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WASTEWATER TREATMENT AND PLANT PERFORMANCE IN SURFACE FLOW CONSTRUCTED WETLANDS

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Hend Ibrahim 31st October 2017

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Dedicated to my family and friends

Table of contents

Riassunto

Una parte delle acque reflue industriali e agricole del Veneto, nord Italia, vengono trasportate nella Laguna Veneta attraverso il suo bacino di drenaggio; principalmente azoto (N) e fosforo (P) oltre ad altri inquinanti come metalli pesanti. Nel 2000, il carico totale di azoto era di un terzo superiore al valore di riferimento ammissibile massimo di 3000 t/ anno per gli ingressi della laguna come indicato dal decreto ministeriale (Ministero dell'Ambiente, 1999), mentre il fosforo totale era di 229 t/anno. Sulla base di questo, gli input di azoto nel sistema lagunare Veneziano devono essere ridotti drasticamente nel prossimo futuro. I sistemi di fitodepurazione costruiti hanno offerto soluzioni promettenti per il controllo dell'inquinamento da nutrienti, in particolare dal deflusso agricolo, a costi e input energetici relativamente bassi. Alcuni sistemi semi-naturali e ricostruiti sono presenti in Italia e sono progettati per il trattamento di sorgenti diffuse di inquinamento da raccolti agricoli e civili con maggiore concentrazione nell'Italia centrale e nel nord.

Questa ricerca di dottorato ha inteso determinare alcuni degli effetti positivi che il sistema di fitodepurazione può dare all'ambiente. In particolare, essa mirava a quantificare la riduzione dell'inquinamento da deflusso agricolo in un sistema convenzionale di fitodepurazione all'interno del sistema lagunare Veneziano. Inoltre, essa mirava a verificare e quantificare la capacità di assorbimento e la crescita delle diverse specie vegetali impiegabili in fitodepurazione.

Nel 2014 è stato realizzato un sistema di fitodepurazione ibrido, composto dall’adattamento di un sistema semi-naturale in due sistemi di flusso superficiale (FWS) e da sistemi di trattamento flottanti (FTW). Il sistema è stato monitorato in termini di parametri della qualità dell'acqua e delle prestazioni vegetative per 3 anni consecutivi. La concentrazione di azoto totale (TN) e azoto nitrato ($N\text{-}NO_3^-$) ha mostrato picchi all'entrata del FWS in primavera, a causa della fertilizzazione dei terreni circostanti e del deflusso causato da precipitazioni abbondanti. Un effetto generale di riduzione di entrambi i parametri era chiaro all'uscita del sistema e le prestazioni depurative somo migliorate nel corso degli anni. Nel 2016, l'efficienza di rimozione ha raggiunto valori del 64% e 91% rispetto ai carichi in ingresso, corrispondenti rimozioni di massa di 2327 per TN e 1873 kg per $N\text{-}NO_3^-$.

Per quanto riguarda le specie vegetali utilizzate nel FTW, *Carex spp*. ha mostrato il tasso di sopravvivenza, la produzione di biomassa, l'assorbimento di N e P più elevati in tre stagioni consecutive seguite da *Lythrum salicaria*, mentre *I. pseudacorus* non ha fornito buoni risultati.

Nel 2016 è stato realizzato un esperimento pilota nell'ambito del suddetto sistema integrato applicando un carico eccessivo di $N\text{-}NO_3^-$ a un sottosistema, di 3 bacini con volume e capacità d'acqua noti per testare l'efficienza di fitodepurazione e alcune dinamiche dell'acqua all'interno di questo sistema. La soluzione elevata di $N\text{-}NO_3^-$ è stata omogeneizzata nel primo sottobacino mentre il secondo e il terzo sono stati intesi a monitorare l'effetto di depurazione. Il picco di 66 mg l^{-1} è stato notato all'ingresso del sottobacino controllato (secondo) dopo il trasferimento, indicando l'omogeneità della soluzione nel primo sottobacino. Dopo 12 ore (tempo di detenzione), la concentrazione mediana all'ingresso è stata di 45,34 mg l^{-1} mentre ha raggiunto i 41,5 mg l^{-1} all'uscita. L'efficienza di rimozione del sotto-bacino calcolata nelle 12 ore successive alla detenzione era dell'8,4% con la rimozione di massa di ~ 800 g di $N\text{-}NO_3^-$ (1g m^{-2} d^{-1}). Sulla base delle concentrazioni di $N\text{-}NO_3^-$ nel sottobacino monitorato in tempi di monitoraggio diversi, si evince che sono presenti alcuni flussi preferenziali, ma che tutto il bacino e' comunque interessato da passaggio dell'acqua.

Infine, una valutazione delle prestazioni delle specie di piante macrofite che trattano diversi tipi di acque reflue in FTW è stata fatta recuperando e analizzando dati relativi alla crescita di 20 specie utilizzate nel sistema flottante Tech-IA® in 9 esperimenti diversi nel nord-Italia per un decennio (2006-2016). L'analisi statistica è stata effettuata per le piante frequentemente utilizzate in molti esperimenti, ovvero *Phragmites australis*, *I. pseudacorus*, *Typha latifolia*, C*arex spp*. e *L. salicaria* mentre le specie a doppio scopo (valore ornamentale e trattamento delle acque reflue) sono state valutate separatamente. *I. pseudacorus*, *P. australis* e *T. latifolia* hanno mostrato le migliori prestazioni di crescita, specialmente nel trattamento delle acque reflue comunali, mentre specie ornamentali quali *Canna indica*, *Mentha aquatica* e *Pontederia cordata* si sono rivelate potenzialmente efficienti per il trattamento delle acque reflue in FTWs. Inoltre, le prestazioni delle piante sono state influenzate da fattori quali l'età e le caratteristiche fisico-chimiche delle acque reflue.

In generale, i sistemi di fitodepurazione costruiti con flusso superficiale si sono rivelati una soluzione promettente nel trattamento di molti tipi di acque reflue con particolare attenzione al deflusso agricolo.

Summary

Most of the industrial and agricultural wastewaters in Veneto, north Italy are conveyed to the Venetian lagoon through its drainage basin; mainly as nitrogen (N) and phosphorus (P) in addition to other pollutants such as heavy metals. In 2000, the total N load was one-third higher than the maximum allowable reference value of 3000 t/year for lagoon inputs as indicated by the Ministerial decree (Ministero dell'Ambiente, 1999), while the total P was 229 t/year. Based on this, inputs of nitrogen into the Venetian Lagoon system must be reduced dramatically in the near future, or at least the maximum allowable value should be attained. Constructed wetlands (CW) offered promising solutions for the control of nutrient pollution, specifically from agricultural run-off, at relatively low cost and energy inputs. Few semi-natural (NW) and re-constructed systems (RCW) are present in Italy and designed for the treatment of diffuse pollution sources from agricultural and civil catchments with major concentration in central and north Italy.

This PhD research aimed at determining some of the positive effects that a wetland can give to environment. In particular, it aimed at quantifying the reduction of pollution from agricultural run-off in a conventional cropping system within the Venetian lagoon system. Understanding some water dynamics and improving water quality in a farm channel network was an additional objective. Furthermore it aimed at verifying and quantifying the efficiency of different surface flow constructed wetland systems and the uptake capability and growth performance of different plant species, mainly macrophytes.

A full-scale integrated wetland system was constructed in 2014 restoring a semi-natural wetland into two surface flow systems, free water surface (FWS), and floating treatment systems (FTW). The system was monitored in terms of water quality parameters and vegetative performance for 3 consecutive years. In assumption, total nitrogen (TN) and nitrate nitrogen ($N\text{-}NO_3^-$) concentrations showed peaks at inlet of the FWS during high agricultural seasons in spring as a result of fertilization of surrounding croplands and runoff due to excessive rainfall. A general reduction effect in both parameters was clear at the system outlet over the years with the increased establishment of the wetland system. High removal efficiency was attained by FWS after the establishment of the wetland system in 2016 with removal percentages of 64 and 91 accounting for mass removals of 2327 and 1873 kg for TN and $N\text{-}NO_3^-$, respectively. Regarding plant species used in the FTW, *Carex spp*. showed the highest survival rate,

biomass production, N and P uptake over 3 consecutive seasons followed by *Lythrum salicaria* while, *I. pseudacorus* did not perform well in the FTW in terms of survival, biomass production and nutrient uptake.

In 2016, an event-driven pilot experiment was designated within the aforementioned integrated wetland by applying excessive N-NO_3^- load to a specific isolated sub-basin system comprising 3 sub-basins with known volume and water capacity to test the phytodepuration efficiency and some water dynamics within this system. The elevated NO_3^- solution was homogenized in the first sub-basin while, the second and the third were meant to monitor the depuration effect. A peak of 66 mg l^{-1} was noticed at the monitored (second) sub-basin inlet following the transfer, indicating homogeneity of solution in the first sub-basin. After 12 hours (detention time), median concentration at inlet was 45.34 mg l^{-1} while it reached 41.5 mg l^{-1} at the outlet. Removal efficiency of the sub-basin calculated in the 12 hours following the detention was 8.4% with mass removal of ~800 g of N-NO_3^- (1g m^{-2} d^{-1}). Based on the N-NO_3^- concentrations within the monitored sub-basin at different monitoring times, it could be concluded that, despite some preferential flows caused by some vegetative obstructions, the system eventually distributes the input nutrient volumes across the sub-basin.

Finally, an evaluation of performance of macrophyte plant species treating different types of wastewaters in FTW was done by compiling data related to the growth performances of 20 plant species used in Tech-IA® floating system in 9 different experiments in north Italy over a decade (2006-2016). Statistical analysis was performed for the plants frequently used in many experiments namely; *Phragmites australis*, *I. pseudacorus*, *Typha latifolia*, *Carex* spp. and *L. salicaria* while dual-purpose species (ornamental value and wastewater treatment) were evaluated separately. *I. pseudacorus*, *P. australis* and *T. latifolia* showed the best growth performances, especially in the treatment of municipal wastewater, whereas ornamental species such as *Canna indica*, *Mentha aquatica*, and *Pontederia cordata* proved to be efficient potentials for the treatment of wastewaters in FTWs. In addition, plant performances were affected by factors such as plant age and physicochemical characteristics of wastewaters.

In general, surface flow constructed wetland systems proved to be promising solution in the treatment of many types of wastewaters with special focus on agricultural runoff.

Chapter I

General introduction and review of literature

1. Agricultural runoff

Agricultural runoff is a major non-point source (NPS) pollution of the environment, specifically water resources, worldwide. Agricultural runoff is the water runoff, normally by the effect of rain, melted snow and irrigation, leaving croplands and depositing in different water bodies such as lakes, rivers, ponds, coastal waters and even underground water resources (Ongley, 1996; EPA, 2017). Described as non point or diffused pollution source, Agricultural run-off can carry pollutants of different natures, composition and impacts on water bodies (chemical fertilizers, pesticides, animal manure, plant organic residues, pathogens, heavy metals and soil sediments) (Wiens, 1980; Higgins *et al.*, 1993; Ongley, 1996; EPA, 2005; O'Geen *et al*., 2010; Blankenberg *et al.*, 2015). The threats of agricultural runoff to the environments have been doubled in the last few decades as a result of agricultural intensification to cope with the needs of the growing population where, inefficient use of resources and poor agricultural practices are major contributors to NPS agricultural pollution (Wiens, 1980; Ongley, 1996; O'Geen *et al*., 2010; Ockenden *et al.*, 2014; Blankenberg *et al.*, 2015). Agricultural runoff leading to the loss of nutrients and sediments from crop lands to water bodies is the major cause of a two-sided problem; the first side is the economical loss of resources (soil degradation and fertilizer loss) for farmers from their agricultural lands while the second and the most important is the environmental loss through the diffusion of pollutants to water bodies contributing to further environmental and human health hazards (Griffin and Bromley, 1982; Ongley, 1996; O'Geen *et al*., 2010). Fewer countries including USA and some European countries were able to determine and quantify the implications of agricultural runoff on water bodies while it was hard to evaluate such situation in developing countries, however, all countries worldwide recently share the concern about this growing hazard (Ongley, 1996; Blankenberg *et al*., 2015).

The major pollutants transferred to water bodies through agricultural runoff are nutrients, pesticides, and sediments. A pollutant in itself, sediment is a carrier of other hazardous pollutants; nutrients, especially phosphorus, pathogens and heavy metals (Weins, 1980; Ongley, 1996; O'Geen *et al*., 2010). The major nutrients of concern in agricultural runoff are nitrogen (N) and phosphorus (P) as they are key reasons of water eutrophication which has negative implications on water bodies including the development of algae, depletion of oxygen, shifting of aquatic habitats and extensive human health hazards (Ongley, 1996;

Kadlec and Wallace, 2009; Sorrell, 2010). N (in organic and inorganic forms) is usually more abundant as a primary source of fertilization in croplands (Blankenberg *et al.*, 2015). Dissolved inorganic nitrogen groups (nitrate ($N\text{-}NO_3^-$), nitrite ($N\text{-}NO_2^-$) and ammonium ($N\text{-}NH_4^+$)) are generally in a readily available form for uptake, hence, affecting water quality, human and aquatic life more than organic nitrogen forms (Davis, 1995b; Vymazal, 2007; Lee *et al.*, 2009; O'Geen *et al.*, 2010). $N\text{-}NO_3^-$, the most abundant nitrogen form in agricultural runoff, would cause majorly eutrophication problems rather than toxicity and is the easiest to treat in water bodies by denitrification or plant uptake (Davis, 1995b; Baker; 1998; Peterson, 1998; Ongley, 1996; O'Geen *et al.*, 2010). P is found in many forms such as mineral, organic, inorganic P and soluble orthopohosphates ($P\text{-}PO_4^-$) which are usually associated with sediment particles by adsorption (Davis, 1995b). Although, P is readily taken up by rooted plants, under anoxic conditions, the remaining P associated with sediment particles can be a major source of uncontrollable oligotrophication in water bodies (Davis, 1995b; Ongley, 1996; Sorrell and Gerbeaux, 2004). On the other side, pesticide leaching to water bodies is a major risk to aquatic as well as human life due to its toxic and accumulative nature over time which makes the removal process rather complex and expensive (Ongley, 1996; Blankenberg *et al.*, 2015).

As described previously, due to its diffused nature, Agricultural runoff is somehow hard to determine, measure and control (Weins, 1980; Higgins *et al.*, 1993; Ongley, 1996; Raisin *et al.*, 1997). In addition, it's more periodic and event-driven, affected by factors like weather conditions (mainly rainfall events) and agricultural practices (mainly fertilization events) which in turn lead to intermittent hydrological loading (Weins, 1980; Higgins *et al.*, 1993; Ongley, 1996). Control measures for NPS agricultural pollution are focused on two sides, the first is reducing agricultural runoff from croplands and the second is the treatment of polluted water.

Strict control measures on agricultural lands were proposed to reducing agricultural runoff losses. Improving agricultural practices and land management was the major solution proposed in many studies; these include improvement of irrigation systems, tillage and cropping patterns (Weins, 1980; Ongley, 1996; Mitsch *et al.*, 2001 and 2005; Sorrell, 2010; Ockenden *et al.*, 2014; Blankenberg *et al.*, 2015). Optimization of the use chemical fertilization and pesticides is a key factor in controlling and reducing the amount of pollutants in water bodies; nitrogen-fixing crops and integrated pest management could offer good substitutes (Weins, 1980; Ongley, 1996; Mitsch *et al*., 2001 and 2005; Sorrell, 2010). Agro-forestry is a growing trend in the recent decades to control runoff; it involves the establishment of trees, riparian zones and buffer strips acting as nitrogen sinks in addition to improving chemical and physical properties of soil and decreasing sediment loss and soil erosion (Weins, 1980; Dillaha *et al*., 1989; Mitsch *et al*., 2001 and 2005; Udawatta *et al*., 2002; Jose, 2009; Dosskey *et al*., 2010). Effective legislation, strict regulatory measures and public awareness of increasing hazardous effect of NPS agricultural pollution are very important tools for the control of agricultural runoff, especially in developing countries (Weins, 1980; Shortle and Dunn, 1986; Ongley, 1996).

Conventional wastewater treatment involves a set of chemical, physical and biological processes designated to remove contaminants like solids, organic matter and nutrients from water (Pescod, 1992; Kadlec and Wallace, 2009). Usually, the conventional wastewater treatment process is divided into many stages namely; preliminary, primary, secondary and tertiary treatments. The preliminary stage involves the removal of solids and large materials after which it goes to the primary is stage in which organic and inorganic solids are removed by sedimentation. The secondary treatment is applied for the treatment of dissolved and colloidal organic residuals and suspended solids where as the tertiary (advanced) treatment is used for the removal of individual materials which are not removed by the secondary treatment such as N, P, heavy metals, biological oxygen demand (BOD) and other dissolved solids. The final stage is disinfection of water by application of chlorine (Cl) ((Pescod, 1992; Kadlec and Wallace, 2009). However, although applicable, conventional methods of wastewater treatment are rather expensive and not a practical solution in treatment of agricultural runoff water where contaminated runoff water is directed immediately to water bodies (Pescod, 1992, EPA, 2006). Direct treatment of agricultural runoff water in water bodies became possible by the introduction of wetlands. A wetland is an area of land which is temporarily or permanently saturated with water with characteristic aquatic plants (macrophytes) and hydric soils providing a specific ecosystem with various ecological functions (EPA, 2004; Sorrell and Gerbreaux, 2004; Kadlec and Wallace, 2009). Typical functions of a wetland include majorly water quality improvement and protection, floodwater storage, and providing habitat to a variety of biota (EPA, 2002). As natural wetlands have proved great efficiency in pollutant removal, especially nutrients, replicates were created to simulate the functions of wetlands and became widely known as constructed wetlands (CW) (EPA, 2006, O'Geen *et al*., 2010; Vymazal, 2005; Vymazal, 2010; Vymazal, 2011). Despite history of natural wetland use for water treatment goes back to as old as 100 years, CW are only few decades old (Kadlec and Wallace, 2009; Vymazal; 2010; Vymazal, 2011). The use of CW in wastewater treatment from agricultural runoff was targeted mainly at the removal of nutrients, chemicals and suspended solids (Kadlec and Wallace, 2009; O'Geen *et al*., 2010).

2. CW for the treatment of agricultural runoff

Removal mechanisms of pollutants

As mentioned earlier, the major pollutants of water bodies by agricultural runoff include nutrients, pesticides, BOD, suspended solids (SS) and pathogens. CWs exhibit many interrelated mechanisms for the removal of such pollutants (Davis, 1995b; Vymazal, 2007; Kadlec and Wallace, 2009; Lee *et al.*, 2009; O'Geen *et al*., 2010). Physical sedimentation and settling is the most common mechanism for the removal of most pollutants such as SS, P, pesticides, pathogens and BOD. Another important mechanism for the removal of N, the major target nutrient in agricultural runoff, is the biogeochemical transformations (Figure 1, O'Geen *et al*., 2010) which involve interchanging processes such as ammonification (mineralization), nitrification and denitrification. Leaching is an additional mechanism for removal of $N\text{-}NO_3^-$ and P. Soil sorption, which is the removal of pollutant from the soluble phase and adherence to the sediment particles, is a major pathway through which P is removed. Volatilization is the removal mechanism of gases like ammonia (NH_3), Nitrogen (N_2) and methane (CH_4). Microbial degradation (under aerobic and anaerobic conditions) is important in the removal of pesticides, organic matter and BOD. Additional mechanism for the removal of pesticides, organic matter and pathogens is the direct photodegradation (photolysis) by sunlight UV rays, while some other pesticides are removed by indirect photolysis. One of the most important removal mechanisms in CW is the biotic assimilation (uptake) by plants and algae where it provides a direct removal of nutrients in water body in addition to its indirect effect in the promotion of SS sedimentation and prevention of re-suspension (Brakserud, 2001), as well, they supply organic carbon (OC) through decayed plants which are important for microbial transformation processes i.e nitrification and denitirification, (Brix, 1997) as they provide more surface area for the substrate (Davis, 1995b; Kadlec and Wallace, 2009; Vymazal, 2007; O'Geen *et al*., 2010).

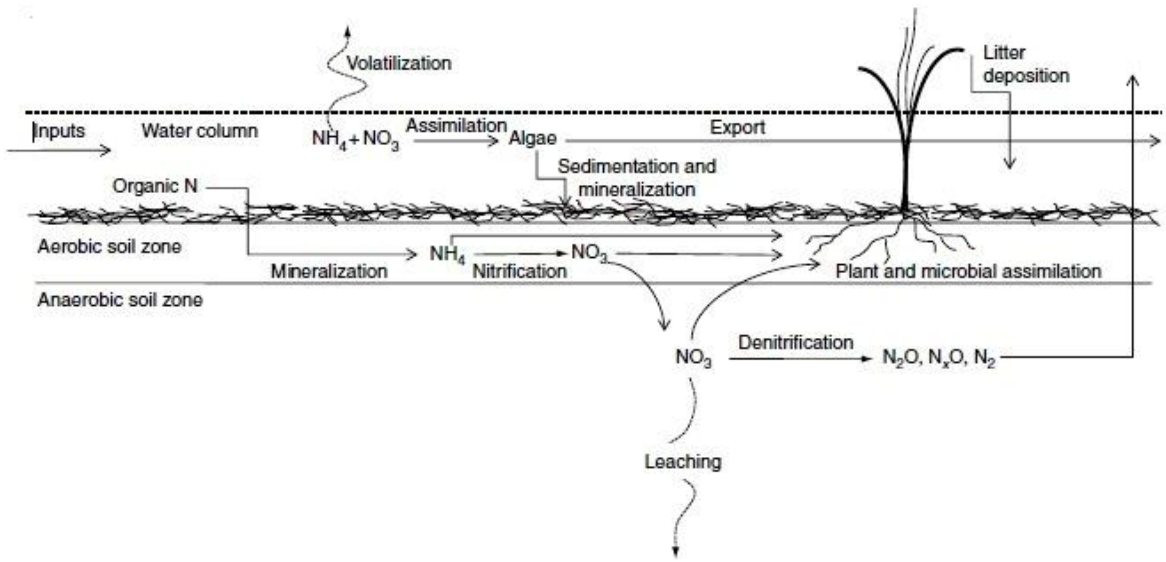


Figure 1: Diagrammatic scheme of the N cycle in CW (O'Geen *et al*., 2010)

Types of CW

Based on the use of floating and emergent rooted macrophytes, CW are generally classified into surface flow (SF) and subsurface flow (SSF) (Figure 2) (Vymazal 2001; Vymazal 2005; Vymazal 2007; Kadlec and Wallace, 2009; O'Geen *et al*., 2010; Vymazal 2010; Vymazal, 2011a). SF CW are also known as free water surface (FWS) CW whereas SSF CW are sub-classified into horizontal and vertical (HSSF and VSSF). In general, FWS CWs are characterized by open waters, floating and emergent vegetation where they are closely related to natural wetlands (Kadlec and Wallace, 2009; Vymazal 2010; Vymazal, 2011a). All possible removal mechanisms of nutrients, organic matter and SS are performed by FWS CW with specific suitability for the removal of all nitrogen forms as they provide good medium for nitrogen transformation processes, hence, they are suitable for the treatment of all types of wastewaters in addition to their ability to deal with pulse flow and different water levels (Kadlec and Wallace, 2009; Vymazal 2010; Vymazal, 2011a). FWS CWs are very cost effective in terms of maintenance and operation compared to other types of CWs (Kadlec and Wallace, 2009; Vymazal 2010). FWS CWs are rarely used for primary or secondary treatment

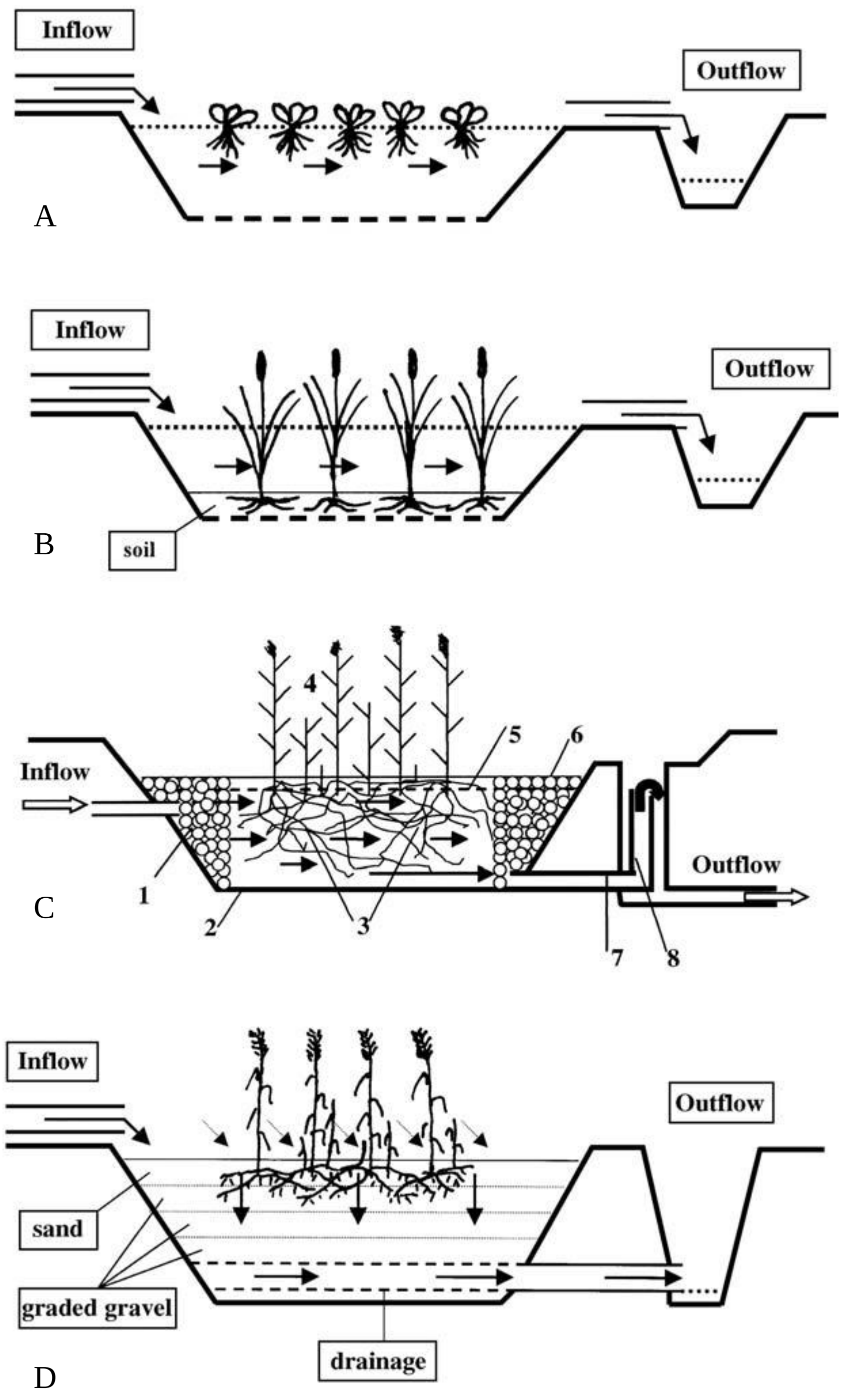


Figure 2: Diagrammatic scheme of the various types of CW (Vymazal, 2007). **A**. FWS CW with floating macrophytes, **B.** FWS CW with emergent rooted macrophytes, **C.** HSSF CW, **D.** VSSF CW

of wastewaters but generally for tertiary treatment or even post-tertiary (Mitsch *et al*., 2001; Kadlec and Wallace, 2009; Vymazal, 2011). On the other hand, HSSF CW consists of gravel or soil beds with macrophyte vegetation; normally, water enters at a horizontal position and flows around the plant roots from inlet to outlet where it is always kept below the surface (Kadlec and Wallace, 2009; Vymazal 2010). HSSF CWs are suitable for removal of organic materials and SS but are very low in nitrogen retention; this is attributed majorly to the poor nitrification ability of this system where constant oxygen availability is minimal (Vymazal 2005; Vymazal, 2007; Kadlec and Wallace, 2009; Vymazal 2010; Vymazal, 2011a). However, the major N removal mechanism performed by HSSF CWs is denitrification (Vymazal, 2005; Vymazal, 2007; Vymazal 2010). In addition, adsorption of N is possible but not common in this type of CWs while volatilization is limited due to limited free water surface (Vymazal 2005; Vymazal, 2010). HSSF CWs are common for secondary wastewater treatment in smaller communities (Kadlec and Wallace, 2009; Vymazal, 2011a). Despite being less susceptible to pathogens, HSSF CWs are more expensive and harder to maintain in comparison with FWS CWs in addition to the major problem of media clogging (Vymazal, 2005; Kadlec and Wallace, 2009; O'Geen, 2010). In VSSF, water is supplied continuously in a vertical position as pulse loading to the surface of sand or gravel and percolates to the roots of macrophytes (Kadlec and Wallace, 2009; Vymazal, 2010; Vymazal, 2011a). Nitrification process is very good in VSSF CWs due to the continuous supply of oxygen allowing the oxidation of ammonia; however, denitirification is very poor in such system (Vymazal 2005; Vymazal, 2007; Vymazal 2010; Vymazal, 2011a). VSSF CWs are very common in primary treatments of wastewater but are characterized by high operational and maintenance costs in addition to the media clogging problems (Vymazal, 2010; Vymazal, 2011a). Although P retention is generally low in all types of CW, good removal is obtained in FWS CW as they provide good conditions suitable for the most important removal mechanisms of P; sorption, sedimentation and uptake, on the other side P removal is poor in HSSF CWs due to its low sorption capacity (Vymazal 2005; Vymazal, 2007; Vymazal 2010; Vymazal, 2011a). In general, hybrid systems of all types of CWs can be a good approach to combine the advantages of each system and achieve best performance (Vymazal 2005; Vymazal 2010; Vymazal, 2011a). However, the most suitable CW for the treatment of agricultural runoff is the FWS CW as it provide high N, especially $N\text{-}NO_3^-$, retention in addition to cost effectiveness and low energy inputs ((Davis, 1995a; Peterson, 1998; Mitsch *et al.*, 2001; Kadlec and Wallace, 2009; Lee *et al.*, 2009; O'Geen *et al.*, 2010)

FWS CW and removal of N

FWS CWs are the most commonly used among CWs for the treatment of agricultural runoff as it provides open surface and intermittent dynamics suitable for all pollutant removal mechanisms (Kadlec and Wallace, 2009; O'Geen *et al.*, 2010; Vymazal 2010; Vymazal, 2011a). In addition, FWS CW are cost effective and devoid of problems of other types of CWs such as media clogging (Kadlec and Wallace, 2009; O'Geen *et al.*, 2010; Vymazal 2010; Vymazal, 2011a). As mentioned earlier, $N\text{-}NO_3^-$ is the most abundant form of N which is to be treated in constructed wetlands (Baker, 1998; O'Geen *et al.*, 2010), hence, anaerobic denitrification is the dominant mechanism of removal in FWS CW where temperature represent a limiting factor controlling the microbial activity necessary for such process (Bachand and Horne, 2002; Poe *et al.*, 2003; O'Geen *et al.*, 2010). Other N removal mechanisms include assimilation, sedimentation and volatilization (Poe *et al.*, 2003; O'Geen *et al.*, 2010)

Comparison of different N ($N\text{-}NO_3^-$) removal efficiencies in different experiments using FWS CW would rather be difficult and unfair as a result of general differences in the agricultural settings, wetland characteristics (hydrology and vegetation), hydraulic and pollutant load for each experiment (O'Geen *et al.*, 2010). However, hydraulic loading, hydraulic retention time (HRT) and surface area of wetland could be defined as key factors affecting the $N\text{-}NO_3^-$ removal efficiency (Kadlec and Wallace, 2009; O'Geen *et al.*, 2010). The $N\text{-}NO_3^-$ removal efficiencies of selected experiments for treatment of agricultural runoff in FWS CW varied between -8 to 99% (Table 1). In colder regions, FWS CW with smaller surface area and shorter HRT generally exhibited lower removal efficiencies (Brakserud, 2002; Koskiaho *et al.*, 2003; Bastviken *et al.*, 2009). On the other hand, increased wetland surface area and HRT increased the removal efficiencies (Hey *et al.*, 1994, Phipps and Crumpton, 1994; Comin *et al.*, 1997; Kovacic *et al.*, 2000; Borin *et al.*, 2001; Jordan *et al.*, 2003; Mitsch *et al.*, 2005; Tanner *et al.*, 2005; Kovacic *et al.*, 2006; Beutel *et al.*, 2009; Mustafa *et al.*, 2009; Moreno *et al.*, 2010; Diaz *et al.*, 2012). However, considering the individual conditions for each experiment is of great interest to assess the specific removal efficiency. For instance,

Table 1. $N\text{-}NO_3^-$ Removal efficiencies in FWS CW for some previous literature arranged in chronological order

Reference	Location	HRT (d)	Area (ha)	Depth (m)	Input (mg l^{-1})	Removal eff. (%)
Hey *et al.* (1994)	Illinois, USA	-	2–3.5	1–1.5	1.22	85.5-98
Mustafa *et al.* (1996)	Florida, USA	-	49	-	1.69	26
Phipps and Crumpton (1994)	Illinois, USA	-	1.9-2.4	0.6-0.7	-	78-95
Comin *et al.* (1997)	NE Spain	-	-	0.1-0.5	-	50-98
Raisin *et al.* (1997)	Victoria, Australia	-	0.045	-	1.3-1.7	11
Hunt *et al.* (1999)	North Carolina, USA	1-111	3.3	0.3-2	6.6	51
Kovacic *et al.* (2000)	Illinois, USA	11-21	0.3-0.8	0.4-0.9	-	34-44
Larson *et al.* (2000)	Illinois, USA	-	0.60-0.78	-	0.1-52	37-65
Woltemade (2000)	Midwest, USA	-	0.03-3.7	-	-	20-80
Borin *et al.* (2001)	NE Italy	-	0.32	-	1.65	90
Braskerud (2002)	Norway	-	0.035-0.09	0.2-0.8	0.75-2.77	3-15
Jordan *et al.* (2003)	Maryland, USA	12-19	1.3	> 1	0-2	52
Koskiaho *et al.* (2003)	Finland	0.25-1.6	0.48–0.6	0.9-2	2.9-7.4	-8-38
Mitsch *et al.* (2005)	Ohio, USA	3-4	1	-	4-6	17-97
Tanner *et al.* (2005)	New Zealand	1.5–51	0.026	0.3	11	11-49
Kovacic *et al.* (2006)	Illinois, USA	7–12	0.16-0.4	0.4-0.5	1.5-8.9	16-43
Moreno *et al.* (2007)	NE Spain	1-4	0.005-0.5	0.1	5.8–20.7	24–43
Bastviken *et al.* (2009)	Sweden	1-3	0.002	0.4		3–15
Beutel *et al.* (2009)	Washington. USA	8	0.7–0.8	0.6	1.3–1.4	93
Mustafa *et al.* (2009)	Ireland	-	0.12–0.24	1–1.5	3.81	74
Van de Moortel *et al.*(2009)	Belgium	-	-	0.5-0.6	8	99-100
Moreno *et al.* (2010)	NE Spain	2-15	0.005–0.5	0.1	-	34–87
Diaz *et al.* (2012)	California, USA	0.9-20	2.3-173	0.5-1	0.28-12.87	22-99
Groh *et al.* (2015)	Illinois, USA	-	0.3,0.6	0.4,0.9	-	56-62

increasing the hydrological loading rates increased removal efficiency by enhanced denitrification up to 95% as described by Phipps and Crumpton, 1994 while, different hydrological loadings and $N\text{-}NO_3^-$ concentrations did not affect the removal efficiency in an experiment conducted by Hey *et al.*, 1994, where the removal efficiency was high in all cases (85.5-98%). On the other hand, higher hydrological loading decrease the removal performance in some other experiments (Jordan *et al.*, 2003). In addition, Continuous flow can also enhance better removal performances than pulse flow (Diaz *et al.*, 2012). Changing climatic conditions and maturation of wetland can be important factors affecting the removal efficiency (Tanner *et al.*, 2005). Some enhancing factors such as the establishment of buffer strips associated with wetlands can also improve the removal performance of FWS CWs (Kovacic *et al.*, 2006). In general, the removal performance of FWS CW is more confined to the individual characteristics of each wetland.

3. Floating treatment wetlands (FTW)

Concept and evolution

Floating treatment wetlands (FTW) is a new eco-trend that outspread extensively in the last decades for the treatment of wastewaters, especially in tertiary stage, in natural and artificial water bodies. The introduction of FTW systems was inspired by the concept of natural floating islands. Floating islands or ‘sud’ generally refers to a mass of floating, usually hydrophyte, plant species growing on a buoyant support which may be organic (roots or remains of other plants) or inorganic (clay, silt, *etc.*) varying between centimeters and several meters to hectares. One of the earliest studies using floating islands was the establishment of a floating fen using *Phragmites communis*, Trin. and β. *flavescens*, Gren. and Godr. (Pallis, 1916). Following this, increasing interest was given to the study of floating islands and their biology, distribution and ecological potentials (Kashyap, 1920; Sahni, 1927; Russel, 1942; Reid, 1952; Lind, 1956; Kaul and Zutshi, 1966; Little, 1969; Junk, 1970, 1973; Scutcliffe, 1974; Varfolomeyeva, 1977; Sasser *et al*., 1995, 1996; Mitsch and Gosselink, 2000; Mallison *et al*., 2001; Adams *et al.*, 2002; Gopal *et al.*, 2003; Azza *et al.*, 2006, John, *et al.*, 2009).

Floating islands, mainly free floating hyrdophytes, were proposed for the natural wastewater treatment from contaminants (nutrients and heavy metals) using plant species such as *Eichhornia crassipes*, *Ipomoea aquatica*, *Lemna spp.*, *Nymphaea alba* and *Pistia stratiotes* (Kranchanawong and Sanijtt, 1995; Kerr- Upal *et al.*, 2000; Zimmels *et al.*, 2006, Li *et al.*, 2007, Mkandawire and Dudel 2007, Tewari *et al.*, 2008; Dhote and Dixit, 2009; Villamagna and Murphy, 2010; Olukanni and Kokumo, 2013; Khan *et al.*, 2016). However, the use of free floating species has some drawbacks; mainly the invasive nature of such species which can oppose and distract many anthropogenic activities (Villamagna and Murphy, 2010). In addition, they may not be adaptive to certain climatic conditions (Villamagna and Murphy, 2010). Another drawback is their free floating nature and fast degradability which can lead them to transferring pollutants from contaminated places to uncontaminated ones (Mkandawire and Dudel 2007). Under such conditions, rooted emergent macrophyte species were preferred in FTWs; where plant species are fixed in floating supporting mats with their aerial parts floating above the water level while their roots submerged in the water column and performing the typical functions (Headley and Tanner, 2006, 2012; Kadlec and Wallace, 2009; Vymazal, 2013; Chen 2016).

Artificial floating mats to support plant species were introduced recently and are prepared from a wide variety of materials, mostly inorganic, varying from simple hand-made to high technology supporting mats. Important criteria regarding the choice of materials for floating mats include buoyancy, flexibility, durability, affordability and suitability to environment (Headley and Tanner, 2006). Polyethylene (PE) and polystyrene foam are among the widely spread used materials that fulfill the previous criteria (Table 2) (Van Acker *et al.*, 2005; Boonsong and Chansiri, 2008; Yang *et al.*, 2008; Xian *et al.*, 2010; Tanner and Headley, 2011; White and Cousins, 2013; Winston *et al.*, 2013; Ebrahimi, 2015, Hartshorn *et al.*, 2016; Zhang *et al.*, 2016). PVC plastic pipes was another commonly used solution in FTW studies (Hubbard *et al.*, 2004; Billore *et al.*, 2008; Zhao *et al.*, 2012a, b; Winston *et al.*, 2013; Ge *et al.*, 2016; Saeed *et al.*, 2016). In the last decade, technology introduced new eco-friendly non-toxic durable floating mats such as Bio Haven® and Tech IA® (Stewart *et al.*, 2008; Tanner and Headley, 2011; Chang *et al.*, 2013; De Stefani *et al.*, 2011, Mietto *et al.*, 2013; Pavan *et al.*, 2015; Pappalardo *et al.*, 2017). In some experiments, organic materials such as timber, bamboo, coconut fiber, rice and barley straw have been supplementary to supporting mats to enhance the FTW establishment and functioning (Smith and Kalin, 2000; Boutwell, 2003; Garbutt, 2005; Billore, 2008; Zhao *et al.*, 2012a, Cao *et al.*, 2016) (Table 2).

Wastewater treatment

Since the 1990s, Focused research was directed to FTWs and their potentiality in the phytodepuration of a wide range of wastewaters with high efficiency, low costs and sustainable environmental value (Table 2). In light of that, the treatment of stormwater was amongst the earliest treatment trials; the use of 1 ha floating reed-beds in Heathrow Airport, England, UK for the removal of glycol and biological oxygen demand (BOD) from stormwater run-off was one of

Table 2. Collection of experiments about the FTWs around the world.

Reference	Location	Floating element	Plant species	Wastewater
Karnchanawong and Sanijtt (1995)	Thailand	Concrete ponds	*Ipomoea aquatica*	University campus wastewater
Van Oostrom (1995)	-	Floating mats	*Glyceria maxima*	nitrified meat processing effluent
Lakatos *et al.* (1997, 2014)	Hungary, Europe	Floating meadow system	*Phragmites australis*	Pertrochemcial waste water
Revitt *et al.* (1997)	U.K., England	Plastic geotextile lattice	*Phragmites australis*	Stormwater
Kerr- Upal *et al.* (2000)	Canada, Toronto	-	*Lemna spp.*	Stormwater
Smith and Kalin (2000)	Canada	Timber, plastic snow fences, fishing net, Styrofoam, plywood panels and Sphagnum spp. Moss on a burlap liner	*Typha spp.*	Acid mine drainage
Revitt *et al.* (2001), Richter (2003)	UK, England	Reed beds	*Phragmites australis*	Stormwater
Boutwell (2002)	USA, Las Vegas	HDPE-shipping pallets, stainless steel and coconut fibres	*Shoenoplectus spp., Typha spp*	Lake water
Ash and Trong (2003)	Australia, Queensland	Floating pontoons	*Chrysopogon (Vetiveria) zizanioides*	Sewerage effluent
Hart *et al.* (2003)	NewSouth Wales, Australia	-	*Chrysopogon zizanioides*	Septic tank effluents
Todd *et al.* (2003)	USA, Vermont, Massachus et	Advanced ecologically engineered system and floating ponds restorer	*200 species: Zantedeschia aethiopica, Carassius auratus, Azolla spp., lemna spp.*	Sewage
Hubbard *et al.* (2004)	USA, Georgia	PVC pipes and fibrous material	*Panicum hemitomon, Typha latifolia, Juncus effuses*	Swine lagoon
Kyambadde *et al.* (2005)	Uganda	-	*Cyperus papyrus, Miscanthidium violaceum*	Stabilization pond
Garbutt (2005)	United Kingdom	Floating reed beds, Barley straw	*Phragmites australis*	Eutrophic water

Table 2. contin. Collection of experiments about the FTWs around the world

Van Acker *et al.* (2005)	Belgium, Europe	PE-net+PE-foam with coconut fibres	*Carex spp., Phragmites australis, Shoenoplectus latifolia, Typha spp., Iris pseudacorus*	Combined sewer overflow
Billore *et al.* (2008)	India	Bamboo, PVC fibres, galvanized iron wire and nylon coconut fibres	*Phragmites Karka*	Lake water
Boonsong and Chansiri (2008)	Thailand	Foamed board with holes	*Vetiveria zizanioides*	Domestic waste water
Stewart *et al.* (2008)	USA	BioHaven® floating islands	Microbes only	Agricultural and municipal wastewater
Yang *et al.* (2008)	China	Foam sheets	*Oenanthe javanica*	River water with chemicals (Simulated agric. Run-off)
Sun *et al.* (2009)	China	Floating beds	*Canna spp.*	River water
Hu *et al.* (2010)	China	Dredged sludge, industrial slag and expanded perlite	*Acorus calamus*	Lake water
Li *et al.* (2010)	China	Polypropylene perforated plate (PPR) frame, buoyancy by sealed empty drinking bottle	*Ipomoea aquatica, Corbicula fluminea*	Eutrophic lake water
Van de Moortel (2010)	Belgium, Europe	Plastic pipes filled with foam and wire netting	*Carex spp., Iris pseudacorus, Juncus effusus, Lythrum salicaria*	Domestic waste water
Xian *et al.* (2010)	China	High density polyethelene foam plates with holes	*Lolium multiflorum*	Swine wastewater
Zhou and wang (2010)	China	Floating beds	*Oenanthe javanica*	River water
Tanner and Headley (2011)	New Zealand	Polyester fibre injected with patches of polystyrene foam (BioHavenTM, Floating Islands)	*Carex dispacia, Carex virgata, Cyperus ustilatus, Eleocharis acutis, Juncus edgarae, Schoenoplectus tabernaemontani*	Stormwater

Table 2. contin. Collection of experiments about the FTWs around the world.

Hubbard *et al.* (2011)	USA	Floating platforms	*Cynodon dactylon, Stenotaphrum secundatum, Panicum dichotomiflorum, Arundo donax*	Swine wastewater
Li *et al.* (2011)	China	-	*Lolium perenne*	Eutrophic lake water
Van de Moortel (2011)	Belgium, Europe	Plastic pipes filled with foam and wire netting	*Carex acutiformis, Iris pseudacorus, Juncus effuses*	Combined sewer overflow
Chang *et al.* (2012)	USA, Florida	Buoyant, interlocked puzzle-cut foam mats joined by nylon connectors	*Canna Flaccida, Juncus effuses*	Stormwater
Dunqiu *et al.* (2012)	China	-	*Phragmites australis, Typha latifolia*	River water
Li *et al.* (2012)	China	Floating beds	*Geophila herbacea, Lolium perenne*	Refinery waste water
Zhao *et al.* (2012a)	China	Bamboos covered with plastic net, PVC pipes with adsorptive biofilms	*Eichornia crassipes, Pistia stratiotes, Jussiaea reppens, Hydrocotyle verticillata, Hydrocharis dubi, Myriophyllum aquaticum, pontederia cordata, Canna indica, Caltha palustris*	Eutrophic river water
Zhao *et al.* (2012b)	China	PVC pipes and bamboo tablets	*Miscanthus sinensis Anderss (sp.), Vetiveria zizanioides*	Hypereutophic pond water
Zhou *et al.* (2012)	China	-	*Rumex acetosa*	Eutrophic river water
Chang *et al.* (2013)	USA, Florida	BioHaven® floating islands	*Pontederia cordata, Juncus effuses*	Stormwater
Ladislas *et al.* (2013)	France, Europe	Polyethylene plot with Puzzolana rocks, polystyrene float.	*Juncus effusus, Carex riparia*	Stormwater

Table 2. contin. Collection of experiments about the FTWs around the world.

White and Cousins (2013)	USA, South Carolina	Beemats of foam mat squares joined using nylon connectors	*Canna Flaccida, Juncus effuses*	Lake water witth fertilizers (simulated stormwater run-off)
Winston *et al.* (2013)	USA, South Carolina	Closed- cell foam and PVC pipes	*Carex stricta, Juncus effusus, Spartina pectinata, Pontederia cordata, Acorus gramineus, Peltandra virginica, Andropogon gerardii, Hibiscus moscheutos*	Stormwater
Borne *et al.* (2014)	New Zealand, Auckland	Floating treatment pond	*Carex virgata*	Storm water
Keizer-Vlek *et al.* (2014)	Netherlands	Styrofoam mats	*Iris pseudacorus, Typha angustifolia*	Eutrophic urban surface water
Wang and Sample (2014), Wang *et al*. (2014, 2015)	USA, Virginia	Floating treatment microcosms	*Pontederia cordata, Schoenoplectus tabernaemontani*	Storm water
Ebrahimi (2015)	Iran	Floating foam	*Juncus effuses*	Eutrophic water
Hartshorn *et al*. (2016)	Florida, USA	Foam mats with nylon connectors for floating system stability	*Canna, Juncus, Iris, Agrostis*	Forest, residential area and stormwater runoff wastewaters
Hartshorn et al. (2016)	Florida, USA	Foam mats with nylon connectors for floating system stability	*Canna, Juncus, Iris, Agrostis*	Agricultural, commercial areas and residential zones wastewaters
Hartshorn *et al*. (2016)	Florida, USA	Foam mats with nylon connectors for floating system stability	*Canna, Juncus, Agrostis*	Stormwater runoff wastewaters derived from cars park.
Cao *et al*. (2016)	China	Perforated polypropylene random copolymer, rice straw and light ceramsite as filling substrates.	*Canna*	Eutrophic river
Zhang *et al*. (2016)	China	Polyetilene foam boards	*Canna indica*	Domestic wastewater and tap water
Ge *et al.* (2016)	China	Polyvinyl chloride pipes, plastic mesh, and pot holders	*Canna indica, Thalia dealbata, Lythrum salicaria*	Storm water

Table 2. contin. Collection of experiments about the FTWs around the world.

Saeed *et al*. (2016)	Bangladesh	UPVC pipes, nylon fiber mesh as medium and macrophytes support	*Phramites australis, Canna indica*	River water
Olguin *et al*. (2017)	Mexico	FTW: Low-cost rigid plastic containers with empty plastic bottles. Plastic bottles perforated at the bottom and filled with volcanic gravel as plants support	*Cyperus papyrus, Pontederia sagittata*	Eutrophic urban water

the first large scale processes reported for this type of treatment (Revitt *et al.* 1997 and 2001; Richter, 2003). FTWs also proved high efficiency in the removal of metals like Cu, Cd, Ni and Zn from urban and artificial stormwater (Tanner and Headley, 2011; Ladilas *et al.*, 2013; Bourne *et al.*, 2014). The use of FTWs for the removal of COD and nutrients (TN, TP, NO_3^-, NH_4^+, PO_4^-) in stormwater was reported by many authors with removal rates ranging between 16-70%, 9-76%, 8-79%, and 51-100% for TN, NO_3^-, TP and NH_4^+, respectively (Chang *et al.* 2012 and 2013; Winston *et al.* 2013; Wang and Sample, 2014; Wang *et al.*, 2014 and 2015 Ge *et al.* 2016, Hartshorn *et al.*, 2016; Olguin *et al.* 2017). Another example of wastewater treated by FTWs was combined sewer flow; two experiments were conducted in Belgium for pollutant removal (Van Acker, 2005; Van de Moortel, 2011). Smith and Kalin (2000) used FTWs for the removal of Cu, Zn and sulphates from acid mine drainage water in Toronto, Canada. Removal of COD and nutrients from swine wastewater was reported by Hubbard (2004) and Xian *et al.* (2010). The treatment of sewage water with FTWs varied between using the simple floating pontoons (Ash and Troung, 2003) and the complicated, Advanced Ecologically Engineered System (AEES) introduced by Todd *et al.* (2003). In China, many researches in the last decade focused on the use of FTWs in the treatment of eutrophic lake and river water bodies for the removal of nutrients and COD with removal rates ranging 31-78%, 26-97% and 8-86% for TN, NO_3^- and TP , respectively (Table 3) (Yang *et al.*, 2008; Sun *et al.*, 2009; Hu *et al.*; 2010; Li *et al.*, 2010; Zhou and Wang, 2010; Li *et al.*, 2011; Dunqiu *et al.*, 2012; Zhao *et al.*, 2012a, 2012b; Zhou *et al.*, 2012; Zhang *et al.*, 2016; Cao *et al.*, 2016).

Table 3. Removal rates of pollutants (%) using FTWs in China in the last decade.

Reference	TN	NO_3^-	NO_2^-	NH_4^+	TP	PO_4^-	COD	Chl-a
Yang *et al.* (2008)	31-64	71-97	-	-	8-15	-	-	-
Sun *et al.* (2009)	72	76	96	-	-	-	95	-
Hu *et al.* (2010)	36	-	-	44	36	-	-	48
Li *et al.* (2010)	53	-	-	34	54.5	-	-	80
Xian *et al.*, (2010)	84	-	-	-	90	-	83	-
Li *et al.* (2011)	32	-	-	81	73	-	-	-
Chang *et al.* (2012)	61	73	-	100	53	79	-	-
Dunqiu *et al.* (2012)	-	-	-	88	83.5	-	-	-
Zhao *et al.* (2012a)	-	59	82	50	86	-	-	-
Zhao *et al.* (2012b)	37	26	53	45	43	-	-	64.5
Chang *et al.* (2013)	16	21	-	51.5	48	79	-	-
Zhang *et al.* (2015)	-	-	-	85	83	82.5	-	-
Cao *et al.* (2016)	65-78	42-62	-	71-81	-	-	-	-
Ge *et al.* (2016)	70	-	-	-	82	-	71	-

Plant species and growth performance

Being favorable in FTWs, rooted emergent macrophytes belonging to different botanical families were used extensively for the treatment of wastewaters (Table 2, 4). However, despite the great variety, choices are limited to a specific group of macrophytes which are frequently used for the treatment of wide range of wastewaters namely, *Carex spp*., *Canna spp*., *Cyperus spp.*, *Iris pseudacorus*, *Juncus effusus*, *Phalaris arundinacea*, *Phragmites australis*, *Typha spp.*, *Scirpus spp.* (*Schoenoplectus spp*.) and *Vetiveria zizanioides* (Kadlec and Wallace, 2009; Vymazal, 2013; Chen *et al.*, 2016).

Many studies have reported the growth performances of vegetation installed in FTWs. Tanner and Headley (2011) assessed the performance of 4 macrophytes in a 365-day experiment for the treatment of heavy metals and phosphorus in a stormwater retention pond. In this experiment, *Carex varigata* exhibited the highest above biomass production (2350 g m^{-2}) followed by *Cyperus ustulatus* (1528 g m^{-2}) while *Schoenoplectus tabernaemontani* had the lowest above mat biomass production (834 g m^{-2}). *C. ustulatus* showed higher overall uptake rates for Cu, Zn and P than *C. Varigata* and *S. tabernaemontani*. White and Cousins (2013)

Table 4. List of macrophyte plant species with their correspondent botanical aspects.

Species	Common name (s)	Family	Origin	Botanic description	Habitat
Acorus calamus L.	Sweet flag, beewort, bitter pepper root, calamus root	*Acoraceae*	Asia	Perennial, rhizomatous; linear leaves; triploid forms more common, infertile.	Lakes or ponds, marshes, rivers or streams and wetland margins
Alnus glutinosa L.	Common alder, black alder, European alder	*Betulaceae*	Europe, southwest Asia and northern Africa	Tree, 20-30 m, adventitious roots, main axial stem branched, monoecious, wind pollinated	Moist soils, near rivers, ponds and lakes
Artemisia caerulescens L.	Mugwort, wormwood, sagebrush	*Asteraceae*	Euro-mediterranean region	Perennial, woody stems, erect branches with inflorescences, linear leaves, fruit; achene	Saline soils, lagoons
Arundo donax L.	Giant cane, spanish cane, wild cane, giant reed	*Poaceae*	Mediterranean Basin, middle east Asia, parts of Africa and southern Arabian Peninsula.	Perennial, 6 m, rhizomatous, hollow stems, linear alternate leaves, seedless or infertile	Fresh or moderately saline soils, wetlands and riparian habitats
Aster tripolium L.	Sea aster	*Asteraceae*	Eurasia and northern Africa	Perennial, 50 cm tall, fleshy lanceolate leaves, purple ray florets	Salt marshes, estuaries
Calamagrostis epigejos (L.) Roth	Wood small-reed, bushgrass	*Poaceae*	Eurasia and Africa	Perennial grass, lengthy rhizomes, erect, 60–200 cm, large inflorescence, flowers form dense, narrow spikes	Salt marsh and wet habitats
Caltha palustris L.	Marsh-marigold, kingcup	*Ranunculaceae*	Temperate regions of the Northern Hemisphere	Perennial herbaceous, 10–80 cm height; thick branching roots; flowering erect stems.	Marshes, fens, ditches and wet woodland
Canna indica L.	Indian shot, African arrowroot, edible canna, purple arrowroot	*Cannaceae*	South America, Central America, southeastern United States	Perennial, rhizomatous, 0.5 -2.5 m height; hermaphrodite flowers; small, globular, black pellets seeds.	Swamp and wetland edges, streambanks and other moist areas
Carex elata Gooden. (*Carex stricta* Lam.)	Upright sedge	*Cyperaceae*	Universal	Perennial, rhizomes, stolons or short rootstocks; flower-bearing stalk; unbranched, erect, leaf blade long and flat; spikes combined into a large inflorescence.	Marshes, calcareous fens, bogs, peatlands, pond and stream banks, riparian zones, ditches
Chrysopogon zizanioides (L.) Robert.	Vetiver	*Poaceae*	India	Perennial bunchgrass, 1 m height; long leaves; long, rigid roots grown downward; flowers in spiklets.	Floodplains, banks of streams and rivers, rich moist soils

Table 4. **contin.** List of macrophyte plant species with their correspondent botanical aspects.

*Cladium mariscus (*L.) Pohl.	Swamp sawgrass, great fen-sedge, saw-sedge	*Cyperaceae*	Temperate Europe and Asia	Perennial, 2.5 m, leaves with hard serrated edges, flowers; hermaphrodite collected in inflorescences, fruit; achene	Boggy areas and lakesides
Cynodon dactylon (L.) Pers.	Dūrvā grass, Bermuda grass, dog's tooth grass, Bahama grass, devil's grass	*Poaceae*	Middle East	Perennial grass, deep root system; 2 m, erect stems; 1–30 cm, leaves, short blades with rough edges	Roadsides, overgrazed and uncultivated areas, lands high nitrogen levels, moist sites along rivers
Cyperus papyrus L.	Papyrus sedge, paper reed, Indian matting plant, Nile grass	*Cyperaceae*	Africa	Perennial, herbaceous, rhizomatous, 4-5 m, triangular green stems; Each topped by a dense cluster of thread-like stems, greenish-brown flower clusters, nut like fruit	Flooded swamps, shallow water.
Dactylis glomerata L.	Cock's-foot, orchard grass, or cat grass	*Poaceae*	Europe, temperate Asia, and northern Africa	Perennial grass, 20–140 cm height; long, grey-green leaves; distinctive triangular flower head, spikelets 2 to 5 flowers.	Meadows, pasture, roadsides, rough grassland
*Elytrigia atherica (*Link) Kerguélen	Sea couch grass	*Poaceae*	Old World in Europe, Asia, and northwest Africa	Perennial grasses	Sandy, and saline environments
*Glyceria maxima (*Hartm.) *Holmb.*	Great Manna Grass, Reed Mannagrass, and Reed Sweet-grass	*Poaceae*	Europe and Western Siberia	Perennial, rhizomatous	wet areas riverbanks and ponds
Halimione portulacoides (L.) Aellen	Sea purslane	*Amaranthaceae*	Temperate Eurasia and parts of Africa	Evergreen, halophyte, 75 cm, flowers; monoecious, pollinated by wind.	Salt marshes and coastal dunes
Inula crithmoides L.	Golden samphire	*Asteraceae*	Western and southern Europe and the Mediterranean	Perennial, tufted habit, 1 m, fleshy leaves, large flower heads, six yellow ray florets, flowers; self-fertile or pollinated by insects	Salt marshes or sea cliffs
Iris laevigata Fisch.	Japanese iris, rabbit-ear iris, kakitsubata	*Iridaceae*	Japan	Perennial, rhizomatous; blue, purple or violet flowers.	Shallow waters, marshy and still ponds, damp soils

Table 4. **contin.** List of macrophyte plant species with their correspondent botanical aspects.

Iris pseudacorus L.	Yellow flag, yellow iris, water flag, lever	*Iridaceae*	Europe, western Asia and northwest Africa	Perennial, herbaceous, 1-1.5 m height, rhizomatous, erect, long leaves, flower; bright yellow, fruit; dry capsule.	very wet conditions, common in wetlands
Juncus effusus L.	Common rush, soft rush	*Juncaceae*	Europe, Asia, Africa, North America, and South America	Perennial herbaceous, 1.5 m, stems; smooth cylinders with light pith filling; yellowish inflorescence emerge from one side of the stem.	Wet areas; wetlands, riparian areas, marshes, ditches, fens
Juncus maritimus Lam.	Sea rush	*Juncaceae*	Europe, Asia, Africa	Perennial, herbaceous, 40-100 cm stems; green, cylindrical, leaves; pointed, inflorescence; green or yellow flowers	Sandy , moist and saline soils, coastlines
Limonium narbonense Mill.	Sea lavender	*Plumbaginaceae*	Southern Europe, North Africa and in Southwest Asia	Perennial, herbaceous, 30–70 mm, leaves; lanceolate-spatulate, in a basal rosette, inflorescence; large, few or absent sterile branches, flowers; white to pale violet	Coastal habitat; beaches, salt marshes, coastal prairie, sandy saline habitats
Lythrum salicaria L.	Purple loosestrife, spiked loosestrife, purple lythrum	*Lythraceae*	Europe, Asia, northwest Africa, and southeastern Australia	Perennial, herbaceous, rhizomatous, 1–2 m height; numerous erect stems, 1.5 m width from a single woody root mass; lanceolate leaves; reddish or purple flowers; fruit: capsule.	Ditches, wet meadows and marshes, along sides of lakes
Mentha aquatica L.	Water mint	*Lamiaceae*	Europe, northwest Africa and southwest Asia	Perennial, herbaceous; fleshy with fibrous roots (90 cm); ovate to lanceolate leaves; tiny flowers, densely crowded, purple, form a terminal hemispherical inflorescence.	Shallow margins, channels of streams, rivers, pools, dikes, ditches, canals, wet meadows, marshes and fens
Phalaris arundinacea L.	Reed canary grass	*Poaceae*	Europe, Asia, northern Africa and North America	Perennial bunchgrass; thick underground rhizomes; stems 2 m height; green variegated leaf; spikelets: light green, streaked with darker green or purple.	Floodplains, riverside meadows, wetland habitat types

Table 4. **contin.** List of macrophyte plant species with their correspondent botanical aspects.

Phragmites australis (Cav.) Trin. ex Steud.	Common reed	*Poaceae*	Cosmopolitan	Perennial grass; horizontal runners roots; erect stems, average 2 m height; linear leaves; flowers: dense, sharp pointed grey hairy spikelets.	Helophyte, alkaline habitats, brackish water, upper edges of estuaries and on other wetlands
Pontederia cordata L.	Pickerel weed	*Pontederiaceae*	American continent	Aquatic, rhizomatous, aerenchyma tissues to carry oxygen into the roots; leaves vary across population; tristylous flowers.	Wetlands, pond and lake margins
Puccinellia palustris (Seen.) Hayek	Alkali grass, salt grass	*Poaceae*	Temperate to Arctic regions of Northern and Southern Hemispheres	Perennial bunchgrass, inflorescence; spreading array of a few branches containing spikelets.	Wet environments, saline or alkaline conditions
Salix eleagnos Scop.	Bitter willow, olive willow, hoary willow	*Salicaceae*	Central and southern Europe, south west Asia, north Africa	Erect bushy deciduous shrub, 3 m, leaves; narrow grey-green ,20 cm long, turn yellow in autumn, green catkins, appear with the leaves in spring, male catkins having yellow anthers, species is dioecious	River banks, streams and mountain streams, gravel and floodplains of watercourses
*Sarcocornia fruticosa (*L.) A. J. Scott	Samphires , glassworts , saltworts	*Amaranthaceae*	Cosmopolitan	Perennial herbs, sub-shrubs or shrubs, erect or prostrate, creeping form, leaves; opposite, blades form small, triangular tips with narrow scarious margin, inflorescences; terminal or lateral, spike-like, paired cymes, cyme; 3-5 flowers	Wet saline habitats; estuaries, salt marshes, tidal flats, seacliffs, salt pans, saline sediment in seasonal desert waterways
Schoenoplectus lacustris (L.) Palla	Lakeshore bulrush, common club-rush	*Cyperaceae*	Europe, North Africa	Perennial, rhizomatous, 3.5 m height; stems: erect, 5 cm thick; leaves: bladless sheaths, blades underwater 100 cm; inflorescence: top of stem, branches.	Fresh water
S*parganium erectum* L.	Simplestem bur-reed, branched bur-reed	*Typhaceae*	Temperate regions of both the Northern and Southern Hemispheres.	Perennial, aquatic, rhizomatous, emergent stems with aerenchym; strap-like leaves; flowers: borne in spherical heads, hermaphrodite.	Shallow marshes, ponds and streams
Spartina maritima (Curtis) Fernald	Small cordgrass	*Poaceae*	Western and southern Europe and western Africa	Perennial, herbaceous, 20-70 cm, leaves; slender, broad at the base, tapering to a point, flowers and seeds on all sides of the stalk, flowers; greenish	Coastal habitat

Table 4. **contin.** List of macrophyte plant species with their correspondent botanical aspects.

Symphytum officinale L.	Common comfrey, true comfrey	*Boraginaceae*	Europe	Perennial, herbaceous, 30-120 cm, rhizomatous, stems; errect, leaves; large rough, strong and hairy, inflorescence; panicle pseudo dense clusters of flowers, fruit; achene	Marshy places, ditches, canals and bogs, damp meadows and edges of woods.
Thalia dealbata Fraser ex Roscoe	Powdery alligator-flag, hardy canna, powdery thalia	*Marantaceae*	Southern and central United States	Aquatic plant, 1.8 m height; leaves: blue-green, ovate to lanceolate; flowers: small, violet.	Swamps, ponds and other wetlands
Typha latifolia L.	Broadleaf cattail, bulrush, common bulrush, common cattail	*Typhaceae*	North and South America, Europe, Eurasia, and Africa	Perennial, herbaceous, rhizomatous, 1.5-3 m height; leaves: linear, broad, erect, monoecious; stems: bear flowering spikes; seeds: minute, hairy.	Obligatory wetland species, fresh water, slightly brackish marshes
Zantedeschia aethiopica (L.) Srengel	Calla lily	*Araceae*	Southern Africa	Perennial, herbaceous, evergreen, rhizomatous, 0.6–1 m height; leaves: arrow shaped, dark green; inflorescences: large with a pure white spathe and a yellow spadix.	Moist, shady areas with plenty of water

used 2 species for the treatment of stormwater runoff; *J. effusus* retained up to 28.5 g N m^{-2} and 1.69 g P m^{-2} versus 16.8 g N m^{-2} and 1.05 g P m^{-2} for *Canna flaccida.* Additionally, In a storm water retention pond, *Thalia dealbata* showed the highest performance (maximum above mat biomass 1989 g/plant, maximum N uptake 5.4 g/plant) while *Lythrum salicaria* L. exhibited the lowest (566 g biomass/plant, 2.7 g N/plant) (*Ge et al.*, 2016). Another example for the use of machrophyte species in the treatment of stormwater involves the use of *P. cordata* and *Scirpus californicus* with average uptake rates of N and P of 36.39 and 1.48 mg m^{-2} d^{-1}, respectively (Chang *et al.*, 2012). Moreover, Plant species in FTWs proved great efficiency in the treatment of swine wastewater. In a swine wastewater lagoon, *T. latifolia* yielded 16511 g m^{-2} total biomass and removed 534, 79 and 563 g m^{-2} of N, P, K, respectively while total biomass for *Panicum hemitomon* was 9751 g m^{-2} and nutrient removal was 323, 48 and 223 g m^{-2} of N, P, K, respectively (Hubbard, 2004). *Cynodon dactylon* Tifton 85, *C. dactylon* and *Panicum dicotomiflorum* were used also in the treatment of swine wastewater and yielded 3600, 3200 and 3100 g m^{-2} of above mat biomass, respectively after 6 cuttings. *C. dactylon* Tifton 85 exhibited the highest annual uptake of N and P; 69 and 25 g m^{-2}, respectively while *P. dicotomiflorum* exhibited the highest K annual uptake; 78 g m^{-2} (Hubbard, 2011). Smith and Calin (2000) investigated the use of *Typha angustifolia* in the removal of suspended solids (SS) from ponds where it removed 290 g m^{-2} of SS and yielded 180 g m^{-2} root biomass in Kitimat lagoon, British Colombia, Canada after the 2nd season. *T. angustifolia* and *I. pseudacorus* were introduced for the removal of TN and TP by Keizer-Vlek *et al.* (2014); the best performance was exhibited by *I. pseudacorus* (277 and 9.32 mg m^{-2} d^{-1} of N and P, respectively). *P. cordata* produced 10.44 g dry weight and absorbed 7.58 mg P per plant in the treatment of urban run-off wet pond (Wang *et al.*, 2015). In general, increasing research is directed recently to the study of the plant growth performance as an important tool for the assessment of wetland treatment systems.

Research objectives

The main objective of this research is to evaluate the overall performance of two types of surface flow constructed wetlands used in north Italy; FWS CW and FTW, in terms of water quality improvement and vegetative performance of different machrophyte plant species on 3 different levels; full and pilot scale experiments, and a review study.

The specific objectives of the research include:

Chapter II

1. Assessment of the water-purification capacity of integrated surface wetland system to control diffused nutrient pollution from a conventional cropping system within the Venetian Lagoon drainage system.

2. Testing the wetland performance in reducing $N\text{-}NO_3^-$ and TN in the water flow.

3. Quantifying the survival rate of plant species, and screening the biometrics, biomass production and nutrient uptake of seven macrophytes adapted to FTWs.

Chapter III

1. Evaluation of $N\text{-}NO_3^-$ retention in a pilot scale event- driven experiment simulating excessive $N\text{-}NO_3^-$ load to draw some conclusions on the overall specific performance of the FWS CW within the Venetian Lagoon system.
2. Prediction of some water dynamics of the FWS CW in a designed event- driven experiment simulating excessive agricultural $N\text{-}NO_3^-$ load.

Chapter IV

1. Reporting the biometric characteristics, biomass production and nutrient uptake of 20 different wetland species installed in 9 different FTWs during 10 years of research in North Italy.
2. Introduction of some correlations between different plant growth parameters and between these and other physico-chemical parameters of treated wastewater.

Chapter II

Surface flow constructed wetlands for the treatment of agricultural surface run-off within the Venetian lagoon system (Full scale)

Introduction

In 2000, Italy recorded one of the highest values among the EU Member States for utilised agricultural area (UAA); 13.1 million hectares (ha), accounting for 43 % of the whole territory (Eurostat, 2015). This area decreased by 1.6% in 2010 (12.9 million ha). Veneto region (northeast Italy) contributes to this area with 6.3% (811.4 thousand ha). Most of the agricultural lands in Veneto region lie in the lower plain (rich in water resources and arable land) with 57% in the Po Valley. Water resources in Veneto include; rivers flowing through the region: the Po, Adige, Brenta, Bacchiglione, Livenza, Piave, and Tagliamento, lakes: the eastern shore of Lake Garda, the largest in Italy, belongs to Veneto. As well, The Venetian Lagoon is an enclosed bay in the northern part of the Adriatic Sea forming a flat terrain with ponds, marshes and islands.

Anthropogenic activities, agricultural and industrial, generate wastes and pollutants with high negative impact on the physicochemical and biological parameters of water resources, thus, declining the quality of water (Zonta *et al.,* 2005). In Veneto, most of the industrial and agricultural wastewaters are conveyed to the Venetian lagoon through its drainage basin; loads of nitrogen (N) and phosphorus (P) are discharged through 12 tributaries divided into sub-basins (Collavini *et al.,* 2005; Zonta *et al.,* 2005; Zuliani *et al.,* 2005). N and P in addition to other pollutants, mainly heavy metals, were evaluated within the framework of the DRAIN project (1998-2000) to determine the pollutant input from the drainage basin to the lagoon. The total nitrogen load was one-third higher than the maximum allowable load of 3000 t/year stated by the Ministerial decree (Ministero dell'Ambiente, 1999) as a reference value for lagoon inputs, while the total phosphorus was 229 t/year, which is lower than the maximum allowable load of 300 t/year (Collavini *et al.*, 2005). In light of this, inputs of nitrogen into the Venetian Lagoon system must be reduced dramatically in the near future, or at least the maximum allowable value should be attained.

Constructed wetland technology was not officially considered as a water treatment technology by the Italian legal framework until 1999 (Masi *et al.,* 2000). The use of constructed wetlands (CW) was officially enforced by the new law about municipal wastewater treatment D.Lgs 152/99 "for urban centers with populations in the range of 10-2000 PE discharging into freshwater, in the range of 10-10.000 PE discharging in sea water, and for tourist facilities and other point sources with high rates of fluctuation of organic and/or hydraulic loads". Most

CW systems were concentrated in central and northern Italy (Masi, 2000); out of 145 systems, 106 (74%) are located in Veneto, Emilia-Romagna and Toscana where local conditions are favorably better. CW varied between sub-surface flow (horizontal (HF) and vertical (VF) flow), with HF systems prevailing over VF, and surface flow (mainly free water surface (FWS), floating treatment wetlands (FTW) were introduced later in 2006). Few semi-natural (NW) and re-constructed systems (RCW) are present in Italy and designed for the treatment of diffuse pollution sources from agricultural and civil catchments (Masi, 2000). In northeast Italy, CW targeted the treatment of many types of wastewater; municipal domestic water in tertiary treatment had the greatest focus (De Stefani, 2012; Mietto *et al*., 2013). Other treated types of wastewater include aquaculture and stream water (De Stefani *et al.*, 2011), sewage water (De Stefani *et al.*, 2012) and digestate liquid fraction (Pavan *et al.*, 2015). Fewer experiments dealt with agricultural runoff (Borin and Tocchetto, 2007; Maucieri *et al*., 2014).

The general aim of the present study is to assess the water-purification capacity of a 3.2-ha integrated wetland system within the Venetian Lagoon drainage system designed to control diffused nutrient pollution from a conventional cropping system. The specific aims focus on two different phytoremediation systems, namely a FWS CW system and an FTW system, so as to estimate their performance in reducing $N\text{-}NO_3^-$ and TN in the water flow, to quantify the survival rate of FTW species, and to screen the survival, biometrics and biomass production of seven macrophytes adapted to FTWs.

Materials and Methods

Geographical framework and the integrated agricultural wetland

The study area is located within the Venetian Lagoon drainage system (north-eastern Italy), a dense minor hydrographic network directly managed by the *Adige Euganeo Land Reclamation Authority*. This hydrographic network plays two crucial roles: draining water from vast 'lowlands' lying below the mean sea level into the Venetian Lagoon system and providing water to the farms there (Pappalardo *et al*. 2015). The experiment was conducted on 'Tenuta Civrana' farm (365 ha), 45.166°N and 12.066°E, in the Province of Venice (Cona, VE). The land was reclaimed by draining the 'Cavarzerano' marshes in the 1930s and contains natural environments, such as lowland forests and wet environments (Figure 1).

The climate is subhumid (Köppen climate classification), with a mean annual rainfall of 850 mm, which is fairly uniformly distributed throughout the year. Temperatures range from an average minimum of –1.5°C in January to an average maximum of 27.2°C in July.

The integrated agricultural wetland covers 3.3 ha and was created in 2014 by restoring a semi-natural wetland and incorporating five sub-basins into a FWS CW. At the outlet, the water flows through a subsurface pipe into a vegetated 470-m-long channel, which has been used to create a second phytoremediation system, the FTW (Figure 1). The farm and integrated agricultural wetland are fed by diverting water from the 'Canale dei Cuori', one of the main canals draining water from the surrounding territory.

GIS analyses and weather data

A preliminary dGPS survey was conducted in 2013 to investigate the micro-topography and drainage system of the area. The experimental site was set up for agro-environmental monitoring by analysing aerial (satellite and UAV) images and processing digital terrain models (DTM) in the GIS environment. Sixteen geo-referenced spots were identified for sampling and for measuring the physical parameters of water. Sampling points follow the water flow from the inlet to the outlet in both CWs. In addition, qualitative and quantitative data from fieldwork, such as pictures of the basins and riparian zones, the floating barriers and the agglomeration of plants, were geo-referenced to analyse the spatial evolution of the system and its components. So as to obtain the most reliable climate dataset, the nearest official weather station 4.2 km from the experimental site was referred (Cesia, ARPAV station, Veneto Region). Validated weather data, such as daily cumulative precipitation and temperature, were collected between 2014-2016 for the analysis of rainfall events and thermic trends.

Figure 1. A. Map of free water-surface constructed wetland (FWS CW): white dots are sampling points and narrow white lines represent the flow direction (high-resolution imagery, Digital Globe, winter 2015). **B.** Unmanned aerial vehicle image during spring. **C.** The floating-treatment wetland system, flow direction and sampling points. **D.** *Lythrum Salicaria* flowering in the floating system (F2).

The free water-surface constructed wetland

The FWS CW system covers 2.4 ha and the hydraulic system is managed such that it feeds five sub-basins by gravity during the crop season (March–November). Water flows through a set of sequential basins connected by subsurface pipes. The mean detention time is ~8–10 days. Because of the climate regime and geomorphology of the area, in winter, the water flow from the channel is intentionally interrupted at the inlet, resulting in the partial drying out of the basins. In spring (mid-March), the main channel is re-opened to feed the downstream basins and fill the FWS CW system. The system is structured in two main sub-trapezoidal basins (B1 and B2) obtained by restoring a semi-natural wetland; their surface areas are 0.5 and 1 ha respectively, with a water depth of ~0.6 m in B1 and 0.4 m in B2. Further three sequential downstream basins (B3, B4, and B5) with shallower depths (0.3–0.4 m) have been created to complete the water-purification treatment. Wetland vegetation has been restored and integrated with several local macrophytes that have become established along riparian zones and within the basins, including *Phragmites australis*, *Typha latifolia*, *Iris pseudacorus*, *Phalaris arundinacea*, *Menta aquatica* L., *Carex* spp. and *Juncus* spp. The creation of four islands vegetated with *P. australis*, *Juncus* spp. *and Carex* spp. in B1 and B2 has basically provided these basins with the task of slowing down the water flow, thereby allowing initial stabilisation of suspended solids. Basin B2 is the most densely vegetated, with *P. australis* having fully colonised the banks (Figure 1A, B). The last three basins (B3, B4 and B5) were planted with *M. aquatica*, *Carex* spp., *P. arundinacea* and *P. australis* in 2014, and the vegetation is still in the process of establishment. However, 3 years after implementation, the vegetation in B1 and B2 is becoming gradually naturalised, especially *P. australis.*

The floating-treatment wetland

Water flows from the FWS CW basins and enters into the FTW system, established along the channel (Figure 1C). The FTW is an open system and probably receives drainage water from croplands on its northern border. It consists of a set of rectangular (50 × 90 cm) self-buoyant mats with eight windows, with grids to support plants. The combined morpho-functional floating system is a 'TECH-IA', a technology of PAN Ltd, (PD), Italy a Padua University spin-off. The rectangular structure, which provides support for aquatic macrophytes, is made from a recyclable material, ethylene vinyl acetate (EVA), and weighs ~2 kg (De Stefani *et al*. 2011; Mietto *et al*. 2013; Pavan *et al*. 2015). Single units were assembled to create three vegetated floating barriers of 120 units each (F1, F2 and F3), which are divided into six modules (20 units per module). The floating units were tied together with plastic strips and maintained *in situ* by means of ropes securely anchored to the shore with stakes. Flexibility of the barrier movement was ensured to allow the barriers to follow the water level in the main downstream channel, without incurring damage to the root systems. Two plants were transplanted into each unit, for a total of 40 plants per module and 240 per floating barrier.

The uppermost floating barrier (F1), the first to meet water from the FWS CW, was vegetated in May 2014 with 240 plants of *Carex* spp. The F2 barrier was vegetated in May 2014 with 240 plants of the following six different macrophytes: *Sparganium erectum* L., *Schoenoplectus lacustris (L.) Palla*, *M. aquatica* L., *Caltha palustris* L., *P. arundinacea* L. and *Juncus effusus* L. This barrier was re-vegetated in April 2015 with 240 plants of *L. salicaria* L. (Figure 1D). The F3 barrier was vegetated with 240 plants of *I. pseudacorus* L. in 2014 and was re-vegetated with plants of same species in 2015 (Figure 2). The three barriers are ~30 m apart and are kept at a certain distance. In 2016, the three barriers (F1, F2 and F3) were translocated together towards the end of the channel.

Fieldwork: water sampling, physicochemical parameters and plant survey

Representative water samples were collected periodically during the 2014, 2015 and 2016 crop seasons, generally twice a month and after significant rainfall events, in the spring, summer and autumn of the 3 years at 10 different points at the inlets and outlets of the FWS CW and the FTW (Figure 1A, D). Each representative sample consisted of three replicates obtained at the same point 30 min apart.

Selected physicochemical parameters of water were measured to determine water quality and the efficiency of the depurative systems. Electric conductivity (μS cm^{-1}), dissolved oxygen (mg L^{-1}), pH and temperature (°C) were measured at the inlets and outlets of the wetland sub-basins and in the main channel containing the floating systems by using HQD (HACH Lange HQ 40d, Hach, CO, USA), a portable multitasking device used to assess some of the physical and chemical properties of water. Water turbidity was measured using a portable turbidimeter (HACH 2100P Turbidimeter) and expressed in mean values of nephelometric turbidity units (NTU). Normality of data was checked by the Kolmogrov-Smirnov test. Since the data were not distributed normally, Kruskal-Wallis non parametric test was used to check significance of values between inlet and outlet of the system ($p<0.05$). Results of the analyses are presented as box and plots and line trends for inlets and outlets of FWS CW and FTW.

Figure 2. F3 barrier re-vegetated manually with 240 plants of *I. Pseudacorus* in April 2015, 2 plants per unit with total of 120 units

The survival rate of plants in the FTW system (F1, F2 and F3) was assessed periodically during the three vegetative seasons, by counting the number of living plants in each of the three barriers once a month from May to August 2014, April to October 2015 and from May to October 2016. The total survival percentage of each species was calculated at the end of each season.

Plant height and root-system length and width were used as parameters to monitor the performance of plants in the floating systems and test their capacity for adaptation and establishment. No plant measurements were taken in 2014 because the plant species had not had enough time to become established and exhibit sufficient growth in the newly implemented floating systems. In 2015, plant height (above the mat) and root length (below the mat) were measured twice, namely in June and October, whereas the root system width was measured once in October. In 2016, plant height, root length and width were measured only once in October (Figure 3). Results were analysed and are presented as means of medians, and 1st and 3rd quartiles.

Laboratory work: biomass production and chemical analyses for N and P determination

A biomass-production survey was conducted on plants established in the FTW system. In October 2015 and 2016, 12 random plant samples, for each year, were taken from each of *Carex* spp. and *L. salicaria*, and divided into aerial and root systems. Samples for *I. Pseudacorus* were taken in October 2015 only due to the insufficient number of surviving plants. Total fresh weight was measured on site (Figure 4). Fresh-matter samples were dried in a force-draught oven at 65°C for 35 h and milled at 2 mm (Cutting Mill SM 100 Comfort, Retsch, Germany). Ground subsamples of 10 g each were dried at 130°C, so as to measure the residual moisture content. Biomass-production data are expressed in grams per square metre (g m^{-2}).

Above- and below-ground dry matter of each plant sample was analysed using the standard Kjeldahl method to determine total Kjeldahl N (TKN), and spectroscopic methods (inductively coupled plasma–optical emission spectroscopy (ICP–OES), SPECTRO ARCOS) to determine TP concentrations (AOAC International 2005; Arduino and Barberis 2000). Uptakes of N and P by plants were calculated and expressed as dry matter per square metre of floating mat (above and below mats separately).

For the water samples, TKN was determined using the standard Kjeldahl method (AOAC International 2005; Benedetti et al., 2000) and nitric N (N-NO_3^-) was determined according to Cataldo et al. (1975) while ammonium N-NH_4^+ was detected by colourimetric flow-rate injection analyser FIAstar 5000 Analyzer (FOSS Analytical, Denmark) (detection limits of

Figure 3. Root length and width measurement on site for randomly selected samples of species in each system of the FTW, October 2016

Figure 4. Fresh weight measurements on site for random samples taken from each species of the FTW and preparation for drying, October 2015

0.05 mg l^{-1}). The TN content of each sample was calculated by summing TKN and N-NO_3^-. TP was negligible because it did not reach the instrument detection threshold. Orthophosphate (P-PO_4^{-3}) was determined in each of the samples by using the standard colourimetric ascorbic acid method (Murphy and Riley 1962; Edwards et al. 1965) and was expressed in milligrams per liter (mg l^{-1}) (detection limits of 0.01 mg l^{-1}). Like in physico-chemical parameters, normality of data was checked using the Kolmogrov-Smirnov test. Data were not distributed normally, so, Kruskal-Wallis non parametric test was used to check significance of concentration values between inlet and outlet of the system ($p < 0.05$). Results of the analyses are presented as box and plots and line charts for inlets and outlets of FWS CW and FTW.

Mass balance and abatement calculations

The mass balance is the balance between the mass of different nutrients (TN, N-NO_3^- N-NH_4^+ and P-PO_4^{-3}) entering into the FWS CW inlet and the mass of same nutrients exiting at its outlet and the abated nutrients per monitoring season were calculated in kilograms (kg) as the difference between the two masses. The mass of nutrients at the inlet was calculated as the product of nutrient concentration (kg m^{-3}) at the inlet and the water inflow (m^3) while the mass of nutrients at the outlet was calculated as the product of nutrient concentration (kg m^{-3}) at the outlet and the water outflow (m^3). The daily water inflow was estimated approximately based on the time required to fill the known volume of the sub-basins in the FWS CW with water (lateral losses were almost negligible) while the outflow was calculated as the difference between the inflow and the estimated total evapotranspiration for the wetland (ET_t). Wetland evapotranspiration (ET_t) was the sum of total crop evapotranspiration under standard conditions (ET_c) and open water surface (ET_w) evaporation The crop evapotranspiration (ET_C) for common reed, the prevailing macrophyte in the FWS CW was calculated as the product of reference evapotranspiration (ET_0) and the tabulated crop coefficient (K_C) for common reed (Allen *et al.*, 1998). Due to the lack of sufficient meteorological data, the ET_0 was calculated using the Hargreaves equation. Based on the previous calculations, the abatement percentage based on mass removal for different nutrients was calculated using the following equation:

$$\text{Abatement}\ (\%) = \frac{(\text{M inlet} - \text{M outlet})}{\text{M inlet}} * 100$$

Where, M inlet is mass of nutrient at inlet and M outlet is the mass of nutrient at the outlet.

Results and discussion

A. Water quality

1. Physicochemical parameters

Temperature

Median air temperatures obtained from the nearest official weather station on the selecting sampling dates followed the seasonal weather trend and varied between a minimum temperature of 3.3 °C in December 2015 and a maximum temperature of 26.2 °C in June 2014 (Figure 5).

Water temperatures for the sub-basins in the FSW CW and in FTW on the selected sampling dates and points varied between minimum temperatures as 4.5 °C in December 2015 and maximum temperatures as 31 °C in May 2015. The water temperature trend over time followed the seasonal weather trend and was generally consistent between different basins and with that of the air temperature with slight differences between both resulting from the difference in specific heat capacity between air and water (Figure 6). Seasonal changes in air and water temperatures or any temperature-driven process are an important factor affecting chemical and biological activities of water, and in turn water quality (Michaud and Noel, 1991, Reichwaldt *et al.*, 2015)

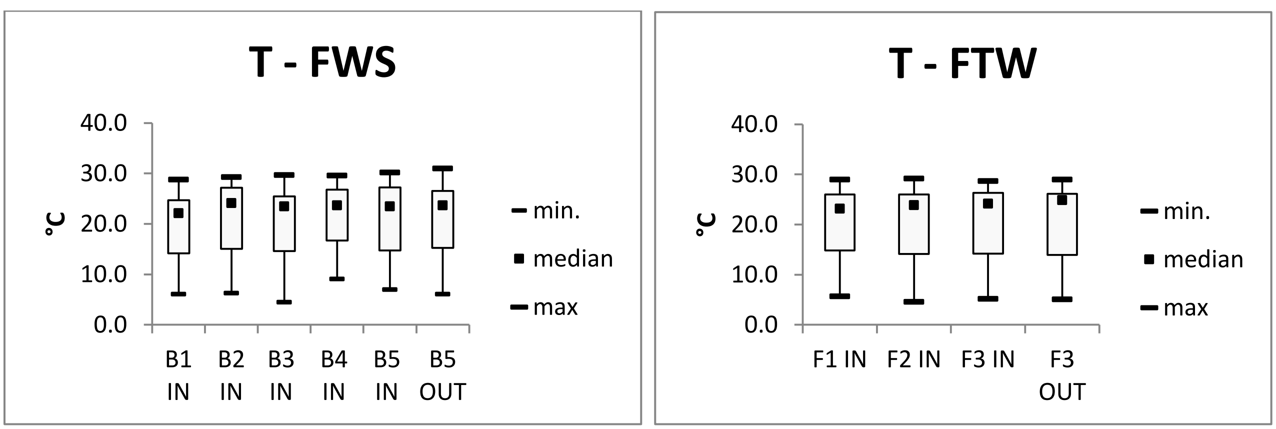
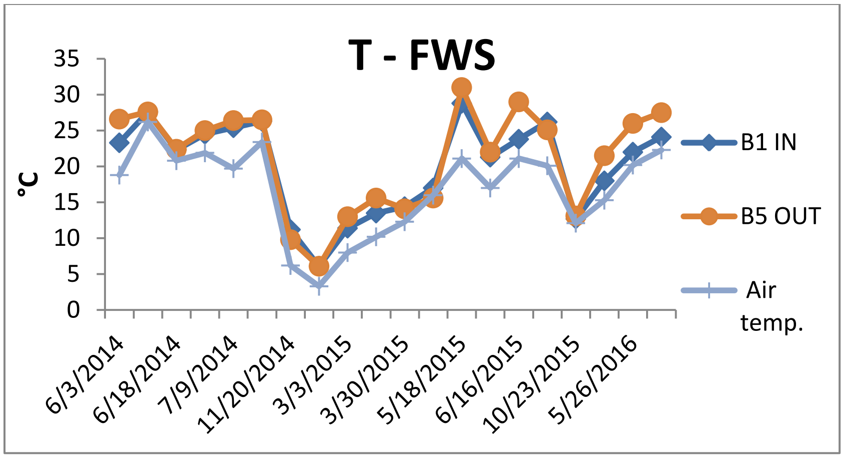


Figure 5. Box and whisker plots showing median, minimum and maximum temperatures in sub-basins of FWS (B1-B5) and FTW (F1-F3)



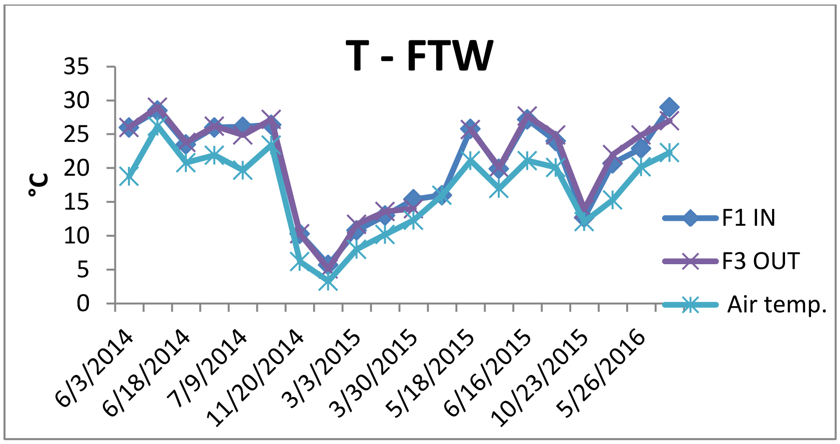


Figure 6. Line charts showing the dynamics of air and water temperature at inlets and outlets of FWS and FTW over the whole monitoring period (2014-2016)

pH

pH of water in the FSW CW did not show uniformity between sub-basins and was fluctuating between different sampling dates (Figure 7). Results showed that the pH in sub-basins is slightly alkaline with a minimum value of 6.9 in B5 IN in September 2015 and a maximum of 10.1 in B4 IN in June 2014. Median values varied between 7.9 in B1 IN and 8.3 in B5 IN with no significant differences (Kruskal-Wallis, $p< 0.05$) between values at system inlet and outlet over the monitoring period (Figure 8). In the FTW, pH of water exhibited more uniformity but still slightly alkaline with a minimum value of 6.9 in F2 IN in September 2015 and a maximum of 8.7 in F1 IN in November 2014 while the median value was 8.1 (Figure 8). Alkalinity of water maybe an indicator of accumulation and sedimentation of mineral salts like calcium carbonate or others in the wetland system, higher de-nitrification processes in water favoured by increased photosynthesis of plants and in all cases refers to a good buffering system (Michaud and Noel; 1991; Murphy, 2007; Kadlec and Wallace, 2009; EPA, 2012a).

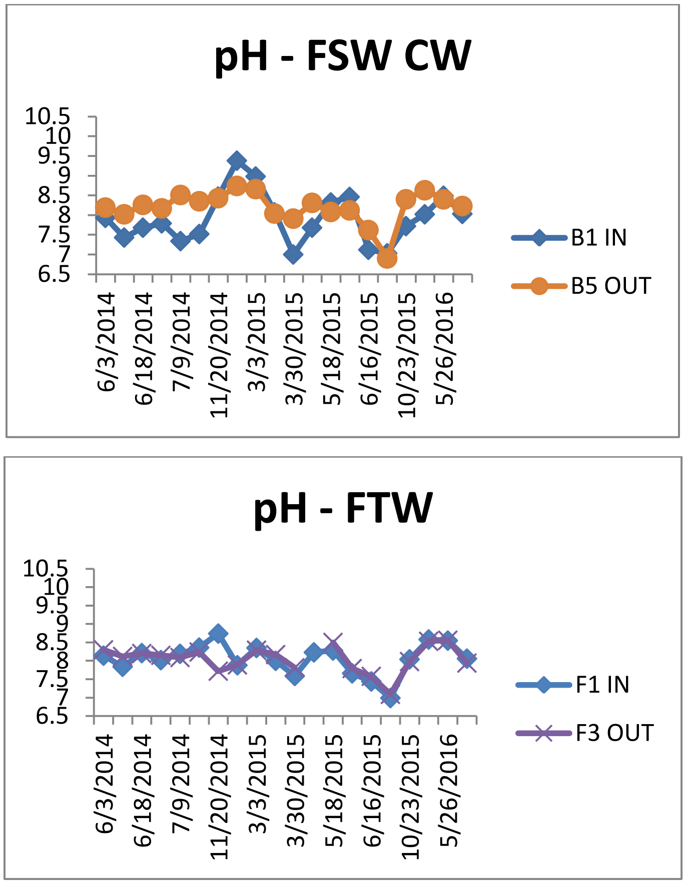


Figure 7. Line charts showing dynamics of pH values at inlets and outlets of FWS and FTW (2014-2016)

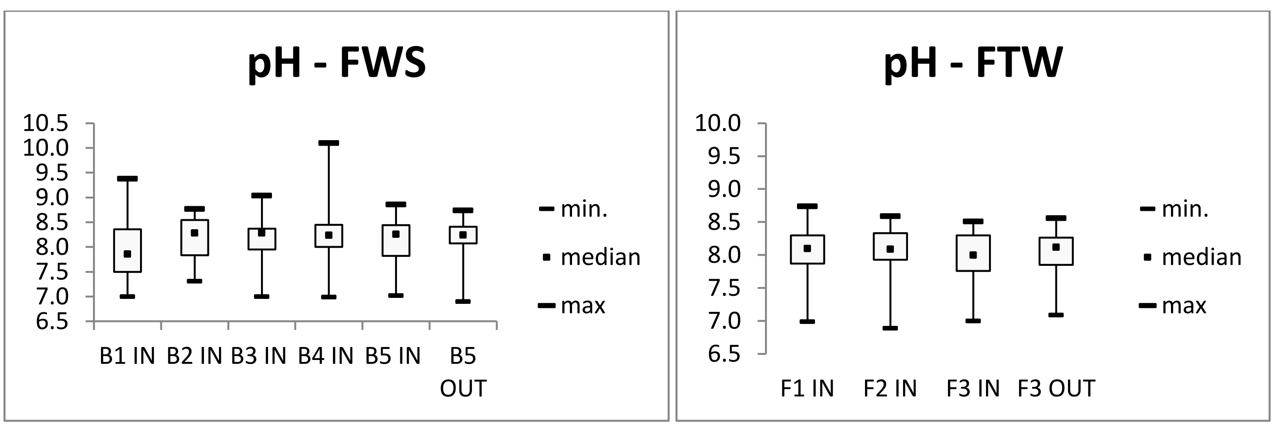


Figure 8. Box and whisker plots showing median, minimum and maximum values of pH in FWS sub-basins and FTW (2014-2016). No significant differences between system inlet and outlet ($p<0.05$)

Dissolved oxygen (DO)

DO in water exhibited irregular dynamics between different sub-basins of the FWS CW as well as the FTW (Figure 9), fluctuating between values as high as 19.1 mg l^{-1} in June 2014 and as low as 4.6 mg l^{-1} during the same month in FWS CW, while the highest value in FTW was 16.9 mg l^{-1} in F2 IN in March 2015 and the lowest was 4.2 mg l^{-1} in F2 IN in November 2014 (Figure 10). Median values for the FWS CW ranged between 8.8 mg l^{-1} in B5 OUT and 11.12 mg l^{-1} in B2 IN while those of the FTW ranged between 8.2 mg l^{-1} in F1 IN and 9.4 mg l^{-1} in F3 IN with no significance difference between concentrations at inlet and outlet of the system over the monitoring period (Kruskal-Wallis, $p< 0.05$). High DO at the beginning of the experiment may be attributed to water supply flowing to the system. Newly established and restored macrophyte species can contribute to this increase by photosynthesis process. Despite fluctuating dramatically, DO values were generally higher during summer 2015 than those during summer 2014 indicating higher water and plant activities resulting from new water supply to the system, rainfall and the revival of the macrophyte species (Watt, 2000; EPA, 2012b). Ranges of DO values were in general accordance with those obtained by Díaz *et al*. (2012), always higher than the levels of anaerobic conditions (< 1 mg l^{-1}).

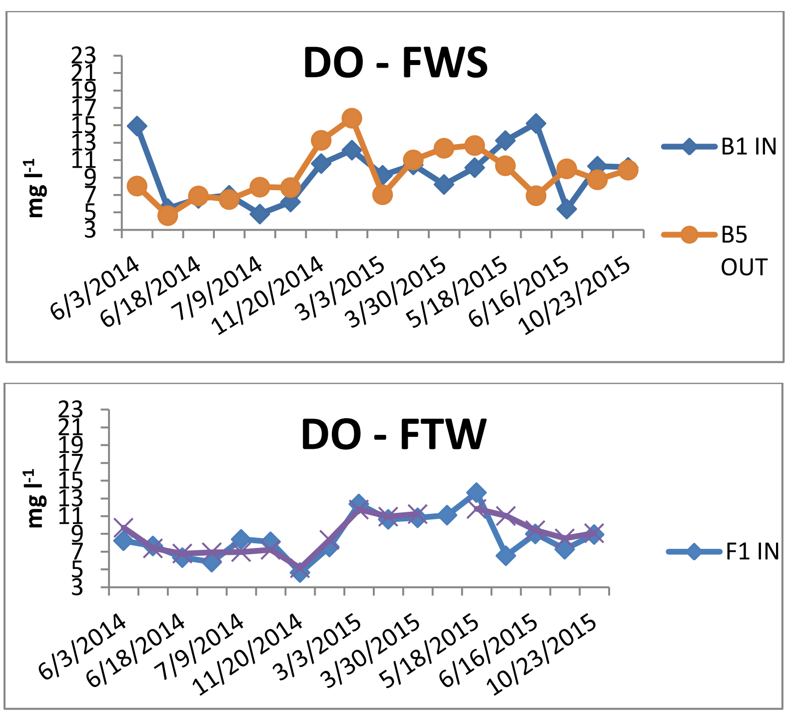


Figure 9. Line charts showing dynamics of DO concentrations at inlets and outlets of FWS CW and FTW (2014-2016)

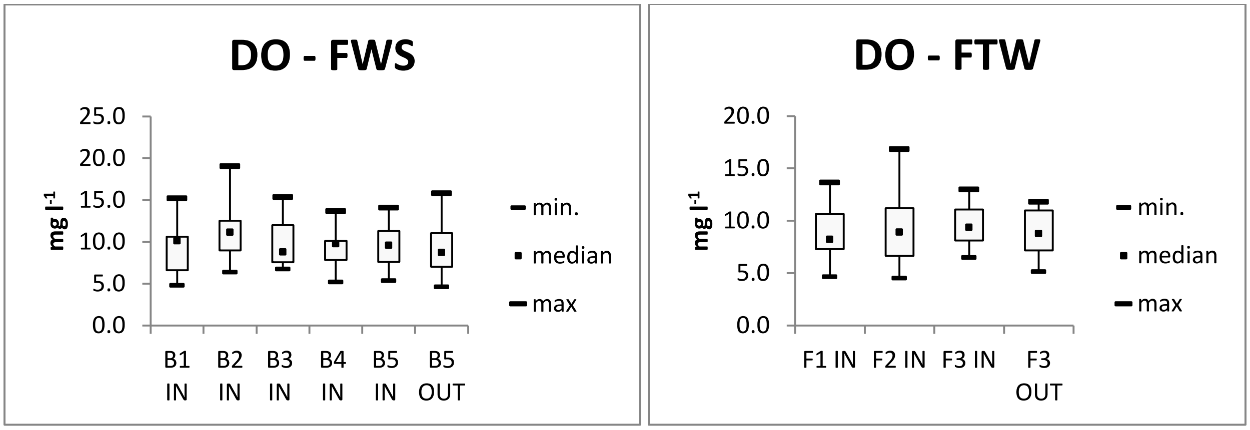
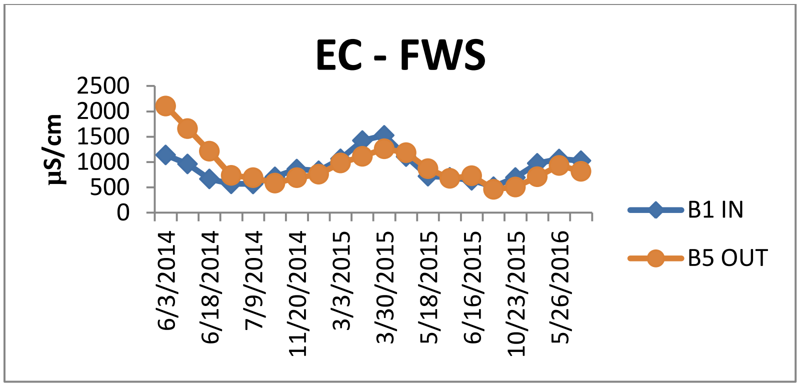


Figure 10. Box and whisker plots showing median, minimum and maximum DO concentrations in FWS sub-basins and FTW (2014-2016). No significant differences between inlet and outlet concentrations ($p<0.05$)

Electric conductivity (EC)

The line trend of electric conductivity (EC) dynamics showed consistency and regularity between different sub-basins in the FSW CW as well as the FTW (Figure 11). The maximum values were 2106 and 2310 µS/cm in B5 OUT and F2 IN, respectively in June 2014 while the minimum values were 458 and 484 µS/cm in B4 IN and F1 IN, respectively in September 2015 (Figure 12). Median values for the the FWS CW ranged between 727 µS/cm in B4 IN and 845 µS/cm in B1 IN while those for the FTW ranged between 1056 µS/cm in F1 IN and 1150 µS/cm in F3 OUT with no significant difference between EC values at system inlet and outlet over the entire monitoring period (Kruskal-Wallis, $p< 0.05$). Higher conductivity at the beginning of the experiment can result from the instability of soil particles in the newly established system where it decreased gradually during summer 2014 (Figure 11). EC values showed a peak during March 2015 which can be attributed to agricultural run-off and leaching resulting from the fertilization of the cropland surrounding the wetland as well as excessive rainfall events contributing to the increase in ionic and total dissolved solids (TDS) concentration in water (Welcomme, 1985; EPA, 2012c; Perlman; 2014). The values exhibited the same trend in 2015 and 2016; decreasing gradually from spring to summer (Figure 11). Ranges of EC at inlets and outlets of wetland were in general accordance with those obtained by Díaz *et al*. (2012) during irrigation times treating river waters receiving agricultural runoff. In addition, significantly indifferent EC between inlets and outlets can be an indicator of a shorter hydraulic retention time (HRT) (Díaz *et al*., 2012).



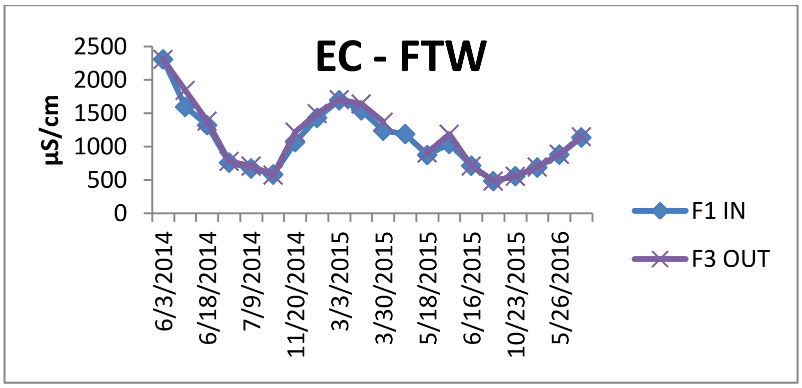
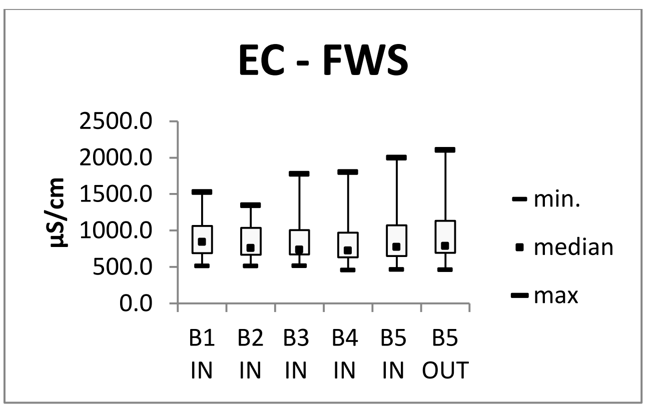


Figure 11. Line charts showing dynamics of EC at inlets and outlets FWS CW and FTW (2014-2016)



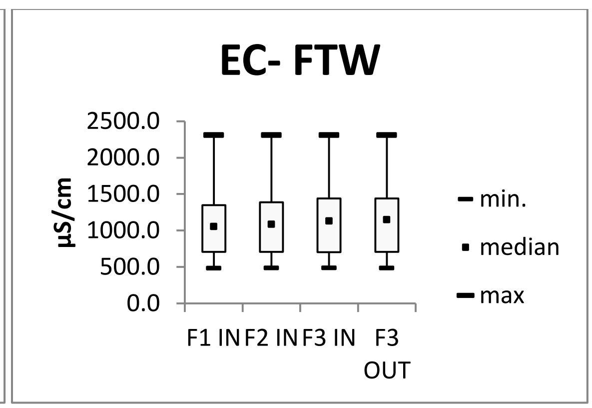
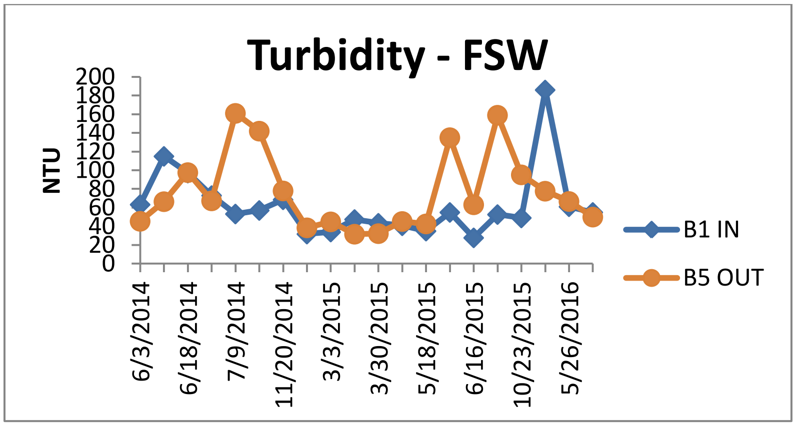


Figure 12. Box and whisker plots showing medians, maximum and minimum values for EC in FWS sub-basins and FTW (2014-2016). No significant differences between inlet and outlet ($p < 0.05$)

Turbidity

In FWS CW, Turbidity dynamics did not show uniformity during 2014 and 2016 but was rather stable in 2015 (Figure 13). The maximum value for turbidity was 209 NTU in B4 IN in June 2014 while the minimum value was 14 NTU in B5 IN in December 2014 (Figure 14).



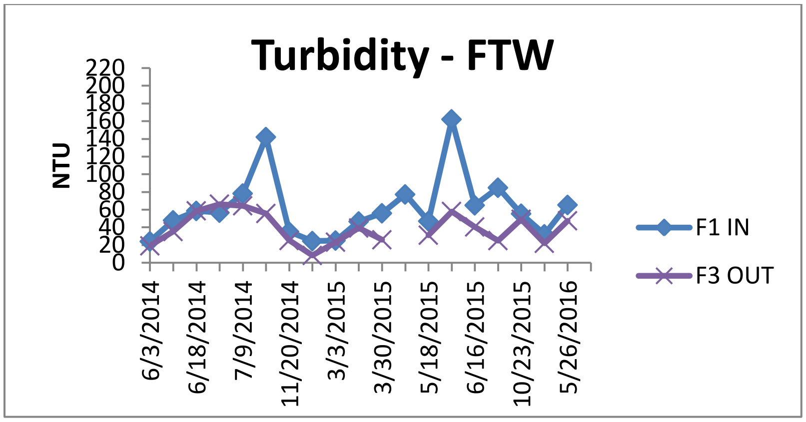
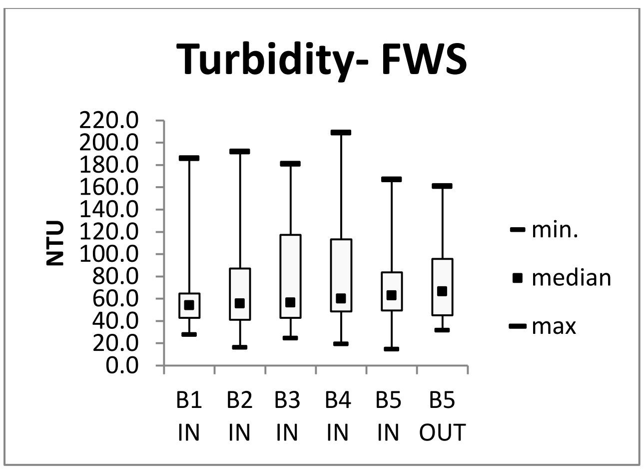


Figure 13. Line charts showing dynamics of turbidity values at inlets and outlets of FWS CW basins and FTW (2014-2016)



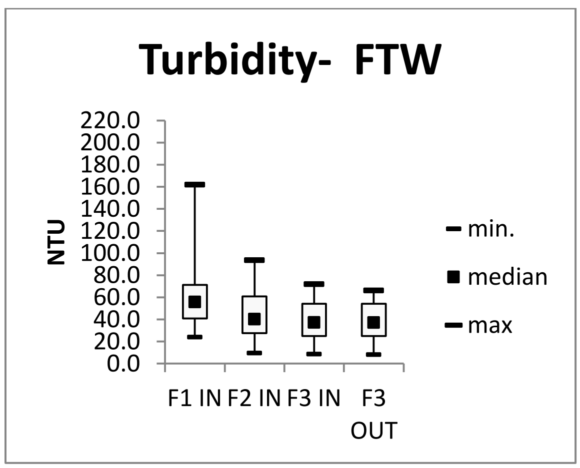


Figure 14. Box and whisker plots showing medians, minimum and maximum values for turbidity in FWS sub-basins and FTW (2014-2016). No significant differences between system inlet and outlet ($p < 0.05$)

Median values ranged between 54.2 NTU in B1 IN and 66.6 NTU in B5 OUT with no significant differences between the values at the inlet and the outlet of the system over the monitoring period (Kruskal-Wallis, $p < 0.05$). Fluctuations in turbidity values during 2014 may indicate instability of soil particles in the newly established wetland system, when water was newly introduced to the system, while the decrease and stability of values in 2015 may be indicative of better establishment and consolidation of the wetland system leading to precipitation of sediments, low re-suspension of particles (Petticrew and Kalff 1992, Horppila and Nurminen 2001, 2003, 2005) and an improvement in water quality (O'Geen *et al.* 2010). In addition, low water velocity in the FWS sub-basin system encouraged sedimentation of TSS (Kadlec and Wallace 2009). In 2016, Basins were emptied in early winter and refilled during summer leading to the re-suspension of particles and fluctuation of turbidity values.

In the FTW system, values varied between a maximum of 162 NTU in F1 IN in May 2015 and a minimum of 8.2 NTU in F3 OUT in December 2014. Median values ranged between 34.7 NTU in F3 OUT and 55.9 in F1 IN. Lower turbidity values downstream in the channel are evidences of better soil stability and better establishment of the root systems of floating plants in the FTW system (Figure 14).

2. Nutrient concentration

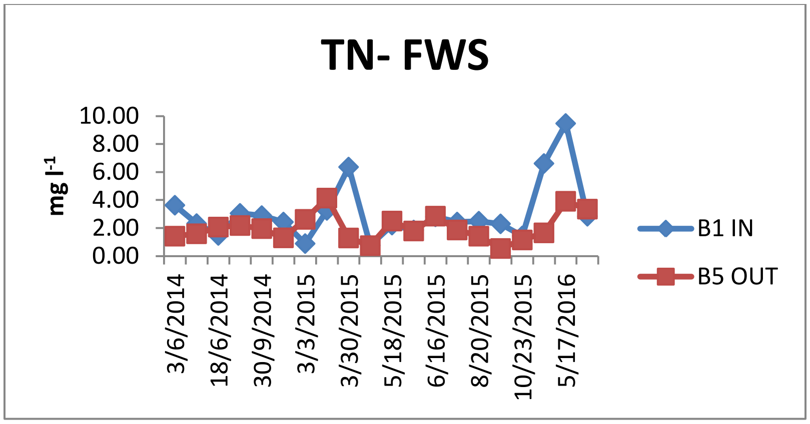
Total Nitrogen (TN)

In FSW CW, dynamics of concentrations of TN did not exhibit a regular trend throughout the three years of experimentation (Figure 15). However, fluctuations of values between dates and years are explainable and give good indications about the efficiency of the wetland system. In 2014, TN concentrations were rather stable with no notable differences between basins on different dates. Maximum concentration value was 7.41 mg l^{-1} in B4 IN in June while the minimum value was 0.70 mg l^{-1} in B5 IN during the same month. Median values ranged between 1.57 mg l^{-1} in B4 IN and 2.66 mg l^{-1} in B1 IN. Fluctuation in concentration values was notable in 2015; the highest value was 16.37 mg l^{-1} in B5 IN in May while the lowest was 0 mg l^{-1} in B4 IN on the same date. Median values ranged between 1.46 mg l^{-1} in B4 IN and 2.30 mg l^{-1} in B1 IN. In 2016, the highest value was in B1 IN (9.47 mg l^{-1}) while the lowest was in B5 IN (0.06 mg l^{-1}). Median values ranged between 1.33 mg l^{-1} in B5 IN and 6.61 mg l^{-1} in B1 IN. No significant difference in TN concentration was notable between inlet and outlet over the total monitoring period (Kruskal-Wallis, $p< 0.05$) (Figure 16) while concentration values were significant between inlet and outlet only in 2016, when years were assessed separately. TN concentrations in FSW CW during the three years were generally low and within the acceptable level for water (WHO, 2004a and b) due to initial low concentrations at inlet, except for few occasions, disaccording with results obtained by Diaz *et al*. (2012) for agricultural runoff reporting input concentrations always > 5 mg l^{-1}. Lower concentrations during 2014 are generally attributed to low rainfall events leading inturn to low agricultural runoff and leaching. In addition the wetland was established in late summer when almost no fertilization processes for the surrounding cropland took place. Higher concentrations at inlet during spring 2015 and 2016 can be attributed to run-off and leaching resulting from persistent rainfall during these dates associated with intensive fertilization in the surrounding cropland (Borah *et al*., 2003; Kato *et al.*, 2009; Lang *et al.*, 2013). Lower concentrations through the wetland sub-basins can be attributed to nitrification and de-nitrification processes, reduction to ammonia as well as assimilation by plants (Kadlec and Knight 1996; Vymazal 2007 and 2010; Kadlec and Wallace 2009; Maltais-Landry *et al*. 2009; Mthembu *et al*. 2013).

In a similar manner, the FTW exhibited higher TN concentrations in spring 2015 and 2016 resulting from fertilization of cropland and intensive rainfall (Figure 15). The maximum value in March 2015 was 6.66 mg l^{-1} in F3 OUT compared to 3.16 mg l^{-1} in F3 OUT in May 2016, while the lowest values were 0.49 and 1.02 mg l^{-1} in F3 OUT and F3 IN in September 2015 and March 2016, respectively. Median values ranged between 1.15 and 1.64 mg l^{-1} in F2 IN and F1 in 2015 and between 1.40 and 1.80 mg l^{-1} in F3 IN and F2 IN in 2016 with no significant difference between inlet and outlet over the entire monitoring period (Kruskal-Wallis, $p< 0.05$) (Figure 16). The decrease in TN concentrations suggests an interesting depurative effect of the integrated wetland system.

Nitrate Nitrogen (N-NO_3^-)

The detection of N-NO_3^- in water is one of the most important determinants of water quality as it is the most abundant and soluble form of nitrogen in water. In FWS CW, similar to TN, N-NO_3^- concentrations were more stable and low during 2014 with median values ranging between 0.31 mg l^{-1} in B5 IN and 1.51 mg l^{-1} in B1 IN. There was more fluctuation in concentrations within basins in 2015 (Figure 17); the maximum values were 15.31 and 13.28 mg l^{-1} in May in B5 IN and B3 IN, respectively. The minimum value was 0 mg l^{-1} in most of



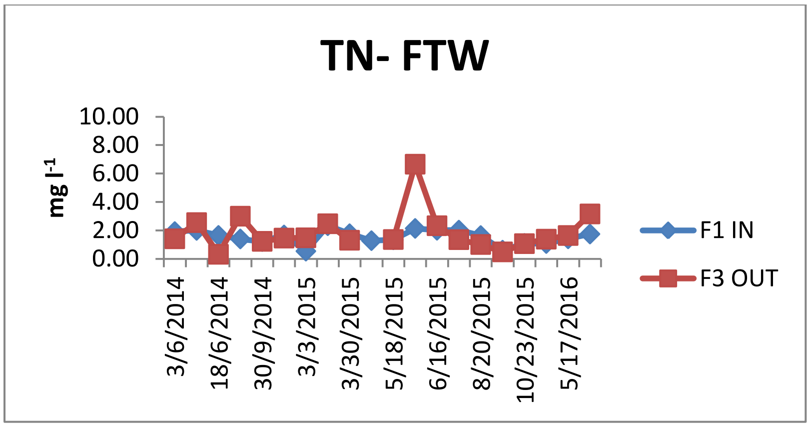
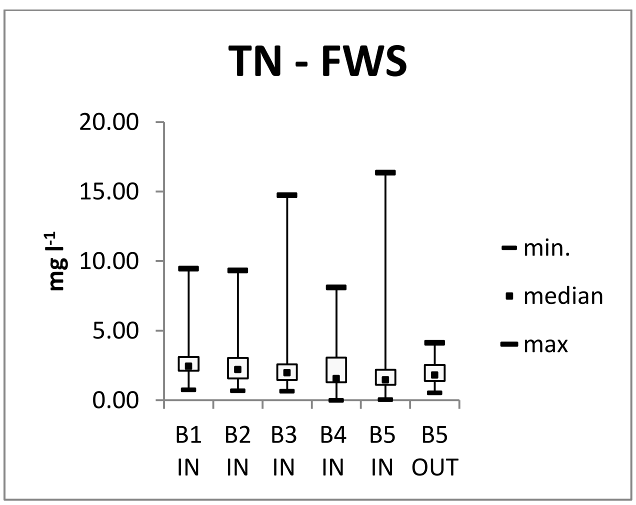


Figure 15. Line charts showing dynamics of TN concentration at inlets and outlets of FWS CW basins and FTW (2014-2016)



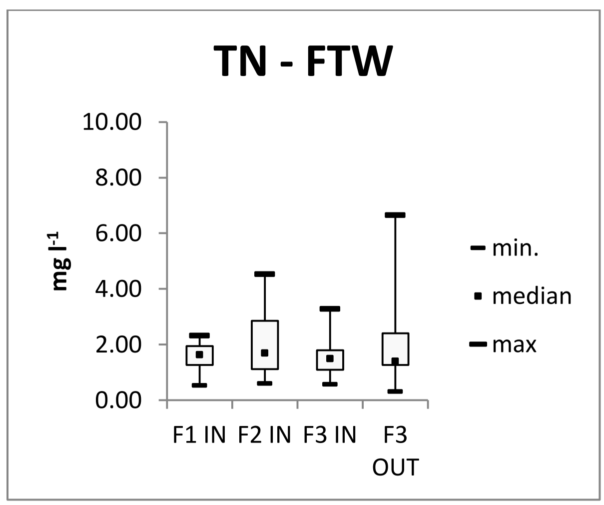


Figure 16. Box and whisker plots showing medians, minimum and maximum concentrations of TN in FWS sub-basins and FTW (2014-2016). No significant difference between system inlet and outlet ($p<0.05$)

basins in September and October. Median values ranged between 0 mg l^{-1} in B4 IN and 0.98 mg l^{-1} in B1IN. Maximum concentrations in 2016 were 7.79 and 6.42 mg l^{-1} in May in B1 IN and B4 IN, respectively while the minimum was 0 mg l^{-1} in rest of basins also in May. Median values ranged between 0.26 mg l^{-1} in B5 IN and 2.04 in B1 IN. As in TN, no significant difference in N-NO_3^- concentration was notable between inlet and outlet considering the total monitoring period (Kruskal-Wallis, $p < 0.05$) (Figure 18) while values were significant between inlet and outlet only in 2016, when years were assessed separately. Higher concentrations of N-NO_3^- at inlet during spring 2015 and 2016 can be related to persistent rainfall with intensive fertilization in the surrounding cropland resulting in run-off and leaching to the system (Borah *et al*., 2003; Kato *et al.*, 2009; Lang *et al.*, 2013), while lower concentrations at outlet may be attributed to depurative effect resulting from nitrification-denitrification processes, reduction to ammonia as well as assimilation by plants (Kadlec and Knight 1996; Vymazal 2007 and 2010; Kadlec and Wallace 2009; Maltais-Landry *et al*. 2009; Mthembu *et al*. 2013).

In FTW, less fluctuation in N-NO_3^- concentrations was notable during 2014 and 2016 in comparison to those of 2015 (Figure 17). The maximum value was 4.69 mg l^{-1} in F3 OUT in May 2015 while the minimum value was 0 mg l^{-1} over the whole FTW on different sampling dates. Median values ranged between 0.40 mg l^{-1} in F1 IN and 0 mg l^{-1} in F3 OUT with no significant difference between inlet and outlet concentrations during the monitoring period (Kruskal-Wallis, $p < 0.05$) (Figure 18). Generally, N-NO_3^- concentrations were low in FTW except on one occasion in May 2015 due to excessive rainfall associated with fertilization of cropland. Although N-NO_3^- concentrations are initially low in the integrated wetland system, decrease in concentrations at outlets after rain fall and fertilization events could give a hint about the performance of the system (Figure 17). Input and output concentration ranges for N-NO_3^- are closely related to values obtained by Kovacic *et al*. (2002) (7.5-14.5 mg l^{-1} for input, 4.6-14.5 mg l^{-1} for output), Borin and Tocchetto (2007) (5-15 mg l^{-1} for input) and Diaz *et al*. (2012) (0.28-12.87 mg l^{-1} for input, <0.01-7.87 mg l^{-1} for output) treating agricultural drainage and runoff waters.

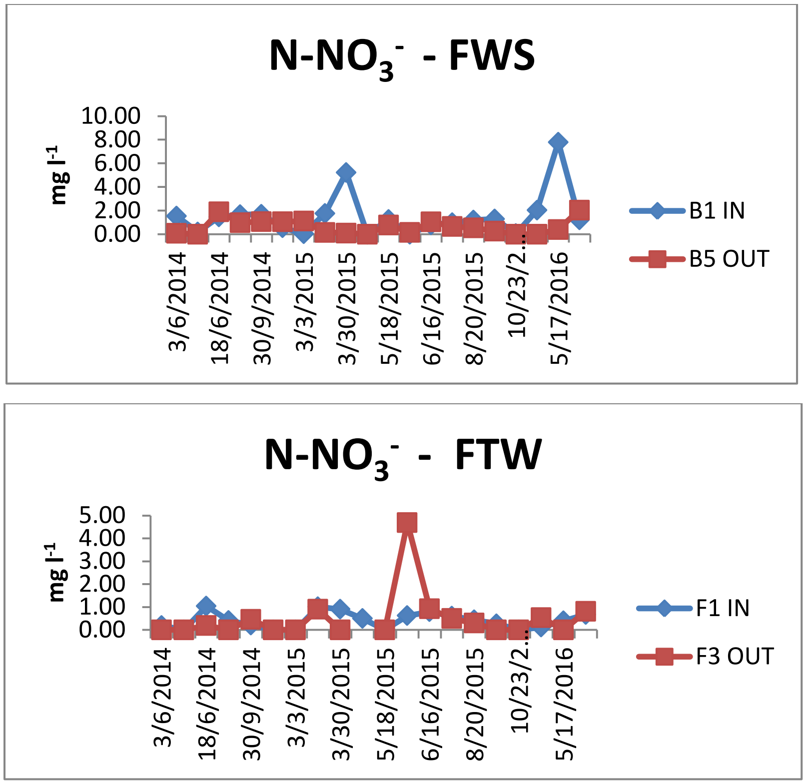


Figure 17. Line charts showing dynamics of N-NO$_3^-$ concentration at inlets and outlets of FWS CW basins and FTW (2014-2016)

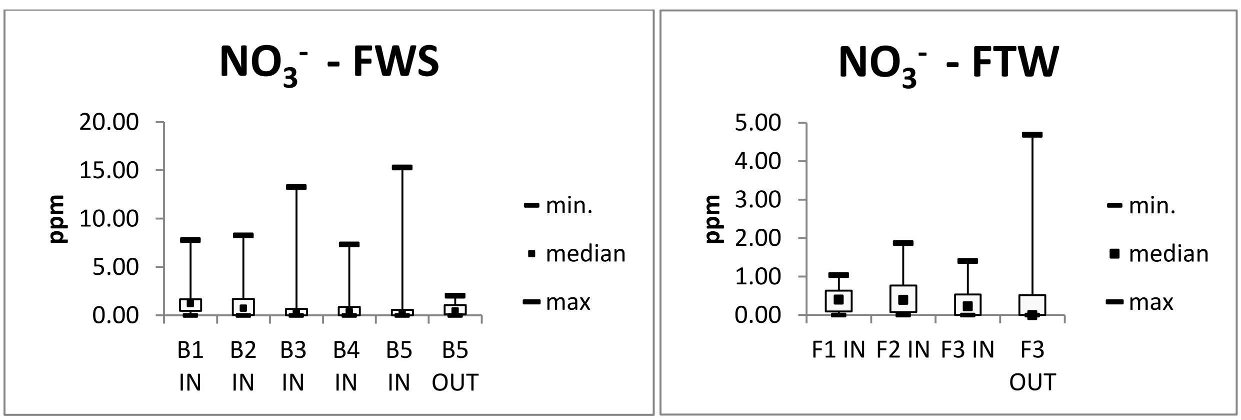


Figure 18. Box and whisker plots showing median, minimum and maximum concentrations of N-NO$_3^-$ in FWS sub-basins and FTW (2014-2016). No significant difference between system inlet and outlet ($p < 0.05$)

Ammonium nitrogen ($N\text{-}NH_4^+$)

$N\text{-}NH_4^+$ concentration was generally low throughout the wetland system in comparison with NO_3^-, except for 2016 (Figure 19). In FWS CW, the maximum concentration was 1.02 mg l^{-1} in B4 IN in May 2016 while the lowest value was 0 mg l^{-1} in B2 IN and B5 OUT in April and June 2015, respectively, whereas the maximum value in the FTW was 4.11 mg l^{-1} in F1 IN and the minimum was 0 mg l^{-1} throughout the FTW in June 2016. Median values for the FWS CW ranged between 0.16 mg l^{-1} in B4 IN and 0.26 mg l^{-1} in B1 IN while those for the FTW ranged between 0.17 mg l^{-1} in F2 IN and 0.22 mg l^{-1} in F1 IN with no significant difference between concentrations at inlets and outlets during the monitoring period (Kruscal-Wallis, $p< 0.05$).

The low input of $N\text{-}NH_4^+$ can be explained by the fact that most of the wetland input from the surrounding cropland is in the form of $N\text{-}NO_3^-$ in addition to the continuous nitrification and plant adsorption of $N\text{-}NH_4^+$ under favorable conditions in spring and summer while occasional higher values indicates increased ammonification process induced by various biological processes (Vymazal *et al.* 1998, Vymazal 2007). Lower $N\text{-}NH_4^+$ input is in general accordance with that reported by Kovacic *et al*., (2002), Borin and Tocchetto (2007), and Diaz *et al*., (2012) (0.4 mg l^{-1}, < 0.3, and <1, respectively) treating agricultural drainage and runoff waters.

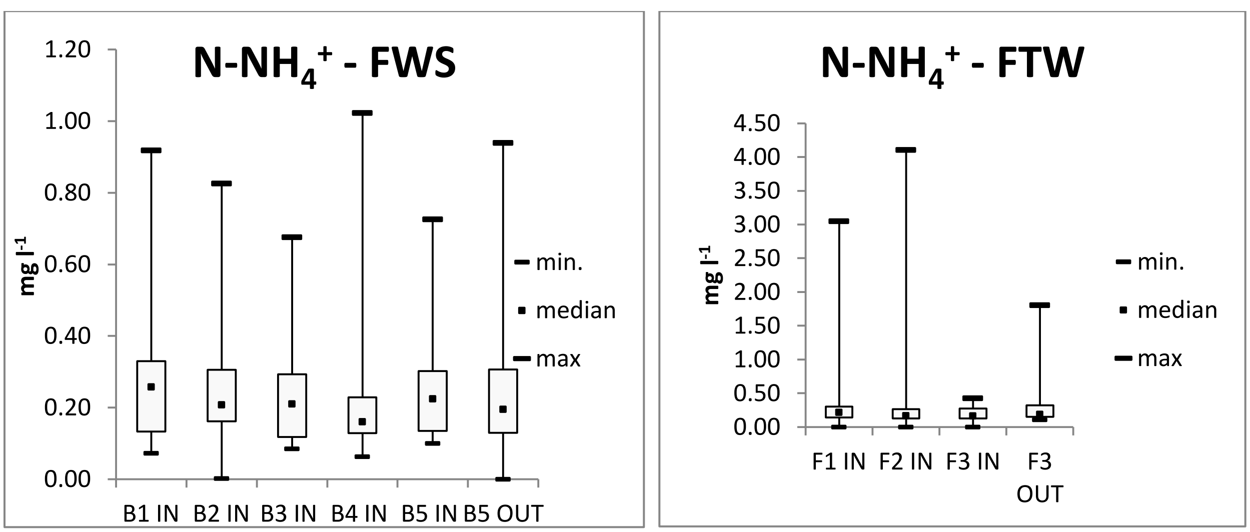


Figure 19. Box and whisker plots showing median, minimum and maximum $N\text{-}NH_4^+$ concentrations in FWS sub-basins and FTW (2014-2016). No significant difference between system inlets and outlets ($p<0.05$)

Orthophosphates ($P\text{-}PO_4^{-3}$)

TP was not detectable in any of the samples obtained during the early stages of the study. Available traces of P forms were identified by determining concentrations of orthophosphates ($P\text{-}PO_4^{-3}$). In FSW CW and FTW, the maximum values for $P\text{-}PO_4^{-3}$ concentration were 0.24 and 0.07 mg l^{-1} in B5 OUT and F3 IN in May and June 2015, respectively while the minimum value was 0 mg l^{-1} within the two systems on different sampling dates (Figure 20). The median values ranged between 0.01 mg l^{-1} in B5 OUT and 0.02 mg l^{-1} in B1 IN for the FWS CW and between 0 mg l^{-1} in F1 IN and 0.01 mg l^{-1} in F3 OUT for the FTW with no significant differences in concentrations between system inlets and outlets (2014-2016) (Kruskal-Wallis, $p<0.05$). $P\text{-}PO_4^{-3}$ concentration levels over the wetland system were in general accordance with Kovacic *et al.*, (2002) and Diaz *et al.*, (2012) reporting overall $P\text{-}PO_4^{-3}$ concentration always < 0.4 mg l^{-1}.

Despite fluctuation in concentrations throughout the integrated wetland system, $P\text{-}PO_4^{-3}$ is only present as insignificant traces, mostly because it was readily taken up by plants (Ongley, 1996). In addition, treatment of P is rarely the primary target of CWs (Vymazal, 2010).

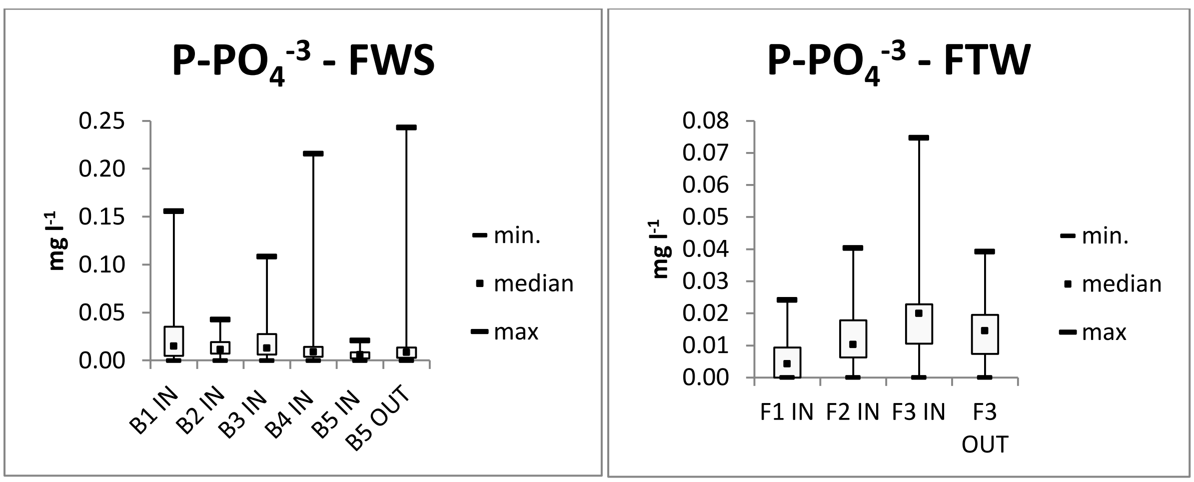


Figure 20. Box and whisker plots showing median, minimum and maximum for $P\text{-}PO_4^{-3}$ concentrations in FWS sub-basins and FTW (2014-2016). No significant difference between system inlets and outlets ($p<0.05$)

3. Mass balance and abatement percentage

The water inflow to the system was approximately 5480 m^3 day^{-1}, and varied over the three consecutive seasons depending on the length of flooding periods (Table 3). The highest inflow was recorded in 2015 (1,342,600 m^3), followed by 2014 (1,002,840 m^3) and finally

2016 (504,160 m^3). Evapotranspiration of the FWS CW (ET_t) was average 3.9 mm day^{-1} in spring and summer season and 1.3 mm day^{-1} during fall and winter, contributing by 1.18% to water outflow throughout the monitoring period.

The cumulative mass balance was calculated for different months during the monitoring seasons over three consecutive years (Figure 21). In 2014, the FWS CW removed approximately 912 kg of TN, 366 kg of $N\text{-}NO_3^-$ and 6 kg of $N\text{-}NH_4^+$ between June and November while the mass abatement in 2015 was 827, 795, 80 and 20 kg for TN, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$ and $P\text{-}PO_4^{-3}$, respectively between March and October. In 2016, the mass abatement increased over a shorter period of time (March – June) to reach 2327 and 1873 kg for TN and $N\text{-}NO_3^-$ respectively while it remained indifferent for $N\text{-}NH_4^+$ (65 kg) (Figure 22).

The highest abatement percentage for TN was attained in 2016 (64%) followed by that in 2014 (33%) which was indifferent from that achieved in 2015 (26%) with a 3 year average removal of 41.7% (46% by Kovacic *et al*., 2000). Similarly, the abatement percentage for $N\text{-}NO_3^-$ was the highest in 2016 (91%) followed by that in 2015 (57%) and finally the lowest was in 2014 (27%) averaging 58% which is in general agreement with similar studies (51 % by Jordan *et al*., 2003) and higher than other studies (19% by Kroeger *et al.*, 2007). Abatement percentage for $N\text{-}NH_4^+$ was rather low for the three consecutive years; 2, 27 and 20% for 2014, 2015 and 2016, respectively with an average of 16%, which is generally low in comparison with other studies (Koskiaho *et al*., 2003). On the other side, average overall P removal was very low (3%) in comparison with similar studies (Braskerud, 2002; Johannesson *et al*., 2011; Jordan *et al*., 2003; Kroeger *et al*., 2007; Lu *et al*., 2009; Yates and Prasher, 2009) while it matched with other studies (Koskiaho *et al*., 2003). (Figure 22).

$N\text{-}NO_3^-$, resulting from fertilization of crop lands and nitrification of $N\text{-}NH_4^+$ under favorable conditions, is the most abundant form of N available in the wetland with the greatest contribution to the available TN. Results showed that the total mass abatement of $N\text{-}NO_3^-$ is consistent with that of TN over the three years of monitoring with the highest abatement for both in 2016 over a shorter period of time despite the high mass input which gives a good indication on the depurative capacity of the FWS CW. Abatement percentage for $N\text{-}NO_3^-$ was always higher than that for TN with gradual increase over time to reach a maximum in 2016. The monthly removals of TN and $N\text{-}NO_3^-$ were rather higher during the monitoring season in spring and summer where the conditions are favorable for nitrification- denitrification processes in addition to plant uptake while they decrease in winter as a result of plant ageing and senescence which results in the release of N back to the FWS CW in addition to providing conditions favorable for nitrification process (Kadlec and Knight 1996, Vymazal *et al.* 1998, Vymazal 2007).

Generally, the mass removal of N-NH_4^+ over the three seasons was very low in comparison with N-NO_3^-. This can be attributed majorly to the initial low concentration and mass input of N-NH_4^+, where most of the N entering into the system by fertilization runoff is in the form of N-NO_3^- in addition to continuous nitrification of N-NH_4^+ under favourable conditions in spring and summer (Vymazal 2007). In winter, lower temperature can limit the nitrification process leading to accumulation of N-NH_4^+ in the system and even negative removal in some cases (November 2014) (Vymazal *et al.* 1998, Vymazal 2007).

Similarly, low phosphorus retention is attributed to utilisation by biota or soil adsorption (Kadlec and Wallace. 2009, Koskiaho et al., 2003, Vymazal 2007, Vymazal 2010) in addition to low intial inputs in this study while negative removal in 2016 can be attributed to decay and translocation of vegetation in addition to algeal and microbial activities leading to the release of P back to the system (Reddy *et al.*, 1999).

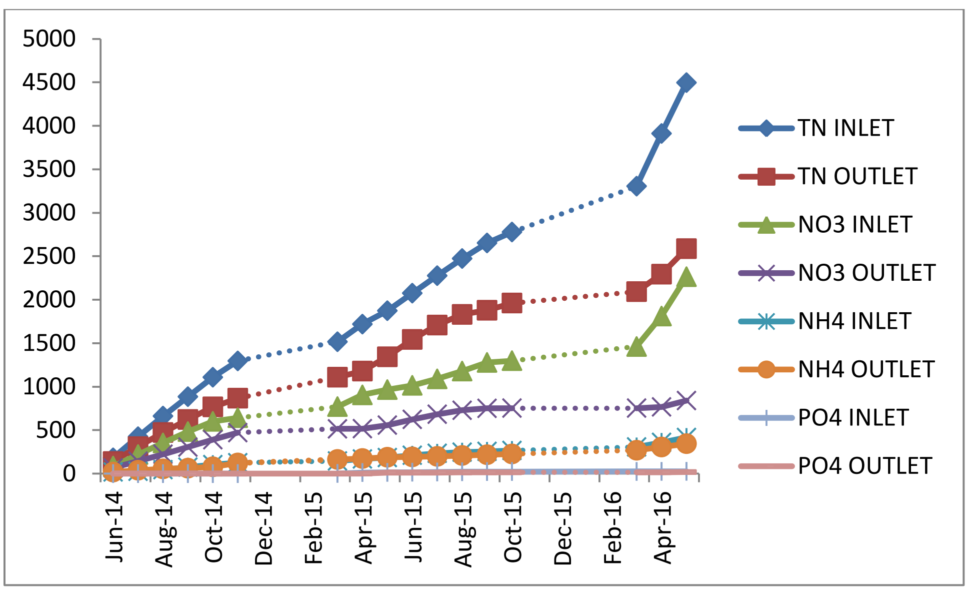


Figure 21. Cumulative mass balance for different nutrients (TN, N-NO_3^-, N-NH_4^+ and P-PO_4^{-3}) at inlet and outlet of FWS CW during the monitoring seasons for the consecutive years 2014, 2015 and 2016. Dots represents periods of inactivity o the FWS CW

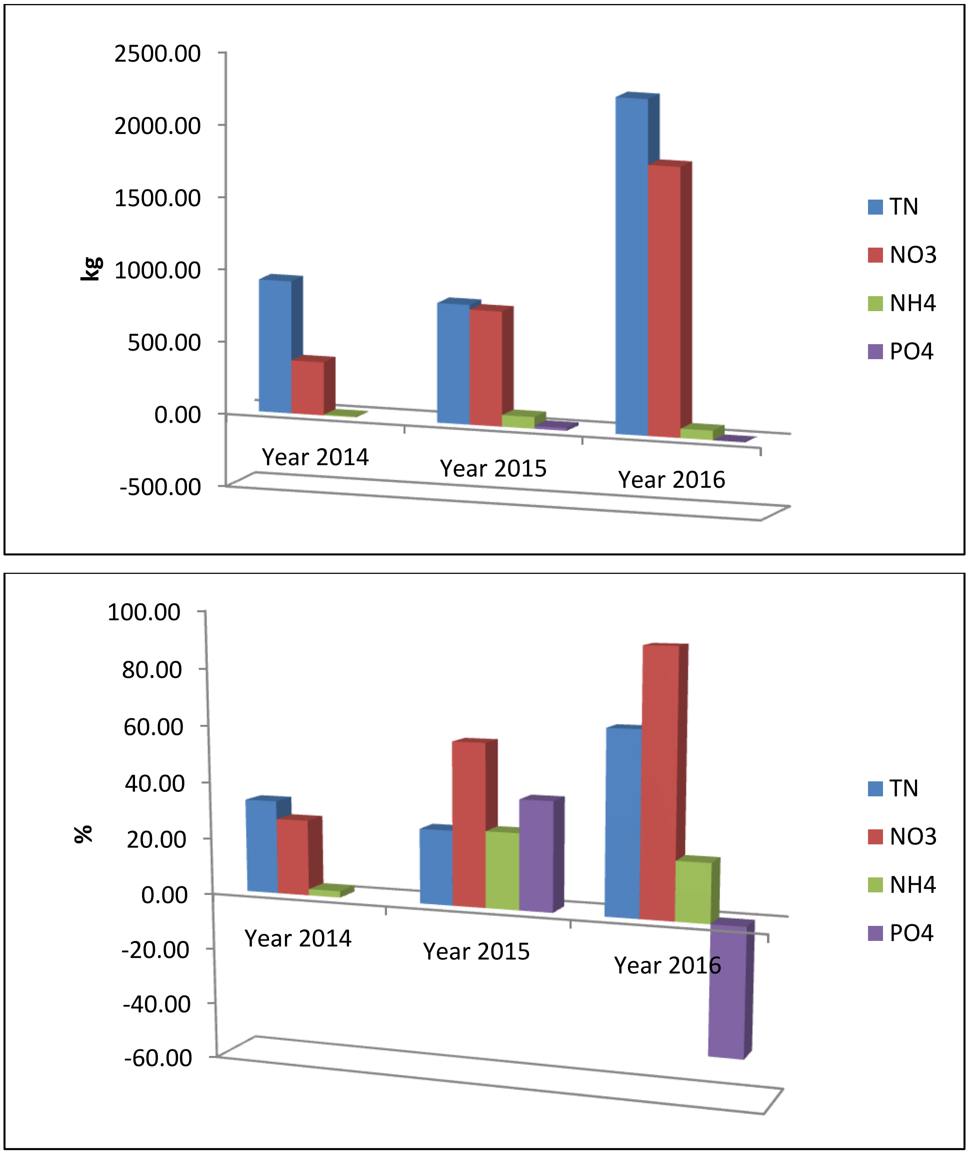


Figure 22. Comparison of mass abatement (upper) and removal percentage (lower) for different nutrients (TN, N-NO_3^-, N-NH_4^+ and P-PO_4^{-3}) in the FWS CW over three consecutive years (2014, 2015 and 2016).

B. Vegetative performance

1. Plant survival in the FTW

Plant species in the FTW system exhibited different survival rates in the three vegetative seasons, 2014, 2015 and 2016 (Table 1). In the first season, the survival rate varied between 3% and 100%, with *P. arundinacea* and *M. aquatica* exhibiting the highest survival rate during August 2014 (100%), followed by *Carex spp.* (98%), *J. effusus* (88%), *C. palustris* (73%) and *I. pseudacorus* (48%). *S. lacustris* and *S. erectum* had the lowest rates at 8% and 3% respectively. In the second season (2015), only *Carex spp.* survived the winter and completely re-grew during spring, whereas other species had to be replaced with new plants. *L. salicaria* had the highest survival rate (95%), followed by *Carex spp.* (82%) and *I. pseudacorus* (40%). In autumn–winter, all three species went into senescence and revived again in spring 2016. Survival rate for *Carex spp.* and *L. Salicaria* was 55% in 2016 while it was 12% only for *I. Pseudacorus*. *Carex spp*. proved to be adaptable and tended to establish well in the FTW (Figure 23), with a high survival rate (55%) over three successive seasons and a large number of living plants (22 of 40 plants per 10 m^2). *L. salicaria* exhibited great stability and steady growth throughout two seasons; similar to *Carex spp*., it had a high survival rate (55%) and large number of living plants (22 of 40 plants per 10 m^2). *Iris pseudacorus* tended not to establish nor grow well in the third season respectively compared with other species (Figure 23), and had the lowest survival rate (12%) and fewest living plants per 10 m^2 (5 of 40 plants). The low survival rate of *I. pseudacorus* may also be related to alien animal species, such as *Myocastor coypus*, feeding on the plants.

Table 1: Survival rate of plant species in the FTW during 3 successive seasons 2014, 2015 and 2016

Plant species	% Survival		
	2014	**2015**	**2016**
Carex spp.	98	82.5	55
Phalaris arundinacea	100	-	-
Sparangium erectum	3	-	-
Schoenoplectus lacustris	8	-	-
Juncus effusus	88	-	-
Caltha palustris	73	-	-
Mentha aquatica	100	-	-
Iris pseudacorus	48	40	12
Lythrum salicaria	-	95	55

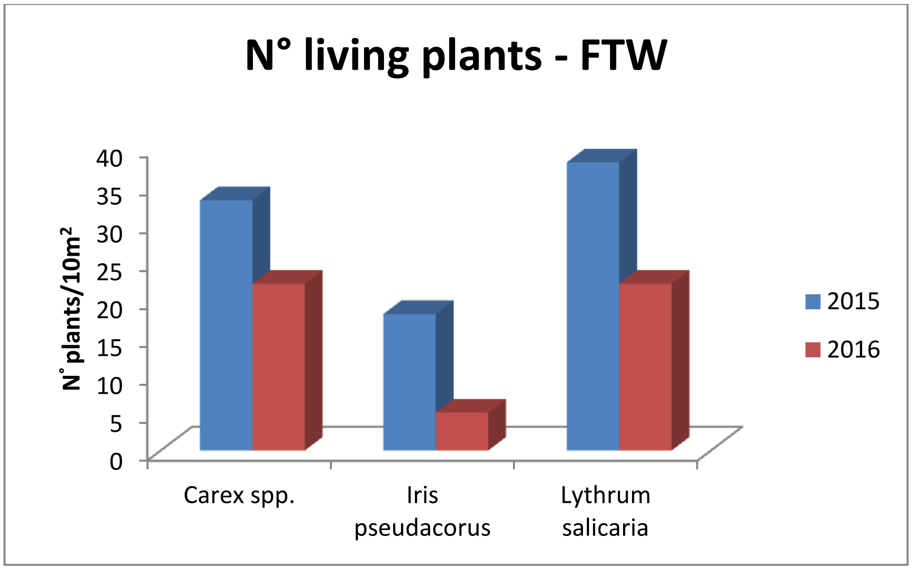


Figure 23. N° living plants per 10 m^2 for 3 species in the FTW in 2015 and 2016

2. Plant growth in the FTW

In 2015, plant height (above the mat) and root length (below the mat) were measured twice, namely in June and in October, whereas root-system width was measured once in October while in 2016, the same parameters were measured only once in October (Table 2). *L. salicaria* exhibited the greatest increase in plant height in 2015, with a median value of 33.5 cm in June rising to 59.5 cm in October. This value dropped to 26.5 cm in 2016. The median values for *I. pseudacorus* were 24 cm in June, and 37.5 cm in October2015, decreasing to 23 cm in October 2016; which were very low compared with values in similar studies. De Stefani (2012) reported median end-of-season plant heights of 136 and 116 cm for *I. pseudacorus* in two different experiments. In contrast, *Carex* spp. did not increase much in height in 2015, with median values of 59.5 cm in June, and 60 cm in October. Slight increase was obvious in October 2016 with median value of 69 cm (Salvato and Borin (2010) recorded 92 cm for *Carex elata* Gooden.). *Carex* spp. most probably increased in density and leaf bulkiness, contributing to plant width, rather than in height. *Iris pseudacorus* exhibited the greatest increase in root depth in 2015, with median values of 16 cm in June and 76 cm in October. This value dropped to 20 cm in October 2016 (end-of-season median root lengths of 46 and 55.5 cm were recorded by De Stefani (2012) in two different experiments, whereas Pavan *et al*. (2015) reported a root length of 16 cm at the end of the season). *Carex* spp. exhibited a moderate increase in root length in 2015 (median values of 36 cm in June, 49 cm in October), decreasing slightly in October 2016 to a median value of 42.5 cm. There was no increase in root length in *L. salicaria* in 2015, the median values being 48.5 cm in June and 42.5 cm in October while decreased to 22 cm in October 2016. Increases in the root lengths of the three species may be related to their growth habits as well as nutrient translocation. *Carex* spp. and *L. salicaria* increased in bulkiness and width, whereas *I. pseudacorus* increased more in root length, exceeding the maximum (30 cm) described by Jacobs *et al*. (2011). This increase may be attributed to nutrients contributing to root length rather than to the height of aerial parts. Another interpretation would attribute excessive increase in root length to scarcity of nutrients in surrounding medium (Borin, 2003). Root-system width was measured in October 2015 and 2016, where representative samples of each species attained maximum width. Median values for a maximum root-system width for *Carex* spp. and *L. salicaria* were similar in 2015 at 16.5 and 15.5 cm respectively, whereas the median value for *I. pseudacorus* was 7.5 cm. Values were indifferent for *Carex* spp. and *I. pseudacorus* in 2016 (15.5 and 7 cm respectively) while it increased for *L. salicaria* (20 cm). Observations showed *Carex* spp. and *L. salicaria* to have bulkier and stouter root systems than that of *I. pseudacorus*, which tended to increase in length rather than width. According to Mthembu *et al*. (2013), the potential rate of nutrient uptake by plants is determined by plant growth rate and the concentration of nutrients in the plant tissues, so that nutrient storage in the plant is dependent on plant-tissue nutrient concentrations and plant biomass accumulation. In light of this, the ideal characteristics for plants to be used as macrophytes in wetland systems are fast growth rate, high tissue nutrient content and the ability to attain a high standing crop (plant sustainability).

Table 2: Plant-growth dimensions for the three species in the FTW system in June and October 2015, and October 2016

Plant character	**Date**	***Carex* L. spp.**			***Lythrum salicaria* L.**			***Iris pseudocorus* L.**		
		Median	**25%**	**75%**	**Median**	**25%**	**75%**	**Median**	**25%**	**75%**
Plant height **(cm)**	June 2015	59.5	40	69	33.5	22	38.25	24	15.15	32.75
	Oct. 2015	60	60	77.25	59.5	37.5	83.75	37.5	34.25	42.75
	Oct. 2016	69	55	76.25	26.5	13.75	37	23	20	30
Root depth **(cm)**	June 2015	36	28	42.25	48.5	38.75	53.25	16	11.25	21.75
	Oct. 2015	49	45	61.5	42.5	40	47	76	63.5	89
	Oct. 2016	42.5	39.5	56.25	22	15.75	41.25	20	14	30
Root-system width **(cm)**	Oct. 2015	16.5	14.25	20	15.5	13.25	20.75	7.5	6.25	9
	Oct. 2016	15.5	13.25	20.75	20	19.25	30	7	5	10

3. Plant biomass production and nutrient uptake

Regarding fresh-biomass, *Carex* spp. exhibited the highest production with average total of 2224.43 ± 409.07 g m^{-2} in 2015 increasing by double in 2016 to a total average of 5402.67 ± 783.22 g m^{-2}. *L. salicaria* came second in terms of fresh biomass with a total average of 1092.84 ± 48.33 g m^{-2} in 2015 increasing to 1913.76 ± 287.12 g m^{-2} in 2016. *I. pseudacorus* had the least fresh biomass with a total average of 534.77± 79.18 g m^{-2} measured in 2015 only. Fresh biomass production was always higher below mat (root system) than above mat (aerial parts) in all three species; *Carex* spp. had averages of 1782.79 ± 344.60 and 3982.67 ± 604.10 g m^{-2} (80 and 73 %) below mat in 2015 and 2016 respectively while above mat averaged 441.64 ± 74.43 g m^{-2} (20%) in 2015 increasing significantly to 1420 ± 227.35 g m^{-2} in 2016 (27 %). The average below mat for *L. Salicaria* was 1010.86 ± 139.35 and 1673.67 ± 270.56 g m^{-2} in 2015 and 2016 respectively (92 and 87%) while the average above mat scored 81.98 ± 14.77 and 240 ± 20.90 g m^{-2} in 2015 and 2016, respectively (8 and 13%). *I. pseudacorus* averaged 463.31 ± 68.25 g m^{-2} (87%) below mat and 71.45 ± 11.53 g m^{-2} (13%) above mat in 2015 (Table 3).

Carex spp. ranked first in terms of dry-biomass production, with a total average of 433.13 ± 84.72 g m^{-2} in 2015 doubled to 1008.32 ± 154.5 g m^{-2} in 2016, followed by *L. salicaria* with a total average of 210.32 ± 27.97 g m^{-2} in 2015 increasing insignificantly to 296.55 ± 38.09 g m^{-2} in 2016. *I. pseudacorus* scored lowest in biomass production, with a total average of 106.95 ± 15.42 g m^{-2} in 2015. Dry biomass production, like fresh biomass, was higher below mat than above mat in the three species. The biomass production of *Carex* spp. was the highest; averaged 266.94 ± 57.36 and 556.73 ± 91.19 g m^{-2} (62 and 55%) below mat in 2015 and 2016, respectively and, 166.19 ± 29.40 and 442.59 ± 74.11 g m^{-2} (38 and 45%) above mat in 2015 and 2016, respectively. 349 g m^{-2} above-mat biomass production was reported by Salvato and Borin (2010) for *C. elata*. *L. salicaria* came second, with an average below-mat biomass of 174.61 ± 24.25 g m^{-2} (83%) in 2015 with insignificant increase to 236.79 ± 35.66 g m^{-2} in 2016 (80%) and an average above-mat biomass of 35.71 ± 6.06 g m^{-2} (17%) in 2015 and 59.76 ± 8.75 g m^{-2} (20%) in 2016, whereas *I. pseudacorus* ranked last (average below-mat biomass 86.73 ± 12.56 g m^{-2} (81%), above-mat biomass 20.22 ± 3.11 g m^{-2} or 19% of total biomass in 2015 (Table 4). *Carex* spp. performed best in terms of fresh and dry, above- and below-mat and total biomass production, demonstrating good stability and establishment in the second season. *L. salicaria* performed well and was highly stable, ranking second for fresh and dry, above- and below-mat, and total biomass production, although it was introduced only during the second season and was already in senescence during sampling. *Iris pseudacorus* did not seem to adapt well in both seasons and had the lowest fresh and dry, above- and below-mat and total biomass production. Results for biomass production of *I. pseudacorus* diverged from those reported by De Stefani (2012) and Pavan *et al*. (2015), which supported the suitability and increased productivity of this species in similar FTWs. De Stefani (2012) reported median values of 3693 and 1516 g m^{-2} for above-mat dry biomass in two different experiments, whereas below-mat dry biomass reached 3346 and 801 g m^{-2} in the same experiments. Pavan *et al*. (2015) recorded median values for above-mat dry biomass of 180 and 500 g m^{-2} in two successive seasons, although it is worth noting that this experiment was set up on an open wetland in an agricultural landscape; during agro-environmental monitoring activities, *M. coypus* was observed feeding on *I. pseudacorus*.

Table 3: Average fresh biomass production (g m^{-2}) with standard deviation for the three species in FTW system in 2015 and 2016

Species	Above-mat		Below Mat		Total	
	2015	2016	2015	2016	2015	2016
Carex L.	441.64 ± 74.43	1420 ± 227.35	1782.79 ± 344.60	3982.67 ± 604.10	2224.43 ± 409.07	5402.67 ± 783.22
Lythrum salicaria L.	81.98 ± 14.77	240 ± 20.90	1010.86 ± 139.35	1673.67 ± 270.56	1092.84 ± 148.33	1913.76 ± 287.12
Iris pseudacorus L.	71.45 ± 11.53	-	463.31 ± 68.25	-	534.77 ± 79.18	-

Table 4: Average dry biomass production (g m^{-2}) with standard deviation for the three species in FTW system in 2015 and 2016

Species	Above-mat		Below Mat		Total	
	2015	2016	2015	2016	2015	2016
Carex L.	166.1 ± 29.40	442.59 ± 74.11	266.94 ± 57.36	556.73 ± 91.19	433.13 ± 84.72	1008.32 ± 154.5
Lythrum salicaria L.	35.71 ± 6.06	59.76 ± 8.75	174.61 ± 24.25	236.79 ± 35.66	210.32 ± 27.97	296.55 ± 38.09
Iris pseudacorus L.	20.22 ± 3.11	-	86.73 ± 12.56	-	106.95 ± 15.42	-

Total N concentrations in total dry biomass were very similar in the three species (1.12 and 0.94% in *Carex* spp., 1.12 and 0.83% in *L. salicaria* in 2015 and 2016 respectively, and 1.02% in *I. Pseudacorus* in 2015*)*, but varied between above-mat and below-mat plant parts, the latter having higher N concentrations, averaging 1.21 and 1.03 in *Carex* spp., 1.19 and 0.85 in *L. salicaria* in 2015 and 2016 respectively and 1.04% in *I. Pseudacorus* in 2015. Average above-mat N concentration in *Carex* was 1.02 and 0.87% (Salvato and Borin (2010) reported 1%), followed by *I. pseudacorus* (0.91% in 2015) and *L. salicaria* (0.64 and 0.82%) in 2015 and 2016 respectively (Table 5). *Carex* spp. had the highest N concentrations in above- and below-mat dry biomass, indicating efficient performance. Although *L. salicaria* had a high N concentration in below-mat biomass, it had the lowest concentration of the three species in above-mat biomass, which could be related to senescence of aerial parts and relocation of N to the root system (Vymazal 2007). Nitrogen concentrations in *I. pseudacorus* were lower than those reported by De Stefani (2012) and Pavan *et al*. (2015), which were, respectively, 4.62% in below-mat biomass and 2.77% in above-mat dry biomass. Regarding N uptake, *Carex* spp. exhibited a total uptake of 4.84 ± 0.93 g m^{-2} in 2015 doubled to 9.43 ± 1.42 g m^{-2} in 2016, with a higher uptake through the roots (3.19 ± 0.66 and 5.62 ± 0.86 g m^{-2}, 66 and 60% of total uptake in 2015 and 2016, respectively), followed by *L. salicaria* with a total uptake of 2.35 ± 0.34 g m^{-2} in 2015 with no significant increase in 2016 (2.46 ± 0.39 g m^{-2}). Uptake by roots was 2.11 ± 0.31 and 2 ± 0.36 g m^{-2} (90 and 81%) in 2015 and 2016, respectively. *Iris pseudacorus* had the lowest uptake (total 1.09 ± 0.17 g m^{-2}, below-mat 0.92 ± 0.14 g m^{-2} or 84% of total uptake in 2015) (Table 6). Nitrogen uptake by *I. Pseudacorus* was also very low compared to results reported by De Stefani (2012) and Pavan *et al*. (2015), with values up to 115 g m^{-2} for below-mat and 70 g m^{-2} for above-mat uptake.

Total P concentrations were not very high compared with N concentrations. The highest concentrations were measured in 2015 in *L. salicaria* (0.09%), followed by *Carex* spp. and *I. pseudacorus* (both 0.07%). In 2016, Concentrations were 0.06 and 0.05 % in *L. Salicaria* and *Carex* spp., respectively. As with N concentrations, P concentrations were higher in the below-mat than the above-mat biomass. *L. salicaria* had the highest P concentration in the roots (0.1%) in 2015, although those of *Carex* spp. and *I. pseudacorus* were nearly the same (0.08 and 0.07%). Concentrations were similar for *Carex* spp. and *L. salicaria* in 2016 (0.064

Table 5: Average N concentration (% per plant DM) with standard deviation for the three species in FTW system in 2015 and 2016

Species	Above-mat		Below Mat		Total	
	2015	2016	2015	2016	2015	2016
Carex L.	1.02 ± 0.09	0.87 ± 0.06	1.21 ± 0.07	1.03 ± 0.11	1.12 ± 0.011	0.94 ± 0.009
Lythrum salicaria L.	0.64 ± 0.12	0.82 ± 0.15	1.19 ± 0.11	0.85± 0.020	1.12 ± 0.012	0.83 ± 0.010
Iris pseudacorus L.	0.91 ± 0.19	-	1.04 ± 0.11	-	1.02 ± 0.010	-

Table 6: Average N uptake (g m^{-2}) with standard deviation for the three species in FTW system in 2015 and 2016

Species	Above-mat		Below Mat		Total	
	2015	2016	2015	2016	2015	2016
Carex L.	1.65 ± 0.28	3.81 ± 0.63	3.19 ± 0.66	5.62 ± 0.86	4.84 ± 0.93	9.43 ± 1.42
Lythrum salicaria L.	0.24 ± 0.043	0.46 ± 0.055	2.11 ± 0.31	2 ± 0.36	2.35 ± 0.34	2.46 ± 0.39
Iris pseudacorus L.	0.18 ± 0.02	-	0.92 ± 0.14	-	1.09 ± 0.17	-

and 0.063%, respectively) (Table 7). Phosphorus concentration in *I. pseudacorus* was low compared with that reported by Pavan *et al*. (2015), which was 0.33%. Total P uptake was highest in *Carex* spp. (0.31 ± 0.07 g m^{-2} in 2015, increasing to 0.52 ± 0.13 g m^{-2} in 2016), with maximum uptake through the root system (0.24 ± 0.057 and 0.36± 0.05 g m^{-2}, ~78 and 70% of total uptake in 2015 and 2016, respectively). *Lythrum salicaria* ranked second, with a total uptake of 0.2 ± 0.03 g m^{-2} (0.185 ± 0.029 and 0.16 ± 0.027 g m^{-2} (93 and 89%) in the roots in 2015 and 2016, respectively). *I. pseudacorus* was the lowest (total 0.074 ± 0.01 g m^{-2}, 0.066 ± 0.013 g m^{-2} (89%) in the roots) in 2015. Only traces of P were taken up through aerial parts by the three species (Table 8). According to Hernández-Crespo *et al*. (2016), nutrient concentrations are inversely correlated with the amount of above-ground biomass, i.e. as above-ground biomass increases, nutrient concentration decreases because most of the nutrients have already been used by the plant for growth and performance at the peak of the season (Mthembu *et al*. 2013). In the present study, the root systems had higher concentrations of nutrients because of translocation of most nutrients as the senescence period approached (Bonaiti and Borin 2000; Vymazal 2007).

Table 7: Average P concentration (% per plant DM) with standard deviation for the three species in FTW system in 2015 and 2016

Species	Above-mat		Below Mat		Total	
	2015	2016	2015	2016	2015	2016
Carex L.	0.04 ± 0.008	0.038 ± 0.005	0.08 ± 0.018	0.064 ± 0.005	0.07 ± 0.0008	0.05 ± 0.0005
Lythrum salicaria L.	0.03 ± 0.008	0.043 ± 0.009	0.10 ± 0.015	0.063± 0.010	0.09 ± 0.0011	0.06 ± 0.0007
Iris pseudacorus L.	0.04 ± 0.018	-	0.07 ± 0.015	-	0.07 ± 0.0009	-

Table 8: Average P uptake (g m^{-2}) with standard deviation for the three species in FTW system in 2015 and 2016

Species	Above-mat		Below Mat		Total	
	2015	2016	2015	2016	2015	2016
Carex L.	0.068 ± 0.013	0.16 ± 0.02	0.240 ± 0.057	0.36 ± 0.055	0.308 ± 0.07	0.52 ± 0.13
Lythrum salicaria L.	0.013 ± 0.002	0.02 ± 0.002	0.185 ± 0.029	0.16 ± 0.027	0.198 ± 0.03	0.18 ± 0.03
Iris pseudacorus L.	0.008 ± 0.001	-	0.066 ± 0.013	-	0.074 ± 0.01	-

Figure 24. Digital photographs for the FWS CW in 2015 (upper) and 2016 (middle, lower)

Conclusion

A generally promising depurative effect was noticeable from the concentration trends throughout the system over three consecutive years of monitoring. This effect was notable during spring 2015 and 2016, as evidenced by the great decrease in TN and NO_3^- concentrations throughout the wetland sub-basins (FWS CW) and the downstream channel (FTW) after the combination of intensive rainfall events and crop fertilisation run-off. Phosphorus concentrations in water were almost negligible. Mass balance and removal percentages for different nutrients, especially TN and N-NO_3^-, were increasing consistently over the years with the continuous establishment of the wetland system to reach 64 and 91 % in 2016 for TN and N-NO_3^-, respectively.

Monitoring of the vegetation in the floating-treatment wetland system showed *Carex* spp. to be the most adaptable, with a high survival rate, hardiness and continuity over three successive seasons, the highest plant parameters, especially biomass production, and the highest N and P uptakes. *L. salicaria* was very stable, exhibited excellent growth performance during the first season and average performance in the second one with a good potential for establishment in the floating system, whereas *I. pseudacorus* lagged behind for the third season, with the lowest survival rate, plant growth parameters and nutrient uptake. A general conclusion is that a crucial role could be played by FWS CWs and FTWs in integrated agro-environmental management to control nutrient runoff from intensive cropping systems.

Chapter III

Performance of free surface constructed wetland in the mitigation of non-point agricultural pollution within the Venetian Lagoon drainage system under intermittent water dynamics (Pilot scale)

Introduction

As mentioned earlier in the last chapter, Nitrogen loads resulting from agricultural wastewaters are discharged through 12 tributaries forming a drainage basin into the Venetian Lagoon; the principal wastewater reservoir for north east Italy (Collavini *et al.*, 2005; Zonta *et al.*; 2005; Zuliani *et al.,* 2005). Assessment of nitrogen loads within the Venetian lagoon drainage system showed that the input loads exceeded the maximum allowed load input (3000 t/year) in the lagoon as given by the ministerial decree (Ministero dell'Ambiente, 1999; Collavini *et al.*, 2005). Based on the previous, real control measures were essential to reduce the nitrogen loads within the lagoon, at least within the accepted levels.

Treatment of non-point agricultural run-off differs from other types of wastewaters as the hydrological loading is intermittent and the organic load is almost absent (Higgins *et al.*, 1993). Constructed wetlands (CW) offered promising solutions for the control of nitrogen pollution resulting from agricultural run-off at relatively low cost and energy inputs (Davis, 1995a; Peterson, 1998; Mitsch *et al.*, 2001; Kadlec and Wallace, 2009; Lee *et al.*, 2009). In general, Dissolved inorganic nitrogen groups including nitrate (N-NO_3^-), nitrite (N-NO_2^-) and ammonium (N-NH_4^+) are more likely to affect water quality and aquatic life rather than organic nitrogen forms as they are readily available for uptake (Lee *et al.*, 2009). Basically, NO_3^- resulting from fertilizer use in the croplands is the most abundant form of inorganic nitrogen and is the major target of the control process using CW ((Baker, 1998; Mitsch *et al.*, 2001; Mitsch *et al.*, 2005; O'Geen *et al.*, 2010). In surface waters, NO_3^- would cause majorly eutrophication problems rather than toxicity due to the effective removal processes mainly by denitrification and plant uptake (Davis, 1995b; Peterson, 1998). Generally, free water surface constructed wetlands (FWS) are more effective in the removal of biological oxygen demand (BOD), total soluble solids (TSS), total nitrogen (TN) and phosphorus (TP) while subsurface flow constructed wetlands, mainly horizontal type (HSSF) is more effective in the removal of tertiary BOD and N-NO_3^- as it favors denitrification process (Vymazal 2007; Kadlec, 2009). However, FWS are more cost effective in treatment of agricultural run-off with lower maintenance requirements than HSSF which has problems with clogging of porous media (Kadlec, 2009; Lee *et al.*, 2009; O'Geen *et al.*, 2010).

Performance of CWs in the removal of nitrogen load is dependent on many factors including climatic conditions like temperature, solar radiation, wind patterns, and precipitation which affect biogeochemical reactions, evapotranspiration, and rate of water inflow to the systems, hence, affecting the removal efficiency (Kadlec, 1999; O'Geen *et al.*, 2010). Hydrological loading is another factor affecting the removal efficiency and is greatly dependent on the design of the wetland and the source of water. In the case of agricultural run-off, water inflow and hydrological loading shows great seasonable variability depending on the different cropping patterns where the contamination fluxes are influenced by fertilization events and pesticide application (Kadlec, 2010; O'Geen *et al.*, 2010). Based on the previous, treatment of nitrogen loads from agricultural run-off in CW tends to be more periodic and event-driven (Kadlec and Wallace, 2009). According to Kadlec (2010), in cases of event driven agricultural run-off, correlation between wetland treatment performance and simple design variables (hydrological loading, detention time and pollutant loading) could not provide comprehensive results to explain such performance. This urged the need to more understanding of the internal water dynamics and their interaction with other factors like vegetation and other biota to be able to understand the internal processes affecting the performance of the wetland.

The aim of this study was to evaluate the $N\text{-}NO_3^-$ retention and give insight to some water dynamics of a FWS CW in a designed event- driven pilot experiment simulating excessive agricultural nitrate load performed in June 2016.

Materials and Methods

Experimental site

The experiment was conducted on the same farm, 'Tenuta Civrana' (365 ha), located in Cona, Venice within the Venetian Lagoon drainage system (north-eastern Italy) with coordinates 45.1668N and 12.0668E. An integrated wetland system of 3.3 ha was created in 2014 by restoring a semi-natural wetland (Pappalardo *et al.*, 2017). The integrated wetland system is composed of a free water surface constructed wetland (FWS CW), divided into 5 sequential sub-basins (B1 to B5) and a floating treatment wetland (FTW), constructed in a vegetated canal perpendicular to the FWS CW and connected to it through a sub-surface pipe system (Chapter II). The wetland system is fed by agricultural run-off water diverted from 'Canale dei Cuori', an important drainage canal within the Venetian drainage system whereas water flows by the force of gravity from inlet of the first sub-basin (B1) to the outlet of the last basin (B5), then to the FTW and finally to agricultural ditches. The detailed description of the FWS CW, in which these experiments were conducted, was given earlier in chapter II and by Pappalardo *et al.* (2017). The fourth sub-basin (B4), which was chosen for monitoring the experiment, has the dimensions 60 x 30 x 0.4 m with a total area of 1800 m^2 (total water area 1720 m^2) holding water volumes ~ 700-1000 m^3. The sub-basin is characterized by the presence of a floating/emergent machrophyte island (80 m^2) at its center, mainly *Phramites australis*; which diverts the main water flow into two different paths before they mix again at the outlet of the sub-basin (Figures 1,2).

Experiment

The experiment started with the isolation of sub-basins B3, B4, and B5 by blocking the sub-surface pipes connecting them with rest of the sub-basins. An elevated nitrate (NO_3^-) solution was prepared by dissolution and addition of 600 kg of calcium nitrate $Ca(NO_3)_2$, N = 15.5% to sub-basin B3 (V=1500-1900 m^3) to obtain a solution of an average N content of 40-60 mg l^{-1}. The homogeneity of solution in B3 was guaranteed by using a motor pump unit connected to a power take-off tractor and an irrigator (used in aspiration systems) (Figure 3). Next, the water with the dissolved solution was transferred from B3 to B4 by the means of the motor pump connected to the power take-off tractor at a flow rate of 1.5 m^3 min^{-1} to allow the total replacement of water in B4 (Figure 4). The hydraulic retention time (HRT) was ~ 11-12 hours. The water flow rate was reduced to 0.3 m^3 min^{-1} on the fourth day depending on the field conditions.

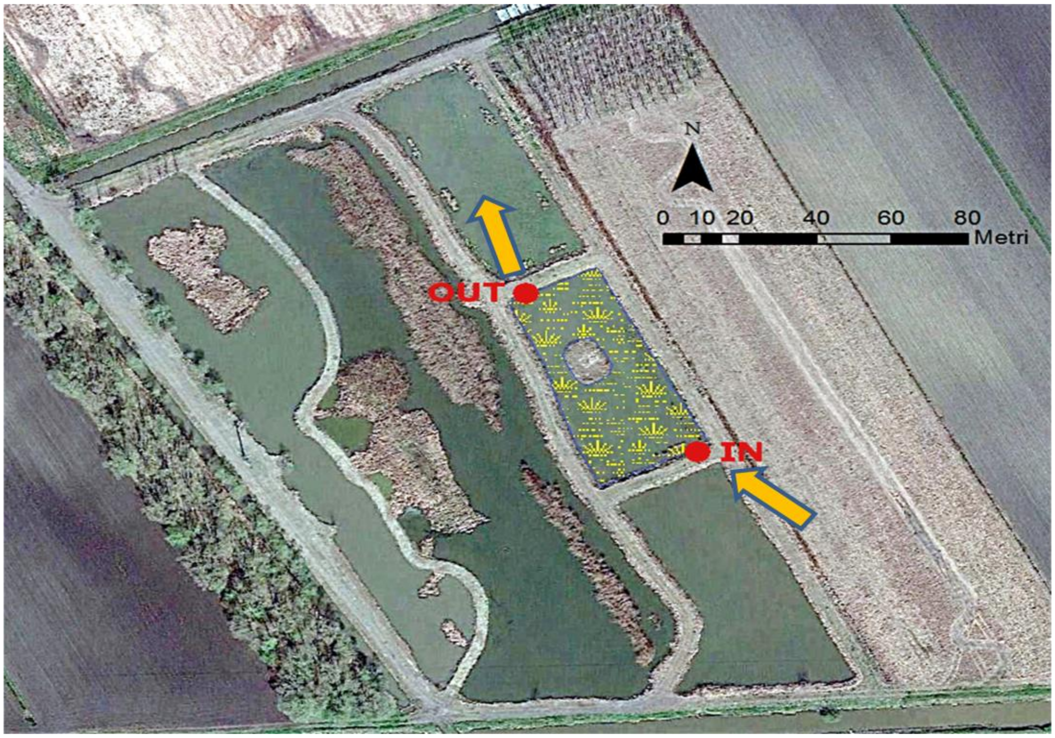


Figure 1: A digital map of the FWS CW with focus on sub-basin 4 (B4) used for the monitoring process with arrows showing the direction of water flow from inlet to outlet

Figure 2: Sub-basin 4 (B4) used for the monitoring process with characteristic *phragmites* island in its center diverting incoming water flow into two paths

Figure 3: Preparation of calcium nitrate solution before adding to B3 (left), homogenization of water in B3 using an irrigator connected to a power take-off tractor (right)

Figure 4: Transfer of dissolved calcium nitrate solution from B3 to B4 using a motor pump connected to a power take-off tractor

Monitoring, sampling, chemical and data analysis

A grid scheme with 30 different sampling points was prepared to monitor the depurative performance in B4 during the experiment (Figure 5). An over-hanging free-moving wire system was set up above B4 at adjusted distances to allow the sampling of the intermediate points in the center of the sub-basin with sampling bottles attached to the free-moving wire at adjusted distances during the sampling procedure. The water sampling process started one hour after the beginning of transfer of the dissolved solution to B4 and continued for 24 hours with a 3-hour time interval between samplings during the first phase of monitoring (7th and 8th June 2016). During the second monitoring phase (10th June 2016), sampling was done only during the day with a 2-hour time interval between different samplings (Table 1). On-site monitoring of some physico-chemical parameters of water (temperature, pH and electric conductivity (EC)) was carried out during using a portable multitasking device; HQD (HACH Lange HQ 40d, Hach, Loveland, CO, USA) at some selected points in B4; majorly inlet and outlet, two points at the sub-basin corners and two points in the middle of the sub-basin. Some samples were taken and some physico-chemical measurements were done for some points at the lateral canal to check that the monitored system is completely isolated. The previous monitoring protocol was designed to be able to evaluate the total volumes entering to the sub-basin, the movement of water and any preferential flows, and the depurative capacity of the sub-basin over space and time both in terms of concentration and quantity. Water samples were analyzed off-site in the Centralized Chemical Laboratory of DAFNAE department (La Chi.), University of Padua, Legnaro (PD), Italy and N-NO_3^- concentration was determined and quantified using standard spectrophotometric methods (Cataldo *et al.*, 1975).

Results of the analyses for NO_3^- concentrations, EC and pH at B4 IN and OUT were presented as box and whisker plots using medians and quartiles. Line charts explained the changes in N-NO_3^- concentrations, EC and pH in B4 IN and OUT during the whole experimentation period. Removal percentage was calculated based on N-NO_3^- concentrations using the formula: $\text{Removal } (\%) = \frac{(\text{C inlet} - \text{C outlet})}{\text{C inlet}} * 100$ Where, C inlet is N-NO_3^- concentration at inlet and C outlet is the N-NO_3^- concentration at the outlet, while total mass removal in 12 hours was calculated as follows: $\text{Mass Removal (kg)} = \text{M inlet} * \%\ \text{Removal} / 100$, Where, M inlet is mass of N-NO_3^- at inlet (water inflow * median concentration at inlet). Daily mass removal in unit area (m^2) was estimated as daily total mass removal/ total sub-basin area.

Approximate prediction of water movement and fluxes throughout the loading experiment was possible by the preparation of some geo-statistical model maps at different sampling times in ArcGIS 10.2 (ESRI, 2013). Based on N-NO_3^- concentrations at different sampling points in the grid scheme, spatial interpolation was performed using kernel interpolation with barriers which takes into account the presence of a vegetative island barrier in the center of the monitored sub-basin.

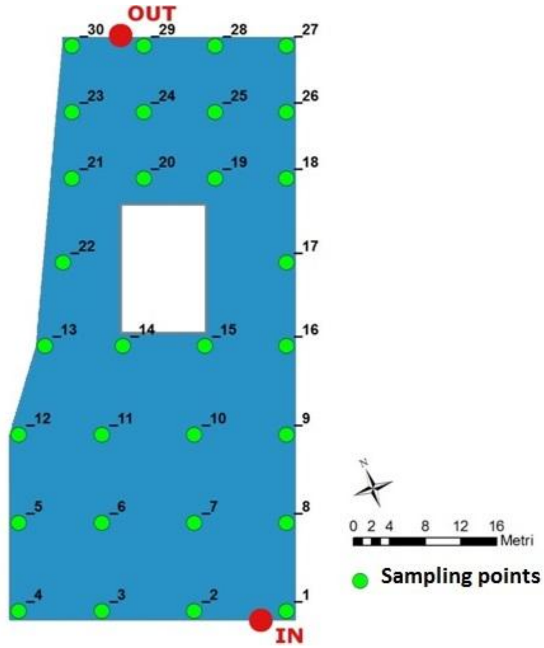


Figure 5: Grid scheme showing 30 different sampling points in the selected sub-basin B4

Table 1: Sampling hours and dates for the loading experiment

	Experiment 1	
Reference	**Sampling hour**	**Sampling date**
A	18.00	7/6/2016
B	19.00	
C	22.00	
D	01.00	8/6/2016
E	04.00	
F	07.00	
G	10.00	
H	13.00	
I	16.00	
J	-	
K	-	
L	09.30	
M	11.10	10/6/2016
N	12.10	
O	14.40	
P	17.10	

Results and discussion

1. N-NO_3^- concentration

Before the start of the loading experiment, N-NO_3^- concentration at the sub-basin inlet B4 IN was very low (0.65 mg l^{-1}) owing to the general low concentration in the inflow, together with the prominent depurative effect of the wetland. During the first phase of the loading experiment (7th and 8th June 2016), N-NO_3^- concentration showed a median concentration of 45.34 mg l^{-1} at B4 IN with a maximum of 66.94 mg l^{-1} reached at the first sampling (7/6/2016, 18.00) indicating that homogenization of dissolved solution in B3 was successful and effective, while the minimum concentration was 25.03 mg l^{-1} achieved on 8/6/2016, 4.00 a.m (point of equilibrium). After the total substitution of water in B4 (poor N-NO_3^- content) with water from B3 (rich N-NO_3^- content) at 4.00 a.m (8/6/2016), median N-NO_3^- concentration at sub-basin outlet B4 OUT reached 41.5 mg l^{-1} with a maximum value of 45.08 mg l^{-1} and a minimum of 20 mg l^{-1} (Figure 6). During the second phase of loading experiment (10th June), after a heavy rainfall event of 76 mm (9th June), median N-NO_3^- concentration reached 10.20 mg l^{-1} at B4 IN and 22.58 mg l^{-1} at B4 OUT while the minimum and maximum values ranged between 6.04 and 28.71, 17.04 and 30.11 mg l^{-1} for B4 IN and B4 OUT, respectively. N-NO_3^- concentrations were very low in the lateral canals (median 0.5 mg l^{-1}) throughout the whole loading experiment indicating that there were no lateral loses from the isolated sub-basin system.

Figure 7 shows the evolution of N-NO_3^- concentration in B4 IN and B4 OUT during the two phases of loading experiment. During the first phase, concentration started very high in B4 IN after the beginning of transfer of the dissolved solution from B3 (7/6/2016, 18.00) and decreased gradually to reach its minimum at the equilibrium point (detention time, 8/6/2016, 4.00 a.m) while it increased gradually in B4 OUT to reach almost the same concentration as in B4 IN at the same point of equilibrium (Kadlec, 2010). After equilibrium, concentrations increased simultaneously in B4 IN and OUT and then they were almost constant till the end of this phase. During the second phase, concentrations were lower in B4 IN than B4 OUT owing to the dilution effect in B3 caused by the heavy rainfall during the preceding day.

The sudden rapid increase in N-NO_3^- concentration after introduction to B4 simulates the "first flush" effect in event-driven wetlands receiving diffused pollution run-off, in which the first inflow is highly loaded with pollutants and then decreases gradually over time (Kadlec and

Wallace, 2009; Kato *et al*., 2009, Kadlec, 2010; Lang *et al.*, 2013). The use of a pump unit to transfer water from B3 to B4 helped to decrease the detention time to a period shorter than a day (Kadlec, 2010). In general, the dilution effect after excessive rainfall is almost negligible due to the subsequent surface run-off (Kato *et al.*, 2009; Lang *et al.*, 2013; Reichwaldt *et al.*, 2015), however, in this case, the system was closed and isolated which allowed the dilution of NO_3^- in B3 and subsequently in B4 with the second water transfer process during the second phase. Increases and decreases in NO_3^- concentration in the simulated experiment followed by rainfall supported the theory that treatment of non- point agricultural run-off in CW is more episodic and event-driven (Kadlec and Wallace, 2009; Kadlec, 2010).

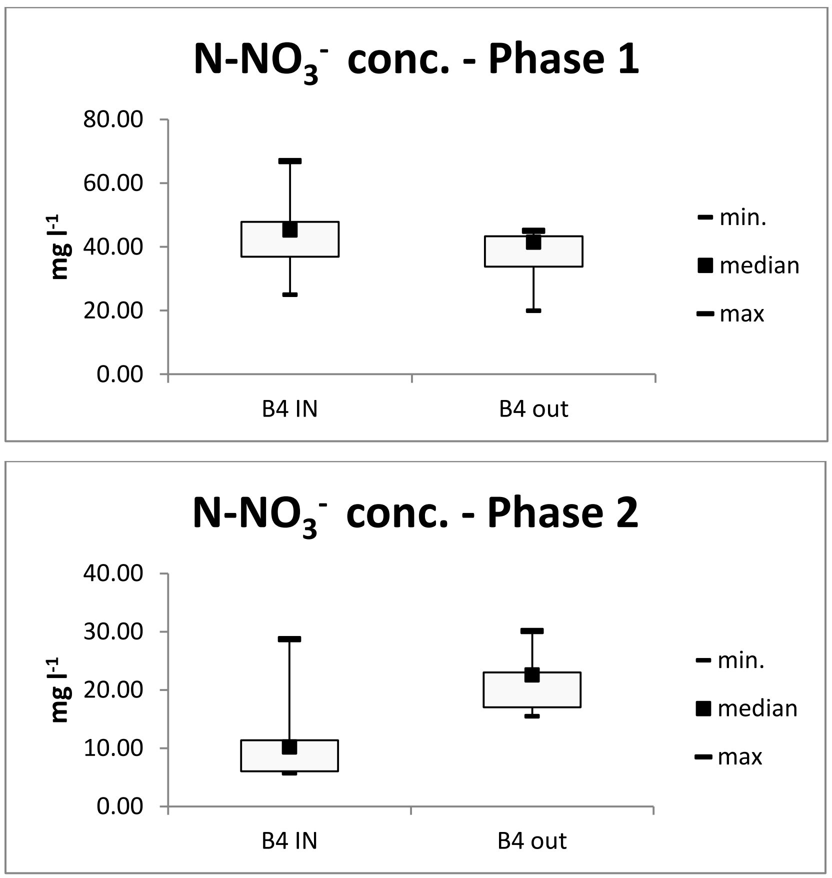


Figure 6: Box and whisker plots showing N-NO_3^- concentration in B4 IN and B4 OUT during the first and second phases of monitoring of the loading experiment

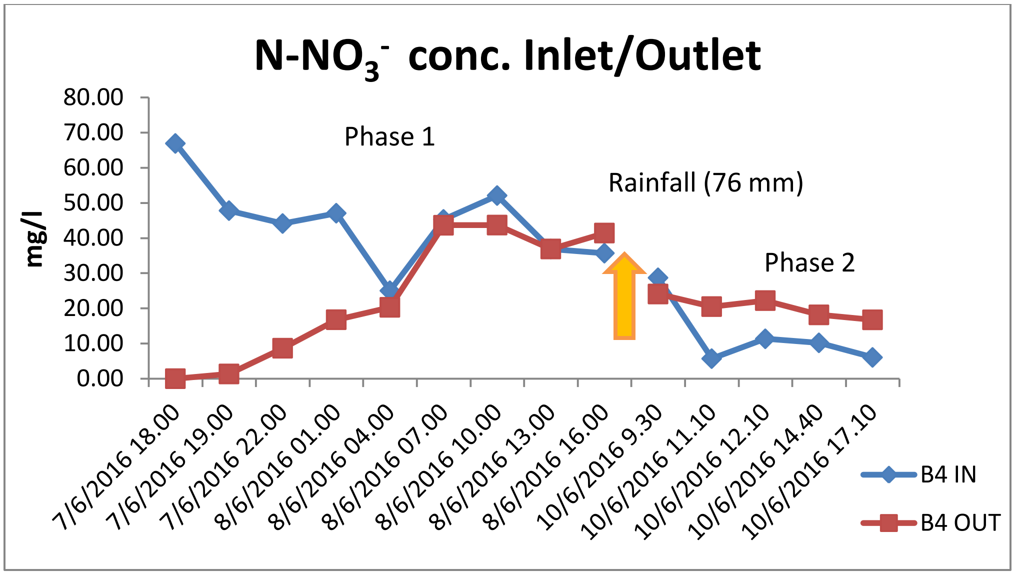


Figure 7: Line chart showing N-NO_3^- concentration in B4 IN and B4 OUT during the first and second phases of monitoring of the loading experiment

2. Mass balance and removal efficiency

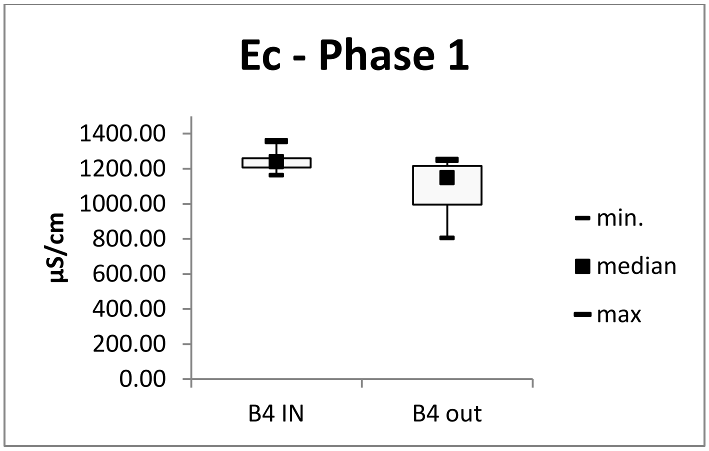
Based on inlet and outlet N-NO_3^- concentrations, a total reduction of 8.4 % was exhibited in B4 after reaching the point of equilibrium i.e. the total substitution of water with the dissolved solution from B3 at 4.00 a.m on 8th June, 2016 till the end of the first phase. The total mass removal of NO_3^- for B4 was 0.82 kg calculated for the period between the detention time (equilibrium) and the end of phase 1 (12 hours). Removal per unit area was estimated to be 1 g NO_3^- m^{-2} d^{-1}. N percent removal was in general lower than other studies (Jordan *et al*., 1999; Kovacic *et al*., 2000; Tanner *et al*., 2003 and 2005; Mitsch *et al*., 2005; Borin and Tocchetto (2007); Kadlec, 2010; Wetland Research, Inc., 2012) due to high nutrient loading within limited experimentation time and sub-basin area, which did not allow enough time and space for the normal biogeochemical cycle and microbial processes to take place (Ballaron, 1988; Braskerud, 2002; Kadlec and Wallace, 2009, O'Geen *et al*., 2010). In addition, increasing the detention time can also be a key factor improving the efficiency of performance of the wetland (Davis, 1995b; Su *et al*., 2009; Wetland Research, Inc., 2012). The presence of vegetative island (obstruction) in the center of B4 could somehow limit the removal efficiency as it creates lower velocity zones preventing the uniform distribution of the flow (Su *et al*., 2009). However, B4 represents only small percentage (~10%) of the total FWS CW area, so it is expected the removal efficiency of the whole wetland would be much higher under similar intermittent conditions.

3. Physico-chemical parameters

Electric conductivity (EC)

Earlier monitoring of EC in B4 (7/6/2016, 10.00 a.m) showed relatively lower values (826 and 823 µS/cm at B4 IN and B4 OUT, respectively) than those achieved after the beginning of the loading experiment. During the first phase, the median conductivity at B4 IN was 1241 µS/cm with a peak of 1358 µS/cm reached at the beginning of the loading of NO_3^- solution from B3 (7/6/2016, 18.00) and a minimum value of 1164 µS/cm (7/6/2016, 22.00). Median conductivity at B4 OUT was 1150 µS/cm with values ranging between a minimum of 806 µS/cm (7/6/2016, 18.00) and a maximum of 1251 µS/cm reached after the detention time (8/6/2016, 7.00 a.m) (Figure 8). Following the second phase of loading, conductivity decreased at B4 IN and B4 OUT after the prominent rainfall (9th June). Median conductivity at B4 IN was 852 µS/cm with values ranging between 819 and 1015 µS/cm. On the other hand, values were higher at B4 OUT ranging between 900 and 1014 µS/cm with a median conductivity of 1004 µS/cm (Figure 8).

Changes in EC between B4 IN and B4 OUT during the two phases were consistent with those of N-NO_3^- concentration (Figure 9). During the first phase of loading, a sudden increase in EC associated with transfer of elevated N-NO_3^- solution from B3 to B4 was noticeable and decreased gradually with passage of time towards the detention time to reach a minimum (7/6/2016, 22.00) then increased again steadily towards the detention time. After equilibrium, EC at B4 IN remained almost constant till the end of the phase. On the other side, EC started low in B4 OUT and increased gradually with the transfer of N-NO_3^- solution through the sub-basin to reach its maximum after the detention time (8/6/2016, 7.00 a.m) after which EC was almost the same throughout the whole sub-basin (B4 IN and OUT). As a result of the dilution effect caused by the heavy rainfall during the preceding day, EC was lower at both B4 IN and OUT during the second phase with lower values at B4 IN than OUT owing to the fast transfer of diluted solution from B3 to B4. Values at both B4 IN and OUT continue to decrease gradually till they reach their minimum towards the end of the second phase.



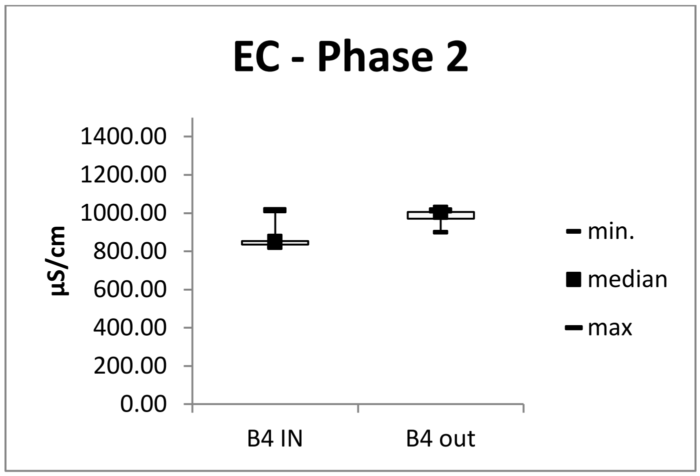


Figure 8: Box and whisker plots showing EC in B4 IN and B4 OUT during the first and second phases of monitoring of the loading experiment

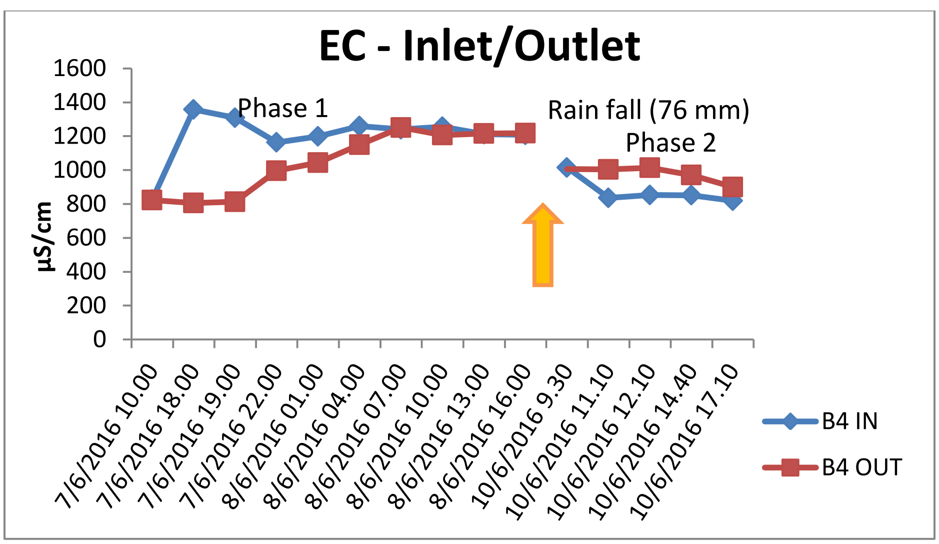


Figure 9: Line chart showing EC in B4 IN and B4 OUT during the first and second phases of monitoring of the loading experiment

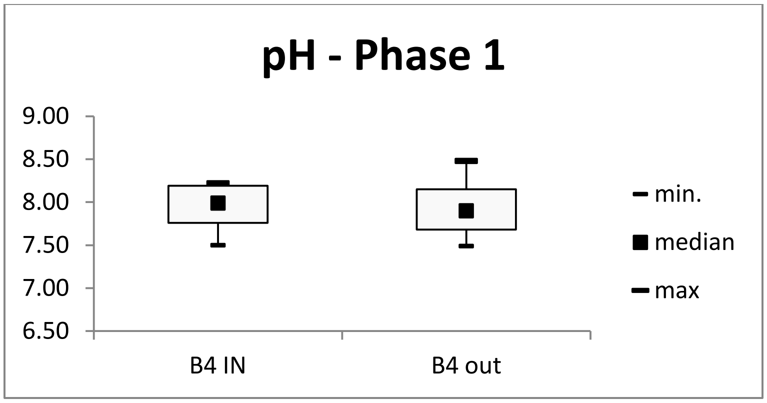
The introduction of high N-NO_3^- concentration to B4 during the first phase greatly increased the ionic and total dissolved solids (TDS) concentrations which in turn massively increased the EC of water (Welcomme, 1985; Kadlec and Wallace, 2009; EPA, 2012c; Perlman; 2014). Diluted waters introduced in the second phase had lower nutrient content, lower TDS and thus lower conductivity (Badve *et al.*, 1993; Gibson *et al.*, 1995; Reichwaldt *et al.*, 2015). EC is a determinant indicator for concentration and dilution of ionic compounds in treatment wetlands (Kadlec and Wallace, 2009).

pH

Despite that the changes in pH in B4 during the loading experiment were small; they could match to some extent with those exhibited by N-NO_3^- concentrations and EC. During the first phase, the median pH at B4 IN was 7.99 with a range varying between a minimum of 7.50 (8/6/2016, 7.00 a.m) and a maximum of 8.22 (7/6/2016, 19.00). At B4 OUT, the median pH was 7.90 with a minimum of 7.49 (8/6/2016, 7.00 a.m) and a maximum of 8.48 (7/6/2016, 18.00). In the second loading phase, pH increased again to reach a median of 8.06 at B4 IN and 8.11 at B4 OUT with minimum and maximum values of 7.69 and 8.12, 7.68 and 8.52 at B4 IN and OUT, respectively (Figure 10).

In B4 IN, during the first phase, pH decreased gradually with the introduction of elevated N-NO_3^- solution from B3 to reach a minimum after the point of equilibrium (8/6/2016, 7.00 a.m), then it increased again gradually towards the end of the phase. On the other hand, pH at B4 OUT remained unchanged before it began decreasing gradually, also to reach its minimum value after the detention time (8/6/2016, 7.00 a.m) where pH became homogenous throughout the whole sub-basin after which it increased again towards the end of the phase, at a rate higher than that of B4 IN (Figure 11). During the second phase, after the rainfall (dilution effect), pH continued increasing again both B4 IN and OUT to almost reach the original values exhibited before the beginning of the loading experiment with a slightly faster rate of increase in B4 OUT than B4 IN.

Normally, the wetland was slightly alkaline (pH $\geq$ 8) due to accumulation of calcium carbonate in soil, photosynthesis and de-nitrification processes, especially during high season (Michaud and Noel; 1991; Murphy, 2007; Kadlec and Wallace, 2009; EPA, 2012a). The introduction of excessive N-NO_3^- solution to the monitored sub-basin led to a gradual slight decrease in pH (alkalinity) as a result of water nitrification, which increased again after the rainfall which



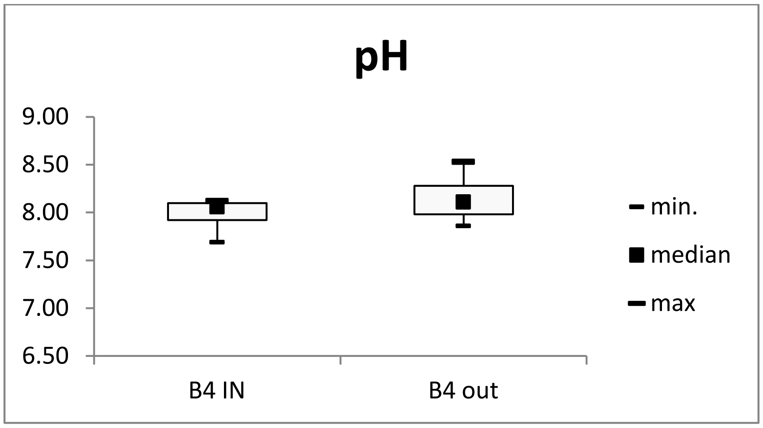


Figure 10: Box and whisker plots showing pH in B4 IN and B4 OUT during the first and second phases of monitoring of the loading experiment

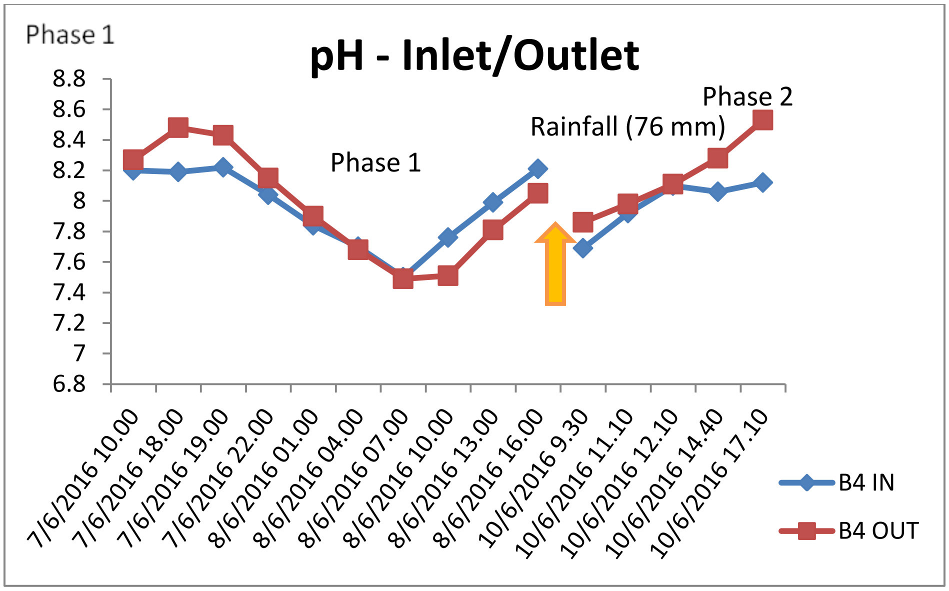


Figure 11: Line chart showing pH in B4 IN and B4 OUT during the first and second phases of monitoring of the loading experiment

caused a dilution in $N\text{-}NO_3^-$ solution in both B3 and B4 (Kadlec and Wallace, 2009; Reichwaldt *et al.*, 2015). Although no clear changes were notable due to the short period of the experiment, decreases and increases in pH could be a good indicator for the changes in $N\text{-}NO_3^-$ concentrations within the wetland.

4. Water movement and fluxes

Spatial interpolation using Kernel with barriers represented a good tool for the prediction of water movement and fluxes during the loading experiment. Before the start of the loading experiment (T Zero), water in B4 was homogenous and nearly static with very low $N\text{-}NO_3^-$ concentrations (0-4 mg l^{-1}) (Figure 12a). On the 7th of June, two hours after the beginning of solution transfer from B3 (T=19.00), concentration gradient was clearly distinct eastwards in B4 indicating major water flow in that direction (Figure 12b). At T=1.00 (8th June), $N\text{-}NO_3^-$ concentrations were increasing gradually at the western side of B4 at a lower rate than the eastern side indicating slower flow in that direction (Figure 12c). After the detention time (T=7.00), the concentration gradient became more homogenous throughout B4 with higher concentrations at the southern and southeastern sides, which can be explained by the presence of vegetative island at the center of the sub-basin acting as a slow-down barrier and creating low velocity zones (Figure 12d). At the last sampling date in this phase (T=16.00), concentration gradient was more prominent at northern and western side of B4 where highly loaded water flow is now directed towards the outlet of the sub-basin (Figure 12e). During the second phase (10th June), following the rainfall event, concentration gradient was more homogenous throughout B4 (T=9.30) with generally lower $N\text{-}NO_3^-$ concentration (20-32 mg l^{-1}) (Figure 12f). After the re-transfer of solution from B3 to B4 (T=11.10), the change in concentration gradient again showed the flow of water towards the eastern side of the sub-basin but this time, $N\text{-}NO_3^-$ concentrations were decreasing as a result of the distinctive dilution effect (Figure 12g). In the same manner of the first phase but with inverted effect, at T=12.10, $N\text{-}NO_3^-$ concentrations declined at a higher rate in the southern and eastern sides of B4 than that at western and northern sides where the vegetative barrier again decreased the water velocity and flow rate (Figure 12h). At T=14.40, water flow was increasing in the western and northern sides of B4 as witnessed by the decrease in the concentration gradient in these sides (Figure 12i). By T=17.10, concentration gradient was completely inverted when

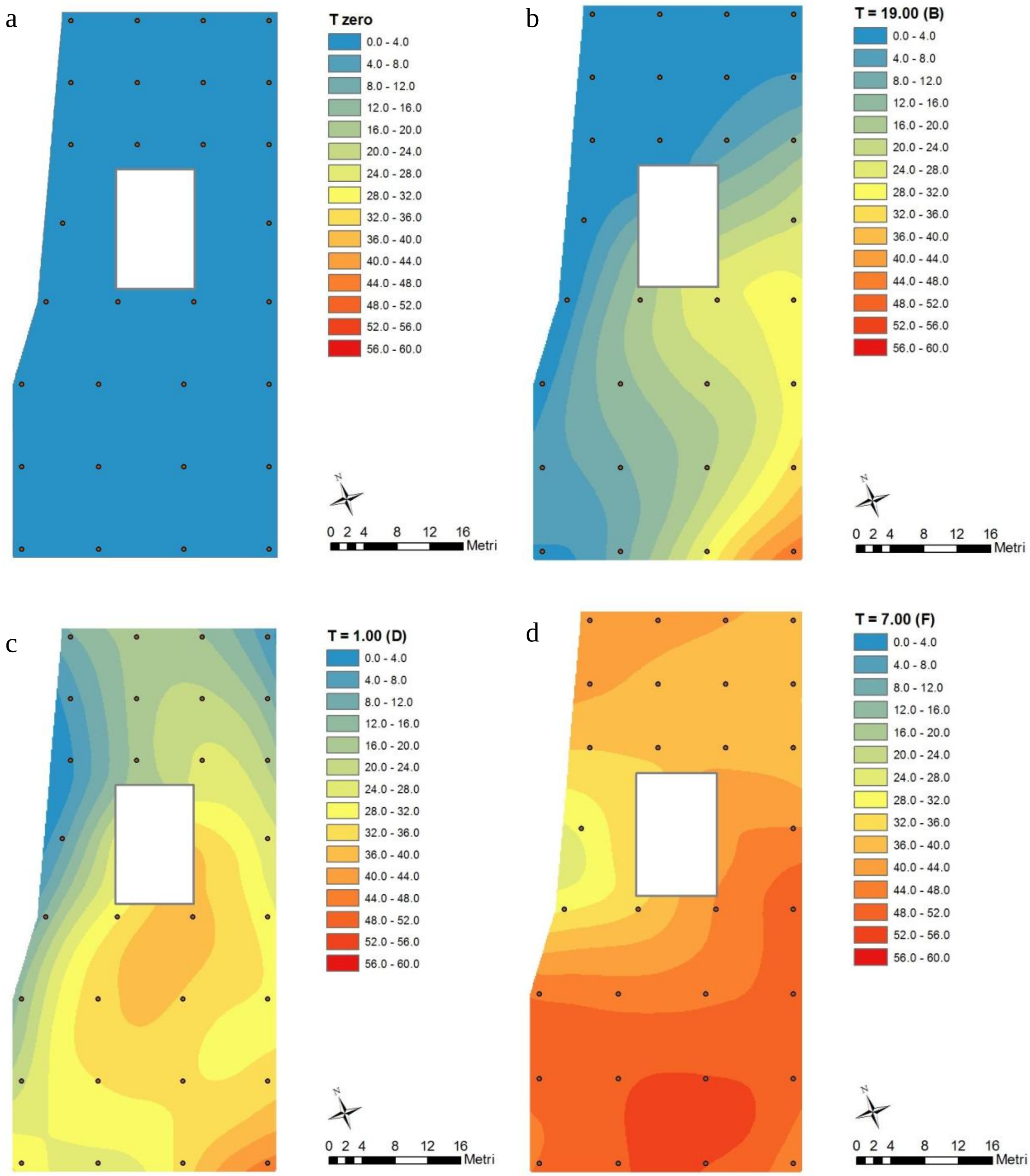


Figure 12: Geo-statistical model maps predicting water flow based on concentration gradients of N-NO_3^- at different sampling times **a**. T Zero **b.** 7/6/2016, T=19.00 **c.** 8//6/2016, T= 1.00 **d**. 8/6/2016, T=7.00

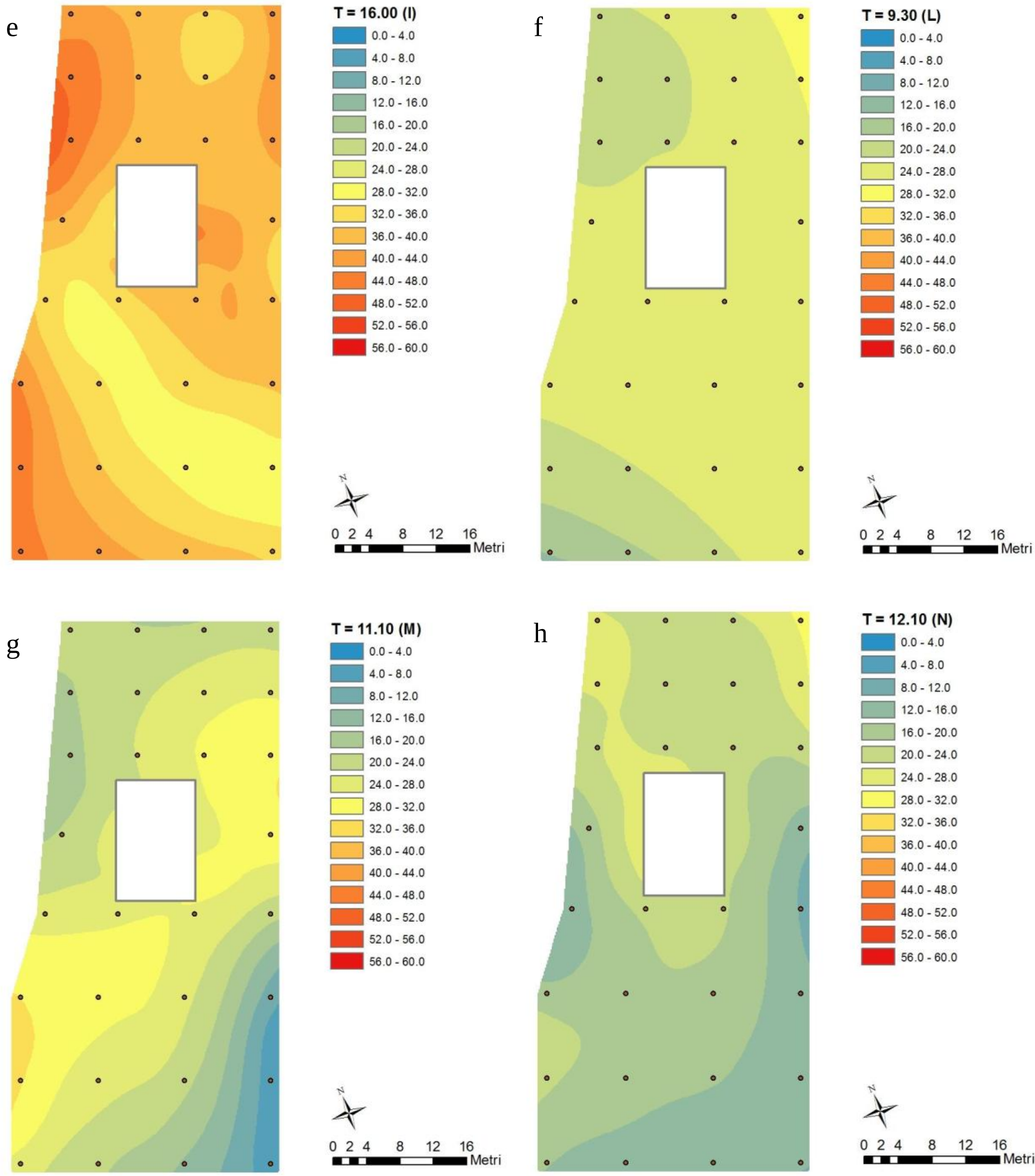


Figure 12 continued: Geo-statistical model maps predicting water flow based on concentration gradients of N-NO_3^- at different sampling times **e**. 8/6/2016, T= 16.00 **f.** 10/6/2016, T= 9.30 **g.** 10/6/2016, T= 11.10 **h**. 10//6/2016, T=12.10

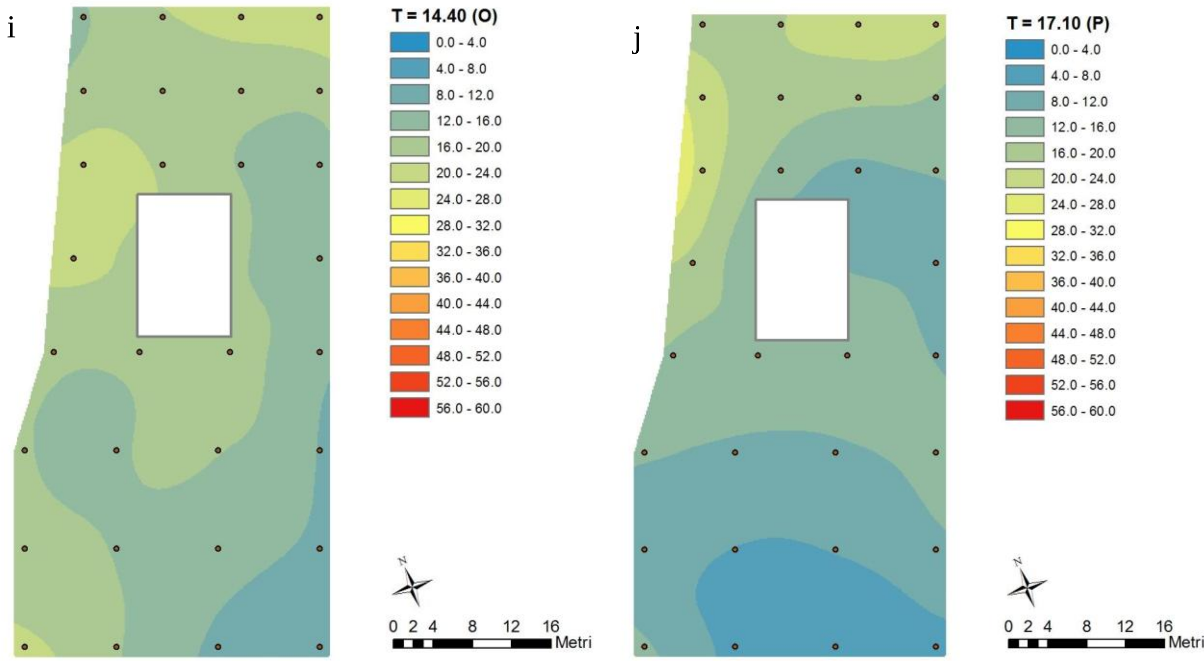


Figure 12 continued: Geo-statistical model maps predicting water flow based on concentration gradients of N-NO_3^- at different sampling times **i.** 10/6/2016, T= 14.40 **j.** 10/6/2016, T= 17.10

compared with the first phase, exhibiting very low N-NO_3^- concentrations in the southern and eastern sides of the sub-basin (0-12 mg l^{-1}) while concentrations were still higher at the northern and western sides indicating lower velocity flow towards the sub-basin outlet (Figure 12j).

Wetland hydrology, water hydraulics and pollutant loadings are very important factors affecting the depurative performance of event-driven wetlands which exhibit dynamic behavior (Somes *et al*., 1999; Somes *et al*., 2000; Kadlec and Wallace, 2009; Su *et al*., 2009, Kadlec, 2010). Inflow and outflow structures are very important considerations to improve the detention and treatment of the wetland (Somes and Wong, 1997; Koskiaho, 2003). In this FWS CW, the water flow from inlet to outlet (corner to corner) is mainly driven by gravitational forces through sub-surface pipes. Based on this, it could be assumed that eventually, all the water entering the system will flow towards the outlet which could be clearly expressed by the N-NO_3^- concentration changes between B4 IN and OUT during the two phases. An initial preferential flow is evident eastwards and northwards in both phases despite the great difference in concentrations where, in the first phase concentrations introduced were very high while they were low in the second phase, the position of the inlet on the south eastern side could contribute to this direction of flow. Vegetation is another major factor affecting the water flow in event-driven wetlands where lower velocity zones are created in wetlands with emergent vegetation which exerts hydraulic resistance to the water flow (Wong and Somes, 1995; Somes *et al*., 1999; Su *et al.*, 2009). In B4, the presence of an emergent vegetative island in the center affected and slowed down the water flow as evident by indicator $N\text{-}NO_3^-$ concentrations where the flow was diverted into two paths with a higher flow rate (preferential flow) on the eastern side than that of the western (Su *et al.*, 2009). Vegetation itself can also be interrelated to wetland hydrology and hydro-periods which can enhance or limit the growth of plant species, affect their productivity and diversity (Tabacchi *et al.*, 1998; Mitsch and Gosselink, 2000; Wetland land, Inc., 2012) and in turn, vegetation can affect the water flow (lower velocity zones) and eventually wetland removal efficiency (Su *et al.*, 2009) In conclusion, flow characteristics, affected by hydraulic and pollutant loads, and vegetation distribution are determinant criteria for the design of an efficient, high removal performance treatment wetland, especially in agricultural run-off.

Conclusion

The introduction of excessive $N\text{-}NO_3^-$ concentration to a pilot isolated sub-basin system within the bigger FWS CW was used as a tool to evaluate the $N\text{-}NO_3^-$ retention in addition to some water dynamics and internal processes. A first flush effect was distinctively clear with the first introduction of the excessive load to the monitored sub-basin increasing the $N\text{-}NO_3^-$ concentrations to the desired limit. In the first monitoring phase, $N\text{-}NO_3^-$ concentrations were higher in B4 IN than OUT until the detention time where concentrations reached a state of equilibrium and uniformity within B4. Similarly, in the second phase of monitoring, decreases in NO_3^- concentrations were faster in B4 IN than OUT as a result of the introduction of diluted water solution from B3 following excessive rainfall. EC and pH changes were consistent with those of $N\text{-}NO_3^-$ concentrations during the two phases where EC increased with the increase in $N\text{-}NO_3^-$ concentrations due to the increase in ionic and TDS concentration while pH decreased with $N\text{-}NO_3^-$ concentrations increase due to nitrification. The removal efficiency was 8.4 % in 12 hours equivalent to mass removal of 0.82 kg of $N\text{-}NO_3^-$ ($1\ g\ m^{-2}\ d^{-1}$).

Using $N\text{-}NO_3^-$ concentrations at different sampling points and times was a good indicator to predict water movement during the loading experiment. The dissolved solution moved from B4 IN to OUT (corner to corner) by gravitational forces with some preferential flows towards the eastern side of the sub-basin, mainly derived by the presence of vegetative obstruction creating lower velocity zones in the center of B4. The sub-basin exhibited similar water flow behavior during the two phases despite the great difference in $N\text{-}NO_3^-$ concentrations between both. In both phases, the water flow was eventually uniformly distributed in B4 over time. Based on this, it could be concluded that wetland hydrology, water hydraulics, pollutant loadings and vegetation morphology and distribution are determinant criteria for the design of effective wetlands. Additionally, the performance of CW in the removal of pollutant loads from agricultural run-off can be described as episodic and event-driven.

Chapter IV

Evaluation of plant species used in floating treatments wetlands: a decade of experiments in North Italy

(Review study)

Introduction

Floating treatment wetlands (FTWs) represent a novel eco-approach for the treatment of various types of wastewater directly in natural and/or artificial water bodies. FTWs were defined as innovative variants of traditional constructed wetlands, which involve rooted, emergent macrophyte plant species growing in hydroponic conditions on floating mats as supports (Headley and Tanner, 2006). According to Headley and Tanner (2012), FTWs are hybridization of all the conventional wetland treatments (Surface and subsurface flow systems). Moreover, FTWs gain advantage over conventional systems because plants are trapped in self-buoyant mats thus, saving huge spaces of water body surface while extending their root system in the water column and performing their typical functions. (De Stefani *et al*. 2011). Important processes for contaminant removal by FTWs include the release of extracellular enzymes, development of biofilms and aggregation of suspended matter at the surface of submerged plant organs (Oliveira and Fernandes, 1998). In addition, other processes include nutrients and metals uptake by plants, enhancement of anaerobic conditions in the water column, settling and sedimentation of contaminants in the water body (Headley and Tanner, 2006).

Over the last decades, FTWs were used extensively for the restoration of water bodies and the treatment of different types of wastewater around the world using different plant species, mainly macrophytes (discussed in details in chapter I). Most of the available literature focused the attention mainly on wastewater quality improvement rather than the plant growth performances in FTWs.

In light of the limited literature dealing with plant growth performance in FTWs (Chapter I), the main aim of this study was to evaluate the growth performance and nutrient uptake of 20 different plant species installed in different FTWs constructed with the Tech-IA® Italian floating support mat in North Italy over 10 years of research. Investigating factors affecting the growth performance in addition to correlations between different growth parameters was an additional interest.

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Materials and Methods

Experiments

Nine experiments were installed in different locations of North-Italy using FTWs during a decade of research (2006-2016) (Table 1). Six different types of wastewaters, whose physico-chemical features are reported in Table 2, were treated in two pilot and six full-scale experiments. The most frequently treated were municipal wastewater in tertiary stage (Mietto *et al.*, 2013; Barco and Borin, 2017) and river wastewater (De Stefani *et al*., 2011; Pappalardo *et al*., 2017). The former consisted of a mixture of domestic, urban run-off and industrial waters that were tertiary treated through a two-stage hybrid constructed wetland (horizontal subsurface flow and floating systems, respectively). The latter is mainly composed of agricultural run-off wastewater (experiment 9), and aquaculture plant-derived wastewater (experiment 1). A detailed study was performed for the treatment of diluted digestate liquid fraction (DLF) (Pavan *et al*., 2015), the sub-product of anaerobic digestion of cattle slurries and manures mixed with energetic crops such as maize silage and flavor. A one-year experiment was conducted under green-house environmental controlled conditions, testing ten different ornamental species using Ferty 3® synthetic nutrient solution (De Stefani, 2012).

Plant support system: Tech-IA®

All the experiments were performed using Tech-IA®, an Italian patented plant supporting floating mat (Figure 1). Tech-IA® is made from ethylene vinyl acetate (EVA), a recyclable and non-toxic formula, with high mechanical, chemical, and biological resistance (De Stefani *et al*., 2011). Each Tech-IA® floating element is rectangular in shape (45 cm x 93 cm), with eight (15 cm x 15 cm) quadrangular grids for plant anchoring. It weighs 1.7 kg and supports more than 20 kg weight. The single elements can be easily connected together and anchored to the basin side by the means of cords and wooden poles.

Plant species

Thirty five different machrophyte species were used in the 9 different experiments; however, focus in this study was given to 20 species belonging to the botanical families *Poaceae*, *Asteraceae*, *Cyperaceae*, *Iridaceae* and *Typhaceae* (Table 3). All the species are perennial, herbaceous and rhizomatous macrophytes, typically found in natural aquatic habitats such as natural marshes or free water surface constructed wetlands (Vymazal, 2013).

Table 1. List of the experiments carried out during the research years (2006-2016)

Experiment code	Year	Coordinates	Location	Wastewater	Treatment stage	Scale plants	Plants m^{-2}	Reference
1	2005 2008	45°38'N 12°10'E	Sile River, Veneto Region	Aquaculture and river wastewater	Single treatment	**Full**: rivers received wastewater from cultivated fields, urban environment and aquaculture plants	16	De Stefani *et al*. (2011)
2	2009 2010	45°35'N, 10°2'E	Cazzago San Martino, Lombardia Region	Municipal wastewater	Tertiary treatment	**Full**: run-off sedimentation pond	8	Unpublished data
3	2010	45°11'N, 11°21'E	Legnaro, Veneto Region	Synthetic nutrient solution	Single treatment	**Pilot**: 3 waterproofed PVC tanks	4	De Stefani, 2012)
4	2010 2011	45°22'N, 11°25'E	Alonte, Veneto Region	Municipal wastewater	Tertiary treatment	**Full**: sedimentation pond	8	Barco and Borin (2017)
5	2011 2012	45°36'N, 11°37'E	Bolzano Vicentino, Veneto Region	Municipal wastewater	Tertiary treatment	**Full**: sedimentation pond	8	Mietto *et al*. (2013)
6	2011 2012	45°25'N, 11°33'E	Montruglio, Veneto Region	Municipal wastewater	Tertiary treatment	**Full**: sedimentation pond	8	Mietto *et al*. (2013)
7	2011 2012	45°44'N, 11°37'E	Pianezze, Veneto Region	Municipal wastewater	Tertiary treatment	**Full**: sedimentation pond	8	Mietto *et al*. (2013)
8	2010 2011 2012	45°14'N, 11°54'E	Terrassa Padovana, Veneto Region	Digestate liquid fraction	Single treatment	**Pilot**: 3 excavated basins waterproofed by PVC plastic mesh	8	Pavan *et al*. (2015)
9	2014 2015 2016	45°11'N, 12°2'E	Cona, Veneto Region	Agricultural wastewater	Single treatment	**Full**: channel receiving wastewater from cultivated fields	4	Pappalardo *et al*. (2017)

Table 2. Physico-chemical characteristics of wastewaters used in the experiments (TN: total nitrogen, PO_4-P: orthophosphate, COD: chemical oxygen demand, EC: electrical conductivity)

Wastewater	Experiment code	Quart (%)	TN (mg L^{-1})	PO_4-P (mg L^{-1})	COD (mg L^{-1})	EC (µS cm^{-1})
Municipal wastewater	2, 4, 5, 6, 7	25	7.2	2.73	36.15	770.0
		Median	22.8	4.31	56.0	900.0
		75	41.7	6.01	96.0	1130.0
Agricultural wastewater	9	25	1.3	u.m.t.	-	709.3
		Median	1.7	0.004	-	1056.0
		75	1.9	0.009	-	1350.5
Aquaculture wastewater	1	25	6.0	0.03	8.3	-
		Median	6.9	0.06	13.7	-
		75	7.7	0.09	16.1	-
Digestate liquid fraction	8	25	71.3	10.85	963.8	3200.0
		Median	116.5	17.20	1580.0	3770.0
		75	163.3	23.40	2237.3	4260.0
Synthetic nutrient solution	3	25	-	-	-	1007.5
		Median	-	-	-	1210.0
		75	-	-	-	1432.5

u.m.t.: under measurable threshold. -: not available.

Figure 1. Tech-IA® floating element used for plant anchoring and support

Table 3. List of used species in each experiment.

Experiment code	Plant Species used
1	*Carex elata Gooden*. (*Carex stricta* Lam.), *Chrysopogon zizanioides* (L.) Robert., *Dactylis glomerata* L., *Juncus effusus* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Sparganium erectum* L., *Typha latifolia* L.
2	*I. pseudacorus* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Typha latifolia* L.
3	*Acorus calamus* L., *Caltha palustris* L., *Canna indica* L., *Iris laevigata* Fisch., *Juncus effusus* L., *Mentha aquatica* L., *Oenanthe javanica* (Blume) DC., *Pontederia cordata* L., *Sparganium erectum* L., *Thalia dealbata* Fraser ex Roscoe, *Zantedeschia aethiopica* (L.) Srengel
4	*I. pseudacorus* L., *Phragmites australis* (Cav.) Trin. ex Steud.
5	*I. pseudacorus* L., *Phragmites australis* (Cav.) Trin. ex Steud.
6	*I. pseudacorus* L.
7	*I. pseudacorus* L.
8	*I. pseudacorus* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Typha latifolia* L.
9	*Caltha palustris* L, *Carex elata Gooden*. (*Carex stricta* Lam.), *I. pseudacorus* L ., *Juncus effusus* L., *Lythrum salicaria* L., *Mentha aquatica* L., *Phalaris arundinacea* L., *Schoenoplectus lacustris* (L.) Palla, *Sparganium erectum* L.,

Major focus was given to evaluate *P. australis*, *T. latifolia*, *I. pseudacorus*, *Carex* spp., and *L. salicaria*.

A group of ten species was chosen for assessing both depurative performances and aesthetic-ornamental value included; *A. calamus*, *C. indica*, *C. palustris*, *I. laevigata*, *J. effesus, M. aquatica*, *O. javanica*, *P. cordata*, *S. erectum*, *T. dealbata*. All ornamental species were transplanted using pieces of rhizome or stolon (20-25 cm length, 3 living sprouts each), except for *A. calamus*, *C. palustris*, and *O. javanica* which were transplanted as 35 cm height plants.

Vegetative performance parameters

Plant growth and development were monitored at the end of each growing season using a specific parameter scheme for each experiment (Table 4) (De Stefani *et al.*, 2011; De Stefani, 2012; Mietto *et al.*, 2013; Pavan *et al.*, 2015; Pappalardo *et al.*, 2017; Barco and Borin, 2017). Shoot height and root length were manually measured using an extensible meter. Aerial and root fresh biomass productions were determined by harvesting plants in randomly selected areas of each FTW. Dry biomass production was obtained by drying fresh tissues samples in a forced air oven at 65°C for about 48 hours, until constant weight was reached. Dry

Table 4. Vegetative parameters measured in each experiment

Experiment	Vegetation monitoring	Above mat biomass	Below mat biomass	Shoot height	Root length	N%	P%
1	March 2006, June 2008	-	-	-	*	-	-
2	November 2009	*	*	*	*	*	-
	November 2010	*	*	*	*	*	-
3	July 2010	*	-	*	*	*	-
4	November 2011	*	*	*	*	*	*
5	October 2012	*	*	*	*	*	-
6	October 2012	*	*	*	*	*	-
7	October 2012	*	*	*	*	*	-
8	November 2011	*	-	-	-	*	*
	October 2012	*	-	-	*	*	*
9	October 2015	*	*	*	*	*	*
	September 2016	*	*	*	*	*	*

*: measured, -: not available.

biomass was then milled to 2 mm and analyzed to quantify Total Kjeldhal Nitrogen (TKN) and phosphorus concentrations through spectrophotometric analysis (FAO, 2011). The total nitrogen and phosphorus contents in above- and below-mat tissues were obtained as the product between aerial and root dry biomass productions and nutrient concentrations percentage. Plant survival rate was computed at the end of growing season and winter as the ratio between the number of living plants at the moment of measurement and the correspondent number in the previous period.

Statistical analysis

The normality of data was checked with Kolmogorov-Smirnov test. For all studied species, plant biometric characteristics (shoot height and root length), biomass productions (above- and below-mat) and root/shoot ratio were statistically analyzed by one-way analysis of variance test (ANOVA) at $p<0.05$ and the differences between average values were detected by Least Significant Difference, LSD test ($p<0.05$). The relations existing between i) above and below-mat biomass production, ii) shoot height and above-mat biomass production, iii) root length and below-mat biomass production and iv) shoot height and below-mat biomass production were checked by a simple linear regression analysis ($p<0.05$).

The variation of plant biometric parameters and biomass production over the different growing seasons was assessed by one-way analysis of variance (ANOVA) at $p<0.05$. The influence of wastewater chemical parameters (nutrients, organic matter concentrations and electrical conductivity) on plant growth parameters was checked by a multiple regression analysis ($p<0.05$) after a random association between plant growth parameters (monitored at the end of growing season) and wastewater chemical features (monitored during the entire growing season) by a boots-trap statistical method as proposed by Efron and Tibshirani (1986).

Results and discussion

1. Major species: growth performance

Biometrics and biomass production

As mentioned earlier, the most frequently used species in these studies were *Carex* spp., *I. pseudacorus*, *L. salicaria*, *P. australis*, and *T. latifolia*. *I. pseudacorus* was used to treat municipal, agricultural drainage wastewaters and diluted DLF. *T. latifolia* and *P. australis* were used to treat municipal wastewater and diluted DLF, whereas the use of *Carex* spp. and *L. salicaria* was limited to treat agricultural run-off wastewater derived from cultivated fields.

Regarding dry above- and below-mat biomass productions, *T. latifolia*, *P. australis* and *I. pseudacorus* produced statistically comparable above-mat biomasses, which were significantly higher (ANOVA, $p<0.001$) than those obtained for *Carex* spp. and *L. salicaria* (Table 5). In addition, *P. australis* and *T. latifolia* produced significantly higher (ANOVA, $p<0.05$) below-mat biomass than those harvested for the other considered species without any significant differences among them. These results suggested that well-watered conditions of hydroponic culture provide good growth environment for *P. australis* and *T. latifolia* opposing to behavior exhibited by species in FWS-CW characterized by un-constant hydro-period (Borin *et al.*, 2012).

T. latifolia and *P. australis* exhibited significantly highest (ANOVA, $p<0.01$) shoot height while the significantly lowest was for *L. salicaria*. There was no significant difference between species in root length except for *L. salicaria* which showed the significantly lowest (ANOVA, $p<0.001$) expansion of root system in the water column (Table 5). The growth of *P. australis* is advantaged over *T. latifolia* during severe drought conditions as it survives water scarcity through the expansion of an articulated network of roots absorbing water between 50 cm and 100 cm depth. (Borin, 2003).

A lot of studies reported the use of *P. australis* and *T. latifolia* (Revitt *et al.*, 1997, 2001; Lakatos *et al.*, 1997, 2014; Hubbard *et al.*, 2004; Garbett, 2005; Van de Moortel, 2010; Dunqiu *et al.*., 2012; Saeed *et al.*, 2014, 2016; Zhang *et al.*, 2016), *Carex* spp. (Van Acker *et al.*, 2005; Van de Moortel, 2010, 2011; Tanner and Headley, 2011; Ladislas *et al.*, 2013; Winston *et al.*, 2013; Borne *et al.*, 2014) and *I. pseudacorus* (Van Acker *et al.*., 2005; Van de Moortel, 2011, Keizer-Velk *et al.*, 2014; Hartshorn *et al.*, 2016) in FTWs, however, the discussion of their growth parameters has remained limited until now.

Table 5. Comparison of the growth parameters (average value±standard error) of the frequently used species. Different letter for each parameter indicated significant differences according to one-way ANOVA test, $p<0.05$

Species	Above-mat biomass		Below-mat biomass		Shoot height		Root length	
	g m^{-2}	n	g m^{-2}	n	cm	n	Cm	n
I. pseudacorus	1059.7±179.38 a	50	725.0±201.6 b	39	78.6±6.0 c	60	53.1±3.4 a	60
P. australis	1379.9±362.7 a	24	3611.1±702.4 a	24	131.7±11.5 b	42	47.4±3.9 a	42
T. latifolia	1466.0±271.5 a	23	4331.1±571.6 a	11	189.0±11.8 a	21	59.3±5.8 a	17
Carex spp.	304.4±53.5 b	26	416.3±68.3 b	12	65.4±2.0 c	31	48.4±2.0 a	19
L. salicaria	47.7±6.5 b	24	205.7±25.2 b	24	42.3±4.3 d	42	35.1±2.1 b	42
ANOVA results	$p<0.001$		$p<0.01$		$p<0.01$		$p<0.001$	

For all considered species, the above-mat biomass productions were lower than those obtained in other types of CWs. *P. australis* above ground production ranged from less than 2000 g m^{-2} (Tanner, 1996)-2022 g m^{-2} (Borin and Salvato, 2012) in plastic tanks filled with gravel medium to 1652-5070 g m^{-2} in HSSF-CWs (Vymazal and Kropfelova, 2005), and reached the highest biomass production in FWS semi-natural wetland (5450 g m^{-2}) (Maucieri *et al.*, 2014). *T. latifolia* and *C. elata* averagely produced 323 g m^{-2} and 349 g m^{-2} respectively, when transplanted in pilot tanks filled with gravel (Salvato and Borin, 2010). The use of *L. salicaria* was limited (Van de Moortel, 2010 ; Ge *et al.*, 2016) in FTWs, although the species was adapted to colonize natural aquatic habitat (Vymazal, 2011b; Florio *et al.*, 2017) such as marshes or riverbanks characterized by eutrophic wastewaters. In this study, both *Carex* spp. and *L. salicaria* did not perform efficiently in comparison with the results reported in scientific literature for floating systems, since their growth and development were probably penalized by low concentration of available macro-nutrients in wastewater (Pappalardo *et al.*, 2017). In comparison, a single specimen of *L. salicaria* produced 566.7 g of above-mat dry biomass (Ge *et al.*, 2016), about 47.6 times the average production (11.9 g plant^{-1}) obtained in the current study. *L. salicaria* maintained a constant production over the growing seasons (more than 1100 g m^{-2}) when managed with high nitrogen and water supplies (Florio *et al.*, 2017). Similarly, *C. virgata* reached 2350 g m^{-2} of above-mat and 533 g m^{-2} of below-mat biomass (Tanner and Headley, 2011) which were respectively 7.7 and 1.3 times the average productions in this study. Moreover, *C. stricta* averagely produced 131.4 g plant^{-1} of above-mat biomass and 207.6 g plant^{-1} of below-mat biomass (Winston *et al.*, 2013), which were about 1.7 and 2.1 times the average values for this study, respectively. On the opposite side, the biometric parameters obtained in this study are in line with values reported in other FTWs for *C. virgata* (shoot height 81 cm, root length 57 cm) (Tanner and Headley, 2011) and *C. stricta* (shoot height 80 cm, root length 40 cm) (Borne *et al.*, 2014).

Shoot/root ratio

Root/shoot ratio was calculated for both biometric parameters (root length and shoot height) and biomass production (above- and below-mat biomass productions) (Figure 2). *L. salicaria* showed significantly higher root/shoot ratio (ANOVA, $p<0.001$) calculated for biometric parameters than all other species which did not show any significant differences among them. The behavior of *L. salicaria* transplanted under low nutrient availability was interesting, since the species seemed to allocate the energetic compounds produced by photosynthesis in the elongation of the root apexes rather than in aerial tissues. Moreover, late sampling of *L. salicaria* after senescence of aerial parts could contribute to increasing ratio. Under the same experimental conditions, the behavior of *Carex* spp. contrasted with that of *L. salicaria*, but was similar to those observed for *P. australis* and *T. latifolia* cultivated under high nutrient concentration in wastewater.

As for the root/shoot ratio based upon biomass production, *L. salicaria* and *I. pseudacorus* exhibited the significantly highest values (ANOVA, $p<0.001$), whereas *T. latifolia* and *P. australis* had the significantly lowest ones (ANOVA, $p<0.001$). These results contrast with those reported for the same plant species grown in soil or substrate (Gries and Garbe, 1989; Peverley *et al.*, 1995; Tanner, 1996; Borin, 2003; Borin and Salvato, 2012; Maucieri *et al.*, 2014; Barco *et al.*, 2018; Florio *et al.*, 2018). A good explanation is that, soil and substrate are characterized by a cationic-anionic exchangeable capacity attracting oppositely charged ions such as nutrients or salts, providing them for plants absorption. In these conditions, perennial macrophyte species usually form a dense network of propagation organs, the rhizomes (Nassi o Di Nasso *et al.*, 2013; Barco and Borin, 2017; Barco *et al.*, 2018) which increase the root/shoot ratio. Oppositely, in hydroponic culture, the production of rhizomes was limited because nutritive resources are mainly in the available form and not sequestered by soil or substrates. In these conditions, plants root systems are mainly composed of roots, slighter than rhizomes, thus reducing the root/shoot ratio.

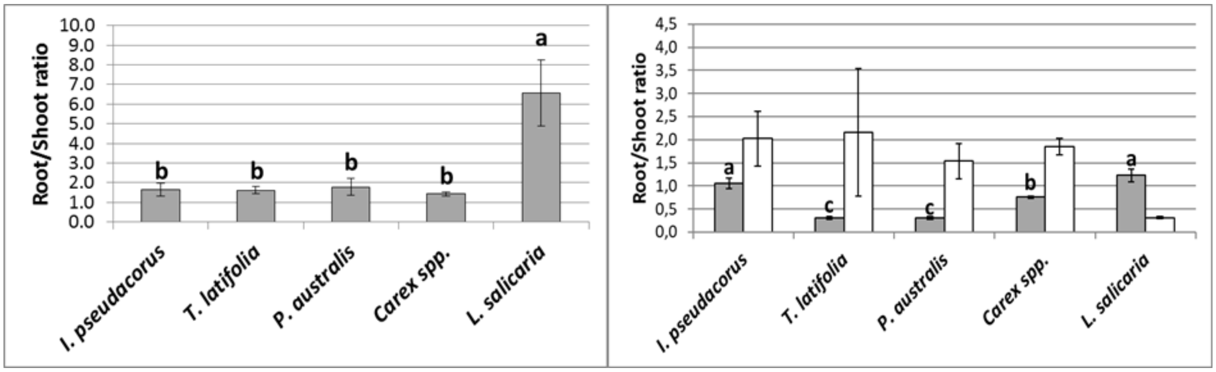

Figure 2. Root/shoot ratio (average value±standard error) calculated on i) biometric characteristics (left) and ii) biomass production (right). White columns represent average root/shoot ratio values derived from scientific literature. Different letters between the species indicated significant differences according to one-way ANOVA test, $p<0.05$.

Correlation between biometrics and biomass production

For all considered species except for *L. salicaria*, the above-mat biomass production was positively correlated with below-mat biomass production (Table 6), matching with results obtained for other wetland species in the same zone with high nitrogen (400 kg ha^{-1} $year^{-1}$) and water supplies (about 40 mm of water twice per week) (Barco *et al.*, 2018; Florio *et al.*, 2018) and with those obtained by Zhu *et al.* (2011) using plant species in artificial floating beds in China.

Regarding above-mat biomass production and shoot length, they were negatively correlated for *I. pseudacorus*, *L. salicaria* and *T. latifolia* whereas a positive correlation was found between them for *P. australis* and *Carex* spp. Similarly, all studied species showed a negative correlation between root system biomass production and root length except for *P. australis* which showed no significance (Table 6). For *I. pseudacorus* and *P. australis* a significant regression was found between below-mat biomass production and shoot height. For the other species, there was an insignificant correlation between the two parameters.

Since the study of plants root system is difficult to perform both in pilot and in full scale FTWs, depending on the plant species, the correct estimation of plant below-mat biomass production by the characterization of above mat biomass can help reducing the labor and the economic investment and avoids serious damages to plant root system (Zhu *et al.*, 2011).

Table 6. Linear regression analysis between i) below-mat (dependent variable, y) and above-mat biomass production (independent variable, x); ii) above-mat biomass production (dependent variable, y) and shoot height (independent variable, x); iii) below-mat biomass production (dependent variable, y) and root length (independent variable, x); iv) below-mat biomass production (dependent variable, y) and shoot height (independent variable, x).

Above-mat biomass-Below-mat biomass		
Species	Equation	Sig
I. pseudacorus	y=1.376+0.299x R=+0.440	**
T. latifolia	y=2.470+0.324x R=+0.270	***
P. australis	y=1.791+0.505x R=+0.590	**
Carex spp.	y=1.956+0.186x R=+0.180	***
L. salicaria	y=2.178+0.003x R=+0.003	ns
Above-mat biomass-Shoot height		
I. pseudacorus	y=-3.360+3.059x R=+0.897	**
T. latifolia	y=-0.883+1.737x R=+0.671	**
P. australis	y=0.743+1.594x R=+0.602	**
Carex spp.	y=2.777-0.308x R=+0.063	**
L. salicaria	y=1.840-0.196x R=-0.193	**
Below-mat biomass-Root length		
I. pseudacorus	y=2.822-0.373x R=-0.132	**
T. latifolia	y=4.537-0.526x R=-0.293	***
P. australis	y=3.494-0.041x R=-0.019	ns
Carex spp.	y=2.903-0.317x R=-0.079	***
L. salicaria	y=2.326-0.091x R=-0.043	*
Below-mat biomass-Shoot height		
I. pseudacorus	y=0.678+0.755x R=+0.302	**
T. latifolia	y=3.616-0.023x R=-0.008	ns
P. australis	y=-0.805+1.869x R=+0.584	**
Carex spp.	y=2.609-0.133x R=-0.025	ns
L. salicaria	y=2.265-0.051x R=-0.048	ns

*: significant at p<0.05, **: significant at p<0.01, ***: significant at p<0.001, ns: not significant.

Factors affecting biometrics and biomass production

Different factors such as plant age and physicochemical characteristics of wastewaters can be determinant for plant biometric parameters and biomass production (Figure 3, Table 7). All considered species increased, although not always significantly, both above- and below-mat biomass productions, between the first year and the second years after transplant (Figure 3). The same behavior has been reported for *P. australis* and *Phalaris arundinacea* grown in sub-surface flow CWs (Vymazal and Kropfelova, 2005) and for other wetland perennial herbaceous species cultivated in soil conditions (Florio *et al*. 2017; Angelini *et al*., 2009).

Shoot height and root length showed a species-specific behavior over the consecutive seasons, with a significant (ANOVA, $p<0.05$) reduction of both parameters between the first and the second growing season for *I. pseudacorus* (-37.0% and -67.5% for shoot height and root length, respectively) and *L. salicaria* (-52.2% and -29.8% for shoot height and root length, respectively) and a significant (ANOVA, $p<0.05$) decrease of root length (-38.2%) for *T. latifolia* (Figure 3). The opposite temporal trend observed between plant biomass production and biometric parameters, suggested a horizontal colonization of the floating platforms by the species, mainly due to the increasing of the number of shoots and roots produced.

For all studied species, the above-mat biomass production, shoot height and root length were statistically modelized by the knowledge of nutrients (TN and $P\text{-}PO_4^-$) and organic matter (COD) concentrations as well as electrical conductivity (Table 7). Based on this, the growth of all species except for *Carex* spp. and *L. salicaria* was significantly influenced by wastewater physico-chemical parameters, showing a species-specific behavior, as already proved by White and Cousins (2013). The aerial biomass and root length of considered species were significantly influenced by all monitored parameters (Table 7). On the other hand, the model of root biomass produced by *I. pseudacorus* and *T. latifolia* included all wastewater parameters except for TN, with a significant influence of COD and EC and an insignificant effect of $P\text{-}PO_4^-$ concentration (Table 7). The root biomass of *P. australis* was significantly influenced by TN concentration and EC whereas the other parameters were not included in the model. The shoot height values of *I. pseudacorus* and *T. latifolia* were significantly influenced by all wastewater parameters, while the shoot elongation of *P. australis* could be modelized considering only the $P\text{-}PO_4^-$ and TN concentrations, without any effect, nor significance, of COD concentration and EC.

In general, *T. latifolia* and *P. australis* were similarly affected by wastewater properties, showing a significant reduction of all growth parameters In this concern, the best performances of the plants were obtained under municipal wastewater characterized with high N, COD and EC (De Stefani, 2012)

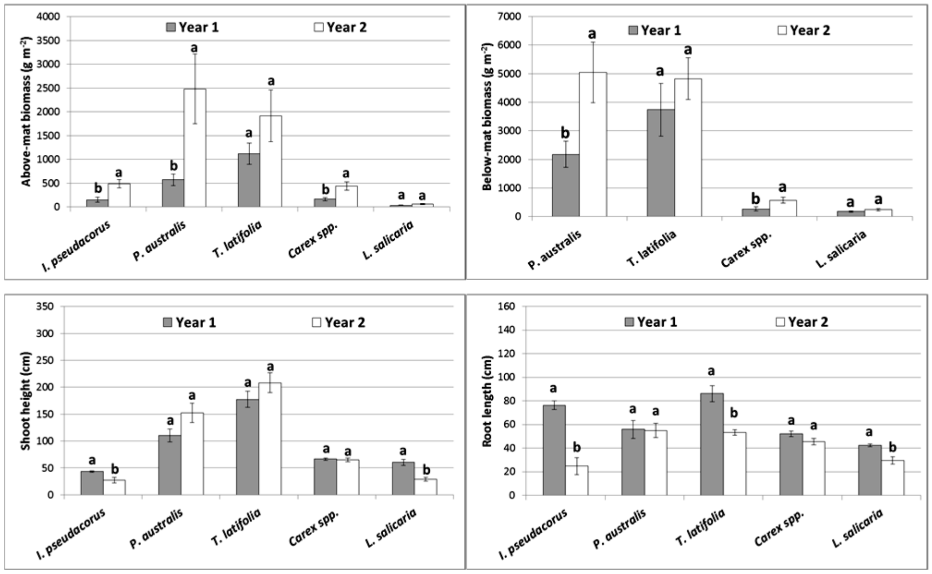

Figure 3. Comparison of biometric characteristics and biomass production between the first and the second growing season for the selected species (average value±standard error). Different letter within the same species indicated significant differences according to one-way ANOVA test, $p<0.05$.

Table 7. Multiple regression analysis between plant growth parameters (biomass production, shoot height, root length) and physico-chemical parameters of wastewater.

Species	Parameter	Multiple regression	R^2	p
I. pseudacorus	Aerial biomass	y=4.421+0.371EC***-0.237COD***+0.162TN***+0.121P-PO_4^-***	0.684	***
	Root biomass	y=2.339-0.240EC***-0.063COD***	0.690	***
	Shoot height	y=2.583+0.309 P-PO_4-***+0.283EC***-0.156COD***+0.104TN***	0.628	***
	Root length	y=2.503-0.301EC***+0.054TN***-0.028P-PO_4***+0.014COD***	0.831	***
T. latifolia	Aerial biomass	y=4.421-0.371EC***-0.237COD***-0.162TN***-0.121P-PO_4***	0.683	***
	Root biomass	y=2.338-0.243EC***-0.063COD***	0.650	***
	Shoot height	y=2.580-0.308 P-PO_4-***-0.280EC***-0.156COD***-0.104TN***	0.627	***
	Root length	y=2.503-0.301EC***+0.054TN***-0.028P-PO_4***+0.014COD***	0.830	***
P. australis	Aerial biomass	y=3.801-0.324 P-PO_4***-0.231 TN***-0.114 COD***-0.091EC***	0.470	***
	Root biomass	y=0.251-1.373EC***-0.145TN***	0.329	***
	Shoot height	y=2.27-0.191P-PO_4 ***-0.052TN***	0.354	***
	Root length	y=1.974-0.084EC***-0.044 P-PO_4 ***-0.022TN***-0.015COD***	0.399	***

*: significant at $p<0.05$, **: significant at $p<0.01$, ***: significant at $p<0.001$

Similar to the other species, *I. pseudacorus* showed the best growth performances when cultivated under municipal wastewater (Barco and Borin, 2017; De Stefani, 2012), whereas the performances of the species progressively decreased under DLF and agricultural run-off wastewater (Pavan *et al*., 2015; Pappalardo *et al*., 2017). The positive relationship obtained between plant height and above-mat biomass with wastewater nutrients concentrations was previously confirmed by White and Cousins (2013).

The below-mat biomass and the root length of all studied species were significantly reduced with the increasing of nutrients, COD and salinity of the wastewater reducing the expansion of root system and the increasing of nutrients absorbing surface (Lopez-Bucio *et al.*, 2003).

The biomass production (above and below-mat) and biometric characteristics of *Carex* spp. and *L. salicaria* were affected only by the age of plants, while wastewater physico-chemical composition did not influence plants growth parameters since the species were cultivated under a relatively constant wastewater composition during the entire experimental period (Pappalardo *et al*., 2017).

2. Major species: Nutrient uptake

N and P concentration in biomass

N and P concentration percentage in both above- and below-mat dry biomass productions highly differed not only between the species but also within the same species (Table 8). In general, all species showed higher N concentrations than P ones reflecting the same trend observed for TN and PO_4-P concentrations of used wastewaters. *P. australis* showed the significantly highest (ANOVA, $P<0.05$) above-mat N and P concentrations, followed by *I. pseudacorus* and *T. latifolia* which did not show any significant difference among them. *L. salicaria* and *Carex* spp. showed the significantly lowest (ANOVA, $p<0.05$) N and P concentrations, without any significant differences among them. Comparable N concentrations were detected in the below-mat biomass of *I. pseudacorus*, *T. latifolia* and *P. australis*, which were significantly higher (ANOVA, $p<0.05$) than those of *L. salicaria* and *Carex* spp. For all studied species N and P concentrations were significantly (ANOVA, $p<0.05$) higher in below-mat biomass than above-mat biomass, matching the results of Keizer-Velck *et al.* (2014) for *I. pseudacorus*. This trend is mainly dependent on the sampling period. In this study, N and P concentrations were determined at the end of the growing season when the translocation of

Table 8. Nitrogen and phosphorus percentage concentrations (N and P%) in above-mat and below-mat biomass of considered species. Different letters within the same parameter indicate significant difference between the species according to one-way ANOVA test at p<0.05.

Species	N%				
	Aerial tissues	**Sig.**	**Root system**	**Sig.**	**Root-Shoot**
I. pseudacorus	1.81±0.09 (50)	b	2.67±0.23 (39)	a	***
T. latifolia	1.67±0.06 (23)	b	2.84±0.14 (11)	a	***
P. australis	2.10±0.09 (26)	a	2.82±0.13 (12)	a	***
Carex spp.	0.93±0.02 (24)	c	1.09±0.03 (24)	b	***
L. salicaria	0.68±0.04 (24)	c	1.72±0.51 (24)	b	*

Species	P%				
	Aerial tissues	**Sig.**	**Root system**	**Sig.**	**Root-Shoot**
I. pseudacorus	0.16±0.03 (23)	b	0.07±0.004 (12)	a	*
T. latifolia	0.14±0.02 (14)	b	-	-	-
P. australis	0.38±0.04 (12)	a	-	-	-
Carex spp.	0.04±0.001 (24)	c	0.07±0.004 (24)	a	***
L. salicaria	0.03±0.002 (24)	c	0.12±0.029 (24)	a	*

*: significant at p<0.05, **: significant at p<0.01, ***: significant at p<0.001, ns: not significant.

nutrients from the aerial tissues to the root system has already occurred (Bonaiti and Borin, 2000; Vymazal, 2007). An opposite trend, with a higher nutrient concentration in aerial tissues than root system, was observed anticipating the sampling period at the beginning of the summer, as proved in a FTW vegetated with *C. virgata* (Tanner and Headley, 2011).

The different chemical composition of wastewaters where plants were transplanted most probably induced variability of N and P concentrations in above and below-mat dry biomass of studied species (Table 9). For *I. pseudacorus*, *P. australis* and *T. latifolia*, the N concentration of both above- and below-mat biomass productions was positively correlated with TN concentration in wastewater, whereas for *Carex* spp. and *L. salicaria* no significant regression between the two parameters was possible. P concentration was positively correlated with PO_4-P concentration in wastewater only for *I. pseudacorus* (above- and below-mat biomass) and *T. latifolia* (above-mat biomass), whereas no significant regressions were calculated for the other species.

Table 9. Linear regression analysis between N and P biomass percentage concentrations (dependent variable, y) and wastewater TN and PO_4-P concentrations (independent variables, x).

Species	Aerial tissues			
	Biomass N vs Wastewater TN	**Sig.**	**Biomass P vs Wastewater P-PO_4^-**	**Sig.**
I. pseudacorus	y=0.050+0.179x R=0.387	**	y=0.132+0.250x R=0.378	**
T. latifolia	y=0.109+0.075x R=0.194	**	y=-0.953+0.075x R=0.046	*
P. australis	y=0.211+0.082x R=0.112	***	y=-0.565+0.033x R=0.014	ns
Carex spp.	y=-0.009-0.103x R=-0.037	ns	y=-1.452-0.015x R=-0.008	ns
L. salicaria	y=-0.193-0.043x R=-0.023	ns	y=-1.549-0.026x R=-0.014	ns

Species	Root system			
	Biomass N vs Wastewater TN	**Sig.**	**Biomass P vs Wastewater P-PO_4^-**	**Sig.**
I. pseudacorus	y=0.765+0.320x R=0.699	**	y=0.252+0.881x R=0.807	**
T. latifolia	y=0.361+0.150x R=0.136	***	-	-
P. australis	y=0.372+0.113x R=0.110	**	-	-
Carex spp.	y=0.048-0.010x R=-0.003	ns	y=-1.033+0.060x R=0.028	ns
L. salicaria	y=0.086-0.047x R=-0.027	ns	y=-0.712+0.146x R=-0.091	ns

*: significant at p<0.05, **: significant at p<0.01, ***: significant at p<0.001, ns: not significant.

N and P content in biomass

I. pseudacorus, P. australis and *T. latifolia* showed the significantly (ANOVA, p<0.05) highest N standing stocks in above-mat biomass, whereas *Carex* spp. and *L. salicaria* exhibited the significantly lowest (ANOVA, p<0.05) ones (Table 10). Except for *I. pseudacorus,* the root systems of all species gave significantly higher (ANOVA, p<0.05) N content than that obtained for the aerial tissues.

The above-mat P content ranged between 0.975±0.210 g m^{-2} for *P. australis* to 0.016±0.002 g m^{-2} for *L. salicaria* with significant differences among the species (ANOVA, p<0.05) (Table 10). Considering the root system, *Carex* spp. exhibited the significantly highest (ANOVA, p<0.05) P content whereas *I. pseudacorus* showed the significantly lowest (ANOVA, p<0.05) one (Table 11). As already reported for N, for the majority of considered species the above-mat P content was significantly higher (ANOVA, p<0.05) than below-mat one. Only *I. pseudacorus* did not show any significant differences between above- and below-mat P contents.

Table 10. Nitrogen and phosphorus content (g m^{-2}) in above- and below-mat biomass of considered species. Different letters within the same parameter indicate significant differences between the species according to one-way ANOVA test at p<0.05.

Species	N (g m^{-2})				
	Aerial tissues	**Sig.**	**Root system**	**Sig.**	**Sig.**
I. pseudacorus	20.21±3.36 (50)	a	25.28± 7.46 (39)	b	ns
T. latifolia	22.73± 4.00 (23)	a	121.16± 16.27 (11)	a	***
P. australis	22.39± 5.11 (26)	a	95.26±15.45 (12)	a	***
Carex spp.	2.64± 0.43 (24)	b	4.26± 0.64 (24)	c	*
L. salicaria	0.32± 0.04 (24)	b	2.30± 0.33 (24)	c	***

Species	P (g m^{-2})				
	Aerial tissues	**Sig.**	**Root system**	**Sig.**	**Sig.**
I. pseudacorus	0.390±0.125 (23)	b	0.070± 0.016 (12)	c	ns
T. latifolia	0.572± 0.115 (14)	b	-	-	-
P. australis	0.975± 0.210 (12)	a	-	-	-
Carex spp.	0.111± 0.019 (24)	c	0.290± 0.045 (24)	a	**
L. salicaria	0.016± 0.002 (24)	c	0.180± 0.024(24)	b	***

*: significant at p<0.05, **: significant at p<0.01, ***: significant at p<0.001, ns: not significant.

3. Ornamental species

Biometrics and biomass production

Significant differences (ANOVA, p<0.01) on all maximum biometric parameters at the end of the season were detected among the species due to their different morphology and adaptability to grow in hydroponic conditions (Figure 4). Regarding this, *C. indica* showed the significantly highest (ANOVA, p<0.01) shoot height without any significant difference if compared with those detected for *P. cordata*, *T. dealbata* and *M. aquatica*. On the contrary, *C. palustris* and *J. effusus* reached the significantly lowest (ANOVA, p<0.01) shoot heights. The shoot height of *J. effusus* was in line with average values of 43.4 cm and 48.7 cm reported by Lynch *et al.* (2015), cultivating the species in Beemat® and BioHaven® FTW plants, respectively while *P. cordata* shoot height was greater than that reported by Wang *et al*. (2015), treating urban wastewater, with an average value of 43 cm. Shoot height of *A. calamus* matched with value reported by Chang *et al.* (2010) (45.2 cm).

P. cordata and *J. effusus* exhibited the significantly highest (ANOVA, p<0.01) root length whereas, *A. calamus* and *O. javanica* had the significantly lowest (ANOVA, p<0.01) ones. Lower root length than these were reported for *J. effusus* by Lynch *et al.* (2015), ranging from 37.4 to 39.1 cm. *A. calamus* root length was in line with values reported by Chang *et al*. 2010 (15.4 cm) and Lai *et al.* (2011) (23.0 cm), whereas *C. indica* and *O. javanica* root length values were respectively 3.4 and 1.7 times those reported by Lai *et al.* (2011) in a pilot-scale plant.

For the majority of species, shoot height was positively correlated with root length during the entire monitoring period (Table 11), suggesting a simultaneous elongation of all plant organs. Only *S. erectum* and *O. javanica* did not show any significant correlation between the considered parameters. In addition, the relation existing between the two parameters followed a species- specific trend during the first part of the vegetative season (sprouting), with a positive linear regression for *C. indica*, *P. cordata* and *T. dealbata* and an insignificant relation for the other species. In the next phase, from the beginning of shoot elongation to the bloom, all studied species similarly behaved, increasing the shoot height and the root expansion in the water column. For the majority of the species, it was not possible to find a significant regression between shoot height and root length at the harvesting time. In fact, during this phonologic phase (June-August), plant root systems continued their expansion through the water column, whereas shoot height remained almost constant since the maximum values were reached at the end of June corresponding with bloom.

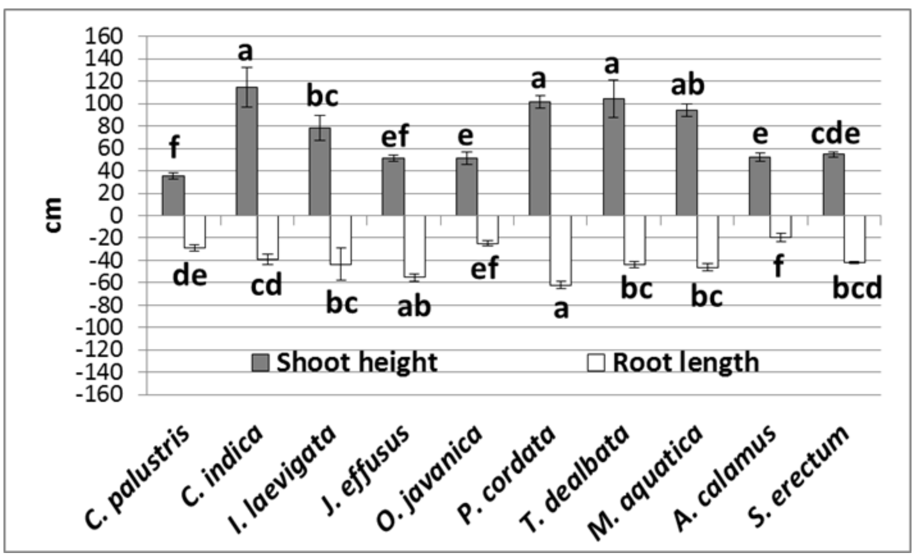

Figure 4. Maximum biometric parameters (shoot height and root length) (average value±standard error) for the ornamental species. Different letters between the species indicate significant differences according to one-way ANOVA test at p<0.05.

Table 11. Linear regression between root length (dependent variable, y) and shoot height (independent variable, x) for the different phonologic phases of ornamental species vegetative cycle.

Species	Sprouting (February 3rd-March 16th)		Shoot elongation (March 24th-May 27th)		Harvesting (June 3rd-July 22th)		Entire cycle	
	Equation	Sig.	Equation	R	Equation	R	Equation	R
I. laevigata	y=8.986+0.203x R=+0.37	*	y=14.407+0.254x R=+0.13	***	y=108.64-0.697x R=-0.13	ns	y=12.774+0.590x R=+0.71	***
C. indica	y=5.424+0.665x R=+0.71	***	y=27.356+0.171 R=+0.35	*	y=22.985+0.118x R=+0.55	ns	y=20.608+0.225x R=+0.53	***
P. cordata	y=15.460+0.511 x R=+0.51	**	y=22.248+0.398x R=+0.76	***	y=53.498+0.455x R=+0.13	ns	y=21.331+0.384x R=+0.83	***
T. dealbata	y=22.680+0.151 x R=+0.03	ns	y=22.876+0.247x R=+0.43	**	y=43.844-0.280x R=-0.11	ns	y=25.032+0.157x R=+0.492	***
S. erectum	y=23.935-0.002x R=+0.71	ns	y=87.571-0.956x R=-0.66	ns	-	-	y=12.104+0.309x R=+0.27	ns
M. aquatica	y=22.686-0.052x R=+0.05	ns	y=30.034+0.147x R=+0.20	*	y=22.206+0.130x R=+0.14	ns	y=22.817+0.105x R=+0.21	*
J. effusus	y=24.610+0.009 x R=+0.01	ns	y=12.151+0.700x R=+0.69	***	y=34.599+0.337x R=+0.19	ns	y=10.878+0.790x R=+0.80	**
C. palustris	-	-	y=-0.9749+0.918x R=+0.48	***	y=21.937+0.215x R=+0.19	ns	y=0.608+0.662x R=+0.50	***
O. javanica	-	-	y=23.033-0.05x R=+0.07	ns	y=18.492-0.017x R=-0.070	ns	y=23.684-0.093x R=-0.238	ns
A. calamus	-	-	y=-3.293+0.411x R=+0.36	**	y=5.049+0.276x R=+0.537	***	y=4.751+0.269x R=+0.469	***

*: significant at p<0.05, **: significant at p<0.01, ***: significant at p<0.001, ns: not significant

Different root length/shoot height ratio values were found among the species (Figure 5). On the average of the vegetative cycle, *T. dealbata*, *J. effusus* and *I. laevigata* showed the highest values (1.23±0.11, 1.21±0.06, 1.19±0.09, respectively) whereas *S. erectum*, *O. javanica* and *A. calamus* exhibited the lowest ones (0.54±0.03, 0.56±0.01, 0.41±0.02, respectively). During the different phases of the vegetative season, *C. indica*, *P. cordata*, *T. dealbata*, *M. aquatica* and *J. effusus* progressively reduced the root length/shoot height ratio from the beginning of the growing season (sprouting) to the harvesting period. The behavior of all other species was different, since their root length/shoot height ratio values were maintained almost constant during the entire monitoring period.

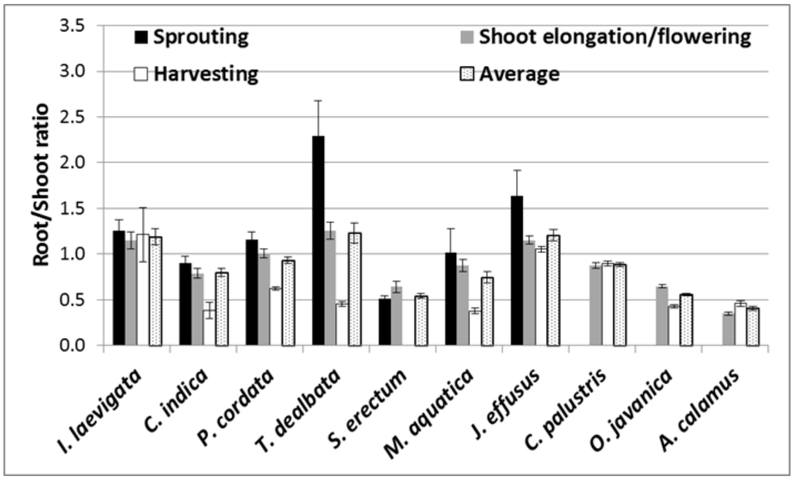

Figure 5. Root/shoot ratio calculated on plants biometric parameters during the vegetative cycle (average value±standard error).

Significantly different biomass production values were detected among the species, reflecting the same statistical trend already observed for shoot height, as testified by the strictly positive correlation existing between plant above-mat biomass production and shoot height (Figure 6). *M. aquatica* and *C. indica* showed significantly higher (ANOVA, $p<0.001$) above-mat dry biomass productions than those obtained for *O. javanica*, *J. effusus* and *C. palustris*, which did not show any significant difference among them (Figure 6). *C. indica* above-mat production obtained in this study was higher than that reported by Zhang *et al.* (2007) (0.5-1.0 kg m^{-2}) in a pilot scale vertical flow system fed with a simulated nutrient solution, whereas it was in line with results obtained by Zhang *et al.* (2008) with high N and P inputs (1682 g m^{-2}). Higher above-mat biomass productions than the currents were obtained in a pilot FTW treating eutrophic wastewater (2.37-2.43 kg m^{-2}), with an equal partitioning between stems and leaves (Zhang *et al.*, 2016).

T. dealbata and *P. cordata* biomass productions were in discordance with the results found in scientific literature, since productions of 1989.0 g $plant^{-1}$ (*T. dealbata*) and 10.4-71.8 g $plant^{-1}$ (*P. cordata*) were reported by Ge *et al.* (2016), Wang *et al.* (2014b) and Winston *et al*. (2013), respectively. In the present study, *J. effusus* above-mat production was lower than those harvested by Borin and Salvato (2012) in mesocosm gravel tanks (3210.0 and 5271.0 g m^{-2}) and by Winston *et al*. (2013) in FTW (66.2-106.3 g $plant^{-1}$) whereas it was higher than those

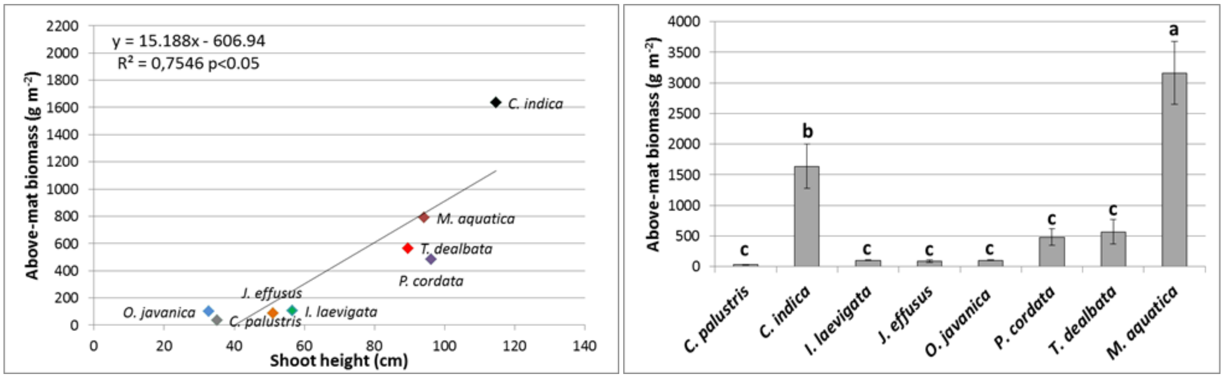

Figure 6. Linear regression analysis between shoot height and above-mat biomass production for the ornamental species (left). Above-mat biomass production for the ornamental species (average value±standard error) (right). Different letters between the species indicate significant differences according to one-way ANOVA test, $p<0.05$.

obtained in hydroponic culture of stromwater run-off (on average 142.9-188.4 g m^{-2}) (Lynch *et al.*, 2015) and DLF (median value 172.0 g m^{-2}) (Pavan *et al.*, 2015).

S. erectum and *A. calamus* maximum shoot heights and root lengths were measured in the late spring (June), whereas their biomass production was not harvested since they did not survive until the harvesting phase (July). The negative adaptability of *S. erectum* contrasted with expectation, where Ennabili *et al.* (1998) assessed a good growth of the species (1293 g m^{-2} and 718 g m^{-2} of above- and below-ground biomass, respectively) in sandy-clay soil typical of coastal wetlands.

N concentration and uptake

Despite similar above-mat nitrogen concentrations percentage between the species (Figure 7), significant differences (ANOVA, $p<0.01$) on their above-mat nitrogen uptakes were detected, mainly depending on above-mat biomass production (Zhu *et al*., 2011). The N concentration percentage values observed in this study were lower than those determined in similar experimental conditions for *C. indica* (1.65-2.75%) (Zhang *et al*., 2016) but were in line with that of *J. effusus* (0.83%) (Lynch *et al*., 2015)

M. aquatica, and *C. indica* showed significantly higher (ANOVA, $p<0.01$) above-mat nitrogen uptakes than those of all the other species, which did not show significant differences (Figure 7). The nitrogen concentrations of the studied species were in line with the values reported for four macrophytes installed in a FTW involved in storm-water run-off treatment (Tanner and Headley, 2011). Double N concentrations than the currents were reported for *C. indica* and *P.*

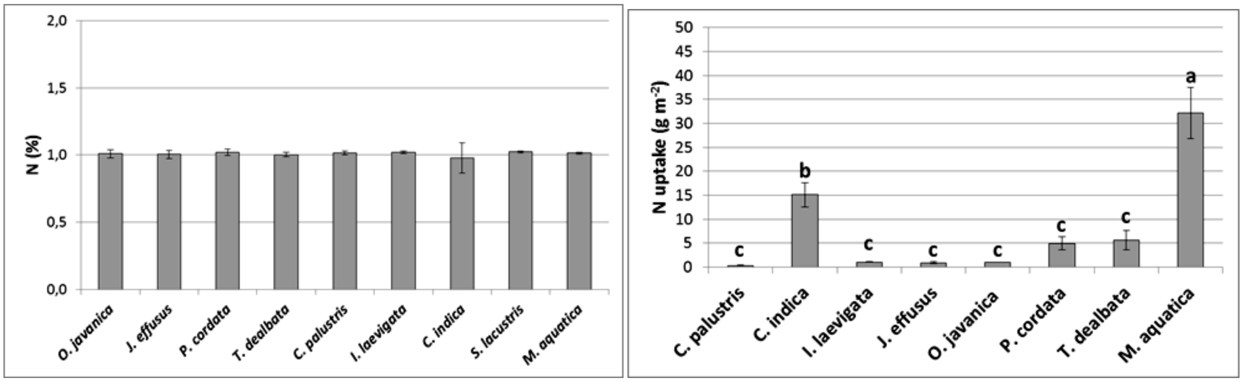

Figure 7. Nitrogen concentration in above-mat dry biomass of ornamental species (average value±standard error) (left). Above-mat nitrogen standing stock for the ornamental species (average value±standard error) (right). Different letters between the species indicate significant differences according to one-way ANOVA test at $p<0.05$.

cordata in a floating island for eutrophic water treatment (Zhao *et al*., 2012a). *C. indica* above-mat N uptake was in line with results reported for *C. flaccida* (16.1 g N m^{-2}) by White and Cousins (2013). Despite White and Cousins (2013) reported good N uptake for *J. effusus* (28.5 g m^{-2}), a contrasting behavior was observed in this study since the average N uptake was almost 1 g m^{-2}.

4. Survival rate

All selected species exhibited different survival rates over the growing season (April-November), and winter (November-March), probably due to their different adaptabilities to hydroponic conditions. In this scope, the selection of native species and plants well-adapted to live under local climatic conditions have to be privileged (Tanner, 1996) with respect to alien species. *Carex* spp., *T. latifolia* and *L. salicaria*, among the most frequently used species, exhibited the greatest adaption to FTWs as shown by the high survival rates over the growing seasons as well as during winter, even at un-favorable growth conditions (e.g. low nutrient availability) (Figures 8). The well-adaption of *L. salicaria* was also confirmed by Wu *et al.* (2011) and by Ge *et al.* (2016) with a survivability of more than 80% and 91.6% of the initial plant investment, respectively. Although *P. australis* and *I. pseudacorus* represent the most adapted macrophytes species employed in CWs (Vymazal, 2011b, 2013), unexpectedly, their performances in FTWs were often contrasting between the different trials. Both species showed good average survival rate during the growing season (72.3% and 53.0%, respectively) and winter (72.2% and 27.5%, respectively), matching the results reported by

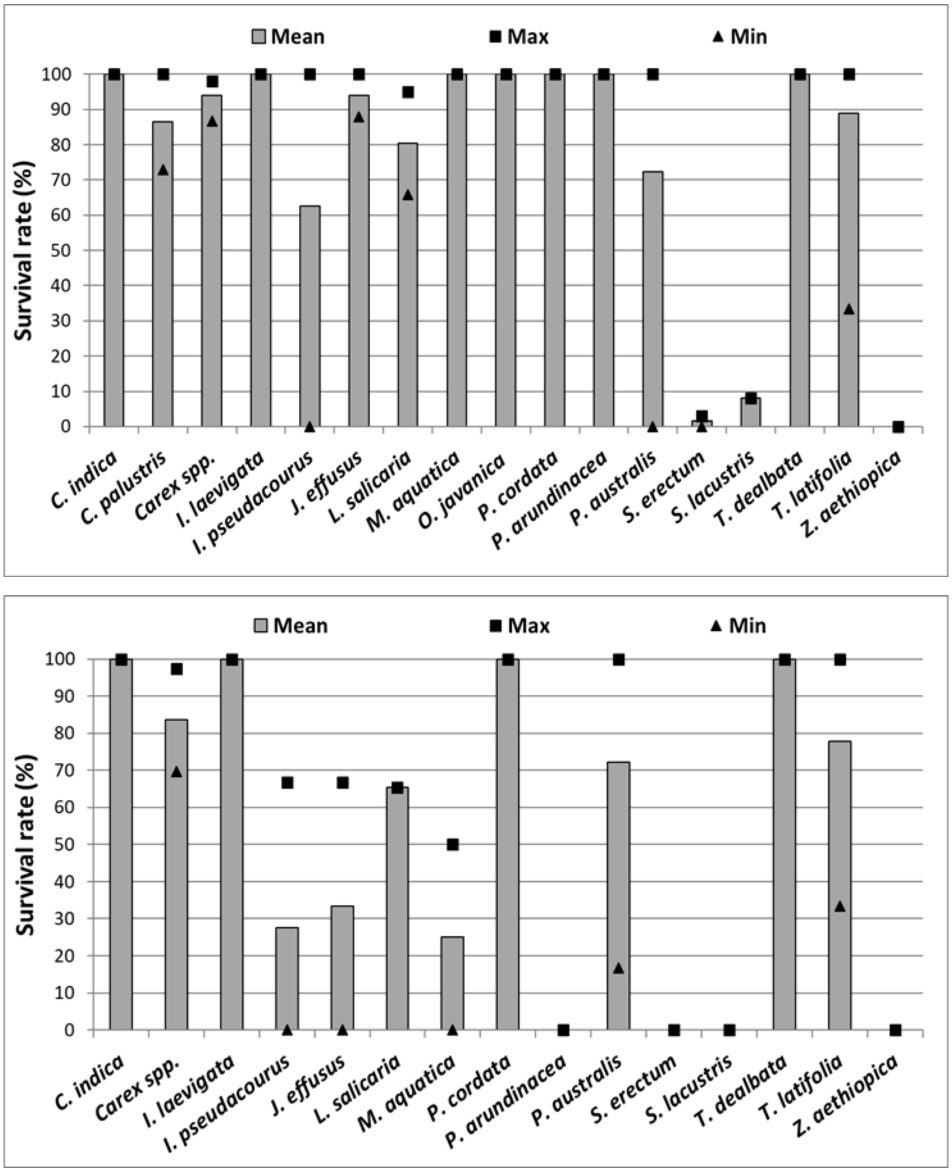

Figure 8. Plants survival rate (%) during the growing season (upper) and during winter (lower).

Wu *et al*. (2011) for *I. pseudacorus* (survival rate of 83.3%). However, in some experiments plants, completely died before the end of summer or did not re-grow after the winter (Figure 8). The low survival rate of both species during the growing season was mainly related to: i) alien animal species, particularly *Myocastor coypus* (commonly called nutria or river rat), living nearby the FTWs and feeding on the aerial parts of plants; ii) extreme meteorological conditions (i.e. excessive rain and wind) which reversed the vegetated floating platforms, thus damaging the plants. The high mortality affecting *I. pseudacorus* during the winter was mainly due to the combined effects of both low temperature and ice formed in the upper part of the FTWs section. In these conditions, plants perennial organs (rhizomes or solons) did not receive a sufficient protection against ice and cold temperatures, hence collapsing.

Among the ornamental species, excellent survivability values were obtained for *C. indica*, *I. laevigata*, *O. javanica*, *P. cordata* and *T. dealbata*, with all plants surviving both winter and growing season as the experiment was set up in a greenhouse, however, the results need to be confirmed as it was a one season study (Figures 8). However, these results can be justified since Wu *et al*. (2011), Zhu *et al*. (2011) and Ge *et al.* (2016) have observed high survival rates for *T. dealbata*, *O. javanica* and *C. indica* respectively. Similarly, *M. aquatica*, *C. palustris, J. effusus, Schoenoplectus lacustris* and *P. arundinacea* exhibited great survival rate during the growing season (average values of 86.5-100%), but they did not overpass the winter except for *J. effusus* which did not completely survive anyway.

S. erectum and *S. lacustris* exhibited the least adaptability in hydroponic conditions; their survival rates reached 1.5 and 8% at the end of the growing season, where clear signs of wilting were observed just at the beginning of the summer (June and July). These species did not survive during the winter, therefore remaining completely senescent at the next vegetative regrowth. Negative performance was similarly detected for *Z. aethiopica* and *V. zizanoides*; although repetitively transplanted over the growing season, the young plants rapidly wilted and died.

Promising results were obtained for *D. glomerata*, which showed a complete colonization and coverage of floating mats all over the year without showing any signs of senescence during the winter. This favorable adaptability, even during the winter, was probably due to the excellent experimental conditions in which the species was installed. At this purpose, the transplant of *D. glomerata* occurred in a resurgence river, characterized by a relatively calm water, with an almost constant water temperature all over the year (average 10-14°C) (De Stefani *et al*., 2011). The correct selection and installation of vegetation in FTWs represent a key factor for better plant establishment (De Stefani *et al*., 2011) and water treatment performances.

Conclusion

This review study provided an analysis of the growth performances (biometric characteristics and biomass production) and nutrient uptake of 20 different plant species installed in the Tech-IA® floating system over 10 years to treat different types of wastewaters. In addition, it established possible inter-correlations between different plant growth parameters, and correlations between plant growth parameters and other factors affecting them (plant age and physic-chemical parameters of wastewater). The results clearly indicated that *I. pseudacorus*, *P. australis* and *T. latifolia* showed the best growth performances when installed in municipal wastewater. The growth of *P. australis* and *T. latifolia* was significantly reduced with the increasing of nutrient and organic matter concentration, with the worst performances at the extreme conditions of DLF. An opposite behavior was recorded for *I. pseudacorus* which increased above-mat biomass production as well as shoot height with the enhancement of nutrients concentration in wastewater. All these species were characterized by relatively high average survival rate, although extreme meteorological events and the presence of nutria population drastically reduced their survivability, especially for *I. pseudacorus* and *P. australis*. *L. salicaria* and *Carex* spp. showed a discrete growth under agricultural run-off wastewater, even though their growth performances were hardly penalized if compared with those reported in scientific literature, probably due to the low availability of nutrient measured in wastewater. For these species, N percentage concentration in both above-mat and below-mat biomass was higher than P one, with greater accumulation in roots than shoots. Most species except for *I. pseudacorus* and *L. salicaria* exhibited an increase in biometrics in the second season. In addition, all species showed an increase in above- and below- biomass production. All species except *Carex* spp. and *L. salicaria* were correlated with the physico-chemical parameters of treated wastewater.

M. aquatica, *C. indica* and *P. cordata* seemed to be the most promising species among the ornamental species to improve the aesthetic-ornamental value of urban water bodies with wastewater treatment simultaneously. On the other side, the use of *S. erectum, Z. aetiophica* and *V. zizanoides* is not recommended since these species exhibited the lowest survival rate during the growing season.

Chapter V

General conclusions

General conclusions

Monitoring a full scale integrated surface flow constructed wetland (FWS CW and FTW) for 3 consecutive years (Chapter II), the following could be concluded:

1. Among selected physico-chemical parameters for evaluating the performance of the integrated system, electric conductivity (EC) and turbidity were the most indicative parameters on the activity and changes within the system.
2. Notable changes could be observed in concentrations of TN and N-NO_3^- between 2014 and 2016, which were mostly dependent on fertilization of croplands and excessive rainfall events leading to surface runoff.
3. Removal efficiency could be enhanced with increased establishment and maturity of wetland system; basically vegetation, as noticed by the increased mass removal in 2016.
4. Assessing the plant growth performance in FTW, a part of the integrated system, *Carex spp.* showed the best performance in terms of survivability, biometrics, biomass production and nutrient uptake while *I. pseudaocorus* lagged behind in all the aforementioned parameters for 3 consecutive years.
5. *L. salicaria* is a good potential for water treatment in FTW with high survivability over seasons, average biomass production and nutrient uptake.

Simulating N-NO_3^- load from agricultural runoff in event-driven pilot experiment (Chapter III), the following conclusions could be drawn:

1. The depurative efficiency of a single sub-basin within the aforementioned FWS CW (Chapter II) reached 8.4% in 12 hours following the detention time representing a mass removal of 0.82 kg (1 g m^{-2} d^{-1}).
2. The previous sub-basin represents only 10% of the total area of the FWS CW, so the depurative effect of the sum of all basins is expected to be much higher and contribute more in the reduction of excessive nutrient load.
3. Despite some preferential flows, mainly driven by vegetative obstructions, the input loads were eventually distributed fully across the sub-basin by normal gravitational forces.
4. The performance of wetlands treating agricultural runoff (NPS pollution) is mainly episodic and event driven.

5. Understanding water dynamics and internal processes can help in designing efficient wetland systems.

Evaluating the performance of plant species used for the treatment of different types of wastewaters in FTWs could draw to light some useful assumptions:

1. FTWs in general represent efficient and cost effective solutions for the treatment of several types of wastewaters in natural and artificial water bodies.
2. *Carex spp*., *I. pseudacorus*, *P. australis* and *T. latifolia* are widely used for the treatment of different types of wastewater with notable performance in the treatment of municipal wastewater.
3. Some ornamental species such as *Canna* indica, *Mentha aquatica*, and *Pontederia cordata* proved to be good dual purpose potentials in FTWs.
4. Factors like plant age and physico-chemical parameters of wastewaters are important determinants of the performance of different plant species in FTWs.
5. Survival rate of plant species, especially over winter, is considered a crucial index of their adaptability and performance in FTWs.

In general, this study fulfilled its aim regarding the evaluation of performance of surface flow wetlands in the treatment of wastewaters, specifically agricultural runoff. In addition, it could give adequate insight to the performance of plant species in an innovative type of surface flow wetlands, the FTWs.

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