralization, nitrification and denitrification) display cyclic seasonal variations (Kadlec and Reddy, 2001). Some studies (Kuschk et al., 2003; Song et al., 2006), stated that nitrogen cycling was inhibited in colder months due to the decrease of oxygen availability. The optimal temperature for nitrifying bacteria in pure cultures ranges from 25 to 35 °C and in soils from 30 to 40 °C (Vymazal, 2005). Cookson et al. (2002) suggested that nitrifying communities can adapt to temperature changes and may maintain their activity at lower temperatures by metabolic adaptation. However, other studies have shown that nitrification is inhibited by water temperatures <10 °C (dropping off rapidly below 6 °C) and >40 °C (Hammer and Knight, 1994; Xie et al., 2003).

The CCW was able to reduce the nitrogen content in 2012, particularly by *T.arundinacea*, while in the second year it was not observed for the increment of the nitrate nitrogen. Removal of total nitrogen in studied types of constructed wetlands varied between 40 and 55% with removed load ranging between 250 and 630 g N m^{-2} yr^{-1} depending on CWs type and inflow loading (Vymazal, 2007). The processes that affect removal and retention of nitrogen during wastewater treatment in constructed wetlands (CWs) are manifold and include NH_3 volatilization, nitrification, denitrification, nitrogen fixation, plant and microbial uptake, mineralization (ammonification), nitrate reduction to ammonium (nitrate-ammonification), anaerobic ammonia oxidation (ANAMMOX), fragmentation, sorption, desorption, burial, and leaching (Kadlec and Knight, 1996;Vymazal et al., 1998; Vymazal, 2007; Kadlec and Wallace, 2008). However, only few processes ultimately remove total nitrogen from the wastewater while most processes just convert nitrogen to its various forms. In coastal and estuarine salt marshes nitrogen appears to be one of the major nutrients limiting the growth of many higher plant halophytes (Valiela and Teal, 1979). The basic characteristics of nitrogen assimilation in halophytes seem qualitatively to be the same as in higher plants (Stewart and Lee, 1974). The sediments of most *H. portulacoides* communities appear to be well aerated and seems likely that NO_3 is the major nitrogen source of *Halimione*. The presence of high levels of nitrate reductase in *H.portulacoides* leaves collected from a salt marsh support this view (Stewart and Lee, 1974).

The phosphorous concentration was reduced in both years (highest reduction in the first year) in 2012 significantly by *M.aquatica* and *T.arundinacea* lines, and in 2013 by all treatments, except those vegetated with *H.portulacoides* and *A.coerulescens.* In particular, in 2013 the increase of the outlet concentration of both TP and PO_4-P was observed over time, probably due to the wastewater temperature decrease. Several studies (Kadlec and

Wallace, 2008; Vymazal, 1995) revealed that wetlands provide an environment for the interconversion of all forms of phosphorus. Soluble reactive phosphorus is taken up by plants and converted to tissue phosphorus or may become sorbed to wetland soils and sediments. Phosphorus transformations in wetlands consists in peat/soil accretion, adsorption/desorption, precipitation/dissolution, plant/microbial uptake, fragmentation and leaching, mineralization and burial (Vymazal, 2007). In organic soils P adsorption has been related to either high Al, Fe or Ca levels and P sorption capacity of wetland soils may be predicted solely from the oxalate extractable (amorphous) aluminum content of the soil (Richardson, 1985). Drizo et al. (1999) reported an estimated maximum phosphorus adsorption of LECA of 0.42 g/Kg, a bit less of zeolite (0.46 g/Kg). LECA systems are known to have an extremely high P-sorption capacity (4 kg /m^3), and have indicated promising P removal (90%) after 2–3 years of operation (e.g. Zhu et al., 2003; Öövel et al., 2007). On the other hand, Farahbakhshazad and Morrison (2003) affirmed that LECA improved equilibrium adsorption characteristics, but uncrushed and within the kinetic constraints of a macrophyte system gave no improvement for P adsorption over sand. Johansson (1997) observed that only a small amount of the applied phosphate was sorbed by the LECA, affirming that it could be considered as chemically non-reactive.

Regarding the organic chemicals, the CCW showed significant reduction only for the BOD_5 concentration in 2012, by *M.aquatica* and *C.divisa* treatments. The COD concentration was lower than the inlet in both years but not significant reduction were observed. Moreover for this parameters the increment over time of the outlet concentration was observed both in 2012 and 2013, especially in the months of November. The high removal rate of BOD_5 and COD is caused by filtration/sedimentation of suspended solids and bacterial oxidation (Babatunde et al., 2008). Lee et al. (2004) reported a median COD concentration reduction from 1,160 to 264 mg/L (77% abatement) obtained with a horizontal subsurface wetland with an HLR of 12 cm/d fed with pig manure after pre-treatment by solid separation followed by anaerobic digestion and aerobic oxidation. During the whole monitored period HCW system reduced the median inlet COD concentration (about 500 mg/L) by 50 to 80%, with a median of 56% and an HLR of 3.8 cm/d. Kantawanichkul and Neamkam (2001) investigated the efficiency of a pilot combined wetland system (VF unit followed by an HF unit) in treating raw swine waste without recirculation of the effluent and a hydraulic loading rate (HLR) of 3.7 cm/d. The combined system reduced the average COD inlet concentration from 2,800 to 43mg/L, with an abatement of 98%. Calheiros et al. (2012) observed mean COD reductions of *S.fruticosa*-planted CWs in the range of 36-66% with an HRT of 60 mm d^{-1} and 48-60% with an HRT of 210 mm d^{-1}, higher values compared to this research. While similar to results obtained in this study, they observed the BOD_5 reduction by 44-73% with an HRT of 60 mm d^{-1} and 62.7-76% with an HRT of 210 mm d^{-1}. Caselles-Osorio et al. (2007) experimented the COD removal of HSSF CWs planted with *P.australis* (gravel medium) fed with high organic loading rates (23 g COD/m^2 day^{-1}) (lower compared to the present research in 2013), founding COD removal rates of over 90%.

The plants more efficient in terms of aerial dry biomass production in the two years-period have been *C.dactylon* (almost 3,300 g/m^2) and *M.aquatica* (1,464 g/m^2). These plants, although the lowest nutrients concentration (especially N) compared to the other plants, gave the highest nitrogen and phosphorous uptakes (77 g N/m^2 of *C.dactylon* vs. 36 g N/m^2 of *M.aquatica*). On the contrary, the plants with lowest dry matter production and nutrients uptake were *C.divisa* (396 g/m^2 dry matter) in 2012 and *P.australis* in 2013 (172 g/m^2 dry matter). However, *P.australis* showed the higher N e P concentrations on aerial part than the all other plants tested in this work.

Some studies (Glenn et al., 1999; Ventura et al., 2011) revealed that halophytes can yield as much as conventional crops, even when irrigated with seawater. The yield potential of a halophyte plant depends on the plant species and the salt concentration to which the crop is subjected during cultivation (Lieth, 2000). The most productive species yield 10 to 20 ton/ha of biomass on seawater irrigation, equivalent to conventional crops. However, growth optimums for most halophytes range from 50 to 250 mM NaCl (Flowers et al., 1986), but for some extreme examples, that value is between 200 and 400 mM NaCl (Flowers and Colmer, 2008). Considering these values, the wastewater used in our research was higher than the minimum salinity required for halophytes growth (around to 7-8mS/cm, or 80mM NaCl). However, studies on halophytes were usually conducted in short term (14–60 days) under controlled environments, and the data provided for yield performance are typically based on extrapolations from small-scale experiments (Flowers and Colmer, 2008; Zurayk and Baalbaki, 1996).

When grown in constructed wetlands, *Sarcocornia* shown to function as efficient biofilters for the removal of nutrients from mariculture effluent while concurrently producing high yields of valuable by-products of vegetables, oilseed crop, or raw materials for the pharmaceutical industry (Shpigel et al.,

2007). Considering the biomass production of *P.australis,* as typical wetland plants, Vymazal et al. (1999) reported values from eight systems in Europe, North America, Australia and Africa between 788 and 6,334 g DM/m$^{2.}$ Maximum dry weight values of aboveground *P. australis* biomass reported in the literature vary between 1.17 kg/m² (Rothman and Bouchard, 1990) to 7.70 kg/m² (Hartzendorf and Rolletschek, 2001). Soetaert et al. (2004) stated that typical production of mature *P. australis* is around 1 kg/m² of dry weight. It is clear that the environmental conditions plays an important role on plant growth and production, and different can be the results. However, values of dry aboveground biomass production similar to those reported by these studies for *P.australis* can be observed for *H.portulacoides* and especially for *C.dactylon* that exceeded 3 kg/m^2.

From the literature the aboveground N concentrations observed in *P. australis* used to vegetate constructed wetlands can vary from 0.5 to 0.27% (Greenway, 1996; Haberl and Perfler, 1990; Vymazal et al., 1999). However, this study accorded with Gries and Garbe (1989), which reported values up to 0.35% and with Vymazal (1995) which informed values up to 0.30%, suggesting the potential nitrogen uptake of this plant species. Regarding the P nitrogen content on aerial part of *P. australis,* similar results were obtained by Greenway (1996) (0.2-0.18%). However other researches (Gries and Garbe , 1989; Vymazal et al., 1999) showed higher P values on aboveground part , till 0.27-0.34%. Similar P contents (0.3%) were observed for all wetland plants used in 2012.

The nutrients uptake of the plants used in this 2 years-experiment was very different according to the plant species. Brix and Schierup (1989) suggested that standing stock in aboveground biomass of emergent macrophytes, and thus available for harvesting, is roughly in the range 3–15 g P/m^2 year^{-1} and 20–250 g/ m^2. Vymazal (1995) informed of yearly aboveground phosphorus standing stock in the range of 0.1–11 g P/m^2 and 22–88 g N/ m^2 for 29 various emergent species. Moreover Johnston (1991) gives the range for nitrogen standing stock in emergent species between 0.6 and 72 g N/ m^2 with an arithmetic mean of 20.7 g N/ m^2. Vymazal (2010) reported for *P.australis* aboveground uptakes of 28-36 g N/m^2 (average 32 g N/m^2), and 1.3- 2.2 g P/ m^2 (average 1.7 g P/ m^2). Considering these values, *M.aquatica* grown in the CCW presented similar N uptake of *P.australis* tested in Vymazal (2010), with 36 g N/m^2 , lower in *T.arundinacea* and

C.divisa. Instead, considering the plants species in 2013, the N uptake of *C.dactylon* exceeded by far the values reported for *P.australis*.

On the base of these considerations, the performances of the phytodepuration system derived from complex interactions between environments, plants, substrates, microbial activities and also type of effluent used. Summarizing, the system received a total N inlet quantity between 89 and 127 g/m^2 in 2012 (1.2-1.8 g/m^2/d), and between 1,064 and 1,295 g/m^2 (7.5-9.1 g/m^2/d) in 2013 (Table 4.32). The wetland plants used in the first year showed the highest N storage in the aerial part, between 12 and 29% of the total N received (Table 4.32), versus 7% on *C.dactylon* plants, and values lower than 3% for the other plants. Similar to this study, Vymazal (2008) defined nitrogen accumulation very low the range 0.51–3.84% (of total nitrogen loading) for *Typha angustifolia*, used to vegetate an up-flow bed, and 0.43–3.68% (of total nitrogen loading) for *Cyperus alternifolius*, used to vegetate a down-flow bed, in a period of 14 weeks. In the present study, a considerable part of the total inlet nitrogen, instead, was retained in the light expanded clay aggregates used as medium, with values between 3.5 and 5.2%, undoubtedly adsorbed as ammonium-nitrogen (Table 4.32).

Table 4.32 Balance of the total nitrogen for the different treatments of the CCW, considering the whole two-years period.

Plant	**Inlet TN**		**N on aerial part***	**N on LECA***
	g/m^2	g/m^2/d	%	%
PHAAP	101	1.4	20	-
MENAQ	127	1.8	29	-
CRXDW	89	1.2	12	-
PUCPA/BLANK	1,154	8.1	-	4.0
HANPO	1,295	9.1	2.4	3.6
SALFR	1,064	7.5	1.6	5.2
PHRCO	1,161	8.2	0.5	3.5
CYNDA	1,155	8.1	6.7	4.1
ARTCO	1,157	8.1	2.8	3.8

**Compared to the total inlet.*

Conclusions

The integrated pilot system tested for almost three years proved to be an efficient economic solution to treat piggery wastewater. The combination of a filtration system to pre-treat the wastewater, composed by economical, easily available and disposable materials, with a phytodepuration system which occupies a restricted area, for the treatment of the filtered wastewater, characterized by low energy demand and low management, produced interesting results for the treatment of piggery wastewater.

The filtration system tested in this research could represent a viable alternative to the other types of pretreatment. The readily available and low-cost materials (gravel, pumice, sand, organic media, tops) proved to reduce the high salinity of the piggery wastewater and the abatement of nitrogen, phosphorous, chemical oxygen demand and biological oxygen demand. Overall the filtration system was able to remove 11,803 g N/m^3, with a daily removal of 48 g N/m^3 and a mass abatement of 36%, with highest performances over time. The oxygenation occurred in the system led to the abatement of the principal pollutants present in the piggery wastewater for at least the 50% , until almost 90% for the ammonia-nitrogen, improved with recirculation. On the other hand, the nitrification occurred. As some studies reported (e.g. Martinez, 1997), nitrification is becoming an increasingly important component in total farm management systems, to the point where the effectiveness of any biological nitrogen removal system that includes a nitrification step treatment depends on the ability of nitrifying organisms to oxidise ammonia. The different materials used as filtering media showed different features, related to their physical and chemical compositions. Pumice for its ion-exchange ability demonstrated the highest performances for the removal of ammonium, COD, and BOD_5, although its high particle size and high organic matter in wastewater. Giant reed and bamboo manifested to be the best materials for the total nitrogen removal, probably obtained from the increment of the internal porosity for degradation and decomposition, which led to an higher N adsorption, combined with microbiological activity. Tops and gravel represented the substrates with no adsorption and ion-exchange properties, however they proved to remove all pollutants, with mass abatements >30% TN, >90% NH_4-N, >40% TP, >50% COD, >80% BOD_5. Filters represented, hence, attached systems for bacteria growth. In filters, biofilm consisting of microorganisms, particulate material, and extracellular polymers is attached and covers the support packing material, allowing the pollutants removal and degradation (Loupasaki and Diamadopoulos, 2013).

Generally, biological filtration uses as biofilm-supporting carriers thermoplastic polymers, which cause potential ecological damage because of the low biodegradation and high accumulation in organisms (Zou et al., 2014). For this reason the use of organic and natural materials for greater sustainability is becoming an important factor in the wastewater treatment, with the employment for example of wheat straw, bamboo, wood chips, peat moss etc. Among the different studies, Zou et al. (2014) tested bamboo in a biological contact oxidation process system with the goal to develop a biodegradable and sustainable biofilm medium, affirming that bamboo represents a viable biofilm carrier for wastewater treatment in the future. Furthermore, the biofilm development was not only affected by the tenacity and antimicrobial activity of bamboo. However, also the all other media tested in this research have good features to allow the growth of microbial activity, as shown by the results.

The wastewater obtained by filtration represented to be ideal for treatment by plants, although the high nitrate-nitrogen content. In this study, than, the increment of the NO_3-N after filtration has not been seen as negative, on the contrary it represented a positivity for the depuration by plants, which require macronutrients like nitrate for their growth, avoiding ammonia nitrogen toxicity at high concentrations. Ammonium , in fact, may cause growth inhibition in many species, particularly in those grown under arable conditions (Marschner ,1999). Under saline conditions, ammonium increases the sensitivity of plants whereas nitrate has been reported to moderate the negative effects of salinity (Khan et al. 1994). Tanner et al. (1995) stated that ammonium toxicity tends to differ between species, although the mechanism of ammonia toxicity to wetland plants remains unclear. Jingtao et al. (2012) reported that *P. australis* grew well at ammonia concentrations of up to 160 mg/L, but growth was inhibited at levels higher than 640 mg/L. In a related study, Szögi et al. (2003) showed that constructed wetlands can remove large amounts of N from pig wastewater through denitrification, but their performance is limited by NO_3^- availability. Using a nitrification pretreatment in constructed wetlands, Humenik et al. (1999) reported total N removal potentials of 14,000 kg ha^{-1}, which was more than five times the N removal obtained without nitrification pretreatment. Than a nitrification pretreatment represented a viable solution for CWs.

The phytodepuration system demonstrated to be able on the treatment of the piggery wastewater, with best results on chemical removal given by the wetland plants tested in 2012. Considering the overall experimental period, the phytodepuration system proved a total N removal of 243 g/m^2 (3.37 g/m^2 per day) in 2012 and of 802 g/m^2 (5.7 g/m^2 per day) in 2013, with total N abatements of 76% in the first year and 11% in the second year. The best plant species on the biomass production and N uptake were *M.aquatica* and *C.dactylon*, although they gave the highest water consumption. The system vegetated with halophytic plants didn't show the ability of these plants to treat the wastewater and *H.portulacoides* and *A.coerulescens* showed the lowest performances on the treatment of the piggery wastewater, however they presented higher N and P contents in the aboveground part; this can be due to a previous accumulation of these nutrients. These results can be related to several factors: 1) the planting in late summer, which caused stress to plants and led to a short time for their development; 2) the planting in LECA medium, very different from the soil found in salt marshes; 3) high nutrients concentrations and high organic load (N>10 times in 2013 compared to 2012), which led probably to plant stress and N release. In this system the medium for plants growth (LECA) proved to interfere with chemical removal and turbidity, giving similar results of tanks vegetated with *P. australis*, *C. dactylon*, *S. fruticosa*. However also the microbial activity must be considered in the chemical removal. The substrate influences the establishment of microbial biofilms and the microbial community structure within complex wetland ecosystems, as well as the treatment performance. A porous matrix, such as expanded clay, provides a greater surface area for treatment contact and biofilm development. Calheiros et al. (2009) investigated the bacterial communities in the CWs with different soil materials, i.e., two types of expanded clay aggregates (FiltraliteMR3-8-FMR and Filtralite NR3-8-FNR) and fine gravel. Higher pollutant removals in terms of COD and BOD_5 were achieved in the expanded clay planted units after a long-term operation (31 months). The similar behavior of the expanded clay systems concerning the pollutant removal may be attributed to the fact that they may have similar functional group of microorganisms (Calheiros et al., 2009).

Considering the emission limits impose by the Italian regulation (Legislative Decree 152/06) for the discharge in surface waters the cascade constructed wetland showed not lower COD values than limits, while BOD_5, TP, NH_4 and $N\text{-}NO_3$ values agreed with the regulation in 2012, and only the NH_4 concentration at the outlet of the treatments was within the limit.

For a future prospective, enhancements on the pretreatment system regard a better study of wastewater load and hydraulic retention time, to increase the contact time of filtering volume with wastewater, enhancing the microbial growth and the chemical-physical processes of the medium. Moreover a more detailed research could be done on recirculation, considering the energy cost and the efficiency of the system. Besides the use of other filtering substrates able to remove phosphorous could be tested for the treatment of piggery wastewater, with particular attention to the grain size, to avoid clogging. Regarding the phytodepuration system, further research on dilution of the piggery wastewater at the inlet, to understand the different plant responses to salinity, organic load and chemical contents, must be carried out, with the aim to minimize it to reduce useless costs. Moreover a comparison between wetland plants and halophytic plants, tested in the same environment and with same experimental conditions (e.g. same wastewater type, load, HRT) could be done to understand the real behaviour of halophytes. Several studies, in fact, report the potentiality of these plants for nutrients removal (Valiela and Teal, 1979; Stewart and Lee, 1974). Finally, an improvement of the phytodepuration system could be done, with technologies which can improve the removal of chemical compounds. Among these, an integration of tanks which could promote anaerobic conditions, than the denitrification process, to enhance nitrogen removal. Effluent recirculation could be another interesting solution to improve the effluent quality of CWs (especially the COD and NH_4 removals), as reported in many studies (e.g. Stefanakis and Tsihrintzis, 2009; Foladori et al., 2013; Huang et al., 2013). The use of recirculation to enhance the performance in CWs depends on many factors, including the CW types and influent loads. Stefanakis and Tsihrintzis (2009) not support the idea that effluent recirculation can improve the removal rates. The effluent recirculation negatively affected wetland performance, which resulted in a reduction of all pollutant removal rates. Moreover, in full-scale operating facilities, this modification may increase operation costs given additional energy consumption for pumping. Moreover others could be the solutions to improve the performances of the CW, like the use of physic-chemical amendments or increase of plant harvesting (Vymazal, 2010).

In conclusion, the piggery wastewater was efficiently treated by the integrated pilot system, considering that not similar studies were previously experimented. All filters, except sand medium which had clogging, demonstrated good performances for its treatment and between the plants tested, wetland plants and especially *Cynodon dactylon* can give excellent performances for the treatment of organic or livestock wastewaters.

Chapter V

TREATMENT OF PRETREATED PIGGERY WASTEWATER WITH *HAEMATOCOCCUS PLUVIALIS* WITH POTENTIAL PRODUCTION OF LOW COST ASTAXANTHIN

THE CASE STUDY

This study was done in collaboration with Gruppo Ricicla - DiSAA, Università degli Studi di Milano (Ledda C., Tamiazzo J., Borin M, Adani F. *In submission*) at the end of the first PhD year (2012). The aim of this work was to evaluate the reduction of the raw piggery slurry organic and mineral pollutants by a two-step filtration-microalgae system, coupled to biomass production. Besides, as an added value of the process, astaxanthin accumulation under nutrients-deprived conditions has been evaluated.

Premise

Managing the input of organic and mineral nutrients derived from livestock activities into the atmosphere and water-bodies poses both technical and economic challenges to the agricultural sector. Storage and land application of animal slurries cause the loss of large amounts of N in the air due to volatilization of ammonia (Clarisse et al., 2009), nitrate leaching and accumulation of P and K compounds in agricultural soil (Ledda et al., 2013). On farm environmentally sustainable manure management is thus crucial to minimize losses of valuable nutrients and to prevent contamination and/or eutrophication of the surrounding environment (Kebede-Westhead et al., 2006). Wastewater-based mass cultivation of algae is a promising solution to contribute to the protection of freshwater ecosystems, providing a more sustainable approach reducing the toxicity potential of pollution point sources than achieved by current treatment practices. Compared to physical and chemical treatment processes, algae based treatment can potentially achieve nutrient removal in a less expensive and ecologically safer way with the added benefits of resources recovery and recycling (Oswald, 2003). Common nitrogen removal methods such as bacterial nitrification/denitrification remove the majority of the nitrogen as N_2 gas, whereas algal treatment retains useful nitrogen compounds in the biomass.

Usually in conventional biological wastewater treatment processes, nitrate reduction requires external carbon sources such as methanol or acetate. Additionally the produced biomass must be treated and disposed safely and in an economically feasible way increasing operating costs. On the other hand nitric nitrogen can be converted into microalgal biomass without any external carbon source, producing bioactive substances, bioenergy, or valuable chemicals (Kang et al., 2006).

Notwithstanding these benefits, microalgae treatment may have application as an integrative process in common nutrient removal mainly due to low rates of growth and nutrients uptake by microalgae leading to an effective difficulty for this process to be used as a principal treatment. In this sense many efforts have been done, since several decades, to study and optimize microalgae cultivation in wastewaters both in stand-alone or integrated systems, mainly regarding nutrients reduction and/or abatement in the final effluent. In particular, piggery manure is characterized by high contents of nitrogen (up to 872 mg L^{-1}, Borin et al., 2013), organic matter (6.7%) (Roche, 1984) and suspended solids (Buelna et al., 2008), so that a pre-treatment system that can reduce these components is often a necessary solution before depuration by algae. As a primary step, filtration could then be used as an economic solution. Among different genera of algae, *Chlorella, Scenedesmus, Athrospira* and *Clamydomonas* have been commonly used for the phytoremediation of industrial, urban and agricultural wastewaters (Ji et al., 2013; Franchino et al., 2013) producing a widely chemically diversified biomass which have claimed to sustain potential applications above all in biofuels and animal feed sectors.

Within the algae-derived products, the ketocarotenoid astaxanthin from green alga *Haematoccoccus pluvialis* is a high-value carotenoid with applications in nutraceuticals, cosmetics, food and feed industries. Astaxanthin sells for US$ 2,500 per kg with an annual worldwide market estimated over US$ 200 million (Del Rìo et al., 2005). Although most of this market is based on synthetically-derived astaxanthin, consumers demand for natural products provides an opportunity for the natural molecule. In this sense, the microalga *H. pluvialis* represents the richest source of natural astaxanthin and it is now cultivated at large scale (Olaizola and Huntley, 2003).

Therefore, *Haematococcus* derived astaxanthin has high application potential in the nutraceutical, pharmaceutical, cosmetics, food, and feed industries (Del Rìo et al., 2005).

The cultivation of *H. pluvialis* represents a highly sensitive process, in the way that vegetative growth phase, prior to astaxanthin accumulation in cells bodies, is susceptible to contamination by fast-growing unicellular green and/or blue-green algae due to *Haematococcus* relative slow growth (Orasa et al., 2000). For this reason, commercial production of *Haematococcus*-derived astaxanthin has been commonly reported by using a two-step culture where the first stage is done photoautotrophically under highly controlled culture condition in either tubular, bubble column or airlift photobioreactors and the reddening stage, less prone to contamination, is done in open cultivation ponds (Hata et al., 2001). Moreover, many works have been conducted on the development of an optimal synthetic growth medium (e.g. Gong and Feng, 1997; Fábregas et al., 2000), but, as far as we are aware, few studies focused on the possibility to use agricultural wastewaters for *H. pluvialis* and subsequent astaxanthin production (Kang et al., 2006).

MATERIALS AND METHODS

Wastewater

The piggery wastewater used for this study came from the pretreatment system tested in 2012 in the experimental plant located in Legnaro (PD) (Chapter IV). A sample of 5L volume filtered wastewater (FW) was picked up in October 2012 to be used as growth medium, and sent in Milano. Afterwards the FW was used as a nutrients source for *H. pluvialis* cultivation and astaxanthin production in a one-step batch system which is considered simple and requires low capital investment and technological know-how (Richmond and Hu, 2013).

Haematococcus pluvialis seeds cultures and cultivation

Green microalga *H. pluvialis* was cultivated in Milano, in a DiSAA laboratory. *H. pluvialis* (strain number 34-1d) was obtained from the SAG Culture Collection of the University of Goettingen (Germany). Pre-cultures were maintained in 200 mL Erlenmeyer flasks in Bold Basal Medium for freshwater microalgae. Constant illumination during seed cultures was provided by fluorescent cool white lamps (Osram L13W/840) with an average irradiance of 50 µE $m^{-2}s^{-1}$. The FW was filtered with 0.45 Whatman GF/C filters and diluted with deionized water to obtain a final growth medium containing 50% FW, 25% FW and 12.5% FW. A preliminary test with 100% FW did not result in any microalgal growth (data not shown).

Each trial was inoculated with seed cultures of *H. pluvialis* to reach a seed dry biomass concentration of around 0.05 g L^{-1}. For each thesis, cells were grown in discontinuous mode, photoautotrophically, in two 500 mL Erlenmeyer flaks containing 300 mL of cell suspension. The flasks were continuously illuminated using two 30W OSRAM-Sylvania GRO-LUX 6400 °K lamps, with an average irradiance on the flasks surface of 150 µE $m^{-2}s^{-1}$. The temperature of the culture was maintained constant at 25°C. Mixing and CO_2 feeding were achieved by air bubbling with a commercial aquarium pump with a nominal flow rate of 250 L air h^{-1}. The specific flow rate for each culture was 2.3 L air L $culture^{-1}$ min^{-1}. Cultures growth was monitored with optical density (OD) measurement through spectrophotometric analysis at 750 nm wavelength. A linear relationship was previously found between absorbance at 750 nm and dry weight of the culture.

Dry weight of biomass in the cultures was measured by drying cells at 85 °C for 24 h after filtration through a pre-weighted GF/C filter (Whatman).

Astaxanthin accumulation did take place in the same experimental conditions of the growth trials. Its content was spectrophotometrically determined after 14 days from the beginning of the stationary phase and after the depletion of nitrogen compounds.

Wastewater physical and chemical analyses

Turbidity (NTU) of the wastewater was measured using a portable HI83414 (HANNA Instruments) turbidimeter and pH were measured on-site using a Hach Lange HQD 40d multi-parameter with interchangeable probes according to standards methods (APHA, 1998). Chemicals concentrations of the wastewater were analyzed in a laboratory immediately after the sample collection. COD, TN, NH_4-N and NO_3-N were determined using Mackerey-Nagel NANOCOLOR® kit for COD (COD 1500), Total Nitrogen (TNb 220), Ammonia (Ammonium 10, Ammonium 50) and Nitrate (Nitrate 50, Nitrate 250). TP, Mg, K, Mn, Ca, Cl, Zn, Fe, Na, Cu contents were determined by inductively coupled plasma mass spectrometry (ICP-MS, Varian. Fort Collins, USA) according to 3051A and 6020A EPA methods (EPA, 2007a,b).

For the algae growth tests, the sample and the acid were placed in a fluorocarbon (PFA or TFW) microwave vessel which is capped and heated in the microwave unit. After cooling, the vessel content was diluted to 50 mL with deionized water, filtered with 0.45

µm cellulose acetate filters and then analyzed. A certified standard reference material (2782 Industrial Sludge) from the National Institute of Standards and Technology (Gaithersburg, US) was used in the digestion and analysis. Average recovery was 92 ± 4% for all the determinations. To ensure the accuracy and precision in the analyses, reagent blanks were run with samples. All analyses were performed in triplicate.

Calculations and data analysis

Nutrients concentration reduction in both filtering system and *H. pluvialis* cultivation assays were calculated as percentage reduction (*R*) and daily removal rate (D_r) by using equations 1 and 2:

$$R(\%) = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \qquad (1)$$

$$D_r = \frac{C_{in} - C_{out}}{t} \qquad (2)$$

where C_{in} is inlet concentration, C_{out} is outlet concentration and *t* is the duration of the run.

Specific growth rate (µ) and daily volumetric productivity of *H. pluvialis* (P_b) were calculated by the equations 4 and 5 respectively:

$$\mu = \frac{1}{t} \times \ln\left(\frac{Cb}{C0}\right)$$

(4)

$$Pb = \mu \times Cb$$

(5)

Where C_b and C_0 are the concentrations of biomass at the end and at the beginning of a growth phase, and *t* is its duration.

Data were processed by one-way ANOVA using the Tukey test to compare means. Statistical analyses were performed by using SPSS software (SPSS v19.0, IBM). The level of significant difference was set at $P<0.05$.

RESULTS

Haematococcus pluvialis growth and nutrients uptake

The growth medium of *H.pluvialis* presented a progressive increment of pH and a decrement of turbidity and chemicals with the increase of the dilution (Table 5.1). The FW showed in particular highest concentrations of COD (410-102 mg L^{-1}), TN (139-35 mg L^{-1}), NO_3-N (122-34 mg L^{-1}), and K (222-63 mg L^{-1}).

H. pluvialis showed a positive growth on all the trials (Fig. 5.1A) in terms of dry weight (DW) increase throughout the experiment with 25% FW and 12.5% FW showing a faster growth reaching stationary phase in 13 days with an average dry weight concentration of 0.97 and 0.81 g L^{-1} for 25% FW and 12.5% FW respectively. On the other hand, 50% FW reached the stationary phase after 24 days, after a lag phase of at least three days after inoculum, with a final dry weight concentration of 1.31 g L^{-1}.

Table 5.1 Chemical characterization of filtered wastewater (FW) used as growth medium for *Haematococcus pluvialis* growth.

	100% FW	50% FW	25% FW	12.5% FW
pH	7.98 ± 0.02	8.25 ± 0.02	8.61 ± 0.03	8.87 ± 0.02
Turbidity (NTU)	732	361	175	91
COD (mg L^{-1})	812 ± 14	410 ± 2	200 ± 4	102 ± 3
TN (mg L^{-1})	272 ± 7	139 ± 8	70 ± 6	35 ± 7
NH_4-N (mg L^{-1})	40 ± 3	21.4 ± 1.4	11.1 ± 2	6.1 ± 0.2
NO_3-N (mg L^{-1})	235 ± 6	122 ± 2	63 ± 2	34 ± 1
TP (mg L^{-1})	22.5 ± 2.1	11 ± 0.1	6.2 ± 0.7	3.1 ± 0.1
K (mg L^{-1})	454 ± 5	222 ± 17	125 ± 5	63 ± 4
Mg (mg L^{-1})	28.4 ± 2.2	13.9 ± 0.1	7.8 ± 0.8	3.9 ± 0
Na (mg L^{-1})	131 ± 3	64 ± 4	36 ± 2	18 ± 1
Ca (mg L^{-1})	95 ± 17	46.1 ± 4.2	26.1 ± 5.3	13.1 ± 1.4
Fe (mg L^{-1})	4.4 ± 1.4	2.1 ± 0.5	1.2 ± 0.4	0.6 ± 0.2
Zn (mg L^{-1})	1.4 ± 0.6	0.7 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Cu (mg L^{-1})	0.2	0.1 ± 0	0.041	0.021
Mn (mg L^{-1})	0.5 ± 0.1	0.26 ± 0.03	0.15 ± 0.03	0.07 ± 0.02

The maximum specific growth rate (μ_{max}) was registered during 12.5% FW assay (0.37 d^{-1}), significantly higher than that obtained in 25% FW trial (0.33 d^{-1}) (Figure 5.1B). On the other hand 50% FW achieved the lowest maximum specific growth rate of 0.26 d^{-1}. Although 25% FW and 12.5% FW trials showed a faster response in terms of cultures growth, regarding biomass productivity all the trials showed a similar average daily volumetric productivity, with the 25% FW trial that was capable of producing up to 63 mg L^{-1} of dry biomass per day of cultivation significantly different from 50% FW and 12.5% FW assays which showed a lower productivity, 45 and 49 mg $L^{-1}d^{-1}$ of dry biomass respectively.

Looking at maximum biomass productivity (Pb; Figure 5.1C) all the trials achieved almost the same value, 110 mg $L^{-1}d^{-1}$ for 50% FW and 25% FW and 100 mg $L^{-1}d^{-1}$ for 12.5% FW with no significant differences between the assays.

The differences in these results are likely to be correlated with the dilution of FW. In fact, although macro and micronutrients concentrations (Table 5.1) were not found inhibiting or limiting, if compared with a synthetic medium, a strong difference between the trials was represented by light incidence in the culture. In fact raw FW, probably, did not support *H.pluvialis* growth due to its high turbidity; consequently, diluting 2, 4 and 8 fold the FW could have overcome this issue, improving light availability in the culture. The result of 50% FW trial supports this hypothesis, showing a much slower growth than that obtained in the other trials although the initial nitrogen and phosphorus concentration would have otherwise improved *H. pluvialis* performance in 50% FW (Figure 5.1A). Beside, these results are also linked with nutrients uptake, above all nitrogen, in the trials; nitrogen compounds were almost completely removed from the cultures by cell growth with high reduction percentages at the stationary phase (Table 5.2). In particular 25% FW and 12.5% FW assays did result in the highest nitrate reduction, up to about 99% of removal at the end of the trials with no significant difference with 50% FW trial. Similarly all the cultures were capable of up taking all the ammonia nitrogen which was totally removed from the medium (Table 5.2).

Nitrogen removal in the cultures is strongly related to cultures biomass productivity (Figure 5.1): 50% FW initial biomass productivity is much related to ammonium uptake even though there is not a direct proportionality between them implying, probably, a certain degree of ammonia stripping from the culture. In this case the culture showed a more affinity for the ammonia form even if light availability at the beginning of the cultivation was probably a limiting factor for cells growth. After ammonia depletion and change in cells metabolism, nitric nitrogen uptake started to increase reaching the highest value when microalga showed the highest biomass productivity at day 13 (Figure 5.1, 50% FW) after seven days of constant nitrogen uptake. On the other hand when light was not limiting, as in the case of 25% FW and 12.5% FW, both nitrogen form were utilized, with no preference, from the beginning of the cultivation, with a parallel strong increase in biomass productivity, indicating the absence of a limiting environment for cells growth (Figure 5.1, 25% FW; Figure 2, 12.5% FW). In this cases, according to FW characterization, nitrogen removal was found faster than 50% FW. Although the N:P ratio in the cultures varied between 12 (25% FW) and 13 (50% FW and 12.5% FW) phosphorus was almost completely removed from the culture mediums (Table 5.2). In this case 50% FW showed a slightly higher efficiency (98.08%) than 25% FW (97.74%) and 12.5% FW (96.95%) even though these data did not shown any significant differences. These results are similar to those obtained by Yin-Hu et al. (2013) that reported high N and P efficiency removal by *H. pluvialis* in a domestic secondary effluent with a slightly higher N:P ratio of 15. In that case both nitrogen (93.8%) and phosphorus (97.8%) were efficiently removed when the stationary phase was reached, but initial NO_3-N plus NH_4-N concentration was much lower (5.3 mg L^{-1}) than that in the present study (Table 5.1). Cell growth, in all cases, was sustained until the nitrogen concentration was depleted. Probably, as already reported by Kang et al. (2006), working with *H. pluvialis* grown in primary-treated piggery wastewater, phosphate concentration in the culture decreased rapidly in the first days of cultivation, so that growth was possible due to phosphate accumulation in polyphosphate granules which have been commonly observed in microalgal cells (Sawayama *et al*., 1992). Unfortunately no analyses about phosphorus trend during cultivation were performed in order to support this hypothesis. According to this, 50% FW culture had the higher dry biomass concentration at the stationary phase than the others assays because of the higher availability of nitrate. On the contrary in 25% FW and 12.5% FW assays, *H. pluvialis* achieved the maximum growth rate, but sustainable growth was rapidly limited by the early exhaustion of nitrogen compounds.

Regarding carbon degradation in the cultures, results showed that in all cases no inhibition of the growth was observed. Travieso et al. (2006) concluded that a COD concentration in the range of 250-800 mg L^{-1} did not affect the growth of *Chlorella vulgaris,* a much more resistant and fast-growing microalga, in piggery waste. In this study, 50% FW was characterized by 410 mg L^{-1} of COD and did not suffer any inhibition phenomena. In all trials, at the stationary phase, up to 26.4% of COD was removed (50% FW) (Table 5.2).

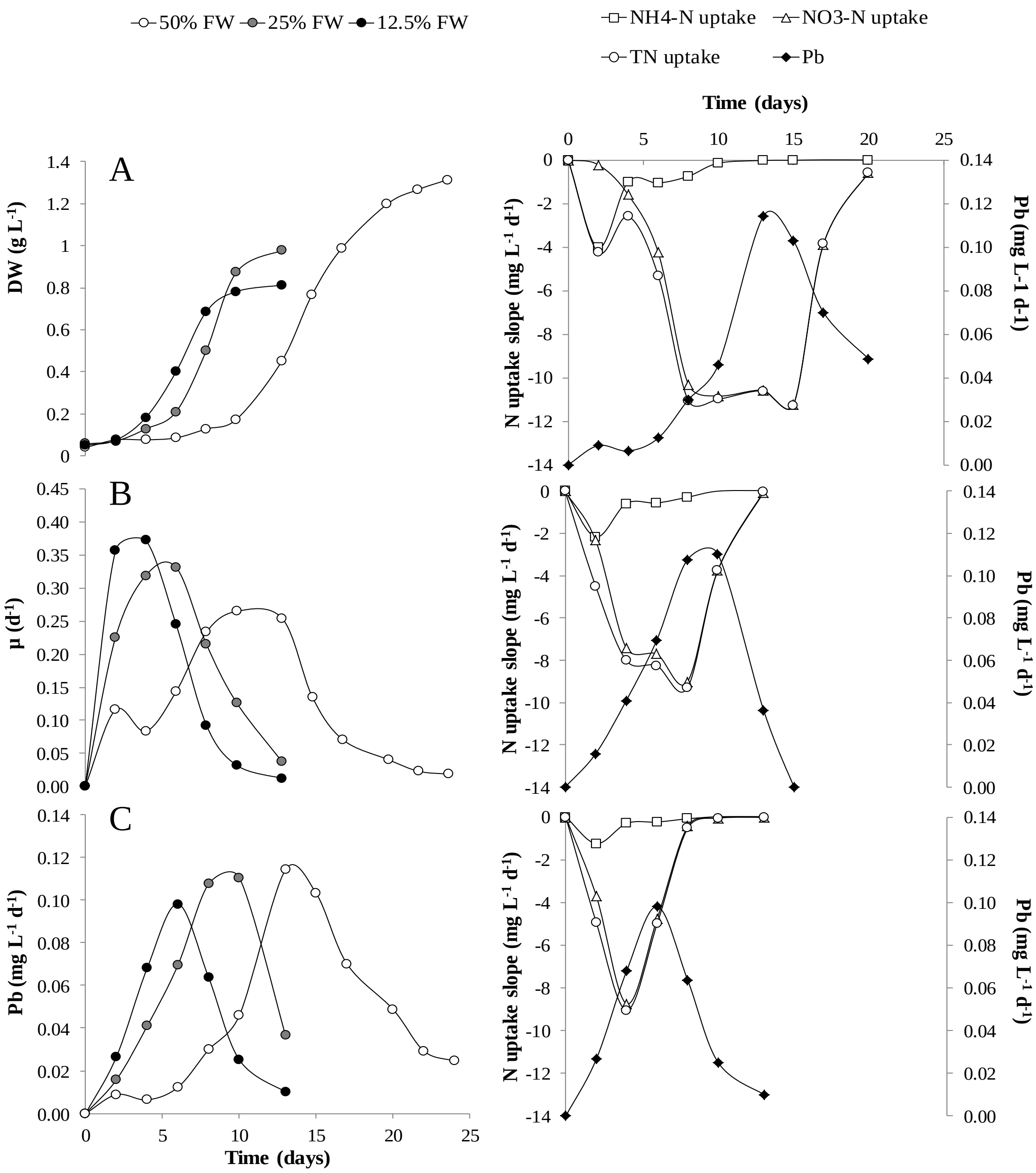

Figure 5.1 Growth of *H. pluvialis* in 50% FW, 25% FW and 12.5% FW: dry weight (A, g L^{-1}), specific growth rate (μ; B, d^{-1}) and biomass productivity (Pb; C, mg L^{-1} d^{-1}) trend during the experiment; NO_3-N, NH_4-N and TN uptake trend against biomass productivity in 50% FW, 25% FW, 12.5% FW trials.

Regarding micronutrients removal all the assays behaved similarly with high reductions rate, in particular in the case of Mg, K, Ca, Fe, Na (> 90% of reduction). A lower removal percentage was obtained in the case of Mn, Zn and Cu. These results showed that *H.pluvialis* grown in a primary-treated swine manure could enhance its remediation with high removal percentages in all cases, except for the COD. In the latter case, the actual

Italian legislation consider a COD limit of 160 mg L-1 for discharging in surface waters (Dlgs 152/2006); 25% FW and 12.5% FW assays did results in a final COD concentration below this limit so that exhausted culture medium can effectively and sustainably be discharged in surface water bodies.

Astaxanthin accumulation

As already reported in material and methods section, astaxanthin accumulation did take place in the same experimental conditions of the growth trials. This was intended to evaluate the possibility to use a system that could reduce the costs of *H. pluvialis* cultivation and astaxanthin production, above all considering the final users of this hypothesized system, i.e. small farmers. The encystment of the cells was spontaneously induced by nitrogen depletion at the stationary phase and was followed by the accumulation of astaxanthin during induction period without any reformulation of the culture medium. Microscopic observations confirmed that green vegetative cells were transformed into red cyst cells during this incubation. Between the trials, 50% FW showed the highest percentage of the carotenoid in the biomass (1.27±0.02%), slightly higher than that observed in 12.5% FW (1.17± 0.02%) and 25% FW (0.92±0.03%). The obtained values are much lower than those reported by Kang et al. (2006), where cultures accumulated up to 5.9% of astaxanthin in the cells, although experimental conditions during induction period were substantially different, i.e. the use of high irradiance and CO_2-mixed gas. In this work high concentrations of astaxanthin in *H.pluvialis* biomass were obtained without any reformulation of the culture medium, a constant low light irradiance and with no use of CO_2 gas for induction of the pigment accumulation, thus improving economic viability of the process.

Overall performances

Coupling preatreament system and *H. pluvialis* growth would allow to strongly reduce organic and mineral nutrients contained in piggery wastewater. In this case, a main result of the process was a net increase in nitric nitrogen concentration, which has not been seen as negative, on the contrary it represented a positivity for the depuration by plants and algae, which require macronutrients like nitrate for their growth, avoiding ammonia nitrogen toxicity at high concentrations. Concerning ammonia a strong reduction was observed during pretreatment (66% of reduction) due to nitrification processes and absorption of the filters. Than FW contained low levels of ammonia which were assimilated by *H. pluvial*is and/or loss in the air due to alkaline pH reached during the growth and continuous aeration of the culture. The treatment with *H. pluvialis* resulted in a more efficient P reduction, causing almost the complete depletion of the element in the cultures. On the other hand the pretreatment resulted in a low abatement (7% of reduction); in this case probably the filter system was not capable of a high adsorption rate of P. Beside this, COD content revealed a similar behavior to that of P. High reductions was achieved by microalga trials (up to 26%), but a lower reduction percentage was observed throughout the filtering assay (19%).

According to the results of the batch cultures it was possible to theorize a continuous culture of *H. pluvialis* using FW as a growth medium coupling it with the filtration system for nitrogen reduction in swine slurry (Figure 5.2). Looking at specific growth rate trend throughout the trials, the optimal dilution rate (D_{opt}) was determined: in continuous cultures, at steady state, it equals to the specific growth rate (Yuan-Ku et al., 2013) in respondance to the maximum biomass productivity . In this case the obtained D_{opt} was 0.25, 0.21 and 0.24 d^{-1} for 50% FW, 25% FW and 12.5% FW, respectively. From maximum biomass productivity (Pb) using equation 5 (see Materials and methods section), it was calculated the optimal working biomass concentration (Cb_{opt}) which was 0.45, 0.51 and 0.41 g L^{-1} for 50% FW, 25% FW and 12.5% FW respectively. According to the daily load in the filtering system (30 L d^{-1}) and the dilution of FW, three photobioreactors with a working volume of 227 L (50% FW), 363 L (25% FW) and 645 L (12.5% FW) were hypothesized to treat all the FW coming from the filtration system.

A main result of theorized continuous system is that in 50% FW reactor it would not be possible to achieve a good reduction of nitrate, working with the optimal dilution rate; in fact the outlet medium would contain still 80.2 mg L^{-1} of nitric nitrogen, meaning the impossibility to discharge the effluent. In this case lowering dilution rate to 0.1 d^{-1} would allow to achieve higher reduction (down to 16 mg L^{-1}, below the discharge limit) but it would imply also a reduction of around 50% of the maximum biomass productivity (down to 60 mg $L^{-1}d^{-1}$).

This system is capable of reducing up to 44%, 99% and 34% of TN, NH_4-N and NO_3-N respectively, producing around 25 g biomass d^{-1}.

The other two systems would behave differently; in fact both reactors (25% FW and 12.5% FW) would allow to reduce nitric nitrogen down below the discharging limit (18.7 mg L^{-1} and 13.2 mg L^{-1} for 25% FW and 12.5% FW respectively) at the optimal dilution rate. This last point being fundamental, as the maximal biomass productivity would be maintained, allowing the production of 40 g and 65 g of dry biomass per day of cultivation.

Regarding nitrogen consumption 25% FW reactor would be the most performant with 74%, 98%, and 70% of TN, NH_4-N and NO_3-N reduction respectively.

CONCLUSIONS

Wastewater treatment and astaxanthin production were performed by a primary treatment filtering system and culture and subsequent carotogenesys induction of *H. pluvialis* on piggery wastewater. High content of nitrate-nitrogen in the wastewater coming from the filtering system seem to have been successful in alga growth. Inorganic wastes in the wastewaters, above all nitrogen and phosphorus, were removed successfully by *Haematococcus* cultivation, after which green vegetative cells were transformed by photoautotrophic induction to red aplanospores with high astaxanthin content.

The proposed method has potential as biological wastewater treatment process because of the combination of inorganic waste removal without any additives and the simultaneous production of high-value added astaxanthin. From the viewpoint of astaxanthin production only, the culture of *H. pluvialis* on primary-treated wastewater could also make algal culture processes more economical by eliminating the need to supply nutrients. Therefore, the proposed system is expected to be an alternative treatment technology in biological wastewater treatment with the additional benefit of a highly valuable compound production.

Table 5.2 Daily removal rate and percentage reduction of nitrogen compounds (NO_3-N, NH_4-N), total phosphorus (TP) and carbon (COD) during *H. pluvialis* growth.

	NO_3-N		NH_4-N		TP		COD	
	D_r (mg L^{-1} d^{-1})	R (%)	D_r (mg L^{-1} d^{-1})	R (%)	D_r (mg L^{-1} d^{-1})	R (%)	D_r (mg L^{-1} d^{-1})	R (%)
50% FW	5.98 ± 0.10	97.75 ± 0.32	1.06 ± 0.07	99.31 ± 0.28	0.54 ± 0.07	98.08 ± 0.03	5.41 ± 0.07	26.40 ± 0.28
25% FW	3.10 ± 0.09	99.12 ± 0.14	0.55	98.66 ± 0.62	0.30 ± 0.02	97.74 ± 0.06	2.47	24.70 ± 0.62
12.5% FW	1.71 ± 0.07	99.06 ± 0.06	0.29 ± 0.02	97.52 ± 1.01	0.15 ± 0.03	96.95 ± 0.01	1.19 ± 0.02	23.40 ± 1.01

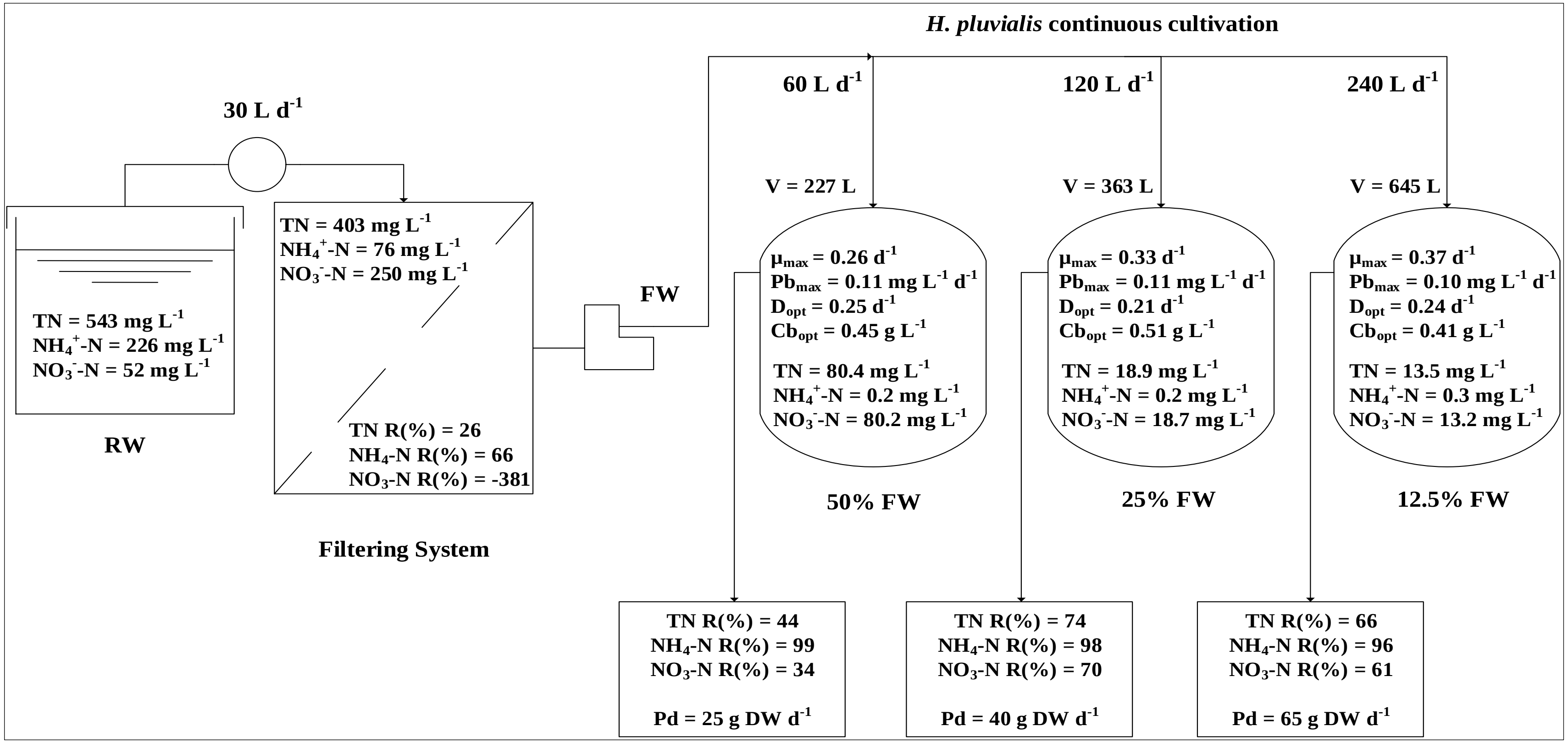

Figure 5.2 Combined pretreatment-microalgae system: *Haematococcus pluvialis* continuous cultivation model and nutrients concentration throughout the process.

Chapter VI

GENERAL DISCUSSION AND CONCLUSIONS

The general aim of this research was to identify a sustainable solution for the treatment of pollutant rich wastewaters, as are digestate and piggery wastewater. The pilot plants provided interesting results on chemical removal, given that there is little information in the literature, especially for digestate treatment.

For the digestate liquid fraction treatment, the granular size of recycled materials played an important role in chemical removal but also for electrical conductivity reduction. In addition, recirculation allowed abatement of the high COD and NH_4-N concentrations, characteristic of the DLF. Porous refractory with lowest granular size (20-30 mm) gave highest performances for total nitrogen, ammonia nitrogen, and COD abatements, while gravel was the better material for total phosphorus and soluble phosphorus reductions. The increase over time of the filters performances for chemical removal suggests that they require an adjustment period to show their behaviour and real depurative efficiencies, so their study in the medium-long term is suggested, also to investigate the clogging over time.

The integrated pilot system represented a viable solution for the treatment of the piggery wastewater liquid fraction, with low energy demand and easy management. Another positive element was the use of a restricted area for the phytodepuration, taking advantage of verticality. The use of economical, reusable, easily available and disposable materials for the treatment of piggery wastewater reduced salinity and removed the main pollutants present in this type of wastewater, which are damaging for phytodepuration plants if not sufficiently reduced. The recirculation improved the reduction of all parameters (in particular COD and NH_4-N), but it increased NO_3-N content at the outlet. Pumice, with its ion-exchange ability, demonstrated the highest performances for the removal of ammonium, COD and BOD_5, while organic media were shown to be the best materials for total nitrogen removal, due to degradation and microbial activity. Tops and gravel were the substrates with lowest performances on the piggery wastewater treatment, however they reduced all pollutants, with mass abatements of NH_4-N and BOD_5 higher than 80%. The filter filled with sand and gravel had clogging after 40 days of operation, however it was the best filter for P removal. The use of these materials is therefore interesting not only for their low-cost and good removal performances, but also for their possibility to be regenerated (pumice), reused (tops or gravel after washing), or strategically applied as soil amendment or for soil fertility enhancement (pumice and organic media). Release rate of NH_4 from zeolites is optimal when compared to synthetic fertilizers, since NH_4 absorbed in zeolite is only marginally leached after intensive rainfall (Onar et al., 1996). However, the relatively short life span of giant reed would tend to reduce its desirability as a biofilter medium.

The cascade constructed wetland presented different results for the treatment of the diluted filtered piggery wastewater, depending on the year. Wetland plants (*Mentha aquatica*, *Carex divisa*, *Typhoides arundinacea*) and *Cynodon dactylon* gave the best wastewater treatment performances. Halophytic plants (*Artemisia coerulescens*, *Halimione portulacoides*, *Sarcocornia fruticosa*) and *Phragmites australis*, instead, seemed to have no effect on wastewater depuration, and *Puccinellia palustris* was not able to survive in the system because of oxic conditions. In 2013 the growth medium (light expanded clay aggregates) had the main effect on depuration, lowering the NH_4-N, PO_4-P and TP contents in particular. Considering both years, *Mentha aquatica* and *Cynodon dactylon* were the species with highest biomass production and highest nutrients uptake (till 33 t/ha of aerial dry biomass and 0.77 t/ha of N uptake for *C. dactylon*). However the literature confirms that these plant species could have higher yield than those obtained in this study, therefore their productivity is probably related to the surface area they have at their disposal. The results obtained in 2013 are probably related to the different conditions compared to the previous year. In that year, in fact, the plants were planted in late summer, in a substrate very different from the soil of salt marshes, and were fed with high nutrients concentrations and high organic load. All these factors contributed to cause stress to plants and probably the release of previously accumulated N by halophytes. *P. australis*, like the halophytes, showed low wastewater treatment performances, and this could be explained by the fact that it did not have enough time to grow and demonstrate its capacity; however the potential of this plant in the removal of nutrients is evident, since it had the highest values of N uptake in the aerial part (3.5%). Although halophytes gave low depurative performances, the advantage of these plants is that they can tolerate the wastewater salinity, which may be high for some wetland plants. Nevertheless the best performances were given by *C. dactylon,* which proved to survive summer planting, to grow exponentially over time, to show the highest biomass production and nutrients uptake, and to give (together with wetland plants) the highest chemical removal. *C. dactylon* could therefore be a promising plant for the treatment of saline and piggery wastewaters, as already experimented (Szögi et al., 2004; Matos et al., 2010; Pavan et al., 2014). Although only one laboratory test was done, *Haematococcus pluvialis* also resulted as interesting for the treatment of filtered piggery wastewater, with daily reduction of NO_3-N, NH_4-N and TP concentrations of almost 100% on undiluted wastewater.

Overall these studies represent viable solutions for the treatment of digestate and piggery wastewater, however it follows that further investigation is needed in order to apply these systems at real scale. More exhaustive studies are required on filters and filter materials for improved wastewater treatment performances, taking advantage of all the available filter volume, and using suitable wastewater volume loads and longer hydraulic residence times to increase the adsorbing surface and microbial biomass responsible for decomposition and nutrient removal. For phytodepuration, examination of wetland plants and halophytic plants with the same experimental conditions and same type of wastewater is necessary to understand their true potentials. Moreover, for an application at real scale, it is necessary to study their treatment of piggery wastewater over time and its dilution.

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