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PHYTOREMEDIATION EFFICIENCY: ASSESSMENT OF REMOVAL PROCESSES AND HYDRAULIC PERFORMANCE IN CONSTRUCTED WETLANDS

SETTORE SCIENTIFICO-DISCIPLINARE DI AFFERENZA: BIO/07

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CONTENTS

ABBREVIATION

ANAMMOX	Anaerobic ammonia oxidation
ANOVA	Analysis of variance
ASTM	American Society for Testing and Material
ATP	Adenosine-5'-triphosphate
BOD_5	Biological oxygen demand (5 days)
CEC	Cation Exchange Capacity
Chl *a* - *b*	Chlorophyll *a* and *b*
COD	Chemical Oxygen Demand
CSTR	Continuously Stirred Tank Reactor
CW	Constructed Wetland
DO	Dissolved Oxygen
DW	Dry Weight
EC_{50}	Effective Concentration 50
EPA	Environmental Protection Agency
EDTA	Ethylenediaminetetraacetic acid
FIP	Fusina Integrated Project
FW	Fresh Weight
FWS	Free Water Surface system
HLR	Hydraulic Loading Rate
HRT	Hydraulic Residence Time
HSF	Horizontal Subsurface Flow system
ICP/AES	Inductively Coupled Plasma/Atomic Emission Spectroscopy
OC	Organic Carbon
PFR	Plug-Flow Reactor
RSD	Residence Time Distribution
TDN	Total Dissolved Nitrogen
TDP	Total Dissolved Phosphorus
TKN	Total Kjeldahl Nitrogen
TIS	Tanks-in-Series model
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus
TSS	Total Suspended Solids
VSB	Vegetated Submerged Bed system
VSF	Vertical Subsurface Flow system
WWTP	Waste Water Treatment Plant

ABSTRACT

Constructed wetlands are now widely recognised as effective treatment facilities for controlling pollution, restoring stream values within urban areas, for their recreational and aesthetic qualities, and for conserving flora and fauna.

The aim of this study was to examine two important functions of wetland systems: removal efficiency and hydraulic performance. The first research line was carried out to evaluate the start-up removal performance of the newly developed pilot surface-flow constructed wetland in the Fusina area in Venice (Italy) receiving Naviglio Brenta river freshwater, and to investigate the nutrients and metals translocation into different tissues of some aquatic plants (*Typha latifolia* L., *Phragmites australis* (Cav.) Trin. ex Steud e *Schoenoplectus lacustris* (L.) Palla) and among plants-sediment-water. The second investigation was performed in 18 pilot wetlands in Halmstad (Sweden) in order to describe the effects of wetland vegetations and different inlets in the hydraulic performance in the 18 experimental wetlands and to understand flow properties.

From this study, removal efficiency of the pilot wetland in Fusina was about 70% for TSS and 80% for nutrients. The high performance of the pilot wetland confirmed the capacity and effectiveness of wetland systems and aquatic plants in reduction of those parameters. Moreover, plants metals content, particularly in roots, was correlated with sediment concentrations. Hydraulic efficiency in the pilot wetlands in Sweden studied by tracer test application was influenced by vegetation type and density, with higher performance in the wetlands planted with mixed vegetation.

The two aspects analysed in this research are strictly connected because a principal controlling factor of contaminants removal in wetlands is water movement patterns. Hydrologic factors are especially important in the ability of both natural and created wetlands to improve water quality and so, based on that, wetland design may play an important role for nutrient and metal retention, as well as for enhancing biodiversity and recreational values in constructed wetland systems.

Keywords: Aquatic plants; Constructed wetlands; Hydraulic performance; Phytoremediation; Removal efficiency; Tracer test

RIASSUNTO

Le aree umide ricostruite sono attualmente riconosciute come un effettivo strumento per il controllo dell'inquinamento, per il ripristino dei corsi d'acqua superficiali nelle aree urbane, per il valore ricreazionale ed educativo e per la conservazione della flora e della fauna.

*Lo scopo del presente studio consiste nell'esaminare due importanti funzioni delle aree umide ricostruite: l'efficienza di rimozione e la performance idraulica. La prima linea di ricerca è stata condotta per valutare l'efficienza di rimozione di un impianto pilota di fitodepurazione a flusso superficiale nell'area di Fusina a Venezia (Italia) alimentato dall'acqua del fiume Naviglio Brenta, e per studiare la traslocazione dei nutrienti e dei metalli nei tessuti di alcune piante acquatiche (*T. latifolia *L.,* P. australis *(Cav.) Trin. ex Steud e* S. lacustris *(L.) Palla) e tra piante-substrato-acqua. La seconda linea di ricerca è stata condotta in 18 impianti pilota di fitodepurazione ad Halmstad (Svezia) per valutare l'influenza della vegetazione e di diversi tipi di alimentazione nelle condizioni idrodinamiche.*

Dal presente studio, l'efficienza di rimozione è risultata pari a circa 70% per SST a 80% per i nutrienti. L'alta performance dell'impianto pilota di Fusina conferma la capacità delle aree umide e delle macrofite nella riduzione di tali parametri. In aggiunta, il contenuto di nutrienti e metalli nelle piante, in particolare nell'apparato radicale, è risultato correlato con le concentrazioni degli stessi nei sedimenti.

L'efficienza idraulica negli impianti pilota in Svezia, studiata utilizzando test con traccianti, è risultata influenzata dal tipo e dalla densità della vegetazione, con una miglior performance nelle celle piantumate con vegetazione mista. I due aspetti analizzati nel presente studio sono strettamente interconnessi in quanto uno dei principali fattori di controllo nella rimozione dei contaminanti nelle aree umide è il movimento dell'acqua. I fattori idrodinamici sono particolarmente influenti per migliorare la qualità dell'acqua e, quindi, il design delle aree umide può avere un ruolo fondamentale nella rimozione di nutrienti e metalli, favorendo la biodiversità e la funzione ricreazionale delle aree umide ricostruite.

Parole chiave: *Aree umide ricostruite; Efficienza di rimozione; Fitodepurazione; Performance idraulica; Piante acquatiche; Test con traccianti.*

1 INTRODUCTION

1.1 Phytoremediation: state of the art

Phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) is a relatively newly evolving field of science and technology that uses plants to clean-up polluted soil, water, or air (Dunbabin & Bowmer, 1992; Salt et al., 1998). Phytoremediation is considered an effective, low cost, preferred cleanup option for moderately contaminated areas.

Plants through several natural biophysical and biochemical processes, such as adsorption, transport and translocation, hyperaccumulation or transformation and mineralisation, can remediate pollutants (Meagher, 2000).

Phytoremediation can be applied to both organic and inorganic pollutants, present in solid (e.g. soil), in liquid (e.g. water) substrates and in the air.

Phytoremediation can be divided according to the processes, applicability and type of contaminant into the following area:

- *Phytoextraction* or *Phytoaccumulation*: the use of pollutant-accumulating plants to absorb, translocate and store toxic compound (metals or organic) from soils by concentrating them in their roots or shoots. The optimum plant for the phytoextraction processes should be able to tolerate and accumulate high levels of toxic substances in its harvestable parts, but also have a rapid growth rate and the potential to produce a high biomass in the field. Some plants, which grow on metalliferous soils, have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibiting symptoms of toxicity.

The proper use of soil amendments can increase or decrease the biological availability of the contaminant for plant uptake (Figure 1.1).

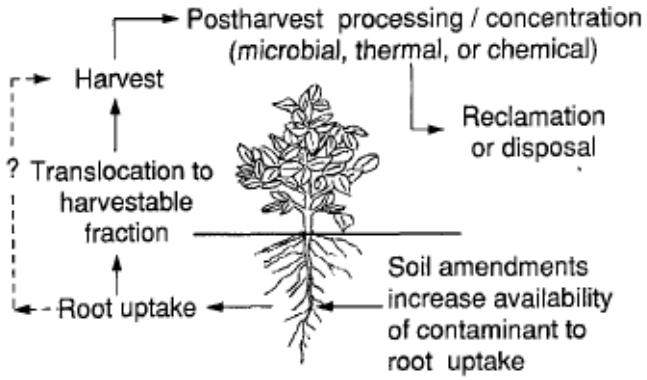


Figure 1.1 Processe involved in phytoextraction (from Cunningham & Ow, 1996).

• *Phytodegradation* and *Rhyzodegradation*: the use of plants or the root system and associated microorganism and the symbiotic associations between the plant and the soil microorganisms to sequester, uptake, store and degrade organic pollutants in their tissue (Figure 1.2).

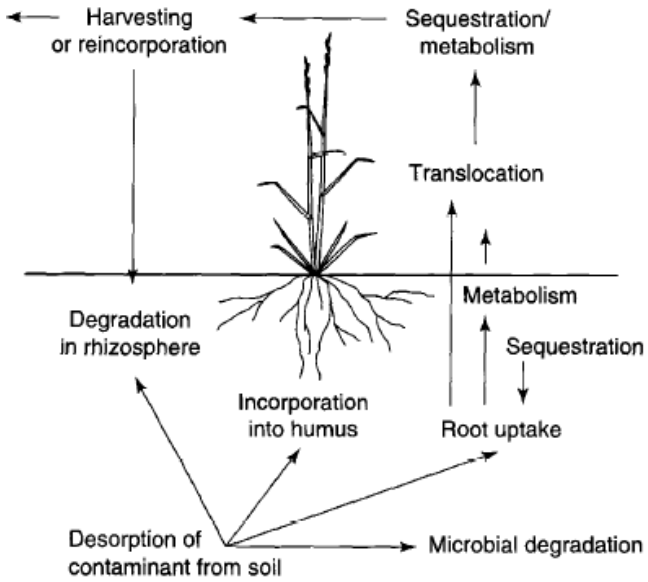


Figure 1.2 Processes involved in the phytodegradation of contaminants from the soil (from Cunningham et al., 1995).

• *Rhyzofiltration*: the use of plants roots to absorb and adsorb pollutants, mainly metals, from water and aqueous waste stream. Mechanisms of toxic metal removal by plant roots depend of different metals. Biological processes, like intracellular uptake, vacuolar deposition and translocation to the shoot, are responsible the removal of metals (Salt et al., 1995).

• *Phytostabilisation*: the use of plants to reduce the bioavailability of pollutant in the environment and to stabilize toxic compounds to reduce leaching of metals from soil into the ground water. Unlike other phytoremediative techniques, the goal of phytostabilization is not to remove metal contaminants from a site, but rather to stabilize them and reduce the risk to human health and the environment.

• *Phytovolatilisation*: the use of plants to volatilize and remove pollutants. Once the plants have taken up contaminants from the substrate, are able to release them as volatile compounds into the atmosphere, like mercury-, selenium- or arsenic-containing compounds (Pilon-Smits & Pilon, 2000) (Figure 1.3).

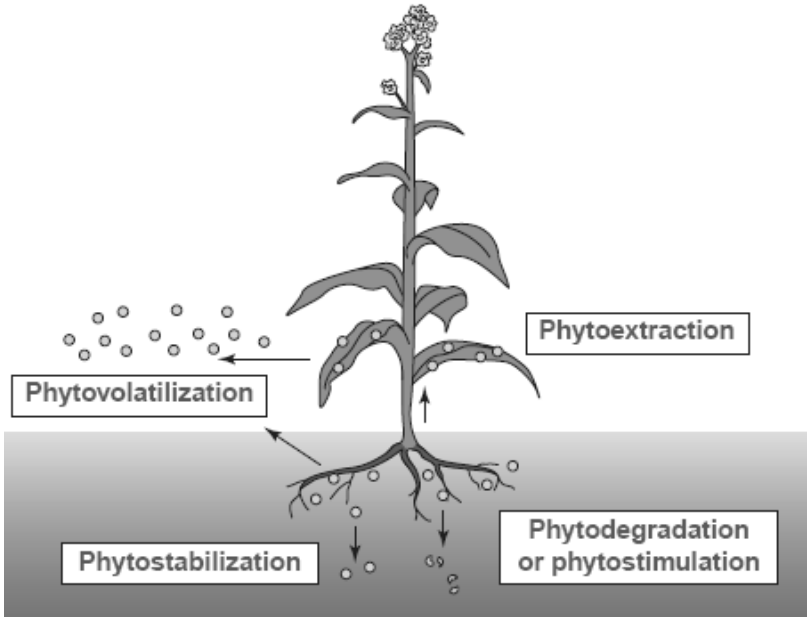


Figure 1.3 Processes involved in phytoremediation strategies (from Pilon-Smits & Pilon, 2000).

The most emphasized advantage of phytoremediation over traditional techniques is cost. Plants can be a cost-effective alternative to physical remediation systems (Cunningham et al., 1995).

At present, plant-based remediation techniques are showing increasing promise for use in contaminates soil, water and air environments (Cheng et al., 2002; Prasad & Freitas, 2003). However, research focused on the basic and applied problems affecting each class of pollutants related to plants are needed to improve

phytoremediation systems and to build a greater understanding of the many and varied processes that are involved.

In light of the above discussion, a complementary and/or alternative method to traditional wastewater treatment for water quality improvement can be played by wetlands, which can be used in concert with other treatment methodologies.

Wetlands can be either saltwater (e.g. mangroves, salt marsh) or freshwater (e.g. sedgelands, reed beds, swamp forests and shallow lagoons) (Odum, 1988). They can be either naturally occurring, for example forebays of lakes, or constructed artificially in support of ecosystem management.

Constructed wetlands are artificial wastewater systems consisting of shallow ponds or channels which have been planted with aquatic plants and which act as biofilters through natural microbial, biological, physical and chemical processes to treat wastewater (USEPA, 2000). A constructed wetland system has the positive characteristics of a natural wetland and it duplicates the physical, chemical and biological processes in the natural system.

Constructed wetlands can be applied for the treatment of domestic wastewater, industrial wastewater (Panswad & Chavalparit, 1997; Mays & Edwards, 2001), agricultural wastewater (Tanner et al., 1995a and b; Kern & Idler, 1999) and stormwaters (Somes et al., 2000; Walker & Hurl, 2002) with the additional benefit of creating lush habitats that support a variety of wildlife and plants. They are furthermore applied to strip nutrients of eutrophied surface waters before these are discharged into vulnerable environment and nature reserves (Arheimer et al., 2004).

Macrophytes in wetlands have been shown to play important roles in marsh biogeochemistry through their active and passive circulation of chemical elements. Through their action as ‘‘nutrient pumps’’ (Odum, 1988), active uptake of elements may promote immobilization in plant tissues, as seen in constructed wetlands for wastewater treatment (Kadlec & Knight, 1996) and in the use of wetland plants in phytoremediation. So, natural and constructed wetlands are effective sinks for nutrients and metals. Processes of metal removal and mobilisation include sedimentation, adsorption, complexation, uptake by plants, and microbially-mediated reactions including oxidation and reduction (Weis & Weis, 2004).

1.2 Constructed wetland types

Constructed wetlands have been classified by the literature, based on the water flow regime, in two types of systems, which are distinguished by the location of the hydraulic grade line (USEPA, 2000) (Figure 1.4):

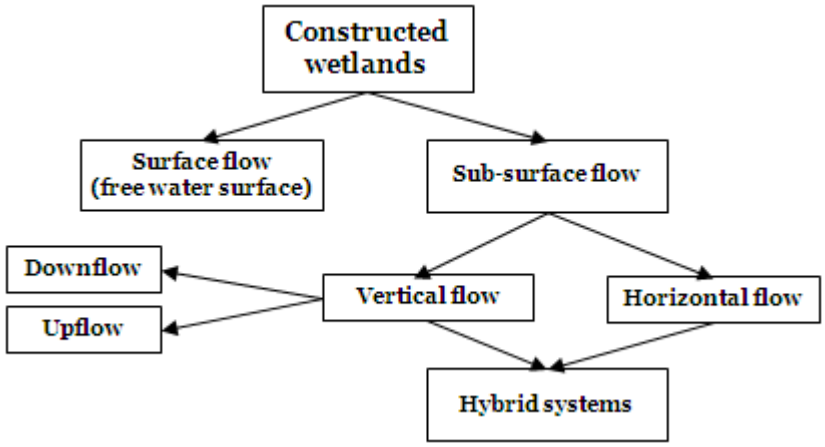


Figure 1.4 Classification of constructed wetlands for wastewater treatment based on the flow (from Vymazal, 2001).

- **Free water surface systems (FWS)** (or **surface flow wetlands**) (Figure 1.5)

This type of system consists of a basin or channels with a barrier to prevent seepage, soil to support the roots of the vegetation, and water at a relatively shallow depth flowing through the system. The water surface is exposed to the atmosphere, and the flow path through the system is horizontal.

FWS closely resemble natural wetland in appearance and functions because they contain aquatic plant that are rooted in a soil layer on the bottom of the wetland and water flow through the leaves and steams of plants, with a combination of open-water areas, emergent vegetation, varying water depths, and other typical wetland features. Shape, size, and complexity of design often are functions of site characteristics rather than preconceived design criteria.

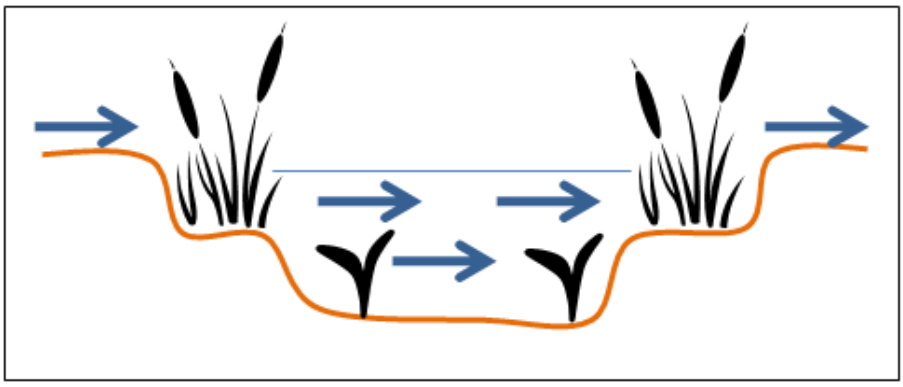


Figure 1.5 Schematic draw of Free Water Surface system (FWS).

- **Vegetated submerged bed systems (VSB)** (or **subsurface flow wetlands**)

This system consists of gravel beds planted with wetland vegetation. In this case, the water level is below the surface of gravel or other media (such as crushed rock, small stones, gravel, sand or soil) placed in wetland bed. The subsurface flow wetland also consists of a basin or channel with a barrier to prevent seepage, but the bed contains a suitable depth of porous media. Wastewater can flow in two ways:

Horizontal subsurface flow systems (HF or HSF) (Figure 1.6):

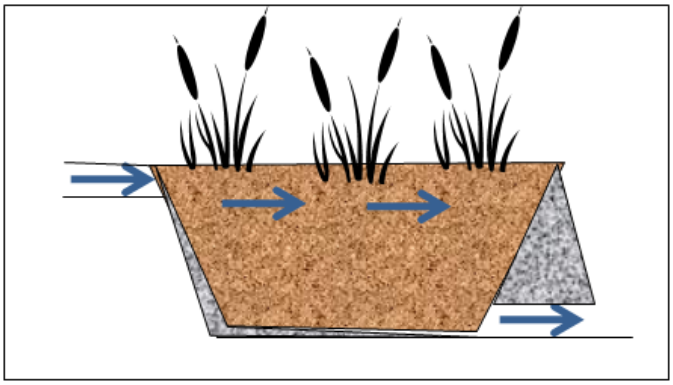


Figure 1.6 Schematic draw of Horizontal Subsurface Flow system (HF or HSF).

The wastewater comes from the inlet, flows slowly through the medium and flows out more or less in a horizontal way. In the passage through the system, the wastewater comes into contact with the soil-organism-plant complex resulting in a reduction of BOD, nitrogen, phosphorus and heavy metals of the treated water.

Vertical subsurface flow systems (VF or VSF) (Figure 1.7)

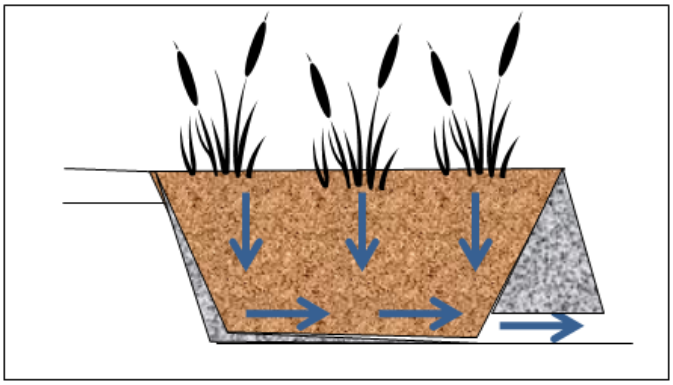


Figure 1.7 Schematic draw of Vertical Subsurface Flow system (VF or VSF).

The wastewater percolates through the gravel, giving better access to the plant roots and rhizomes and exposure to oxygenated conditions in the rhizosphere. The alternating oxidised-reduced conditions of the substrate stimulate nitrification/denitrification processes and phosphorus adsorption. When properly designed and operated, wastewater stays beneath the surface of the media, flows in contact with the roots and rhizomes of the plants, and is not visible or available to wildlife.

Pond and wetland ecosystems comprise (Lawrence & Breen, 1998):

- Primary producers (large rooted plants, floating plants (macrophytes, algae), using nutrients and light fluxes;
- Secondary producers (fungi, bacteria), using decaying organic material;
- Grazers (*Cladocera*, copepods);
- Higher animals (fish and mammals, birds);
- Fluxes of water and its constituents (nutrients, organic material, suspended solids, pollutants);
- Adsorption of nutrients, metals and organic compounds onto suspended particles, and their sedimentation;
- Transformation and transfer of constituents between water column, sediment, atmosphere and biological compartments.

Wetland systems are complex ecosystems where an understanding of the interactions between abiotic and biotic components is fundamental for effective treatment

processes. Constructed wetland design involves the integration of engineering and ecological principles, since hydraulic design as well plant-mediated removal processes appear to be critical to the performance of these systems.

Constructed ponds and wetlands are now widely recognized as effective treatment facilities for controlling pollution, for restoring stream values within urban areas, for their recreational and aesthetic qualities, and for conserving flora and fauna (Verhoeven & Meuleman, 1999). Those systems show promise for application to provide tertiary treatment to reduce biochemical oxygen demand, suspended solids, nutrients and pathogens and metals contents (Thomas et al., 1995).
Recently, wetlands for wastewater purification have been constructed for treatment of sewage and urban runoff, and show good potential for concentrating metals from industrial wastewaters and mine seepage.

1.3 Wetland functions and values

Functions and values are often used synonymously with respect to description of important wetland processes, however it should be noted that only some wetland functions have value. Functions are the normal or characteristic activities that take place within a wetland, whereas values are societal benefits derived from the goods and services provided by wetland functions (Brinson, 1993).

Ecosystem wetland functions include hydrologic transfers and storage of water, biogeochemical transformations, primary productivity, decomposition, nutrient trapping, accumulation of inorganic sediments and organic matter, and maintenance of plant communities as well as vertebrate populations (Dahl, 1990; Brinson, 1993).

Values ascribed to many wetlands include providing habitats for fishing, hunting, waterfowl, timber harvesting, wastewater assimilation, and flood control, to name a few. These perceived values arise directly from the ecological functions found within the wetlands. Many of these functions are beneficial and can prevent flood damage, improve water quality (Reddy & Gale, 1994), and provide habitat for threatened and endangered species (Confer & Niering, 1992) at little or no cost.

1.4 Components of wetland area

A constructed wetland consists of a properly designed basin that contains water, a substrate and plants. Other components of wetlands are the communities of microbes and aquatic invertebrates.

Each component plays an important role and can be manipulated in constructing a wetland in order to promote internal processes and improve the performance.

1.4.1 Water

The presence of surface or near-surface water, at least periodically, is a common characteristic in all wetlands, natural or constructed. Hydrology is one of the most important design factors in constructed wetlands because it links all of the functions in a wetland and can have an influence on treatment effectiveness (Wörman & Kronnäs, 2005). In addition, hydrologic regime of a wetland influences the community composition and the primary productivity in natural wetlands by controlling abiotic factors, such as water and nutrient availability, aerobic and anaerobic conditions in both the soil and water columns, water chemistry, soil salinity, soil conditions, and water depth and velocity (Casanova & Brock, 2000; Gagnon et al., 2007).

In turn, biotic components of a wetland (primarily vegetation) directly influence wetland hydrology through processes, such as transpiration, interception of precipitation, peat building, shading, wind blocks, and development of microclimates within the wetland (Barko & Smart, 1983; Mitsch & Gosselink, 1993).

1.4.2 Substrates, sediments and litter

The substrate in constructed wetlands, that includes soil, sand, gravel, rock and organic materials, is important because it supports many of the living organisms in wetlands and is both the medium in which many of the wetland chemical transformations take place and the primary storage of available chemicals for most wetland plants (Lau & Chu, 2000; Stottmeister et al., 2003).

It is often described as a *hydric soil*, defined by the U.S. Soil Conservation Service (1987) as "a soil that is saturated, flooded, or ponded long enough during the growing season to develop anaerobic conditions in the upper part". Wetland soils are of two types: mineral or organic soils. Organic soil is formed by the accumulation of organic matter where biomass production exceeds decomposition. Generally, mineral wetland soil consists of alluvial material: mainly fluvial, lacustrine, estuarine or marine (Faulkner & Richardson, 1989).

Different physiochemical features of organic and mineral soils are summarised in Table 1.1.

Table 1.1 Comparison of physical and chemical properties of wetland soil (from Vymazal, 1995).

	Mineral soil	**Organic soil**
Organic content (%)	< 12 - 20	> 12 - 20
pH	6.0 – 7.0	< 6.0
Bulk density* (g cm^{-3})	Gravel ca 2.1 Sand 1.2 – 1.8 Clay 1.0 – 1.6	Fibric < 0.09 Hemic 0.09 – 0.20 Sapric > 0.20
Porosity (%)	Gravel 20 Sand 35 - 50 Clay 40 - 60	Fibric > 90 Hemic 84 – 90 Sapric < 84
Hydraulic conductivity (m d^{-1})	Gravel 100 -1000 Sand 1 - 100 Clay < 0.01	Fibric > 1.3 Hemic 0.01 – 1.3 Sapric < 0.01
Water holding capacity	Low	High
Nutrient availability	General high	Often low
Cation exchange capacity (CEC)	Low, dominated by major cations	High, dominated by hydrogen ion

*bulk density = dry mass of soil material per unit of volume

Wetland soils are dominated by anaerobic conditions induced by soil saturation and flooding. However, in many wetland soils, there may be small oxidized pockets in the reduced soil matrix and oxidized streaks corresponding to root channels. Several of the important chemical transformations in wetlands occur upon or within the aerated rhizosphere and roots of wetland plants (Kadlec & Knight, 1996).

In the complex sediment-water system, the movement, availability and possible toxicity of contaminants are influenced by chemical and physical factors like redox gradient, pH, salinity and temperature (Bryan & Langston, 1992; Van Den Berg et al., 1998).

1.4.3 Vegetation

The prevalent vegetation in wetland consists of macrophytes that are typically adapted to areas inundated either permanently or periodically or with the soil saturated to the surface.

A macrophyte is an aquatic plant characterized by visible tissues, which grows in or near water or in soil, which is saturated for most of the growing season. The term includes aquatic vascular plants, aquatic mosses and some large algae (*Cladospora*).

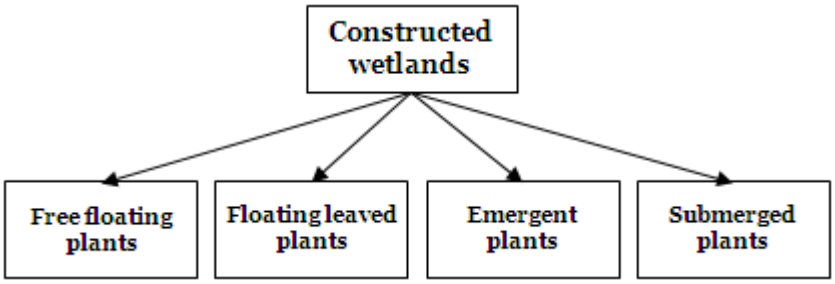


Figure 1.8 Classification of constructed wetlands for wastewater treatment based on the vegetation type (from Vymazal, 2001).

As shown in Figure 1.8, the macrophytes plants could be divided in (Thomas et al., 1995; Stottmeister et al., 2003):

- Emergent, with leaves and/or stems which rise above the water surface, generally anchored to the substrate;
- Free-floating, that float on the surface of the water, and are not attached to the substrate;
- Submerged, that residing below the surface, which may have emergent flowering bodies and may or may not be rooted to the substrate.

The macrophytes most commonly used in constructed wetlands are listed in Table 1.2.

The following criteria should be used in selecting a plant for inclusion in water treatment systems (Reddy & DeBusk, 1987):

- Adaptability to local climate;
- High photosynthetic rates;
- High oxygen transport capability;
- Tolerance to adverse concentration of pollutants;
- Pollutant assimilative capacity;
- Tolerance to adverse climatic conditions;
- Resistance to pests and diseases;
- Ease of management.

Table 1.2 Selection of plant species used in constructed wetlands (from: Engelhardt & Ritchie, 2001; Stottmeister et al., 2003).

Plants - Scientific name	**Common name**
Emergent macrophytes:	
Phragmites australis (Cav.) Trin. ex Steud.	common reed
Juncus spp.	rushes
Scirpus spp.	bulrushes
Typha angustifolia L.	narrow-leaved cattail
Typha latifolia L.	broad-leaved cattail
Carex spp.	sedges
Iris pseudacorus L.	yellow flag
Floating macrophytes:	
Eichhornia crassipes (Mart.) Solms	water hyacinth
Pistia stratiotes L.	water lettuce
Lemna spp.	duckweed
Nymphaea alba L.	water lily
Submerged macrophytes:	
Potamogeton pectinatus L.	sago pondweed
Potamogeton nodosus Poir.	long-leaved pondweed
Potamogeton crispus L.	crisped pondweed
Zannichellia palustris L.	horned pondweed

A major role of the macrophytes growing in anaerobic substrate is to transfer the atmospheric oxygen to the root zone, creating aerobic microzones in the rhizosphere (Figure 1.9).

Thus, aerobic and anaerobic processes are activated, facilitating the breakdown of the organic matter, and removal of nitrogen through nitrification and denitrification (Thomas et al., 1995). In addition, constructed wetlands (CWs) assist in the even

distribution and calming of flows, enhancing sedimentation in the case of fine suspended particulate systems and provide a substrate for algal and microbial biofilm biomass, necessary to absorb fine colloidal and dissolved nutrients and toxicants for wetland systems (Lawrence & Breen, 1998).

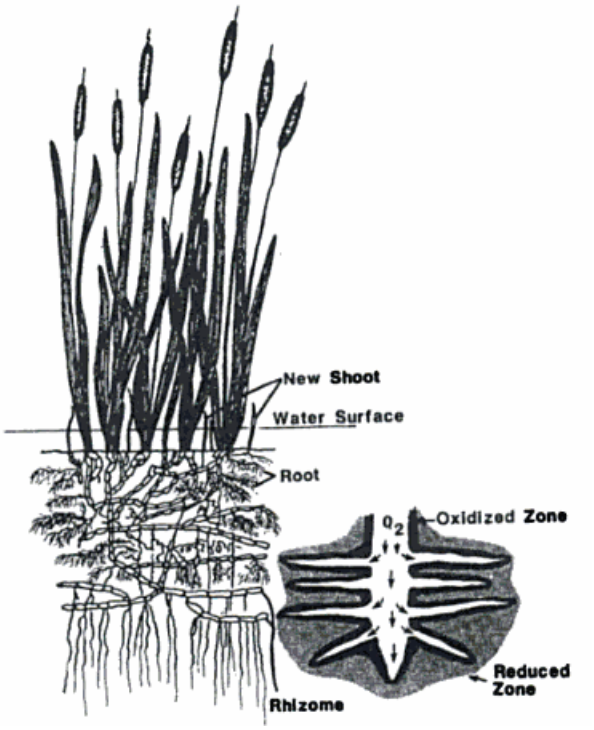


Figure 1.9 Oxygen transport by wetlands plants to support their roots growing in the anaerobic substrates (from Hammer & Bastian, 1989).

1.4.4 Microorganisms

A fundamental characteristic of wetlands is that their functions are largely regulated by microorganisms and their metabolism (Wetzel, 1993). Microorganisms include bacteria, yeasts, fungi, protozoa, algae. Most bacteria in wetland habitats are heterotrophic (i.e. they degrade organic compounds for their growth and nutrition) while others are autotrophic (they metabolise molecules from inorganic carbon).

As schematized in Figure 1.10, in the root zone (or rhizosphere) physicochemical and biological processes take place that are induced by the interaction of plants, microorganisms, soil and pollutants.

Plant rhizosphere enhances microbial density and activity by providing surface for microbial growth, a source of carbon compound through root exudates and a micro

aerobic environment via root oxygenation release (Brix, 1997). Microbial density is also related to the presence of specific plant species (Hatano et al., 1993; Vymazal et al., 2001; Gagnon et al, 2007).

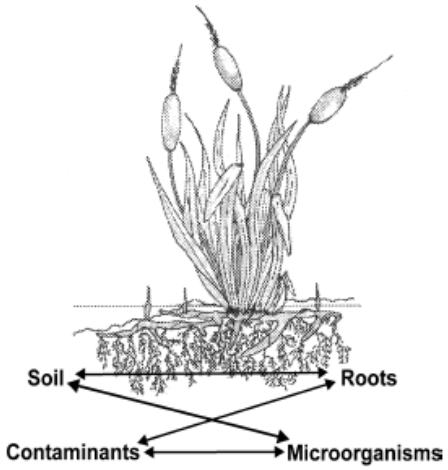


Figure 1.10 Possible interactions in the root zone of wetland plants (from Stottmeister et al., 2003).

Microbial activity transforms a great number of organic and inorganic potential pollutants into innocuous or insoluble substances and it alters the reduction/oxidation redox conditions of the surface and thus affects the processing capacity of the wetland.

Temperature, dissolved oxygen, and pH in association with the chemical substrate regulate the size and the activity of the microbial population. Generally, in a wetland treatment system the microbial activity is more important than the population size and it is measured as BOD_5 (5-days biochemical oxygen demand).

1.5 Removal mechanisms in wetland systems

The level of several types of contaminants, including organics, suspended solids, nitrogen, phosphorus, trace metals, and pathogens may be reduced in wetland systems. Wetlands may not only store contaminants but also transform biologically available forms into non-available forms and vice versa (Reddy et al., 1999).

1.5.1 Total Suspended Solids (TSS)

Total suspended solids (TSS) are both removed and produced by natural wetland processes.

The major processes responsible for removal of settleable suspended solids are sedimentation and filtration (Karathanasis, et al., 2003). Most of the removal probably occurs within the first few meters of travel distance from the inlet zone.

In wetlands, settleable incoming particulate matter usually has ample time to settle and become trapped in litter or dead zones. Once there, soluble organic constituents are reduced to carbon dioxide and low molecular weight organic acids and inorganic constituents can be bound as sulfide complexes or be buried through sediment accretion.

Wetland processes that produce particulate matter include death of invertebrates, fragmentation of detritus from plants and algae, and the formation of chemical precipitates such iron sulfide. Bacteria and fungi can colonize these materials and add to their mass (Tanner & Sukias, 1994).

1.5.2 Organic compounds

Settleable organics (BOD, COD) are rapidly removed in wetlands systems mainly by deposition and filtration. Microbial activity is the main cause of the decrease in soluble organic compounds, by both aerobic and anaerobic degradation.

In aerobic degradation of soluble organic matter, aerobic heterotrophic bacteria consume oxygen to degrade organic compounds by a series of redox reactions, releasing carbon dioxide (CO_2) and water. Both heterotrophic and autotrophic bacteria are able to consume organics but the faster metabolic rate of the first group means that they are mainly responsible for the reduction in the BOD of the system (Cooper et al., 1996).

Anaerobic degradation is a multi-step process that occurs within constructed wetlands in the absence of dissolved oxygen (Cooper et al., 1996). Either facultative or obligate anaerobic heterotrophic bacteria can carry out the process, called

fermentation. The primary products of fermentation are acetic acid, butyric acid, and lactic acid, alcohols and the gases CO_2 and H_2 (Vymazal, 1995). The end-products of fermentation can be utilize by strictly anaerobic sulphate-reducing and methane-forming bacteria.

The physical removal of BOD_5 is believed to occur rapidly through settling and entrapment of particulate matter in the void spaces in the gravel or rock media. Soluble BOD_5 is removed by the microbial growth on the media surfaces and attached to the plant roots and rhizomes penetrating the bed. Some oxygen is believed to be available at microsites on the surfaces of the plant roots, but the remainder of the bed can be expected to be anaerobic.

In wetland system, a fraction of BOD_5 is produced within the system due to the composition of plant litter and other naturally occurring organic materials. As a result, the system can never achieve complete BOD_5 removal and a residual BOD_5 is typical present in the effluent (Stringfellow et al., 2008).

1.5.3 Nitrogen

Nitrogen has a complex biogeochemical cycle with multiple biotic/abiotic transformation involving seven valence states (+5 to -3). The major nitrogen transformations in wetlands are summarized in Table 1.3.

Table 1.3 Natural trasformations in (constructed) wetlands (from Vymazal, 2007).

Process	Transformation
Volatilization	ammonia-N (aq) → ammonia-N (g)
Ammonification (Mineralization)	organic-N → ammonia-N
Nitrification	ammonia-N → nitrite-N → nitrate-N
Nitrate-ammonification	nitrate-N → ammonia-N
Denitrification	nitrate-N → nitrite-N → gaseous N_2, N_2O
N_2 fixation	gaseous N_2 → ammonia-N (organic-N)
Plant/microbial uptake (assimilation)	ammonia-, nitrite-, nitrate-N → organic-N
Ammonia adsorption	
Organic nitrogen burial	
Anaerobic ammonia oxidation (ANAMMOX)	ammonia-N → gaseous N_2

The compounds include a variety of inorganic and organic nitrogen forms that are essential for all biological life.

Ammonia volatilization is a physicochemical process where ammonium-N is known to be in equilibrium between gaseous and hydroxyl forms. This is a temperature–dependent–process, influenced by pH value in soils and sediments, water ammonium concentration, wind speed, and solar radiation.

In the ammonification (mineralization) the organic N is biologically converted into ammonia (NH_4^+), through a complex, energy-releasing, multi-step, biochemical aerobic and anaerobic process. Ammonification rates are dependent on temperature, pH, C/N ratio, available nutrients and soil conditions such as texture and structure (Reddy & Patrick, 1984).

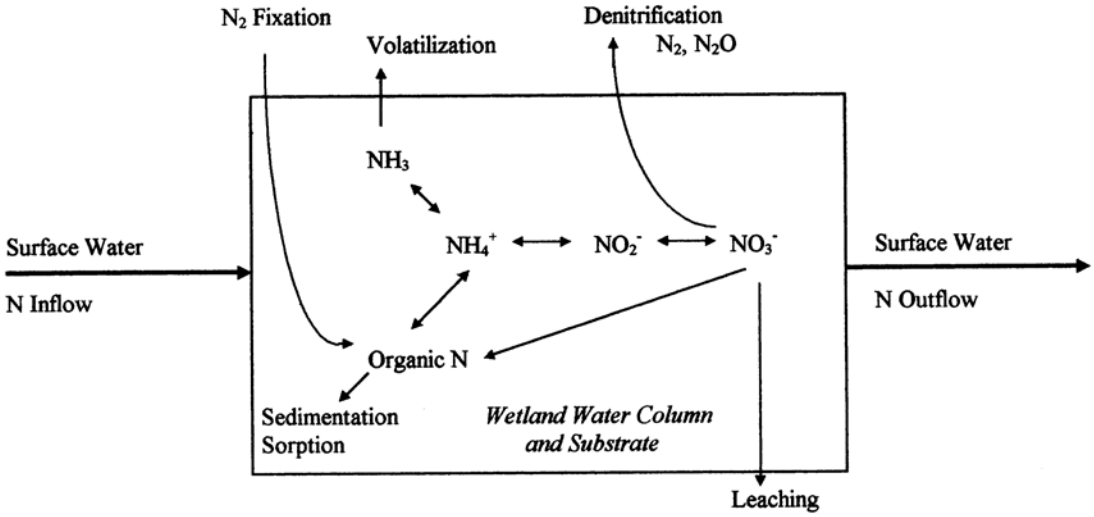


Figure 1.11 Schematic diagram of nitrogen dynamics in a wetland water column and substrate (from Spieles & Mitsch, 2000).

Nitrification is defined as the biological oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) (Figure 1.11). Nitrification is known to take place in two stages as a result of the activity of chemoautotrophic bacteria of the genera *Nitrosomonas* ($NH_4^+ \rightarrow NO_2^-$) and *Nitrobacter* ($NO_2^- \rightarrow NO_3^-$). The nitrifying bacteria derive energy from the oxidation of ammonia and/or nitrite and carbon dioxide is used as a source for synthesis of new cells.

The first step, the oxidation of ammonium to nitrite, is executed by strictly chemolithotrophic (strictly aerobic) bacteria, which are entirely dependent on the

oxidation of ammonia for the generation of energy for growth. Facultative chemolithotrophic bacteria perform the second step, the oxidation of nitrite to nitrate. Those bacteria can also use organic compounds, in addition to nitrite, for the generation of energy for growth (Vymazal, 2007).

Nitrification is influenced by temperature, pH value, alkalinity of the water, inorganic C source, microbial population, and concentration of ammonium-N and dissolved oxygen.

In a wetland system, nitrification can occur in the water column, above wet soils and in the surface-oxidized soil or sediment later of the wetland systems (Willems et al., 1997).

Nitrate may follow several biochemical pathways. Plants and microbes may reduce nitrate to ammonia for incorporation into cellular amino acids (assimilatory nitrate reduction).

Under anaerobic or oxygen-free conditions and in presence of available organic substrate, the denitrifying organisms can use nitrate as an electron acceptor during respiration (Reddy & Patrick, 1984).

Nitrate may function as a terminal electron acceptor during the oxidation of organic matter and thereby supply energy for microbial growth. Nitrate respiration results in the reduction of nitrate to gaseous nitrogen form N_2 and N_2O (denitrification) or ammonia (nitrate-ammonification) (Hargreaves, 1998).

Denitrification produces ATP, which is used by the denitrifying organism to support respiration. This process is carried out by heterotrophic facultative anaerobes bacteria: when oxygen concentration becomes limiting those bacteria shift to nitrate as the terminal electron acceptor. The genera *Pseudomonas*, *Aeromonas*, and *Vibrio* are reported to be the most important denitrifying bacteria in aquatic systems while the genera *Bacillus*, *Micrococcus*, *Pseudomonas*, and *Azospirillum* are more common in soil.

Although denitrification is an anaerobic process, it is largely dependent on oxygen concentration for the production of nitrate through nitrification.

Nitrification and denitrification can occur simultaneously in wetland soils where both aerobic and anaerobic zones exist i.e. the aerobic rhyzosphere microsites in anaerobic soil (Vymazal, et al., 1998)

Nitrogen fixation is the conversion of gaseous nitrogen (N_2) to ammonia. In wetland soils, biological N_2 fixation may occur in the floodwater, on the soil surface, in aerobic and anaerobic flooded soils, in the root zone of plants, and on the leaf and steam surfaces of plants (Buresh et al., 1980). A wide variety of symbiotic (associated with nodulated host plants) and asymbiotic heterotrophic microorganisms can fix nitrogen in wetlands.

Nitrogen assimilation refers to a variety of biological processes that convert inorganic nitrogen forms into organic compounds that serve as building blocks for cells and tissues. The two forms of nitrogen used by plants for assimilation are ammonia and nitrate. Vegetation can play a significant role in N removal, assimilating nitrogen into plant tissue and providing environment in the root zone for nitrification-denitrification (Eriksson & Weisner, 1997). Plants derive most of their N from soil porewater with only a small amount from floodwater. Aquatic macrophytes also promote N removal by lowering the water velocity and thereby enhancing sedimentation of particulate matter, often containing organic N and ammonia (NH_4^+) (Howard-Williams, 1985). Macrophyte growth is not the only potential biological assimilation process: microorganisms and algae also utilize nitrogen. Ionized ammonia may be adsorbed from solution through a cation exchange reaction with detritus, inorganic sediments or soils. When water chemistry conditions change, the adsorbed ammonia can be released easily. Ammonium ion is generally adsorbed as an exchangeable ion to negatively-charged sites on the surface of clay mineral (Hargreaves, 1998).

Most nitrogen entering wetlands and ponds is associated with organic matter. This organic matter can settling and the resistant fraction to decomposition can be accumulated in the sediment and may be eventually become unavailable for additional nutrient cycling through the process of peat formation and burial (Vymazal, 2007).

Another process involving ammonia is the anaerobic ammonia oxidation (ANAMMOX) is the anaerobic conversion of NO_2^- and NH_4^+ to N_2 (Van De Graaf et

al., 1995). In this recently discovered process, nitrite is the electron acceptor (Strous et al., 1997), but research is needed to better understand how the microbes and the ammonia oxidizing reactions compete in the ecology of varied wetland systems (Hunt et al., 2005).

1.5.4 Phosphorus

Phosphorus (P) is one of the major nutrient-limiting in many fresh water ecosystems. It interacts strongly with wetland soils and biota, which provide both short term and sustainable long-term storage of this nutrient.

The ability of wetlands to retain phosphorus discharged from anthropogenic and natural sources depends on the form and concentration of the element and the chemistry of the soil and water column of a wetland (Gale & Reddy, 1995). The chemistry of the wetland will depend upon the type of wetland, hydrology, and management (Mitsch & Gosselink, 1993).

Phosphorous entering a wetland or stream is typically present in both organic and inorganic forms. The relative proportion of each form depends on soil, vegetation, and land use characteristics of the drainage basin, or on the type of effluent treated in the wetland. In wetland soils, phosphorus occurs as soluble and insoluble complexes in both organic and inorganic forms (Table 1.4).

Table 1.4 Major types of dissolved and insoluble phosphorus in wetlands (from Stumm & Morgan, 1970).

Phosphorus	Soluble Forms	Insoluble Forms
Inorganic	orthophosphate ($H_2PO_4^-$, $HPO_4^{=}$, PO_4^{3-})	clay phosphate complexes
	polyphosphates	
	ferric phosphate ($FeHPO_4^+$)	metal hydroxide-phosphate, e.g. vivianite $Fe_3(PO_4)_2$; variscite $Al(OH)_2H_2PO_4$
	calcium phosphate ($CaH_2PO_4^+$)	minerals, e.g. apatite ($Ca_{10}(OH)_2(PO_4)_6$)
Organic	dissolved organics, e.g. sugar phosphates, inositol phosphate, phospholipids, phosphoproteins	insoluble organic phosphorus bound in organic matter

The principle inorganic form is orthophosphate, which includes the ions PO_4^{3-}, $HPO_4^{=}$, and $H_2PO_4^{-}$; the predominant forms are related by the pH-dependent dissociation series:

$H_3PO_4 \leftrightarrow H_2PO_4^- + H^+$
$H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$
$HPO_4^{2-} \leftrightarrow PO_4^{3-} + H^+$

Free orthophosphate is certainly the major form of phosphate to be utilized directly by algae and macrophytes and thus represents a major link between organic and inorganic phosphorus cycling in wetlands. Organically bound phosphorus is present in phospholipids, nucleic acids, nucleoproteins, phosphorylated sugars or organic condensed polyphosphates (Reddy et al., 1999).

Wetlands provide an environment for the interconversion of all forms of phosphorus. The main compartments in wetland P cycling are water, plants, microbiota, litter, and soil.

In wetlands, several processes influence the concentration of P in the overlying flood water. These processes are biological, physical, and chemical. Physical processes of P retention are associated with the movement of water. Flowing waters entering a wetland are slowed, resulting in the settling, or sedimentation, of particles and P associated to these particles. The slowing of the flowing water also provides longer contact times between the water and sediment or biota, thus influencing the removal of P.

Chemical removal mechanisms within the soil occurs mainly as a consequence of adsorption, complexation, and precipitation reactions with aluminium, iron, calcium and clay particles, and by peat accretion (accumulation of organic matter). Of these, peat accretion is the most sustainable process (Brix, 1994).

Phosphate is adsorbed in the solid-liquid interface, and it refers to movement of soluble inorganic P from soil porewater to soil mineral surfaces, where it accumulates without penetrating the soil surface.

Phosphorus adsorption and retention in fresh-water wetland soil is controlled by interaction of redox potential, pH, Fe, Al, and Ca minerals, and the amount of native soil P (Faulkner & Richardson, 1989).

A variety of cations can precipitate phosphate under certain conditions.

In acid soils, inorganic P is adsorbed on hydrous oxides of Fe an Al and may precipitate as insoluble Fe-phosphate (Fe-P) and Al- phosphate (Al-P). Precipitation as insoluble Ca-P is the dominant transformation at pH value greater than 7 (Vymazal et al., 1998). In addition, clay particulates such as organic fractions material can contribute to trap P on their surfaces.

Phosphorus assimilation and storage in plants depends on vegetative type and growth characteristics (Reddy et al., 1999).

Emergent macrophytes have an extensive network of roots and rhizomes and have a greater potential to store P as compared to floating macrophytes (Figure 1.12).

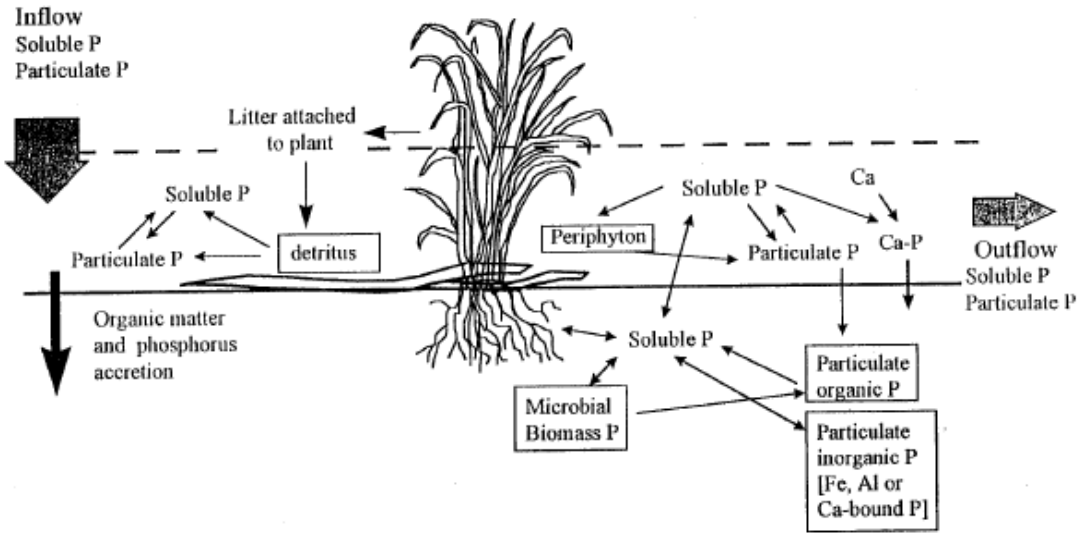


Figure 1.12 Scheme of P cycling as influenced by vegetation components of streams and wetlands (from Reddy et al., 1995).

Plant uptake is usually maximum during the peak growing season, followed by decrease or even cessation in the fall/winter (Howard-Williams, 1985). Other researchers observed that in many aquatic macrophytes the uptake rates of essential nutrients is highest during early spring growth, before maximum growth rate is attained (Boyd, 1979; Garver et al., 1988). Translocation of nutrient within the plant can vary through the vegetative season. Plants absorb phosphorus through their roots and transport it to the growing tissues.

Aboveground plant parts return leached P to the water after death and decomposition and deposit refractory residuals on the soil/sediment surface. The net effect of vegetation on P retention depends on type of vegetation, root-shoot ratio, turnover rates of detrital tissues, C/P ratio of the detrital tissue, type of metabolic pathways, and physicochemical properties of the water column. Biomass increases, however, should not be counted as part of the long term sustainable phosphorus removal capacity of wetland (Kadlec & Knight, 1996). Phosphorus is released back from the biomass of the wetland ecosystem after the plant decay.

1.5.5 Pathogens

Constructed wetlands are efficient at reducing and eliminating pathogen although the very complex mechanisms in these systems have so far only been studied to a limited extent (Gersberg et al., 1987; Stottmeister et al., 2003). Important factors of influence in connection with germ reduction include physical, biological and chemical.

Physical factors include filtration, sedimentation, adsorption and aggregation. Biological removal mechanisms include consumption by protozoa, attack by lytic bacteria, and bacteriophages and natural death. Chemical factors are oxidative damage, UV radiation and influence of toxins from other microorganisms and plants (Ottová et al., 1997; Vymazal et al., 1998).

1.5.6 Heavy metals

Heavy metals are usually removed from industrial wastewater and mine drainage in constructed wetlands by a variety of processes including filtration and sedimentation of suspended particles, adsorption, cation exchange, uptake into the plant material and precipitation by biogeochemical (microbial) (Stottmeister et al., 2003).

Heavy metals released into aquatic systems are generally bound to particulate matter. Part of metal entering an artificial wetland is retained in the substratum as a result of trapping of particulate matter, precipitation and adsorption reactions, part is taken up by plants and the remainder flows out of the system (Gersberg et al., 1986; Peng et al., 2008).

The extent of metal accumulation within aquatic macrophytes is known to vary significantly between species. For example, emergent aquatic vegetation usually accumulates lower amounts of metals than submerged aquatic vegetation. Some species have been found to develop tolerant ecotypes that either are able to survive higher concentrations of metals accumulating within their tissues or have developed more efficient mechanisms to exclude metal ions from their tissues Dunbabin & Bowmer (1992).

Aquatic macrophytes differ both in their capacity to take up metals in root tissues and in the proportion of metals transferred to above-ground parts (Sawidis et al., 1995; Baldantoni et al., 2004).

2 THE CONTEXT OF THE PRESENT WORK

In the light of above, constructed wetland are now widely recognised as effective treatment facilities for controlling pollution, restoring stream values within urban areas, for their recreational and aesthetic qualities, and for conserving flora and fauna. The treatment of water as it flows through a wetland is the result of a complex interaction between the physical, chemical and biological processes (Greenway, 2004). In wetland systems, the vegetation plays an important role in these treatment processes, including the filtration of particles, reduction in turbulence, stabilization of sediments and provision of increased surface area for biofilm growth (Greenway, 2004). Most of these processes are controlled and influenced by the hydraulic interaction between the vegetation and the water movement through the system.

In order to optimize the application of these systems, the knowledge of the biological behaviour of the components involved in the retention processes, as well as the hydrological aspects need to be studied.

Based on that, the aim of the study was to examine two of the more relevant functions of constructed wetland systems: removal efficiency and hydraulic performance. The first research line was performed in a newly developed pilot surface-flow constructed wetland in the Fusina area in Venice (Italy) (Chapter 3).

The second research line was performed in 18 pilot surface-flow constructed wetlands in Halmstad (Sweden) (Chapter 4).

3 CASE STUDY: REMOVAL EFFICIENCY

3.1 Introduction

Venice and its lagoon are a particular sensitive coastal marine habitat under growing stress due to centuries of anthropogenic pollution and continuous terraforming. The Venice Lagoon is a shallow (≈1.0 m) enclosed embayment located on the northwestern Adriatic Sea, it encompasses approximately 540 km^2, of which 62% is open water, 13% are salt marshes, 17% diked fish farms, and 8% islands (Pellizzato, 1996). The Venice lagoon drainage basin extends 2,038 km^2 and has a population of more than 1 million inhabitants. In the last decades, polluted water coming from the watershed has been discharged into the Venice lagoon, causing severe damage to this unique ecosystem.

To improve the health of the lagoon a series of actions have been conducted by the local regional government (Regione Veneto) in the past decades for the collection and treatment of wastewaters in the vicinities of the lagoon, and was progressively extended to include all the pollution sources over the whole lagoon watershed. In particular, the Master Plan set up by the Regione Veneto in 2001 aims to reduce the annual nutrient loads discharged to the lagoon in order to prevent the proliferation of macroalgae and the risk of environmental crises. The Plan also aims to reduce the concentration of micro pollutants in water and sediments to levels that ensure the protection of human beings from adverse effects associated with the consumption of fish and shellfish.

In this context, the Fusina Integrated Project (FIP) has been developed to deal with the most densely populated part of the lagoon watershed and aims to review and upgrade of the existing waste water treatment plant (WWTP) of Fusina, to reuse the water within the Porto Marghera industrial complex with a final discharge to the Adriatic open sea.

One of the major components of the project is the design and construction of a 100ha surface flow treatment wetland system (Figure 3.1).

The project will convert a dredge spoil basin (Cassa di Colmata A) facing the Venice Lagoon in the vicinities of the industrial parts into an ecologically functional wetland habitat while treating wastewater for beneficial reuse. The wetland will provide final polishing treatment of 4000 m^3/h of wastewater effluent of the municipal WWTP, which will be cleaned to standards fit for reuse or discharge.

The wetland will consist of six cells configured to flow in two parallel and independent flow paths. The cells consist of alternating deep open water and shallow emergent marsh zones arranged perpendicular to the path of flow. This configuration will enable operational flexibility and the ability to take individual cells or an entire flow path out of service if required for maintenance and will optimize hydraulics and treatment efficiency. In addition, numerous islands constructed within the open water zones will provide refuge from predators to encourage use of the system by a wide variety of wetland animals.

The wetland will provide potential for leisure and public education related to wetland ecosystems, natural water treatment systems, and passive recreation also enabling views of the historic city of Venice across the lagoon.

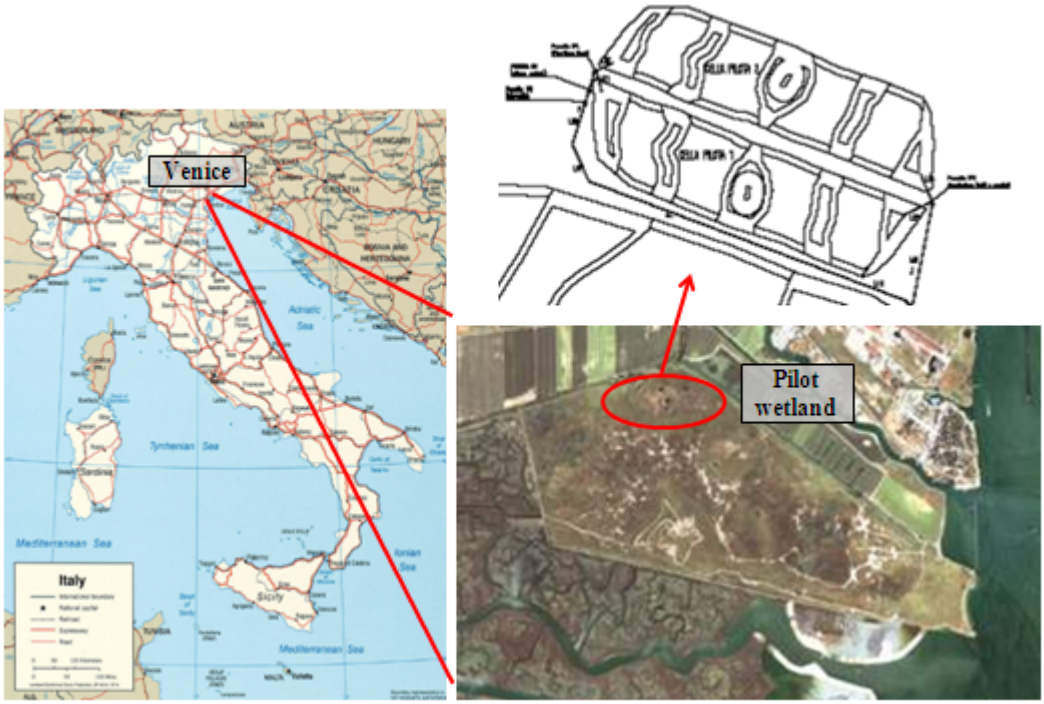


Figure 3.1 Fusina project location (Cassa di Colmata A) and pilot wetland system.

Moreover, a pilot scale wetland (Figure 3.1) with the same features, such as shape, depth, vegetation, hydraulic characteristic like the final one has been constructed with the following aims:

- Work as pilot system/nursery for vegetation species that will be planted in the final wetland;
- Provide operation optimization guidance because it will operate in advance of full scale system;
- Be a research site to improve knowledge concerning the removal mechanisms in wetlands;
- Create an area for educational and promotional potentiality and a venue for public involvement in wetland education and research.

3.2 Aim of the case study

The principal objectives of this study were to evaluate the start-up removal performance of the newly developed pilot surface-flow constructed wetland in the Fusina area, and to investigate the nutrients and metals translocation into different tissues of plants and among plants-sediment-water. In addition, attention has been paid in monitoring the plants growth in the wetland, in particular of *Typha latifolia* L., *Phragmites australis* (Cav.) Trin. ex Steud. and *Schoenoplectus lacustris* (L.) Palla.

To meet the objectives set forth in this study, a monitoring program was developed to follow the system evolution from start-up operation.

A sampling strategy was established considering the three matrices that play an important role in wetland system, such as soil, water and plant. This approach aims at improving our knowledge on patterns and processes of elements and metals uptake, distribution and removal by different species of wetland plants. This information is needed in order to better understand the functioning of the pilot wetland system and use this know-how to optimize the management of the neighbouring full-scale 100 ha constructed wetland.

3.3 Material and methods

3.3.1 Site description

The present study was performed in the pilot wetland system in Fusina. The hydraulic and morphological characteristics of the site are listed in Table 3.1.

Table 3.1 Hydraulic and morphological characteristics of the pilot system studied.

Inlet flow rate	~ 200 m^3/d
Hydraulic loading rate (HLR)	~ 2.2 cm/d
Nominal residence time (t_n)	~ 14 d
Shallow marsh water depth	30 – 50 cm
Deep zone water depth	150 – 200 cm

The pilot wetland was constructed in July 2007 and consists of two parallel Cells (1 and 2), with a total surface area of approximately 1000 m2, where deep open water sections (approximately 1.6 m depth) alternate with shallow water subcells (approximately 0.4 m depth), arranged perpendicularly to the flow path (Figure 3.2 and Figure 3.3).

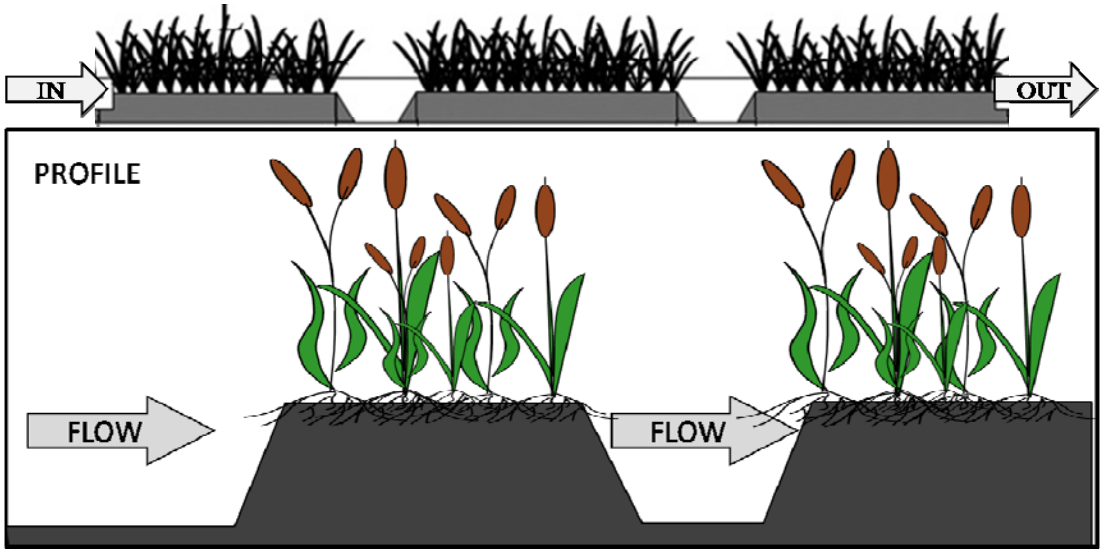


Figure 3.2 Simplified representation of the bathymetry with deep zones alternated with shallow zones within the Fusina pilot wetland.

Such configuration based on the alternation of different depth zones is commonly used in wetlands in order to increase wetland performance, by promoting uniformity of flow and transverse mixing, further minimizing short-circuiting (Gerke et al., 2001; Lightbody et al. 2007, 2008, 2009).

The soil-sediments in Fusina pilot wetland were characterized by high variability and heterogeneity. Cassa di Colmata A, where the pilot wetland was constructed, is an artificial area realized between ’60 e ’70 years with sediment taken from the excavation of channels, and it is constituted by different substrates (sand, silt, clay). In addition, to reach the morphologic design level, some sediment was added on Cell1 during the wetland construction, with the result of high soil variability between Cell1 and Cell2.

In the pilot wetland, shallow zones were planted with emergent vegetation, (e.g. *Phragmites australis* (Cav.) Trin. ex Steud., *Typha latifolia* L., *Schoenoplectus lacustris* (L.) Palla; *Juncus effusus* L.), while deep zones contained submerged vegetation (*Potamogeton crispus* L., *Nymphaea alba* L.). Some subcells, located at the beginning, at the end and in the middle of the two Cells, were not planted and left with free water (Figure 3.3).

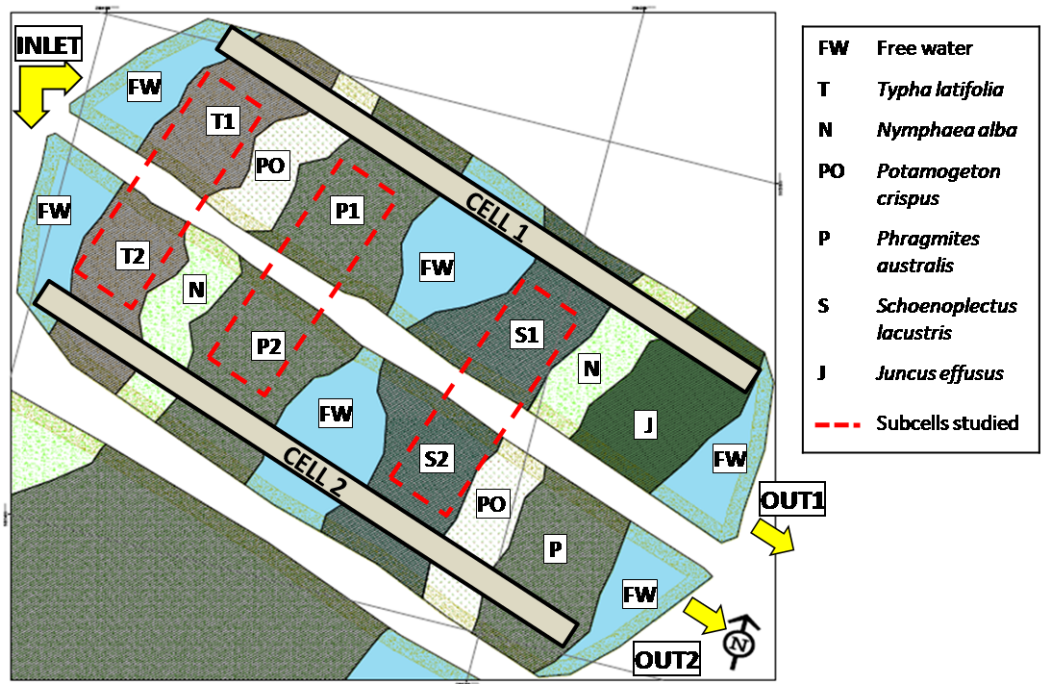


Figure 3.3 Fusina pilot wetland layout.

During the monitoring period the pilot wetland received water pumped from the neighbouring Naviglio-Brenta River, originated from a lateral derivation of Brenta River.

To the aims of the present study our attention was focused on 6 subcells, i.e. the subcells in Cell1 and Cell2, planted respectively with *Phragmites australis* (Figure I. 1 a, b in Annex I), *Typha latifolia* (Figure I. 1 c, d in Annex I) and *Schoenoplectus lacustris* (Figure I. 1 e, f in Annex I).

The considered subcells are identified, referring to Figure 3.3, as shown in Table 3.2.

Table 3.2 Identification codes of studied subcells.

Subcell identification code	Description
T1	Cell1 - Subcell planted with *Typha latifolia*
T2	Cell2 - Subcell planted with *Typha latifolia*
P1	Cell1 - Subcell planted with *Phragmites australis*
P2	Cell2 - Subcell planted with *Phragmites australis*
S1	Cell1 - Subcell planted with *Schoenoplectus lacustris*
S2	Cell2 - Subcell planted with *Schoenoplectus lacustris*

3.3.2 Species Description

Phragmites australis (Cav.) Trin. ex Steudel

The common reed, *Phragmites australis* (Cav.) Trin. ex Steud., grows well in chemical reduced, water-logged soils. Beds of this reed, and others, have been used successfully in the "root zone treatment" of sewage both in America, Australia and in Europe (Armstrong et al., 1999).

Phragmites (common reed) belongs to the family *Poaceae*. It is a robust, tall, perennial emergent grass found on every continent with the exception of Antarctica (Tucker, 1990). It presents a terminal, feathery seed head and is often found as an invader of disturbed wetlands. It has a very long (as long as 12 m), exposed rhizomes and a characteristic seed head. It usually grows rapidly to 1.5-3 m in height. *Phragmites* has a wide range of tolerance for environmental conditions and can grow in fresh, brackish, and salt marsh systems (Lissner & Schierup, 1997). It establishes new stands both by seed and dispersal of rhizome fragments, but expansion of

existing stands is primarily vegetative. *Phragmites* can produce large quantities of seeds, but germination rates are variable and generally low (Galinato & Van der Valk, 1986).

Phragmites has been expanding over the past century from high marsh habitats into lower marshes, replacing *Spartina alterniflora* Loisel (salt marsh cordgrass). *P. australis* most easily invades sites considered ‘‘disturbed’’, including wetlands constructed for mitigation purposes to offset the loss of natural wetlands (Havens et al., 1997).

***Typha latifolia* L.**

The broadleaf cattail belongs to the family *Typhaceae* (*Cattail* family). It is a tall perennial herb growing from thick, white, fleshy rhizomes in early Spring. Stems reach 90-270 cm with alternate pale-green leaves tightly clasping at the base, 2.5 cm wide, sword-like, flat on the inside and rounded on the outside, with a spongy interior.

Broadleaf cattail is a common plant of shallow marshes, swamps, freshwaters ponds, and lake edges, in slow-flowing or quiet water, low to middle elevation. Cattails are found all over Eurasia, North America (except Arctic) and North Africa.

***Schoenoplectus lacustris* (L.) Palla**

Schoenoplectus lacustris (L.) Palla (synonymous with *Scirpus lacustris* L. ssp. *lacustris*) belongs to the *Cyperaceae* family of monocotyledone. It is a perennial grass, growing to 2.5 m. *Schoenoplectus* has long, slender, solid stems tipped with brown spikelets of tiny flowers. The flowers are hermaphrodite and are pollinated by wind. The plant prefers light, loamy and clayey soils. The plant succeeds in any wet to moisture retentive soil, pond margins and shallow water, in full sun or shade.

3.3.3 Experimental design

Sediment (Par. 3.3.3.2), water (Par. 3.3.3.3) and vegetation (Par. 3.3.3.4) sampling was carried out from July to November 2008, as schematized in Table 3.3.

Concerning water, in addition to sampling carried out monthly, an intensive sampling field been performed in July to have a first chemical-physical characterisation of the effluent entering in the pilot system. In November some problems occurred in the analyses, in particular no data are presented concerning nitrogen and phosphorus content and nutrients.

Table 3.3 Schematic sampling design of water, vegetation and sediment in the pilot wetland in Venice.

Sampling date	
Water	*Vegetation / Sediment*
8 July 2008*	2 July 2008
9 July 2008*	
11 July 2008*	
29 July 2008	5 August 2008
8 September 2008	23 September 2008
13 October 2008	
3 November 2008	17 November 2008

*Intensive sampling.

A comprehensive set of analyses was carried out on the collected samples (Figure 3.4) as described in the following paragraphs 3.3.4, 3.3.5 and 3.3.6. In particular, for what concerns the water monitoring plan, ecotoxicological tests were included to comply with the requirements of the Italian Water Directive (D. Leg. N°152, 2006) to assess discharge acceptability to the water bodies (Baudo, 2001).

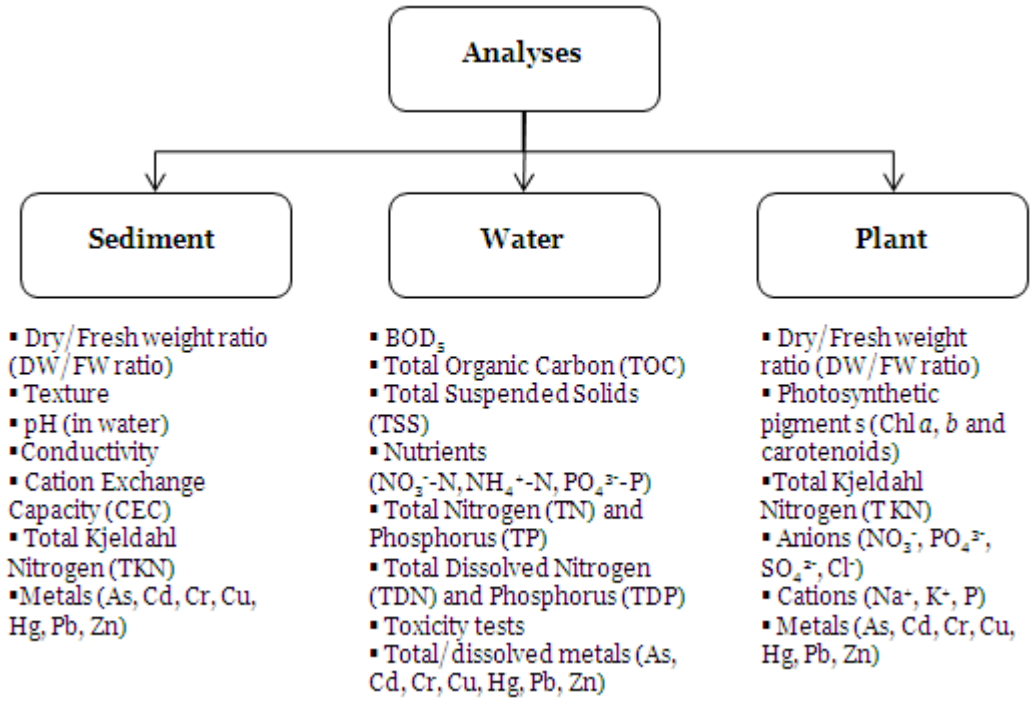


Figure 3.4 Schematic diagram of the different analyses performed in soil, water and plant collected to the Fusina pilot system.

Sediment and plant analyses, have been conducted at the plant physiology laboratory of the Agricultural Biotechnology Department, Faculty of Agriculture, Padua University, whereas water physical-chemical and biological analyses were performed at Environmental Laboratory of Thetis S.p.A in Venice.

3.3.3.1 Weather conditions

Weather conditions were recorded at each sampling time. In addition, weather report was provided by "Ente Zona Industriale Porto Marghera", which registered daily meteorological conditions through a meteorological station (Stat. N°23) located in proximity to the study site (Table I. 1, Table I. 2, Table I. 3, Table I. 4 and Table I. 5 in Annex I).

At each sampling date weather was always characterised by clear sky and sometimes windy. Considering the first water sample performed in July, it is notable that some days before some amount of rainfall occurred (Table I. 1 in Annex I). In November, the weather was generally characterised by higher rainfall, with rain mean value of 4.5 mm and maximum value of 31 mm of rain (Table I. 5 in Annex I).

3.3.3.2 Sediment sampling

Sediment samples (three replicates) from each of the three subcells of Cell1 and Cell2 respectively were collected at a depth of 0 – 5 cm and 5 – 20 cm from the sediment surface. A 5-cm-diameter sediment corer was used. Samples were placed in plastic ziplock bags and stored below 4°C for the transport to the laboratory.

The sampling was performed once per month between June and November. The replicates of sediment layer (0-5 and 5-20 cm) from each subcell and at each date were polled for a single chemical analysis. Part of the samples were weight for the fresh weight determination and part was dried overnight (60°C) and then grinded with a mortar and pestle.

The characterisation of surface and deep layer of the sediments, as schematized in Figure 3.4 and as explained later (Par. 3.3.5) included the determination of dry/fresh weight ratio, texture, pH (in water), conductivity, organic carbon (OC), cation exchange capacity (CEC), total Kjeldahl nitrogen and metals (As, Cd, Cr, Cu, Hg, Pb, Zn).

3.3.3.3 Water sampling

Water samples were collected from the inlet (IN) and from the two outlets (OUT1 and OUT2) of the Cell1 and Cell2 and kept at 4°C in clean polyethylene bottles until analysis (Figure 3.4).

The determinations in the unfiltered samples, as schematized in Figure 3.4, included total nitrogen (TN) and phosphorus (TP), total suspended solids (TSS), total organic carbon (TOC), biological oxygen demand (BOD_5), metals (As, Cd, Cr, Hg, Pb, Zn, Cu, Li), and toxicity tests (*Daphnia magna* and Microtox®). Part of the samples were filtered (0.45 μm Millipore) and then analyzed for nutrients (NO_3-N, NH_4-N, PO_4-P) (Figure 3.5), total dissolved nitrogen (TDN) and phosphorus (TDP), and the toxicity test with *Pseudokirchneriella subcapitata*. Part of water samples (unfiltered) were acidified with HNO_3 to pH<2, and stored at 4°C, for analyses of dissolved metals concentrations (As, Cd, Cr, Hg, Pb, Zn, Cu).

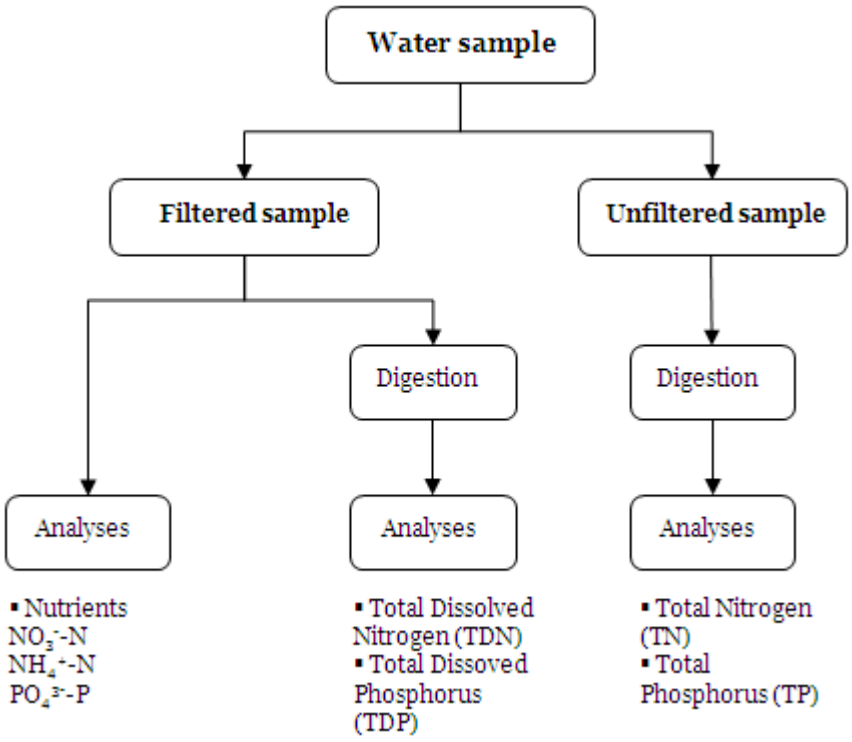


Figure 3.5 Diagram of the different nitrogen and phosphorus analyses performed on water samples (filtered and unfiltered) from the pilot system.

3.3.3.4 Plant sampling

Six specimens of each plant species (*Phragmites australis, Typha latifolia, Schoenoplectus lacustris*) were collected in conjunction with sediment sampling from the six subcells of the wetland (Figure 3.4 and Table 3.2).

The samples were divided into shoot (for *S. lacustris* and *T. latifolia*)/stem/leaves (for *P. australis*) and roots/rhizome components, washed gently with distilled water, cleaned with towel paper and then quickly transferred in plastic bags to the laboratory. Some fresh leaf samples were immediately analysed for pigments contents, while the remaining fresh plant material was frozen at -20°C and then analyzed for total nitrogen (TKN), nitrate (NO_3^-), phosphorus, potassium, sodium and metals as described below. A subsample of each vegetation species was harvested to determine shoot/leaves and root/rhizome dry weight/fresh weight ratio (DW/FW ratio) (Figure 3.4).

No free-floating species were collected from any of the sites (as previously noted in Table 3.1).

Sampling of shoots and leaves was performed monthly during the growing season (June-November), whereas roots/rhizome components were collected in June and November.

3.3.4 Field measurements

3.3.4.1 Plant biomass

Five plants for each selected subcell and each species (*Phragmites australis*, *Typha latifolia* and *Schoenoplectus lacustris*) were randomly selected and marked with a nylon cable tie. At each sampling time, the plant's height was determined with a tape measure and general conditions were reordered for each sample plant.

3.3.4.2 Water pH – Temperature

Water pH and temperature were determined in the field using an appropriate pH-electrode, previously calibrated (WTW Germany-LF 330/SET).

3.3.4.3 Water Dissolved Oxygen (DO)

Dissolved oxygen determination in water was performed directly in the field. Measurements were done using an electronic DO probe, previously calibrate (WTW Germany-Oxi 315i/SET).

Data were expressed as mg L^{-1} and as dissolved oxygen percent saturation, %.

3.3.4.4 Water salinity

Water salinity was measured in the field using electronic salinity probe (WTW Germany -LF 330/SET). Results were expressed using the Practical Salinity Scale, which defines the salinity as a pure ratio, with no dimensions. Data, according with Joint Panel of Oceanographic Tables and Standards were reported as a number with no symbol.

3.3.5 Soil characterisation

3.3.5.1 Dry weight / Fresh weight ratio (DW/FW ratio)

The percentage of water content was calculated as the weight difference between wet and dried sediments (dried for 24h at 105°C) (ASTM, 1990), in three replicates for each sediment sample.

3.3.5.2 Particle size analysis

The soil texture was measured using the Bouyoucos hydrometer method (MIPAF, 2000). This method measures the density of the suspension and it involves calculation of size of particle setting in a suspension at a given time from the hydrometer reading. After removing the >2 mm size fraction by means of dry-sieve with 2 mm meshes, 100 mg of sieved material was added to 100 mL of sodium esametaphosphate solution and approximately 250 mL of milliQ water. The suspension was stirred for 5 min at 14000-16000 rpm (Avanti® J-E – Beckman Coulter). Next, the solution was transferred to the settling cylinder (1000 mL volume) with the addition of milliQ water. After specified periods of time (4 min and 2 h), using the hydrometer, the density of the liquid in the cylinder (volumetric mass) was read. The amount of sand, silt and clay was calculated as follows and expressed as percentage (Eq. 3.1, Eq. 3.2 and Eq. 3.3):

Eq. 3.1 $$\%Silt = T1 - T2$$

Eq. 3.2 $$\%Clay = T2 - blank\ reading$$

Eq. 3.3 $$\%Sand = 100 - (\%Silt + \%Clay)$$

Where:

$T1$ = reading after 4 min;
$T2$ = reading after 2 h.

To determine the textural class of soil, knowing the proportions of sand, silt and clay, the textural triangle was use, which is a graphical representation of the 12 soil textural classes.

3.3.5.3 pH (in water)

This procedure is used to determine the pH of suspended soil in water (MIPAF, 2000). Distilled water (12.5 mL) was added to 5 g of dry soil-sediment and stirred for 1-2 min. The pH was measured in the supernatant after 1 h of standing and a second short stirring, using the pH meter previously calibrated. The result was reported as water pH (pH_W).

3.3.5.4 Conductivity

Conductivity is a measure of the ability of water to carry an electrical current, related to the amount of ions in the solution. Conductivity is typically used to measure the amount of salt present in the water.

Electrical conductivity in sediment was measured by addition of 30 mL of distilled water to 15 g of dry sediment (MIPAF, 2000). The solution was stirred for 2 h, centrifuged (3000 rpm for 10 min) (Avanti® J-E – Beckman Coulter) and filtered (Whatman n°42- 2.5 µm). The conductivity was measured using a cell conductivity meter (HI 8333 – Hanna Instrument).

The results were expressed in µS.

3.3.5.5 Total Organic Carbon (TOC)

According to Walkley-Black method (MIPAF, 2000), the amount of organic carbon present was estimated by a determination of the amount of dichromate consumed by organic carbon by back titration of the excess dichromate with a ferrous solution. In this procedure, 10 mL of potassium dichromate ($K_2Cr_2O_2$) and 20 mL of concentrated sulphuric acid (H_2SO_4) were added to 2 g of sediment previously dried and grounded. The solution was swirled and after 30 min almost 150 mL of deionised water and 5-10 mL of concentrated orthophosphoric acid (H_3PO_4 85%) were added.

Manual titration quantification was performed using diphenylamine as indicator. The excess of Cr_2O_4 was titrated with ferrous sulphate ($FeSO_4$) until colour change

occurs in the sample. The amount of total organic carbon was calculated as follows and expressed as percentage (Eq. 3.4):

Eq. 3.4
$$TOC\% = \frac{(V_B - V_T) * N * P_{eqC}}{W} * 100$$

Where:

V_B = volume (mL) of dichromate;
V_T = volume (mL) of ferrous sulphate used to titrate the sample;
N = normality of standard ferrous sulphate;
P_{eqC} = equivalent weight of carbon;
W = weight (mg) of dry sediment tested.

3.3.5.6 Cation Exchange Capacity (CEC)

Cation exchange capacity was determined following the MIPAF method (MIPAF, 2000). In this procedure, 3 g of dried and grounded sediment were weighted and 25 mL of solution barium chloride ($BaCl_2$) and triethanolammina (TEA) were added. The formed solution was centrifuged (Avanti® J-E – Beckman Coulter) and after each of the two cycle the limpid supernatant was removed and the sediments were washed with deionised water, removed after other two consecutive centrifuged cycles. The sediment was then weighted and 25 mL of sulphate magnesium ($MgSO_4$) were added and centrifuged. After this step, 10 mL of supernatant were titrated, after the addition of 10 mL of tampon solution pH=10 (ammonium chloride, NH_4Cl) and deionised water, using Na-EDTA and eriochrome black T as indicator. CEC was calculated as follows and expressed as meq 100 g^{-1} (Eq. 3.5):

Eq. 3.5
$$CEC = \frac{(V_B - V_T) * 0.25 * (25 + B - A)}{W} * 2$$

Where:

CEC = cation exchange capacity expressed as meq 100 g^{-1};
V_B = volume (mL) of Na-EDTA solution used to titrate the blank;
V_T = volume (mL) of Na-EDTA solution used to titrate the sample;
0.25 = EDTA molarity expressed as cmol L^{-1};

B - A = change of the sample weight after $BaCl_2$ addition;
2 = correction factor.

3.3.5.7 Total Kjeldahl Nitrogen (TKN)

Kjeldahl method was used to quantitatively determine total Kjeldahl nitrogen (TKN) including NH_4 and proteins N in sediments, based on the wet oxidation of organic matter using H_2SO_4 and a digestion catalyst (MIPAF, 2000). Approximately 5 g of dry sediments were weighted and put in the appropriated tubes: 1 catalysts table and 10 mL of H_2SO_4 were added. Tubes were placed on a digested block, preheated to 370 for 2 h. The solution was then distilled with NaOH, which converts the ammonium salt to ammonia. The present amount of ammonia was determined by back titration using HCl 0.1N.

The total Kjeldahl nitrogen % was calculated as follows and expressed as % TKN (or mg/g TKN with the appropriate calculation) (Eq. 3.6):

Eq. 3.6
$$TKN\ \% = \frac{(V_S - V_B) * M * 14.01}{W * 10}$$

Where:

V_S = volume (mL) of standardized acid used to titrate a test;
V_B = volume (mL) of standardized acid used to titrate reagent blank;
M = molarity of standard HCl;
14.01 = atomic weight of N;
W = weight (g) of test portion or standard;
10 = factor to convert mg g^{-1} to percent.

3.3.5.8 Metals

Mineral determination of sediments was determined following USEPA method (1997). Dry sediment was powered in a mortar, weighted (500 mg), mineralised in 10 mL of nitric acid (69% v/v), and transferred to a microwave oven (ETHOS 900-Milestone) with the following program (for 12 fluorocarbon microwave vessels):

$\Rightarrow$ 10 min 250W, 10 min 450W, 10 min 600W, 5 min 250W, 5 min ventilation.

The digest was made to 50 mL final volume with deionised water, filtered (45µm, Millipore) and then analysed for heavy metals (As, Cd, Cr, Cu, Hg, Pb, Zn) using an Inductively Coupled Plasma Spectrometry/Atomic Emission Spectroscopy, ICP/AES (Spectro Ciros^{CCD}, Spectro Italia s.r.l).

3.3.6 Water analyses

3.3.6.1 Ammonia (NH_4^+-N)

Ammonia was determined in water filtered samples (0.45 µm Millipore) following Phenate method (APHA, 2005).

An intensely blue compound, indophenol, was formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside.

To a 25 mL sample were added, thorough mixing after each addition, 1 mL phenol solution, 1 mL sodium nitroprusside solution, and 2.5 mL oxidizing solution. Samples were covered and let colour develop at room temperature in subdued light for at least 24h. The absorbance was measured at 640 nm (UV-1700 SHIMUDZU).

3.3.6.2 Nitrate (NO_3^--N)

Filtered (0.45 µm Millipore) water samples were analyzed following Standard methods APHA (2005). Nitrate was reduced almost quantitatively to nitrite in the presence of cadmium. The produced NO_2^- was determined by diazotizing with sulphanilamide and by coupling with N-(1-naphthyl)-ethylenediamonedihydrochloride to form a highly colored azo dye that was measured colorimetrically.

3.3.6.3 Phosphorus (PO_4^{3-}-P)

Phosphate (PO_4^{3-}-P) in water filtrate samples (0.45 µm Millipore) was determined following ascorbic acid method (USEPA, 1983).

Ammoniummolybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid – phosphomolybdic acid, that was reduced to intensely coloured molybdenum blue by ascorbic acid.

3.3.6.4 Total Nitrogen (TN), Total Dissolved Nitrogen (TDN)

In water samples, the digestion was performed by the addition of 16 mL of persulfate reagent (50 g potassium persulfate ($K_2S_2O_4$), 30 g boric acid (H_3BO_4) dissolved in 1000 mL of sodium hydroxide (NaOH, 0.375 M) to 80 mL water sample (filtered or unfiltered). The persulfate oxidation, converting all N into nitrate and all P into orthophosphate, is a method to determine total nitrogen (TN) and phosphorus (TP) and, in filtered samples (0.45 μm Millipore), total dissolved nitrogen (TDN) and phosphorus (TDP). After adding the persulfate reagent, the labelled vials were placed into autoclave for 30 minutes at 120°C, and then stored until further analysis. At every analysis, at least two replicates respectively of controls, blanks, and samples were prepared.

Filtered (0.45 μm Millipore)/unfiltered samples were analyzed following Standard methods APHA (2005) as previously explained for nitrogen analysis.

The results were expressed as total nitrogen (TN) for oxidized unfiltered samples and total dissolved nitrogen (TDN) for oxidized and filtered ones.

3.3.6.5 Phosphorus (PO_4^{3-}-P), Total Phosphorus (TP), Total Dissolved Phosphorus (TDP)

In water samples, total phosphorus (TP) and total dissolved phosphorus (TDP) were determined after persulfate oxidation, following ascorbic acid method (USEPA, 1983). This method is based on reduction of the ammonium molybdiphosphate complex by ascorbic acid in the presence of antimony. The colour produced is stable for 24 hours.

Filtration through a 0.45 um pore-diam membrane filter separates dissolved from suspended and dissolved forms of phosphorus.

3.3.6.6 Total Suspended Solids (TSS)

Total suspended solids were determined according the APAT IRSA-CNR method (2003c). A known volume of well-mixed sample (300-500 mL) was filtered through a weighted filter (Millipore 0.45 μm). The residual retained in the filter was dried to a constant weight at 105°C for 24 h. The estimation of total suspended solids was calculated by the difference between initial and final weight.

3.3.6.7 Biological Oxygen Demand (BOD_5)

Biological oxygen demand (BOD_5) determination measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present in the sample. The sample was incubated for 5 days at 20°C in dark condition; oxygen concentration was detected at the beginning and at the end of the incubation time. The reduction in dissolved oxygen during the incubation period yields a measure of the biochemical oxygen demand (USEPA, 1985).

3.3.6.8 Total Organic Carbon (TOC)

Total organic carbon (TOC) was determined following USEPA method (1983). Organic carbon in the sample was converted to carbon dioxide (CO_2) by catalytic combustion.

The formed CO_2 was measured directly by an infrared detector (TOC-V CSH, Shimadzu). The amount of CO_2 is directly proportional to the concentration of carbonaceous material in the samples.

3.3.6.9 Metals

For the mineral determination of unfiltered water, 15 mL of water were mineralised in 5 mL of nitric acid (69% v/v) and transferred to a microwave oven (ETHOS 900-Milestone) with the following program (for 6 fluorocarbon microwave vessels):

$\Rightarrow$ 5 min 500W, 5 min 350W, 5 min ventilation.

The digest was filtered (45µm, Millipore) and then analysed, as well as filtered water samples, for heavy metals (As, Cd, Cr, Cu, Hg, Pb, Zn) using an Inductively Coupled Plasma Spectrometry/Atomic Emission Spectroscopy, ICP/AES (Spectro Ciros^{CCD}, Spectro Italia s.r.l.) (APAT IRSA-CNR, 2003a).

3.3.6.10 Toxicity tests

Studies published in the last decades and the Italian legislation (D. Leg. N°152, 2006) suggest that acute tests with invertebrates, green algae and bacteria may be used as first screening methods for the assessment of the short-term toxicity of chemicals and new chemicals (Gaggi et al., 1995; Ward et al., 2002) and to assess the toxicity of effluent and/or wastewater discharges in the environment and receiving waters (Janssen & Persoone, 1993; Rosen & Lennox, 2001; Mansour & Sidky, 2003; Pehlivanoglu & Sedlak, 2004; Hernando et al., 2005). Bioassays are rapid, sensitive, reproducible, ecologically relevant and cost effective tools used as standard procedures for aquatic toxicity testing.

***Daphnia magna* Straus**

Tests with *Daphnia magna* Straus were carried out following the APAT IRSA-CNR method (2003b). *D. magna* belongs to the family *Daphniidae* of the order cladoceran (Figure 3.6). It is widely used as a standard bioindicator, and several protocols exist for its use in assessing the toxicity of substances under acute and chronic experimental conditions (Baird et al., 1989).

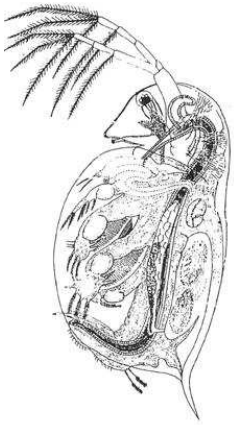


***Figure 3.6* Daphnia magna.**

The test is used to assess if wastewater discharges and effluents are "acceptable" according to the Italian Law (D. Leg. N°152, 2006). Discharges and effluents are considered acceptable if the percentage of mobile *D. magna* in the sample resulted higher than 50% in respect to control.

All experiments were carried out with newborn daphnids (< 24-h old) from Thetis. Test medium was OECD hard water (OECD, 2004) and the animals were not fed during the tests. Ten animals per treatment were exposed in 70 mL PE vessels filled with 50 mL of test solution. In addition, a negative control with dilution water and a positive control using a reference toxicant (Potassium dichromate, $K_2Cr_2O_7$) in order to check the sensitivity of the tested organisms were carried out simultaneously with each testing session. The resulting EC_{50} values obtained with $K_2Cr_2O_7$ were included in the range of control charts produced by the laboratory. The data were accepted only if they fell within the range reported by ISO 16341 (1996).

Test was run in triplicate for 24 hours under controlled photoperiod (16-h light and 8-h dark) at a temperature of 20±1°C.

Oxygen concentrations (WTW Germany-Oxi 315i/SET) and pH levels (WTW Germany-LF 330/SET) were measured at the beginning and at the end of the test (24 h) both in the controls and in the samples. At the end of the test, the Daphnis unable to swim after gentle agitation of the sample for 15 s were considered as immobilized, even if they can still move their antennae.

Data were expressed as mean percentage of immobile *D. magna* after 24 h, derived from the following formulas (Eq. 3.7; Eq. 3.8):

Eq. 3.7 $$Mob\,\% = \frac{N_{mob}}{N_{cont}} * 100$$

Eq. 3.8 $$I\mathrm{m}\% = 100 - Mob\,\%$$

Where:

Mob% = mobilisation of individuals (%);
Im% = immobilisation of individuals (%);
N_{mob} = average number of mobile individuals;
N_{cont} = average number of mobile individuals in the controls.

Number of immobilized individuals were determined for each replicate separately and average value was determined afterwards.

The results could be expressed, according to Italian Legislation, as schematized in the following table (Table 3.4):

Table 3.4 Correspondence for* D. magna *test results and immobilization percentage (Im%).

Im%	Result
I% > 50%	Toxic effect Sample not acceptable
I% < 50%	No toxic effect Sample acceptable

Reference toxicant potassium dichromate ($K_2Cr_2O_7$) was used as positive control at each *D. magna* test.

Pseudokirchneriella subcapitata (Korshikov) Hindak (ex *Selenastrum capricornutum*)

The freshwater chlorophyte *Pseudokirchneriella subcapitata* (Korshikov) Hindak (ex *Selenastrum capricornutum*) was used as test organism for the determination of possible effects on algal growth (Figure 3.7).

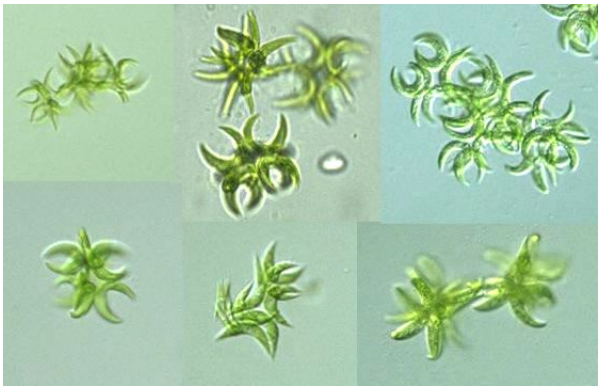


***Figure 3.7* Pseudokirchneriella subcapitata.**

Algae tests were performed according to ASTM Standard Guide (ASTM, 2004) and EPA method (USEPA, 2002).

To eliminate false negative results due to low nutrient concentrations, nutrient solutions were added into both the water sample and the control with dilution water. Salinity (WTW Germany-LF 330/SET) and pH levels (WTW Germany-LF 330/SET) were measured at the beginning and at the end of the test (72 h) both in the controls and in the samples. The initial algal culture was prepared from the stock culture maintained in the enriched medium and incubated at 24±2°C. A pre-culture was started two to four days before the beginning of the test.

The sample concentrations (three replicates) and negative controls (enriched medium) were inoculated at an initial density of 10^6 cells ml^{-1} and then incubated at 24±2°C for 72 hours under continuous illumination. At the end of the exposure, inhibition of algal growth in the samples as compared with controls was determined using a coulter counter instrument (Beckman Coulter- Multisizer 3).

For each test, a positive control was performed using a reference toxicant (Potassium dichromate, $K_2Cr_2O_7$) in order to check the sensitivity of the algae *P. subcapitata*. The resulting EC_{50} values were included in the range of control charts produced by the laboratory.

Data were expressed as EC_{50} (Effective Concentration 50) or as percent of inhibition I% of algal growth with respect to control over the test duration. EC_{50} is the experimentally derived concentration of test substance that is calculated to affect 50 percent of a test population during continuous exposure over a specified period of time. EC_{50} is calculated using Probit (Probability units) analyses. The results could be expressed, according to Italian Legislation, as schematized in the following table (Table 3.5):

Table 3.5 Correspondence for* P. subcapitata *test results and inhibition percentage (I%).

I%	Result
I% > 50 %	Toxic effect EC50 calculation using Probit analyses Sample not acceptable
I% < 50%	No toxic effect Sample acceptable
I%: negative	No adverse effect but biostimulation Possible presence of eutrophization

Reference toxicant potassium dichromate ($K_2Cr_2O_7$) was used as positive control at each *P. subcapitata* test.

Microtox®

The experimental procedure for conducting the bacterial bioluminescence assay was based on the ISO 11348 standard protocol (ISO, 1993), integrated by the protocol provided by Microbics Corporation (1999).

Salinity in freshwater samples was adjusted to 20, by addition of sodium chloride (NaCl). The procedure employs the bioluminescent marine bacterium, *Vibrio fischeri*, as the test organism (Figure 3.8 – b). For each test, a negative and positive control was performed using a reference toxicant ($ZnSO_4$ solution) in order to check the sensitivity of the tested organisms. The resulting EC_{50} values were included in the range of control charts produced by the laboratory.

The freeze-dried *V. fischeri* (Microtox® Acute Reagent - SDI) were reconstituted with 1 mL of reconstitution solution (SDI-Strategic Diagnostic), and incubated at 4°C for 20-30 min before use. In the screening test provided by the instrument (81.9 % Screening test), the bacteria were exposed to the sample to be tested. The reduction of the intensity of bacterial bioluminescence after 5, 15 and 30 min was measured using AZUR Environmental M500 Analyzer along with standard solution and control samples (Figure 3.8).

a) **b)**

***Figure 3.8 (a) Instrument utilized for Microtox® test; (b)* Vibrio fischeri.**

The results were expressed as average inhibition percentage of bioluminescence (I%), according to the following equation (Eq. 3.9):

Eq. 3.9
$$I\% = \frac{I_b - I_c}{I_b} * 100$$

Where:

I_c = sample light emission;

I_b = control light emission.

The results could be expressed, according to Italian Legislation, as reported in the following table (Table 3.6).

Table 3.6 Correspondence for* Microtox® *test results and Inhibition percentage (I%).

I%	Result
I% > 50%	Toxic effect EC_{50} calculation by Basic test Sample not acceptable
I% < 50%	No toxic effect Sample acceptable
I%: negative	No adverse effect but biostimulation

Reference toxicant ($ZnSO_4 \cdot 7H_2O$) was used to control *V. fischeri* batch quality according to the Basic Test procedure (two replicates) (Azur Environmental, 1999).

3.3.7 Plant tissues analyses

3.3.7.1 Nutrient and chloride contents

In plant tissues, the nitrate (NO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}) and chloride (Cl^-) contents were determined by the extraction in hot water (at 85°C for 2 h).

Fresh foliar and root tissues (500 mg) were ground in liquid nitrogen and 10 ml of distilled water were added. The samples were placed in hot water (at 85°C for 2 h) and the extracts obtained were filtered (Millipore 0.45 μm).

After filtration (Millipore 0.45 μm), the solutions were then analysed by High Performance Liquid Chromatography (HPLC) using IonPac AS14 column/AG14 guard column (Dionex), with $NaHCO_3/Na_2CO_3$ eluent (1mM: 3.5 mM) and a flow rate of 0.9 mL/min.

Nitrate, phosphate and sulphate content were expressed in μg g^{-1} F.W.

3.3.7.2 Total Kjeldahl Nitrogen (TKN)

Kjeldahl method was used to quantitatively determine Total Kjeldahl Nitrogen (TKN) including NH_4 and proteins N in plant tissues, based, as previously described (Par. 3.3.5.7) on the wet oxidation of organic matter using H_2SO_4 and a digestion catalyst (APHA, 2005). Approximately 500 mg of powered plants dry sample were weighted and put in the appropriated tubes: 2 catalysts tables and 12 mL of H_2SO_4 were added. Tubes were placed on a digested block, preheated to 370 for 2 h. The solution was then distilled with NaOH, which converts the ammonium salt to ammonia. The present amount of ammonia was determined by back titration using HCl 0.1N.

The total Kjeldahl nitrogen % was calculated as previously explained (Par. 3.3.5.7) and expressed as % TKN (or mg/g TKN with the appropriate calculation) (Eq. 3.6):

3.3.7.3 Plant weight

Fresh and dry (60°C for 48h) weights of roots, leaves and stems of *Phragmites* and roots and areal parts of *Typha* and *Schoenoplectus* were measured in triplicate (Campbell & Plank, 1998). Dry/fresh weight ratio (DW/FW ratio) was then calculated.

3.3.7.4 Photosynthetic pigments

The content of chlorophyll *a* (Chl *a*), *b* (Chl *b*) and carotenoids (Car) was determined by the extraction of fresh leaf samples (approx. 300 mg, stored at -80°C), previously powered in a mortar, with 10 mL of ethanol (96% v/v). The samples were stored at +4°C for 48 h in the dark, and then measured spectrophotometrically (JASCO V-

530) at three different wavelengths 665, 649 and 470 nm (Welburn & Lichtenthaler, 1984). The concentrations were calculated as follows (Eq. 3.10, Eq. 3.11 and Eq. 3.12):

Eq. 3.10 $$Chla = \left(13.95 * A_{665} - 6.88 * A_{649}\right)$$

Eq. 3.11 $$Chlb = \left(24.96 * A_{649} - 7.32 * A_{665}\right)$$

Eq. 3.12 $$Car = \left(1000 * A_{470} - 2.05Chla - 114.8Chlb\right) * 245$$

3.3.7.5 Metals

For the mineral determination of plant tissues (USEPA, 1997), dry plant material was powered in a mortar, weighted (300-400 mg), mineralised in 5 mL of nitric acid (69% v/v), 1 mL of H_2O_2, and finally transferred to a microwave oven (ETHOS 900-Milestone) with the following program:

$\Rightarrow$ 5 min 250W, 5 min 450W, 5 min 650W, 2 min 250W, 5 min ventilation.

The digest was made to 25 mL final volume with deionised water, filtered (45µm, Millipore) and then analysed for anion (Na^+) and heavy metals (As, Cd, Cr, Cu, Hg, Pb, Zn) using an Inductively Coupled Plasma Spectrometry/Atomic Emission Spectroscopy, ICP/AES (Spectro Ciros^{CCD}, Spectro Italia s.r.l).

3.3.8 Statistical analysis

Statistical analysis was computed using standard statistical packages (STATISTICA® for Windows). A statistical comparison of means was done with analysis of variance (ANOVA) followed by Post-Hoc test ($p<0.05$).

When normality and homogeneity of variance range did not occur, Kruskal Wallis non–parametric test was applied to evaluate the significance of difference between groups with a level of significance set to $p<0.05$. When the Kruskal Wallis test gave a significant result a pair wise Mann Whitney U-test was carried out to evaluate differences between each pair.

3.4 Results and discussion

3.4.1 Soil characterisation

3.4.1.1 Chemical-physical parameters

The surface (0-5 cm) and sub-surface (5-20 cm) sediment layers of the study site were characterized separately.

The measured chemical-physical parameters, in particular pH (H_2O), electrical conductivity, organic carbon, organic matter, cation exchange capacity and total Kjeldahl nitrogen, were all affected both by the sampling point (Cell1 and Cell2) and the soil horizons (0-5 and 5-20 cm). Differences in sediment texture were observed, with a remarkable variation in sand composition between the subcells. In particular, Cell1 presented a high proportion of sand, with respect to sediments in Cell2. The subcells in Cell2 planted with *P. australis* (P2) and S. *lacustris* (S2) were characterised by clayey soil (Figure 3.9). In addition, Cell2 clearly displayed higher values of all the above chemical-physical parameters (Table 3.7).

The bottom sediments were more alkaline than the surface layer, with a mean pH value of 8.02 in Cell1 and 8.03 in Cell2 – values ranged from 7.80 to 8.19 in Cell1 and 7.94 to 8.22 in Cell2 (Table 3.7).

a) **b)**

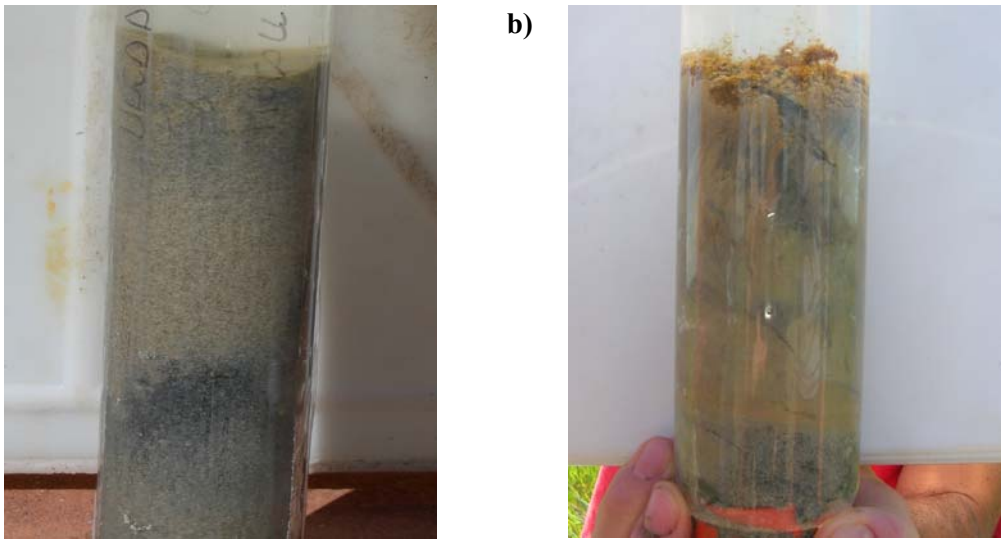


Figure 3.9 Particular of sediment cores collected from S1 (a) mainly sandy and from S2 (b) mainly characterized by clayey soil.

Electrical conductivity in the surface sediments was higher with respect to the deeper layer in both the Cells. Conductivity measured in surface sediments ranged from 288 to 437 µS (Cell1) and from 520 to 1140 µS (Cell2), whereas in 5-20 cm layer varied between 220 and 282 µS (Cell1) and between 300 and 740 µS (Cell2) (Table 3.7).

Total organic carbon and organic matter were higher in the surface sediments with greater values in Cell2 (P2 and S2), characterised by high content of clay (Table 3.7).

Cation exchange capacity (CEC) was higher in Cell2 than in Cell1, and generally higher in the surface layer. The mean values were of 5.16 meq 100 g^{-1} and 14.37 meq 100 g^{-1} respectively in Cell1 and in Cell2, with maximum value of 17.67 meq 100 g^{-1} measured in P2, subcell characterised by clayey texture (Table 3.7). The same trend was observed for total nitrogen (TKN). Mean values of 0.26 and 0.92‰ have been observed in surface sediments in Cell1 and Cell2 respectively, and mean values of 0.10 and 0.34‰ in the bottom layer in Cell1 and Cell2 respectively.

Table 3.7 Chemical- physical characteristics of surface (0-5 cm) and bottom soil-sediment (5-20 cm) in subcells planted with* T. latifolia *(T1 – T2),* P. australis *(P1 – P2),* S. lacustris *(S1 – S2).

Subcell	Layer (cm)	Texture	pH (H_2O)	Electric conductivity (µS)	Total organic carbon (%)	Organic matter (%)	CEC (meq 100 g^{-1})	TKN (‰)
T1	**0-5**	sandy	7.53	288	0.24	0.41	4.73	0.18
	5-20	sandy	7.80	220	0.10	0.17	3.87	0.10
T2	**0-5**	sandy loam	7.73	520	0.97	1.68	10.06	0.72
	5-20	sandy	7.94	325	0.28	0.48	5.34	0.19
P1	**0-5**	sandy	7.99	308	0.30	0.51	4.01	0.20
	5-20	sandy	8.19	241	0.09	0.15	4.91	0.06
P2	**0-5**	clay loam	7.99	572	1.45	2.51	17.67	0.83
	5-20	sandy	8.22	300	0.22	0.38	6.84	0.14
S1	**0-5**	sandy loam	7.77	437	0.47	0.81	6.73	0.39
	5-20	sandy soil	8.07	282	0.12	0.21	4.99	0.13
S2	**0-5**	sandy clay loam	7.69	1140	1.91	3.30	15.39	1.20
	5-20	clay	7.94	740	1.24	2.13	14.67	0.70

Dry weight/fresh weight (DW/FW) ratios in soil-sediments in Cell1 and Cell2, planted with the three macrophytes, *T. latifolia*, *P. australis* and *S. lacustris*, during

the sampling period – from July to November 2008, are reported in Table 3.8.

Sediment DW/FW ratio did not present significant variations among the sediments of the different Cells (Cell1 and Cell2), and the soil layers (0-5 and 5-20 cm) for the whole period, even though DW/FW ratios measured in the subcells characterised by clay loamy and clayey soils were generally lower than in sandy soils. Surface sediments collected in Cell2 were slightly lower than the other ratios, with a mean value of 0.62.

Table 3.8 Dry weight/fresh weight ratio of surface (0-5 cm) and bottom soil-sediment (5-20 cm) in subcells planted with* T. latifolia *(T1 – T2),* P. australis *(P1 – P2),* S. lacustris *(S1 – S2) during the sampling period (from June to November).

Subcell	Layer (cm)	*Jun*	*Aug*	*Sep*	*Nov*
T1	**0-5**	0.75±0.04	0.73±0.02	0.70±0.02	0.64±0.02
	5-20	0.80±0.02	0.82±0.002	0.74±0.004	0.79±0.004
P1	**0-5**	0.76±0.05	0.78±0.01	0.69±0.01	0.65±0.03
	5-20	0.79±0.05	0.82±0.001	0.79±0.01	0.76±0.03
S1	**0-5**	0.86±0.03	0.66±0.01	0.72±0.003	0.66±0.04
	5-20	0.79±0.01	0.78±0.05	0.79±0.004	0.80±0.004
T2	**0-5**	0.65±0.04	0.58±0.05	0.58±0.04	0.59±0.04
	5-20	0.82±0.04	0.78±0.01	0.79±0.004	0.75±0.01
P2	**0-5**	0.75±0.04	0.53±0.05	0.60±0.03	0.62±0.03
	5-20	0.82±0.03	0.74±0.01	0.71±0.01	0.76±0.01
S2	**0-5**	0.62±0.03	0.64±0.02	0.61±0.02	0.63±0.02
	5-20	0.75±0.04	0.73±0.002	0.75±0.01	0.74±0.001

3.4.1.2 Metals content

Heavy metals concentration was determined in sediment collected in Cell1 and Cell2 from the subcells planted with *T. latifolia*, *P. australis* and *S. lacustris* in surface (0-5 cm) and deep (5-20 cm) layers.

The soil-sediment collected in Cell1 and Cell2 did not show a significant variability in time.

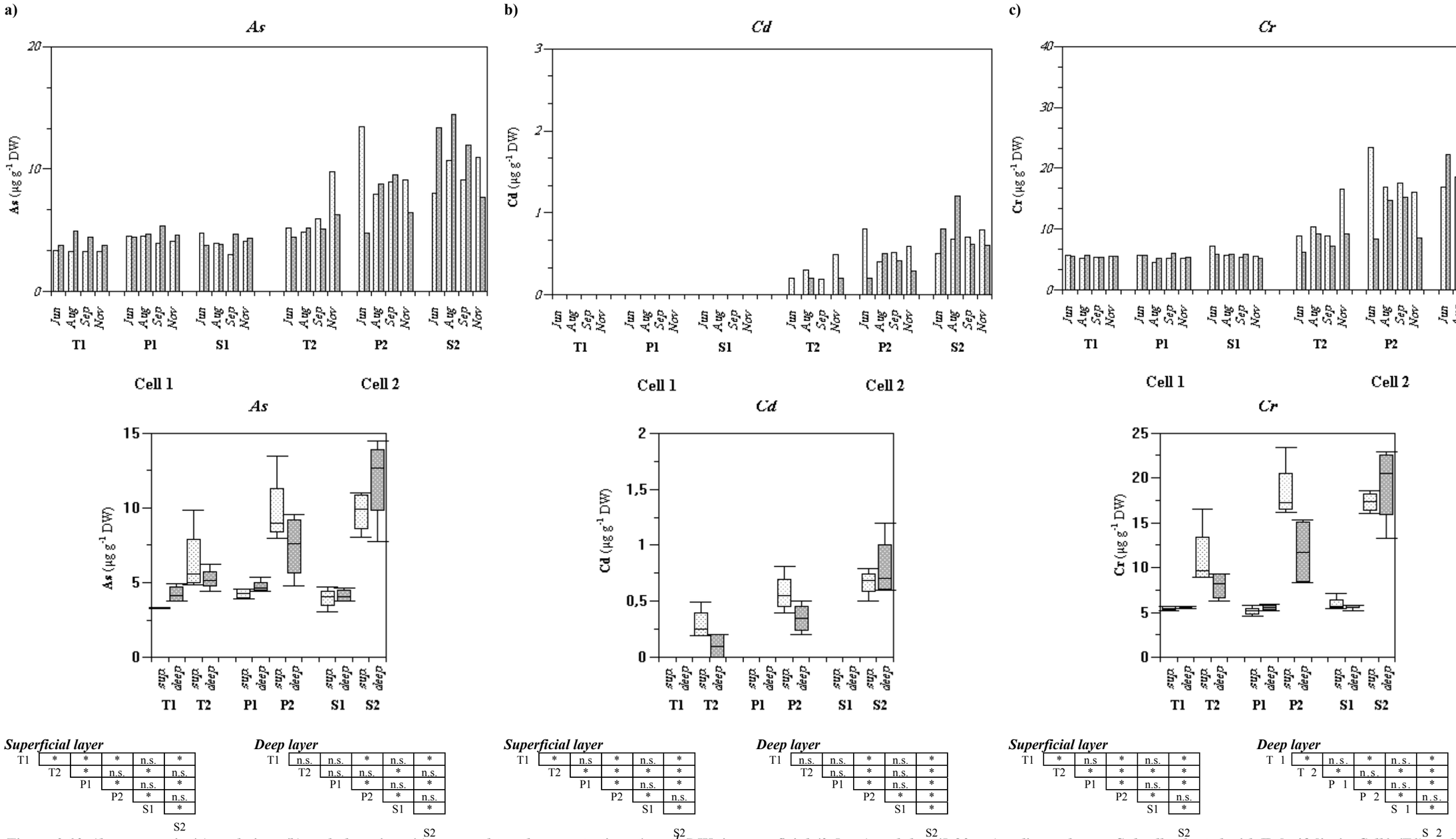
In most of the subcells, metals concentration in surface and deep layer were not significantly different, exception made for the subcell P2, where Cr, Cu, Pb and Zn

concentration in 0-5 cm layer was higher compared to 5-20 cm ($p<0.05$) (Figure 3.10, Figure 3.11).

Differences between Cell1 and Cell2 have been highlighted for all the considered metals, with values in Cell2 higher than in Cell1.

As pointed out from the box–plot representations (Figure 3.10, Figure 3.11), Cell2 presented a remarkably higher concentration values than Cell1 for most of the metals ($p<0.005$). Exceptions occurred for As, Cd and Pb in the deep layer, where the difference was not significant.

The mean heavy metal content of sediments are described in descending order of Zn>Cu>Cr>Pb>Ar>Cd.



Superficial layer

	T1	T2	P1	P2	S1	S2
T1	*	*	*	n.s.	*	
T2		*	n.s.	*	n.s.	
P1			*	n.s.	*	
P2				*	n.s.	
S1					*	
S2						

Deep layer

	T1	T2	P1	P2	S1	S2
T1	n.s.	n.s.	*	n.s.	*	
T2		n.s.	n.s.	*	n.s.	
P1			*	n.s.	*	
P2				*	n.s.	
S1					*	
S2						

Superficial layer

	T1	T2	P1	P2	S1	S2
T1	*	n.s	*	n.s.	*	
T2		*	*	*	*	
P1			*	n.s.	*	
P2				*	n.s.	
S1					*	
S2						

Deep layer

	T1	T2	P1	P2	S1	S2
T1	n.s.	n.s.	*	n.s.	*	
T2		n.s.	*	n.s.	*	
P1			*	n.s.	*	
P2				*	*	
S1					*	
S2						

Superficial layer

	T1	T2	P1	P2	S1	S2
T1	*	n.s	*	n.s.	*	
T2		*	*	*	*	
P1			*	n.s.	*	
P2				*	n.s.	
S1					*	
S2						

Deep layer

	T 1	T 2	P 1	P 2	S 1	S 2
T 1	*	n.s.	*	n.s.	*	
T 2		*	n.s.	*	*	
P 1			*	n.s.	*	
P 2				*	n.s.	
S 1					*	
S 2						

***Figure 3.10** Above, arsenic (a), cadmium (b) and chromium (c) temporal trend concentrations (µg g^{-1} DW) in superficial (0-5 cm) and deep(5-20 cm) sediment layers. Subcells planted with* **T. latifolia** *in Cell1 (T1) and Cell2 (T2), with* **P. australis** *in Cell1 (P1) and Cell2 (P2) and* **S. lacustris** *in Cell1 (S1) and Cell2 (S2).*
Below, box charts of metal sediment concentrations in superficial (sup) and deep layers. The top, bottom and middle line of the box corresponds to the 75th percentile (top quartile), 25th percentile (bottom quartile), and 50th percentile (median), respectively. The whiskers extend from the 10th percentile and the top 90th percentile.
*Significant differences in superficial and deep layers are indicated in tables below (Kruskal-Wallis test: *p<0.05; n.s. not significant).*

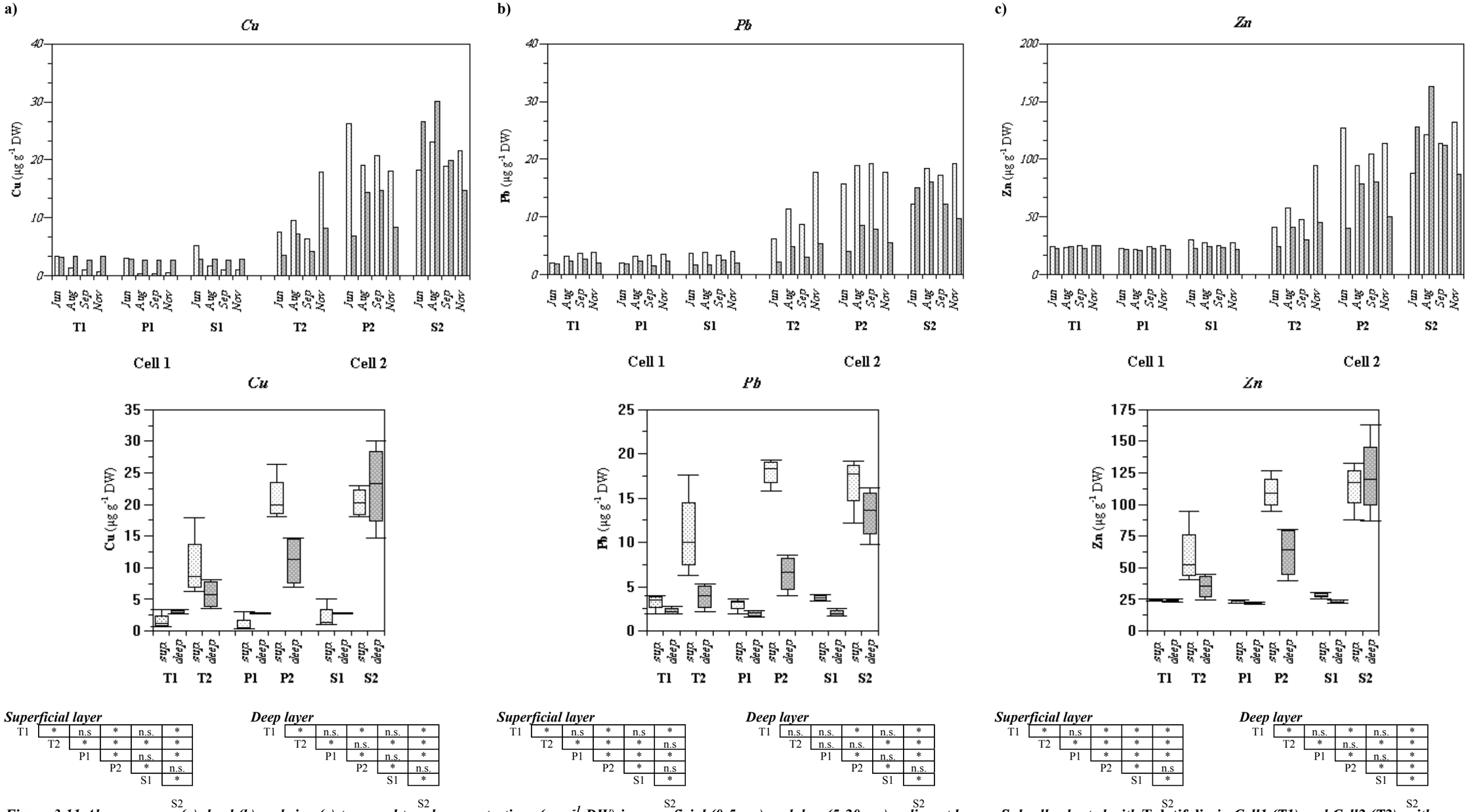


***Figure 3.11** Above, copper (a), lead (b) and zinc (c) temporal trend concentrations (µg g^{-1} DW) in superficial (0-5 cm) and deep(5-20 cm) sediment layers. Subcells planted with* **T. latifolia** *in Cell1 (T1) and Cell2 (T2), with* **P. australis** *in Cell1 (P1) and Cell2 (P2) and* **S. lacustris** *in Cell1 (S1) and Cell2 (S2).*
Below, box charts of metal sediment concentrations in superficial (sup) and deep layers. The top, bottom and middle line of the box corresponds to the 75th percentile (top quartile), 25th percentile (bottom quartile), and 50th percentile (median), respectively. The whiskers extend from the 10th percentile and the top 90th percentile.
*Significant differences in superficial and deep layers are indicated in tables below (Kruskal-Wallis test: *p<0.05; n.s. not significant).*

3.4.1.3 Discussion

The chemical-physical parameters analysed in the sediments collected from Cell1 and Cell2 did not show a temporal variation.

The studied sites Cell1 and Cell2 were well discriminated by the soil texture and chemical-physical characteristics, as well as metals concentration. Cell2 texture was mostly clayey, whereas Cell1 was mostly sandy. This difference in texture linked to higher levels of chemical-physical parameters and heavy metals concentrations in Cell2 with respect to Cell1. In fact, all metal concentrations were lower in subcells characterised by sandy soil than in clayey soils, which is probably due to the attraction of the clay micelle for metal cations (Stumm & Morgan, 1981; Sparling & Lowe, 1998).

The difference in texture of sediments between Cell1 and Cell2 can be attributed to the addition of sandy soil to the basin, during the wetland construction to obtain the right cells morphologic design profile. The additional soil came from the neighbouring Cassa di Colmata A site, where various and heterogenic soils were present, such as sandy loamy and clayey soil.

The organic carbon, organic matter, and heavy metals (As, Cd, Cr, Cu, Pb, Zn) concentration in soil were almost all significantly affected both by site and soil layers, whereas no temporal trend was evidenced. The surface layer of subcells T1, T2, P1 and P2 had the highest concentrations of As, Cd, Cr, Cu, Pb, Zn, whereas in S1 and S2 this behaviour was observed only for Pb. Sediments collected in Cell2 displayed the most pronounced differences among 0-5 and 5-20 cm layers.

3.4.2 Water quality

3.4.2.1 Chemical and physical parameters

Water samples were manually collected during July, August, September, October and November from the inlet (IN) and from the two outlets (OUT1 and OUT2) of the pilot wetland system.

Results of the field measurements – temperature and pH – are reported in Table 3.9.

The mean pH values in the incoming water for the whole period was about 8.00, whereas in the effluents was about 7.84 for OUT1, and 7.68 for OUT2 (Table 3.9).

Table 3.9 Temperature (°C) and pH of incoming and outcoming water during July – November 2008. *Intensive sampling performed in July, in particular on a) 08/07/08, b) 09/07/08, c) 11/07/08.

Sampling data	IN		OUT1		OUT2	
	T	pH	T	pH	T	pH
Jul*a	25.8	7.70	27.7	7.70	26.2	7.69
Jul*b	24.8	7.65	24.6	7.78	24.6	7.70
Jul*c	26.8	8.01	28.6	7.78	27.8	7.65
Aug	26.5	8.05	28.0	7.94	27.2	7.58
Sep	23.9	7.96	23.9	7.83	23.9	7.68
Oct	16.8	8.17	16.6	7.90	15.4	7.82
Nov	15.3	8.46	15.5	7.94	16.4	7.66
Average	22.8	8.00	23.5	7.84	23.1	7.68
St. dev.	4.8	0.28	5.4	0.09	5.1	0.07

Seasonal changes in dissolved oxygen concentration were observed in the inlet and in the two outlets, although dissolved oxygen percent saturation during the sampling period was higher than 50%.

The biological oxygen demand (BOD_5) ranged between 0.6 and 2.7 mg L^{-1} in the pilot system (Table 3.10). Those values are relatively low if compared with other systems (Tanner et al., 1999; Vanier & Dahab, 2001).

Table 3.10 Biological oxygen demand (BOD_5) concentration of incoming and outcoming water during July – November 2008. Data is expressed as mg L^{-1}. *Intensive sampling performed in Jul: a) 08/07/08, b) 09/07/08, c) 11/07/08.

Sampling data	IN	OUT1	OUT2
Jul*a	1.8	1.2	0.6
Jul*b	1.7	1.6	1.0
Jul*c	1.9	1.1	1.1
Aug	1.8	1.5	1.2
Sep	0.9	1.8	1.7
Oct	0.8	1.2	1.2
Nov	2.7	1.8	2.5
Average	1.6	1.4	1.3
St. dev.	0.6	0.3	0.6

BOD concentration was usually higher in incoming water (except in September and October) than the effluents.

Removal efficiency was positive in almost all the sampling period, except in September and October (Figure 3.12). Mean removal efficiency values were about 26% in Cell1 and 37% in Cell2, without considering the negative values. Higher removal capacity occurred in July in both Cell1 and Cell2, with maximum value of 44% and 65% respectively.

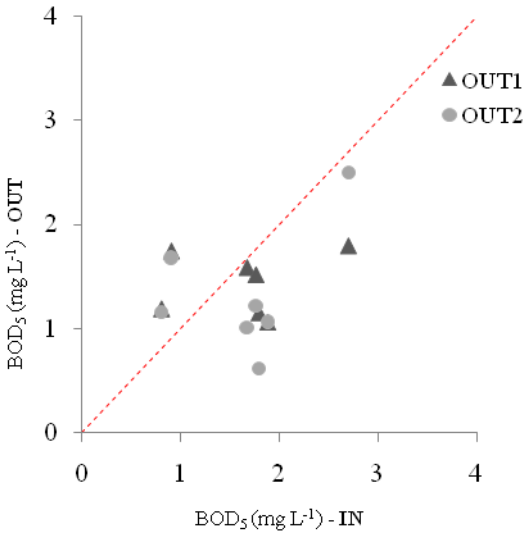


Figure 3.12 Inlet BOD_5 concentration versus outlets concentrations at each sampling time. Dashed red line indicates the 1:1 regression, corresponding to 0 removal efficiency. Data is expressed as mg L^{-1}.

Total suspended solid (TSS) concentration in the pilot system ranged between 0.6 and 74.9 mg L^{-1}.

In the incoming water, the values resulted higher compared to the outlets, whit the exception in November, in particular in OUT2 (Table 3.11; Figure 3.13). At this sampling time the higher TSS concentration measured in OUT2 was probably due to the turbidity in the cell caused by the high algae productivity occurred in Cell2 that could have affected sampling collection and results.

Table 3.11 Total suspended solid (TSS) concentration of incoming and outcoming waterduring July – November 2008. Data is expressed as mg L^{-1}. *Intensive sampling performed in July: a) 08/07/08, b) 09/07/08, c) 11/07/08.

Sampling data	IN	OUT1	OUT2
Jul*a	74.93	6.50	14.14
Jul*b	23.10	5.93	0.91
Jul*c	19.02	4.43	0.57
Aug	43.10	1.40	2.42
Sep	41.70	4.48	1.06
Oct	41.26	1.80	1.30
Nov	3.66	3.07	6.14
Average	35.25	3.94	3.79
St. dev.	22.81	1.95	4.95

The higher value in the influent measured at the first sampling time (7th of July 2008) was probably due to the turbidity caused by the rainfall occurred some day before (31.4 mm on the 6th and 1.2 mm on the 7th of July 2008) the sampling collection (Table I. 1 in Annex I). Removal efficiency was positive during the sampling time, with mean value of 74% in Cell1 and 67% in Cell2. The maximum removal occurred in correspondence of the maximum inlet TSS concentration. As shown in Figure 3.13, the removal was almost constant during the sampling time, even with increasing inlet TSS concentration.

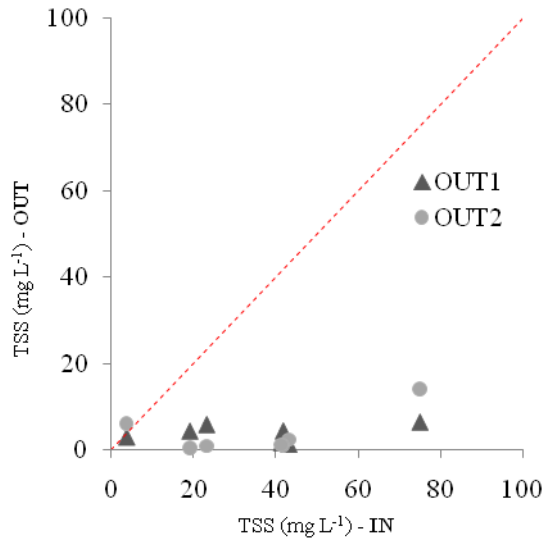


Figure 3.13 Inlet TSS concentration versus outlets concentrations at each sampling time. Dashed red line indicates the 1:1 regression, corresponding to 0 removal efficiency. Data is expressed as mg L^{-1}.

Total organic carbon (TOC) ranged between 1.57 and 4.10 mg L^{-1} in the inlet, whereas ranged between 3.78 to 12.14 mg L^{-1} in OUT1 and between 3.26 to 5.95 mg L^{-1} in OUT2 (Table 3.12).

Table 3.12 Total organic carbon (TOC) concentration of incoming and outcoming water during July – November 2008. Data is expressed as mg L^{-1}. *Intensive sampling performed in July: a) 08/07/08, b) 09/07/08, c) 11/07/08.

Sampling data	IN	OUT1	OUT2
Jul*a	3.08	5.05	3.71
Jul*b	3.89	12.14	3.26
Jul*c	2.57	5.04	3.52
Aug	3.96	4.83	5.95
Sep	2.96	6.11	5.73
Oct	1.57	3.78	4.11
Nov	4.10	3.77	5.31
Average	3.16	5.82	4.51
St. dev.	0.91	2.90	1.12

TOC concentration was usually lower at inlet than at outlets, probably due to the internal sources of TOC (soil and vegetation) (Figure 3.14). Aquatic plants modify the system by their own production of organic matter (Carpenter, 1981; Barko & Smart, 1983).

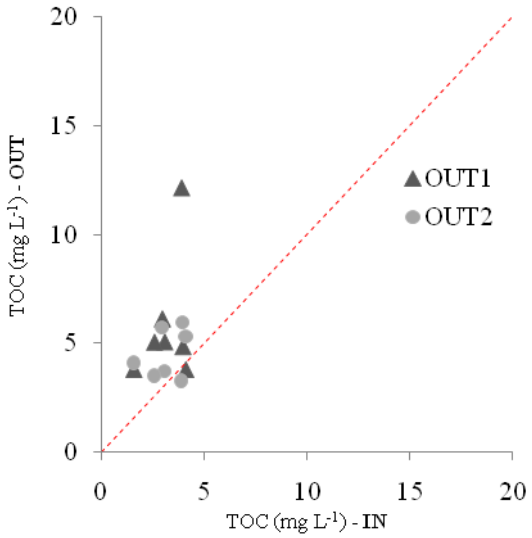


Figure 3.14 Inlet TOC concentration versus outlets concentrations at each sampling time. Dashed red line indicates the 1:1 regression, corresponding to 0 removal efficiency. Data is expressed as mg L^{-1}.

3.4.2.2 Nutrients content

The nitrate concentration was always higher in the inlet water, with a mean value about 1183 μg L^{-1} and a maximum value (1900 μg L^{-1}) in October (Table 3.13). At the outlets, nitrate in water was relatively constant for the whole period, with mean values of about 270 μg L^{-1} (OUT1) and 120 μg L^{-1} (OUT2).

The mean ammonia concentration in the incoming water was 133 μg L^{-1}, with a maximum peak in July (420 μg L^{-1}). The minimum values occurred in October and November (10 μg L^{-1}). Ammonia concentration in OUT1 and OUT2 was relatively constant during the whole sampling period, with values lower than the inlet concentration, with the exception of values measured in October and November, with ammonia concentration slightly higher in the outlets in respect to the inlet.

The mean phosphate concentration in water at the inlet was 81 μg L^{-1}. The maximum value occurred in July (140 μg L^{-1}) and the minimum in October (26 μg L^{-1}). The phosphate concentration in water at the outlets was relatively constant, with mean value of about 9 and 5 μg L^{-1} in OUT1 and OUT2, respectively.

Table 3.13 Nutrient concentration of the incoming and outcoming water during the period July – November 2008. Data is expressed as μg L^{-1}. <d.l.: below detection limit (10 and 2 μg L^{-1} for NH_4^+-N and PO_4^{3-}-P, respectively); n.d.: no data.*Intensive sampling performed in July, in particular on a) 08/07/08, b) 09/07/08, c) 11/07/08.

Sampling data	NH_4^+-N			NO_3^--N			PO_4^{3-}-P		
	IN	**OUT1**	**OUT2**	**IN**	**OUT1**	**OUT2**	**IN**	**OUT1**	**OUT2**
Jul[*a]	290	26	48	900	400	200	110	<d.l.	6
Jul[*b]	420	28	26	800	100	100	140	10	3
Jul[*c]	63	<d.l.	12	1000	400	100	100	4	4
Aug	63	14	<d.l.	1400	300	100	57	6	4
Sep	73	27	14	1100	100	100	56	6	6
Oct	10	14	27	1900	300	100	26	20	4
Nov	10	12	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Average	153	22	25	1183	267	117	81	9	5
St. dev.	163	7	14	407	137	41	42	6	1

As shown in Figure 3.15, removal efficiency was positive during the sampling time, with mean values of about 84%, 76% and 82% in Cell1 and 87%, 89% and 92% in Cell2 for ammonia, nitrate and phosphate, respectively. In all three cases, the

removal was almost constant during the sampling time, when increasing the inlet TSS concentration.

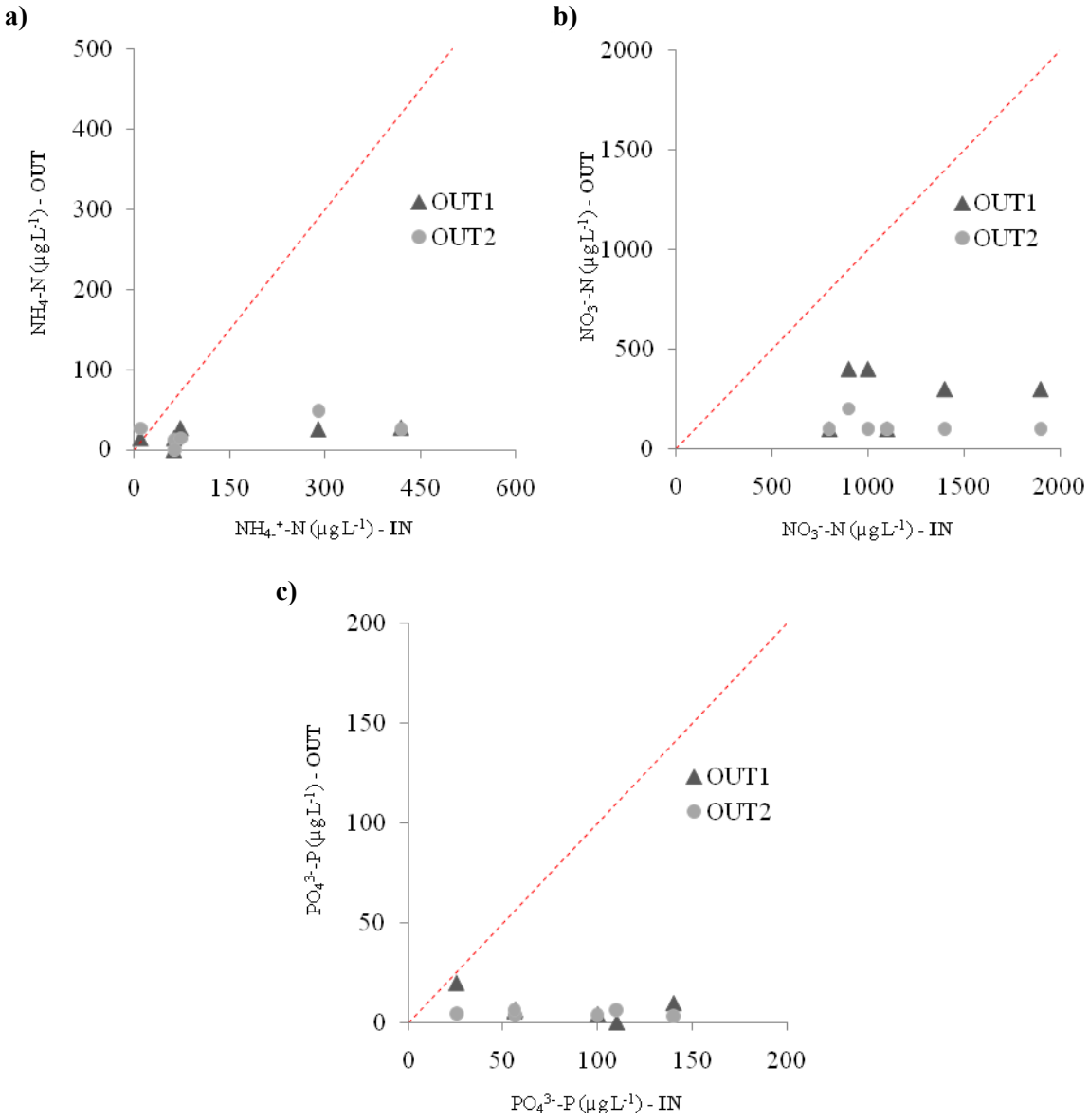


Figure 3.15 Inlet a) NH_4^+-N, b) NO_3^--N and c) PO_4^{3-}-P concentrations versus outlets a) NH_4^+-N, b) NO_3^--N and c) PO_4^{3-}-P concentrations at each sampling time. Dashed red line indicates the 1:1 regression, corresponding to 0 removal efficiency. Data is expressed as µg L^{-1}.

The concentrations of nitrogen and phosphorus (total and dissolved) were always higher in incoming water (IN) than in the outcoming water (OUT1 and OUT2) (Table 3.14).

The mean total and total dissolved nitrogen content in the incoming water for the whole period were about 2400 μg L^{-1} and 2225 μg L^{-1} respectively, with a maximum peak – 2900 μg L^{-1} (TN) and 2700 μg L^{-1} (TDN) – measured in October. In OUT1 and OUT2, total and dissolved nitrogen was relatively constant, with mean values of TN of 575 μg L^{-1} in OUT1 and 513 μg L^{-1} in OUT2 and with mean values of TDN of 518 and 491 μg L^{-1} in OUT1 and OUT2, respectively (Table 3.14).

Table 3.14 Total nitrogen (TN), dissolved nitrogen (TDN), phosphorus (TP) and dissolved phosphorus (TDP) concentration of incoming and outcoming water during July – November 2008. Data is expressed as μg L^{-1}. n.d.: no data.*Intensive sampling performed in July: a) 08/07/08, b) 09/07/08, c) 11/07/08.

Sampling data	TN			TDN			TP			TDP		
	IN	OUT1	OUT2	IN	OUT1	OUT2	IN	OUT1	OUT2	IN	OUT1	OUT2
Jul[*a]	2500	610	530	2500	540	520	230	28	35	130	9	17
Jul[*b]	2300	580	440	2100	570	430	200	25	23	160	18	15
Jul[*c]	2200	660	400	1900	500	380	190	26	20	110	13	13
Aug	2300	590	590	2200	560	580	140	25	23	69	15	20
Sep	2200	600	550	2100	560	470	140	28	25	65	17	15
Oct	2900	550	530	2700	480	500	110	28	18	39	10	31
Nov	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Average	2400	598	507	2250	535	480	168	27	24	95	14	18
St. dev.	268	37	72	295	37	70	45	1	6	46	4	6

Mean total nitrogen and total phosphorus removal efficiency was about 75% and 83% in Cell1 and 78% and 85% in Cell2, whereas mean total dissolved nitrogen and total dissolved phosphorus removal efficiency was about 76% and 82% in Cell1 and 79% and 83% in Cell2 (Figure 3.16).

As previously noted, the removal efficiency was influenced by inlet nitrogen and phosphorus concentrations, with constant removal during the sampling time, correspondent to increasing values of inlet concentrations.

Total and total dissolved phosphorus concentration measured in the incoming water decreased from July to October, whit mean values of 168 μg L^{-1} (TP) and 95 μg L^{-1} (TDP). In OUT1 and OUT2 water total and dissolved phosphorus were relatively constant, with TP mean values of 27 μg L^{-1} (OUT1) and 24 μg L^{-1}L (OUT2) and TDP mean values of 14 μg L^{-1} (TP) and 18 μg L^{-1} (OUT2).

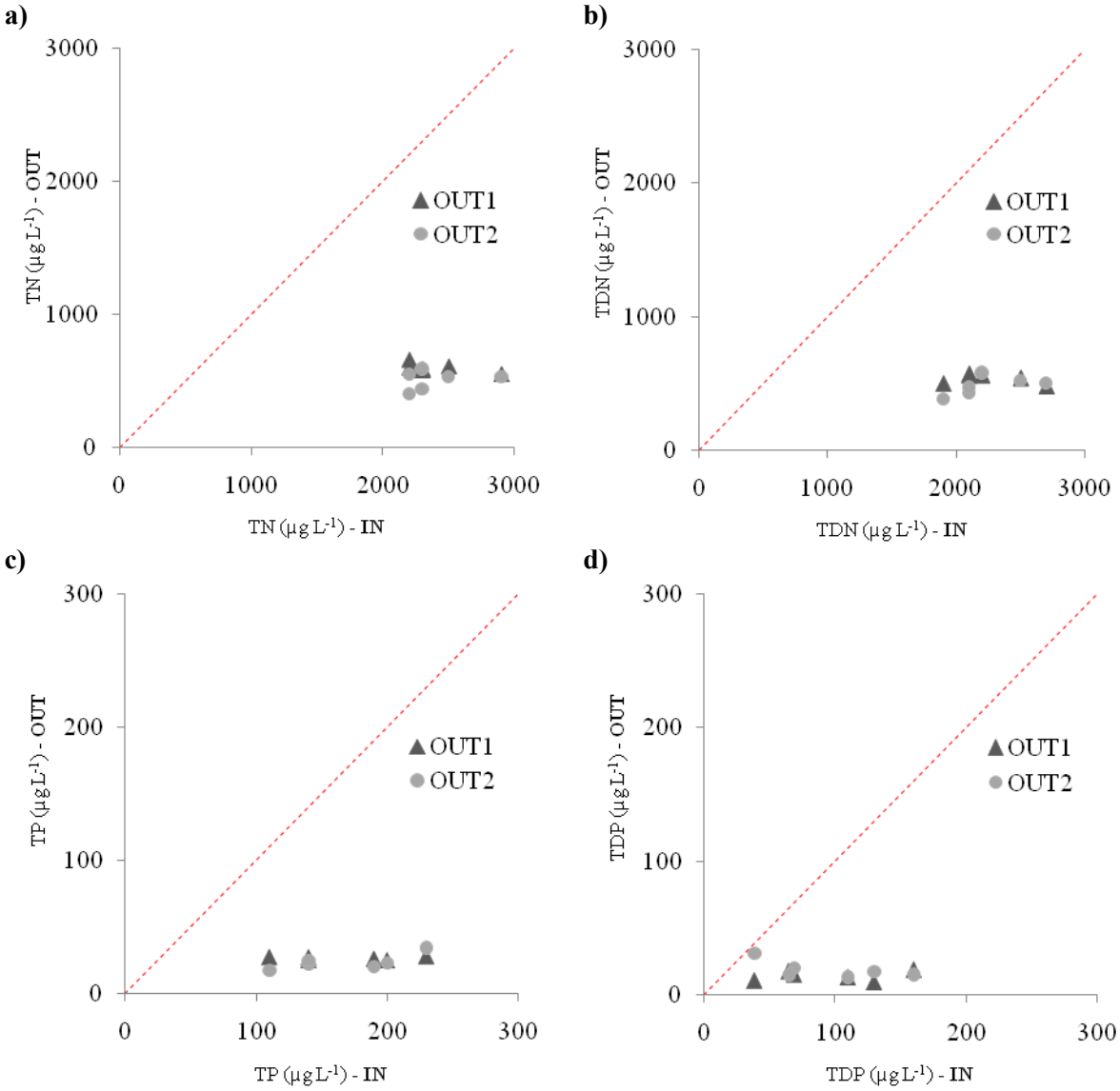


Figure 3.16 Inlet a)TN, b)TDN, c)TP concentrations versus outlets a)TN, b)TDN, c)TP and d)TDP concentrations at each sampling time. Dashed red line indicates the 1:1 regression, corresponding to 0 removal efficiency. Data is expressed as µg L^{-1}.

3.4.2.3 Metals content

The incoming and outcoming water (filtered and unfiltered) was also analysed for heavy metals concentration (As, Cd, Cr, Cu, Hg, Pb, Zn). Due to the low concentration in the water, only Cu and Zn were detected in the unfiltered water, whereas no heavy metals, in concentration above to the detection limit were detected in filtered water samples.

Copper concentration in water was higher at the inlet than at the outlets for the whole period, with maximum values (32.00 µg L^{-1} at IN, 22.67 and 18.67 µg L^{-1} at OUT1 and OUT2, respectively) in July (09/07/08) (Table 3.15).

Mean zinc concentration was about 28 µg L^{-1} in the incoming water. Maximum value of zinc occurred in July (08/07/2009) in the inlet (106.67 µg L^{-1}) and in OUT1 (77 µg L^{-1}), whereas in November in OUT2 (37 µg L^{-1}) (Table 3.15).

Table 3.15 Total heavy metal concentration of incoming and outcoming water during July – November 2008. Data is expressed as µg L^{-1}. < d.l.: below detection limit (4 µg L^{-1}); As, Cd, Cr, Hg and Pb were always below the detection limit (6, 1, 2, 1 and 9 µg L^{-1}, respectively) and are not included. *Intensive sampling performed in July: a) 08/07/08, b) 09/07/08, c) 11/07/08.

Sampling data	Cu			Zn		
	IN	OUT1	OUT2	IN	OUT1	OUT2
Jul*[a]	9.33	< d.l.	6.67	106.67	77.30	< d.l.
Jul*[b]	32.00	22.67	18.67	4.00	< d.l.	< d.l.
Jul*[c]	17.33	13.33	13.33	4.00	< d.l.	< d.l.
Aug	2.67	< d.l.	< d.l.	9.33	< d.l.	< d.l.
Sep	2.67	< d.l.	< d.l.	13.33	< d.l.	< d.l.
Oct	2.67	< d.l.	< d.l.	14.67	< d.l.	< d.l.
Nov	< d.l.	< d.l.	4.00	42.67	< d.l.	37.00

Mean copper removal efficiency was about 34% and 28% in Cell1 and Cell2, respectively and concerning zinc, was about 63% in Cell1 and 75% in Cell2, respectively.

3.4.2.4 Toxicity tests

According with current legislation a battery of toxicity tests – *Daphnia magna*, *Pseudokirchneriella subcapitata* and Microtox® – was applied in order to evaluate the acceptability of the effluents water of the pilot wetland.

As previously reported (Par. 3.3.6.10), *Daphnia magna* test is based on the observation of organism immobilisation after 24 hours of exposition (APAT IRSA-CNR, 2003b). Microtox® is an acute toxicity test based on the inhibition of bioluminescence of the marine bacterium *Vibrio fischeri* (Microbics Corp., 1999).

As regard the freshwater chlorophyte *Pseudokirchneriella subcapitata*, the endpoint is based on the adverse effects on cell growth after 72 hours exposure period of test algae (USEPA, 2002; ASTM, 2004). According to standard Italian guidelines, an effluent can be considered acceptable and dischargeable to a water body if the effect percentage in toxicological tests is less than 50% (D. Leg. N°152, 2006).

Considering the battery of toxicity tests applied in this study, no toxicity was identified in all the samples. Mean percentage inhibition (or effects) of biological tests during whole period was <50%, and so, following Italian Legislation standard quality, the water at inlet (IN) and outlets (OUT1 and OUT2) of the pilot wetland resulted acceptable (Table 3.16).

The *Daphnia magna* test results, expressed as immobilization percentage, shown that the water at inlet and at both effluents was not toxic to *D. magna*. Mean percentages of inhibition were 3% in the inlet and 1% in the two outlets (Table 3.16).

Table 3.16 Biological test results (*D.magna *24-h,* P.subcapitata *72-h, Microtox® 30-min) of incoming and outcoming water during July – November 2008. Data is expressed as percent inhibition (I%) on the test organism over the test duration with respect to control.

Sampling data	IN			OUT1			OUT2		
	D. magna	*P. subcapitata*	Microtox®	*D. magna*	*P. subcapitata*	Microtox®	*D. magna*	*P. subcapitata*	Microtox®
Jul	0%	-432%	-16%	0%	-206%	14%	0%	-167%	20%
Aug	10%	-90%	26%	3%	-95%	21%	0%	32%	0%
Sep	3%	-282%	27%	3%	-136%	9%	3%	-85%	6%
Oct	0%	-152%	27%	0%	-64%	7%	0%	-121%	0%
Nov	3%	-58%	15%	0%	-92%	23%	0%	-56%	-5%

Green algae test results shown growth stimulation in all the samples – highlighted by the negative value – with lower I% in the inlet than in the outlets (Table 3.16). Mean values were about -200% in the inlets and -120% and -80% in OUT1 and OUT2, respectively. The toxicity detected in August in Cell2 could be due to the salinity occurred in the sample (2), considering that *P. subcapitata* can be used only for freshwater samples with salinity less than 1 (Sbrilli et al., 2003).

Microtox® test revealed a low toxicity, with mean I (%) of 16% in the inlet, 15% and 4% in OUT1 and OUT2 respectively (Table 3.16).

3.4.2.5 Discussion

The constructed wetland removal efficiencies for the various water quality parameters analysed in these study are presented in Table 3.17. Removal efficiencies were very similar between Cell1 and Cell2. Removal efficiency obtained in the pilot wetland was high and close to efficiency found in literature (Vanier & Dahab, 2001; Karathanasis et al., 2003).

Water BOD_5 concentration measured in September and October at the outlets was slightly higher than at inlet. BOD in wetland systems may derive from inputs of organic matter such as sewage effluent, surface runoff, and living organisms and litter decomposition as well as from internal BOD production. Wetlands BOD production can be attributed to various processes, such as algal growth, leaching of organic carbon from live and decaying plant material, and ammonia production to anaerobic sediments (Stringfellow et al., 2008). As previously explained (Par. 1.5.2), in wetlands, BOD is mostly removed through bacterial decomposition of organic matter.

Table 3.17 Mean and maximum removal capacity (%) relative to the two Cells (OUT1 and OUT2) to the different chemical parameters. *indicates that some values were omitted to the calculation (see explanation in the text).

	OUT1		**OUT2**	
	removal	*max*	*removal*	*max*
BOD_5*	26%	44%	37%	65%
TSS	74%	96%	67%	97%
$N\text{-}NH_4$*	84%	95%	87%	95%
$N\text{-}NO_3$	76%	91%	89%	95%
$P\text{-}PO_4$	82%	99%	92%	98%
TN	75%	81%	79%	82%
TDN	76%	82%	79%	81%
TP	83%	88%	85%	89%
TDP	82%	93%	73%	91%
Cu	34%	79%	28%	42%
Zn	63%	86%	66%	98%

Total organic carbon in the water entering the wetland was lower than concentration measured in the two effluents during whole the sampling time, even though values were comparable with those measured in other wetlands (Stringfellow et al., 2008). This result suggests that processes involved in internal carbon cycle, such as decomposition of vegetation were present (Kadlec & Knight, 1996).

Total suspended solids (TSS), nitrogen and phosphorus removal capacity was higher, with value about 70-80%, as confirmed in other studies (Tanner et al., 1995a; Karathanasis et al., 2003; Greenway, 2004).

During the whole period a considerable reduction in both total and dissolved inorganic nutrients occurred in both Cell1 and Cell2, demonstrating the important roles of macrophytes and periphyton in the wetland. The presence of dense macrophyte zones, improves biological uptake to the phytoplankton community and the attached periphyton in the narrow littoral zone.

Metals concentration for almost all the metals considered (As, Cd, Cr, Hg and Pb) were always below the detection limit and only copper and zinc were detected in the unfiltered water. Removal efficiency of Cu and Zn was positive, with mean values about 30% and 60% for Cu and Zn, respectively.

Considering total suspended solids and nutrients, we found that removal efficiency was affected by inlet concentration, with higher removal corresponding to higher values of inlet concentrations.

Toxicity tests integrated the chemical results and, in this study, shown no toxic effects in the effluent. Stimulation in algae growth indicated a grade of eutrophication, common in closed pond and wetlands, characterised by low flow conditions.

In conclusion, by observing the performance and test results we demonstrated that Fusina pilot wetland shown quick and efficient start-up behaviour. The reason for this might be correlated to the efficient design of the pilot-scale wetland, with the alternation of deep and shallow zones.

3.4.3 Vegetation

3.4.3.1 Weight growth dynamics

Fresh weight (FW) of the aerial part of *T. latifolia*, *P. australis* and *S. lacustris* did not present an evident seasonal trend, with a high variability during sampling period (Table 3.18). Plant FW values seem not to be affected by the location (Cell1 and Cell2), even though some punctual significant differences between Cell1 and Cell2 were measured.

Higher *T. latifolia* and *S. lacustris* FW mean values were measured in September in Cell1 and in August in Cell2, whereas in *P. australis* higher mean weight occurred in August in both Cell1 and Cell2.

Table 3.18 Seasonal change of fresh weight (g) of aerial part in* T. latifolia, P. australis, S. lacustris *in Cell1 and Cell2 (mean±SD, n=5).

Sampling data	Plant species	Cell1 *aerial part*	Cell2 *aerial part*
Jul	*T. latifolia*	35.2±11.6	54.8±17.2
	P. australis	13.6±2.4	12.6±5.7
	S. lacustris	19.2±8.9	38.69±12.6
Aug	*T. latifolia*	26.8±10.9	84.0±17.5
	P. australis	19.2±7.0	20.2±6.0
	S. lacustris	25.4±13.4	48.2±19.7
Sep	*T. latifolia*	87±17.7	61.6±1.6
	P. australis	10.6±5.5	11.0±4.2
	S. lacustris	85.8±29.5	29.2±12.0
Nov	*T. latifolia*	86.4±41.4	88.8±17.2
	P. australis	13.2±6.0	16.2±5.6
	S. lacustris	38.9±12.4	37.5±9.7

The seasonal trend in shoot length of *T. Latifolia*, *P. australis* and *S. lacustris* measured directly in the field in July, August, September and November is reported in Table I. 9 in Annex I.

No significant differences in shoot length of plants species growing in the same cell occurred during the sampling time.

As represented in Figure 3.17 (a), *T. latifolia* plants were significantly higher in Cell1 than in Cell2 in September and November ($p<0.05$), whereas *P. australis* shown a higher growth rate in Cell2 during all the sampling period ($p<0.05$), in particular in September and November ($p<0.01$) (Figure 3.17 (b)). No significant differences in length of *S. lacustris* plants between Cell1 and Cell2 were found, although plants growing in Cell2 shown a higher growth rate than in Cell1 (Figure 3.17 (c)).

The maximum plant length occurred in August in *T. latifolia* (159 cm in Cell1 – 162 cm in Cell2) and *S. lacustris* (118 cm in Cell1 – 120 cm in Cell2), and in September for *P. australis* (226 cm in Cell1 – 252 cm in Cell2).

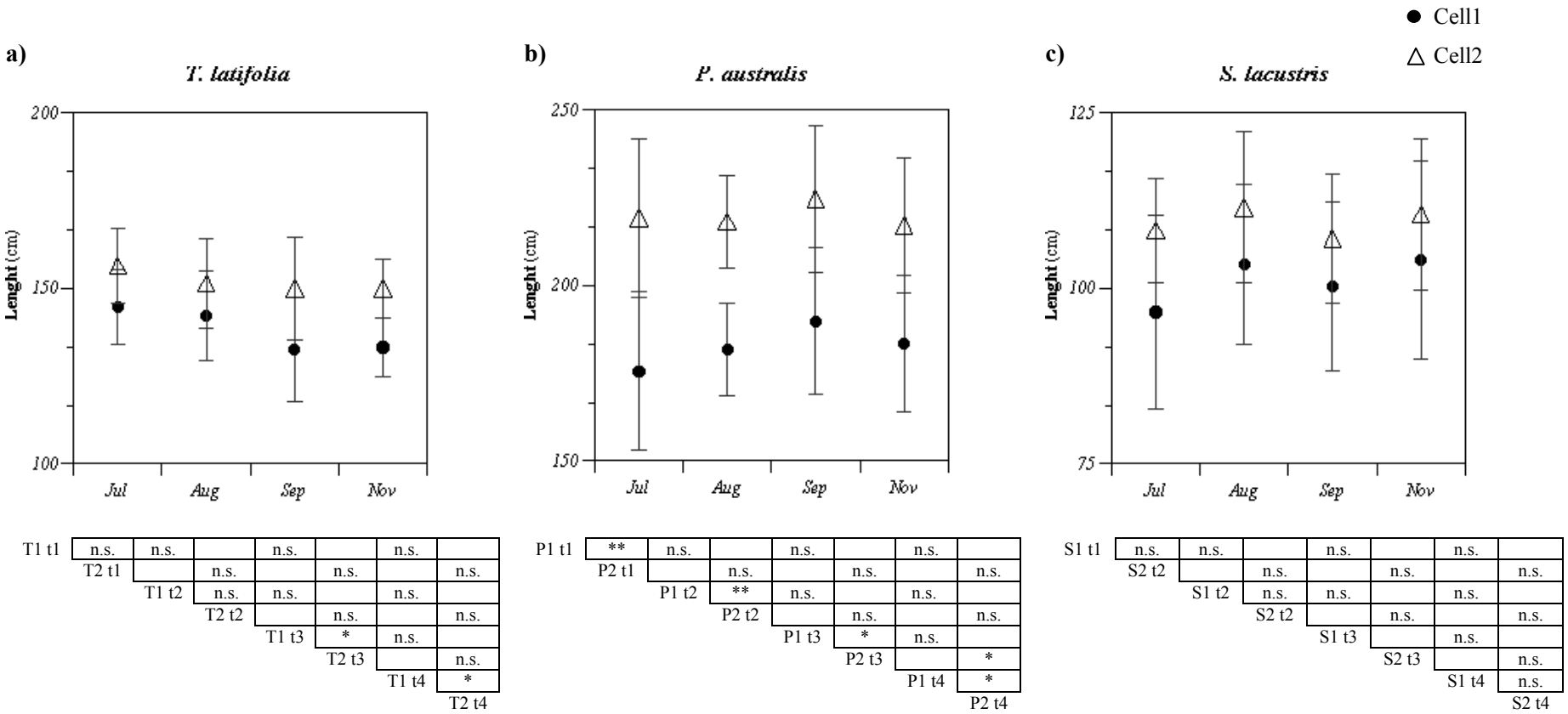


Figure 3.17 Above, seasonal trend of shoot length (cm) of* T. latifolia *(a),* P. australis *(b) and* S. lacustris *(c) in Cell1 (black point) and Cell2 (white triangle) (mean±SD, n=5).
Below, statistical analyses (Post Hoc test: *p<0.05; **p<0.01, n.s. not significant) indicating statistically significant shoot length differences between Cell1 and Cell2 during the time: July (t1), August (t2), September (t3), November (t4); subcells planted with* T. latifolia *in Cell1 (T1) and Cell2 (T2), with* P. australis *in Cell1 (P1) and Cell2 (P2) and* S. lacustris *in Cell1 (S1) and Cell2 (S2).

The dry weight/fresh weight ratio (DW/FW ratio) of shoot or stem was determined in *T. latifolia*, *P. australis* and *S. lacustris* collected in Cell1 and Cell2 during the sampling period (July-August-September-November), whereas DW/FW ratio of root and rhizome was determined only at the beginning and at the end of the growing season (July and November) (Table 3.19).

The rhizome DW/FW ratios in *T. latifolia* and *P. australis* in Cell1 and Cell2 were not significantly different during the sampling period. In *T. latifolia* the ratio ranged 0.13÷0.25 and 0.13÷0.21 in Cell1 and Cell2 respectively, whereas in *P. australis* it was 0.18÷0.39 in Cell1 and 0.19÷0.31 in Cell2. The root DW/FW ratio was significantly higher in *T. latifolia* ($p<0.05$) in November in Cell1, with a maximum value of 0.29, whereas for *P. australis* the highest value was reached in July ($p<0.01$) in Cell1.

In *S. lacustris* root DW/FW ratio was not different during sampling period, with a minimum value in July (0.13 and 0.10 in Cell1 and Cell2, respectively) and a maximum value in November (0.21 and 0.31 in Cell1 and Cell2, respectively). In *T. latifolia*, no significant difference in rhizome/root DW/FW ratio between Cells was observed.

The shoot DW/FW ratio in *T. latifolia* was lower in July both in Cell1 and Cell2, with a significant increase from July to August (Table 3.19) ($p<0.05$ and $p<0.001$ in Cell1 and Cell2 respectively). In *S. lacustris* there was a significant difference ($p<0.05$) only in Cell1 between July and August and between September and November, with maximum value in September (Cell1) and July (Cell2) and minimum value in July (Cell1) and in August (Cell2) (Table 3.19). No difference between the plants of *T. latifolia* of the Cell1 and Cell2 was evidenced, whereas for *S. lacustris* in July there was a significant difference between Cells ($p<0.05$).

The stem DW/FW ratio in *P. australis* was significantly different during the sampling period in both Cells, and ranged from 0.39 in November to 0.68 in July in Cell1and from 0.33 in August to 0.66 in September in Cell2. Maximum values for shoot DW/FW were reached in July in Cell1 and in September in Cell2. No difference in stem and shoot DW/FW ratio were detected between Cell1 and Cell2 during the sampling period.

Table 3.19 Dry/fresh weight (DW/FW) ratio of the three plant species in Cell1 and Cell2 during the sampling period (mean±SD, n=3, n.d.: no data).

Sampling data	Plant species	Cell1				Cell2			
		rhizome	*roots*	*stems*	*leaves/shoot*	*rhizome*	*roots*	*stems*	*leaves/shoot*
Jul	*T. latifolia*	0.15±0.02	0.15±0.01	-	0.22±0.04	0.16±0.03	0.16±0.04	-	0.20±0.08
	P. australis	0.21±0.02	0.33±0.09	0.64±0.04	0.78±0.13	0.25±0.05	0.14±0.02	0.59±0.01	0.67±0.03
	S. lacustris	-	0.16±0.02	-	0.17±0.02	-	0.12±0.02	-	0.24±0.07
Aug	*T. latifolia*	n.d.	n.d.	-	0.31±0.01	n.d.	n.d.	-	0.35±0.01
	P. australis	n.d.	n.d.	0.47±0.08	0.78±0.03	n.d.	n.d.	0.42±0.13	0.50±0.12
	S. lacustris	-	-	-	0.25±0.03	-	-	-	0.25±0.02
Sep	*T. latifolia*	n.d.	n.d.	-	0.34±0.04	n.d.	n.d.	-	0.34±0.02
	P. australis	n.d.	n.d.	0.62±0.01	0.70±0.17	n.d.	n.d.	0.61±0.02	0.79±0.17
	S. lacustris	-	-	-	0.27±0.05	-	-	-	0.27±0.02
Nov	*T. latifolia*	0.22±0.05	0.23±0.08	-	0.32±0.09	0.18±0.03	0.20±0.01	-	0.26±0.05
	P. australis	0.26±0.11	0.36±0.03	0.43±0.04	0.55±0.08	0.23±0.04	0.27±0.04	0.38±0.06	0.46±0.07
	S. lacustris	-	0.19±0.02	-	0.21±0.01	-	0.22±0.08	-	0.21±0.02

Table 3.20, Table 3.21 and Table 3.22 report the Na^+ e Cl^- concentrations in the different parts of the plants grown in the pilot wetland.

The sodium accumulation in the considered plant species did not vary with the sampling point (Cell1 and Cell2), while some differences have been observed among the species. In fact, *T. latifolia* and *S. lacustris* accumulated more sodium than *P. australis*.

The Na^+ content in *T. latifolia* did not show important variation during the season in both above- and underground tissues (Table 3.20). On the contrary, a temporal trend seems to characterise *P. australis* and *S. lacustris*.

Sodium accumulation in *P. australis* slightly increased during the sampling period in all the analysed plant parts (Table 3.21). A similar behaviour has been observed in the roots of *S. lacustris*, while the Na content decreased in the shoots (Table 3.22).

All plant species accumulated more chloride in the underground than aboveground parts.

Moreover, in *T. latifolia* the chloride content was significantly higher in rhizomes than in roots ($p<0.05$) (Table 3.20). The same behaviour occurred in *P. australis* in Cell2, whereas in Cell1 chloride content was significantly higher in root than in rhizome ($p<0.05$).

Table 3.20 Aboveground (shoots) and belowground (roots and rhizomes) Na^+ and Cl^- content (expressed as mg g^{-1} DW) in* T. latifolia (for Cl^- mean±SD, n=3), *(n.d.: no data).

T. latifolia		Cell1			Cell2		
		roots	*rhizomes*	*shoots*	*roots*	*rhizomes*	*shoots*
Na^+	**Jul**	6.93	4.21	3.94	3.45	5.38	8.17
	Aug	n.d.	n.d.	3.24	n.d.	n.d.	2.17
	Sep	n.d.	n.d.	2.26	n.d.	n.d.	3.41
	Nov	5.04	2.61	2.46	3.19	4.95	5.80
Cl^-	**Jul**	0.14±0.01	0.17±0.005	1.22±0.07	0.10±0.02	0.31±0.03	0.91±0.05
	Aug	n.d.	n.d.	1.15±0.05	n.d.	n.d.	1.31±0.15
	Sep	n.d.	n.d.	1.40±0.10	n.d.	n.d.	1.35±0.14
	Nov	0.19±0.03	0.34±0.005	0.58±0.02	0.14±0.02	0.38±0.09	1.40±0.05

Leaves in *P. australis* accumulated more chloride than stems ($p<0.05$) (Table 3.21). The highest accumulation of Cl^- in below-ground tissues was measured in November

in all plant species, whereas opposite trend was observed in aboveground parts, except for *T. latifolia* in Cell2 and *S. lacustris* in Cell1.

Table 3.21 Aboveground (stem and leaves) and below-ground (roots and rhizomes) Na^+ and Cl^- content (expressed as mg g^{-1} DW) in* P. australis *(for Cl^- mean±SD, n=3), (n.d.: no data).

P. australis		Cell1				Cell2			
		roots	*rhizomes*	*stem*	*leaves*	*roots*	*rhizomes*	*stem*	*leaves*
Na^+	**Jul**	2.19	0.69	0.20	0.12	1.01	0.86	0.21	0.08
	Aug	n.d.	n.d.	0.25	0.05	n.d.	n.d.	0.31	0.05
	Sep	n.d.	n.d.	0.32	0.09	n.d.	n.d.	0.62	0.18
	Nov	2.17	1.14	0.63	0.38	3.07	1.10	1.34	0.59
Cl^-	**Jul**	0.19±0.02	0.14±0.002	0.99±0.13	3.44±0.11	0.09±0.02	0.19±0.01	0.82±0.05	4.37±0.06
	Aug	n.d.	n.d.	0.95±0.27	1.41±0.13	n.d.	n.d.	0.87±0.02	2.36±0.30
	Sep	n.d.	n.d.	0.93±0.07	1.91±0.43	n.d.	n.d.	0.16±0.02	2.52±0.04
	Nov	0.52±0.06	0.15±0.001	0.95±0.46	2.35±0.12	0.12±0.01	0.25±0.01	1.09±0.05	1.75±0.19

The *S. lacustris* chloride content between Cell1 and Cell2 was significantly different in both the above- and below-ground parts. Plants growing in Cell2 had higher Cl^- concentration than plants in Cell1 ($p<0.05$) (Table 3.22). On the contrary, no clear difference between the Cells was evident for *T. latifolia* and *S. lacustris* plants.

Table 3.22 Aboveground (shoots) and below-ground (roots) Na^+ and Cl^- content (expressed as mg g^{-1} DW) in* S. lacustris *(for Cl^- mean±SD, n=3), (n.d.: no data).

S. lacustris		Cell1		Cell2	
		roots	*shoots*	*roots*	*shoots*
Na^+	**Jul**	3.41	3.41	3.63	7.18
	Aug	n.d.	3.11	n.d.	3.36
	Sep	n.d.	3.18	n.d.	3.24
	Nov	5.40	2.98	5.59	5.40
Cl^-	**Jul**	0.11±0.02	0.18±0.01	0.09±0.003	1.26±0.10
	Aug	n.d.	2.05±0.05	n.d.	2.31±0.16
	Sep	n.d.	1.29±0.05	n.d.	2.18±0.08
	Nov	0.16±0.01	1.17±0.07	0.42±0.01	0.89±0.03

3.4.3.2 Nutrients content

The total Kjeldahl nitrogen (TKN) content was higher in *P. australis* and *T. latifolia* with respect to *S. lacustris*. More nitrogen was accumulated in the aerial parts (shoots and leaves) than in the underground parts, in the three macrophytes (Table 3.23, Table 3.24 and Table 3.25).

All the plant species shown significantly higher underground TKN accumulation in November (in particular, rhizomes for *T. latifolia* and *P. australis* and roots for *S. lacustris*) ($p<0.05$). Opposite trend was observed in aboveground parts, in *T. latifolia*, and *S. lacustris* with a decreased in TKN content in November, in both Cell1 and Cell2. In *P. australis*, TKN content in stem was relatively constant during the season. TKN accumulation in Cell2 leaves increased until September, whereas in leaves of Cell1 plants higher value was measured in August, followed by a negative ($p<0.05$) (Table 3.24).

Total nitrogen (TKN) content in shoot of *T. latifolia* ranged from 6.52 to 18.26 mg/g DW and from 5.19 to 18.26 mg/g DW in Cell1 and Cell2 respectively (Table 3.23).

In November, the shoot of plants in Cell1 shown a significantly higher TKN content than the shoot of Cell2, and the opposite behaviour occurred in roots ($p<0.05$).

In Cell1 *P. australis,* TKN in leaves varied between 16.45 to 21.51 mg/g DW, while in Cell2 plants was between 15.29 to 22.95 mg/g DW (Table 3.24).

In shoot of *S. lacustris*, TKN content ranged from 5.35 to 12.80 mg/g DW in Cell1 and from 5.93 to 12.09 mg/g DW in Cell2 (Table 3.25).

The nitrate (NO_3^-) and sulphate (SO_4^{2-}) contents were higher in aboveground than underground parts. In general, NO_3^- and SO_4^{2-} were more accumulated in the aerial part of *P. australis* than in the others plants species. No significant differences in the tissues content of the two anions were found between plants grown in Cell1 and Cell2.

Similarly to TKN, nitrate content in shoot of *T latifolia* increased until September, followed by a significant decrement in November, both in Cell1 than Cell2 ($p<0.05$). Rhizome nitrate contents shown the opposite trend in Cell2, with slightly higher

value reached in November, whereas a decrement between July and November was observed in Cell1.

In *P. australis* as well, nitrate content in shoots increased until September, followed by a significant decrement in November in Cell1 ($p<0.05$) and an opposite trend in roots accumulation ($p<0.05$). Nitrate shoot accumulation in Cell2 plants increased from June to November, whereas not significant difference in roots between June and November was determined.

Nitrate in leaves of *P. australis* ranged from 14.5 to 24.7 µg g^{-1} DW and from 17.7 to 39.9 µg g^{-1} DW in Cell1 and Cell2, respectively.

The sulphate content (SO_4^{2-}) in shoot of *T. latifolia* ranged from 0.28 to 0.60 mg g^{-1} DW and from 0.32 to 0.49 mg g^{-1} DW in Cell1 and Cell2, respectively.

Sulphate stem concentration in *P. australis* was relatively constant during the sampling period, whereas in leaves, sulphate content varied between 0.79 and 2.5 mg g^{-1} DW in Cell1 and between 0.83 and 2.0 mg g^{-1} DW in Cell2.

In *S. lacustris* shoots accumulation increased in August-September and then decreased in November, with values ranged from 0.06 to 1 mg g^{-1} DW and from 0.16 to 1.0 mg g^{-1} DW in Cell1 and Cell2, respectively.

Table 3.23 Total Kjeldahl nitrogen (TKN), sulphate (SO_4^{2-}) (expressed as mg g^{-1} DW) and nitrate (NO_3^-) (expressed as µg g^{-1} DW) content in aboveground (shoot) and belowground (divided in root and rhizome) parts of* T. latifolia *in Cell1 and Cell2, for the different sampling time, (n.d.: no data).

T. latifolia		Cell1			Cell2		
		root	*rhizome*	*shoot*	*root*	*rhizome*	*shoot*
TKN	**Jul**	4.87±0.08	3.32±0.08	6.52±0.17	4.32±0.04	2.71±0.14	5.19±0.12
	Aug	n.d.	n.d.	13.13±2.15	n.d.	n.d.	15.05±0.18
	Sep	n.d.	n.d.	18.26±0.34	n.d.	n.d.	18.36±0.22
	Nov	4.6±0.14	5.30±0.04	12.05±0.11	5.99±0.18	9.24±0.04	8.00±0.22
NO_3^-	**Jul**	5.77±0.59	5.32±0.96	5.69±1.37	3.52±0.76	2.79±0.15	5.03±0.93
	Aug	n.d.	n.d.	8.59±1.62	n.d.	n.d.	11.78±1.56
	Sep	n.d.	n.d.	11.89±1.68	n.d.	n.d.	14.42±2.60
	Nov	3.96±0.59	3.50±0.97	5.21±1.77	3.30±0.69	3.79±1.74	4.66±1.34
SO_4^{2-}	**Jul**	0.08±0.004	0.06±0.001	0.55±0.048	0.04±0.004	0.05±0.004	0.34±0.027
	Aug	n.d.	n.d.	0.29±0.015	n.d.	n.d.	0.39±0.057
	Sep	n.d.	n.d.	0.44±0.020	n.d.	n.d.	0.46±0.028
	Nov	0.10±0.011	0.12±0.006	0.33±0.045	0.09±0.013	0.07±0.009	0.38±0.017

Table 3.24 Total Kjeldahl nitrogen (TKN), sulphate (SO_4^{2-}) (expressed as mg g^{-1} DW) and nitrate (NO_3^-) (expressed as µg g^{-1} DW) content in aboveground (divided in stem and shoot) and belowground (divided in root and rhizome) parts of* P. australis *in Cell1 and Cell2, for the different sampling time, (n.d.: no data).

P. australis		Cell1				Cell2			
		root	*rhizome*	*stem*	*leave*	*root*	*rhizome*	*stem*	*leave*
TKN	**Jul**	6.19±0.05	2.19±0.05	3.78±0.40	16.89±0.03	5.08±0.13	2.17±0.05	2.49±0.12	15.29±0.04
	Aug	n.d.	n.d.	6.51±0.48	21.51±0.63	n.d.	n.d.	6.18±0.14	19.39±0.22
	Sep	n.d.	n.d.	4.92±0.44	19.61±0.12	n.d.	n.d.	5.22±0.07	22.85±0.16
	Nov	4.70±0.65	7.80±0.14	4.32±0.07	16.45±0.49	4.33±0.06	2.98±0.02	4.99±0.14	21.99±1.52
NO_3^-	**Jul**	10.96±1.40	3.96±0.51	37.86±4.29	23.55±1.94	5.14±0.16	6.11±1.99	15.17±1.29	17.66±3.42
	Aug	n.d.	n.d.	22.31±1.94	24.24±2.97	n.d.	n.d.	8.33±1.31	31.72±5.09
	Sep	n.d.	n.d.	12.38±7.12	24.67±4.94	n.d.	n.d.	8.66±0.46	30.29±3.37
	Nov	5.55±1.67	5.96±1.00	16.24±2.39	14.52±11.45	5.39±0.29	5.64±0.50	7.06±0.67	39.86±3.72
SO_4^{2-}	**Jul**	0.41±0.018	0.09±0.034	0.95±0.095	2.37±0.039	0.15±0.010	0.13±0.019	0.74±0.024	1.96±0.040
	Aug	n.d.	n.d.	0.34±0.044	0.85±0.057	n.d.	n.d.	0.61±0.049	1.22±0.151
	Sep	n.d.	n.d.	0.62±0.065	1.40±0.276	n.d.	n.d.	0.61±0.022	1.54±0.102
	Nov	0.23±0.015	0.25±0.004	0.25±0.150	0.95±0.127	0.14±0.017	0.18±0.005	0.36±0.015	0.92±0.071

Table 3.25 Total Kjeldahl nitrogen (TKN), sulphate (SO_4^{2-}) (expressed as mg g^{-1} DW) and nitrate (NO_3^-) (expressed as µg g^{-1} DW) content in aboveground (shoot) and belowground (divided in root) parts of* S. lacustris *in Cell1 and Cell2, for the different sampling time, (n.d.: no data).

S. lacustris		Cell1		Cell2	
		root	*shoot*	*root*	*shoot*
TKN	**Jun**	3.86±0.04	6.61±0.13	3.89±0.10	8.10±0.70
	Aug	n.d.	12.80±0.15	n.d.	8.27±0.08
	Sep	n.d.	12.62±0.13	n.d.	12.09±0.03
	Nov	4.93±0.17	5.35±0.21	4.33±0.10	5.93±0.16
NO_3^-	**Jun**	6.50±0.74	5.70±1.69	3.53±0.76	6.53±2.11
	Aug	n.d.	16.16±3.86	n.d.	7.10±0.74
	Sep	n.d.	7.63±1.41	n.d.	7.94±0.68
	Nov	2.84±0.34	4.20±1.52	3.58±0.17	5.96±0.60
SO_4^{2-}	**Jun**	0.05±0.002	0.07±0.004	0.03±0.003	0.43±0.016
	Aug	n.d.	0.97±0.023	n.d.	0.94±0.063
	Sep	n.d.	0.84±0.038	n.d.	0.92±0.025
	Nov	0.06±0.002	0.15±0.005	0.14±0.019	0.16±0.004

3.4.3.3 Mineral content

The potassium concentration in the above- and below-ground parts of *T. latifolia*, *P. australis* and *S. lacustris* was relatively constant during the sampling period and no differences were evidenced among plants growing in Cell1 and Cell2 (Figure 3.18, Figure 3.19 and Figure 3.20).

T. latifolia rhizomes accumulated generally more K than roots, with mean values of 12.2 mg g^{-1} DW in Cell1 and 10.6 mg g^{-1} DW in Cell2. Potassium mean concentration in roots was 7.9 mg g^{-1} DW in Cell1 plants and 5.1 mg g^{-1} DW in Cell2 plants (Figure 3.18). The level of K in shoots ranged between 9.4 and 15.1 mg g^{-1} DW in Cell1 and between 8.5 and 12.4 mg g^{-1} DW in Cell2 (Figure 3.18).

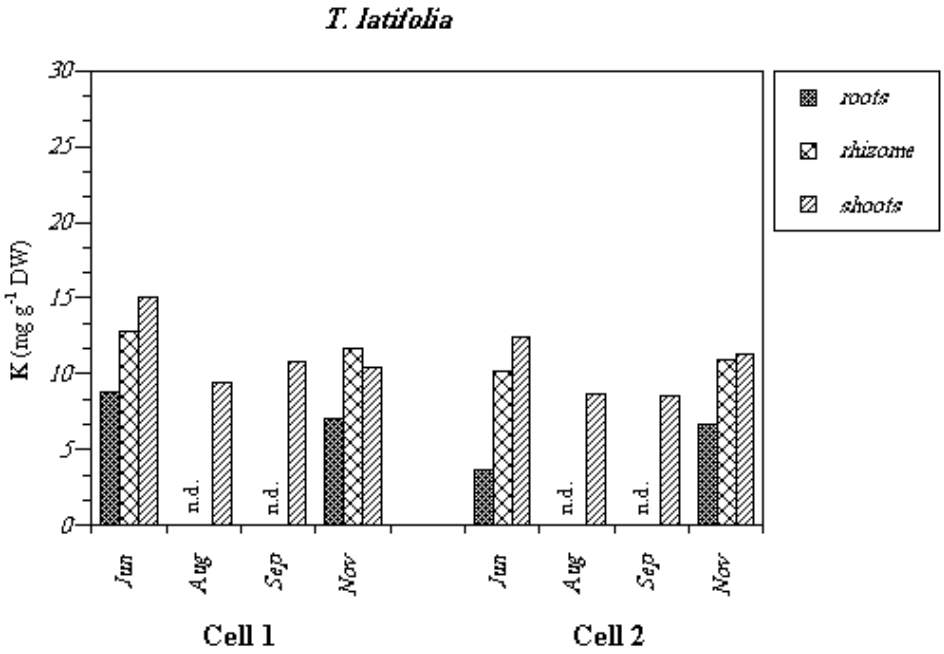


Figure 3.18 Potassium (K^+) (expressed as mg g^{-1} DW) content in aboveground (shoots) and belowground (roots and rhizome) parts of* T. latifolia *in Cell1 and Cell2, for the different sampling time, (n.d.: no data).

The same behaviour was observed in *P. australis*, in which K content was higher in rhizome than in roots, expected in July. Mean values were 11.8 mg g^{-1} DW in Cell1 and 8.3 mg g^{-1} DW in Cell2. Mean roots K concentration was 6.5 mg g^{-1} DW and 7.8 mg g^{-1} DW in Cell1 plants and Cell2 plants, respectively (Figure 3.19).

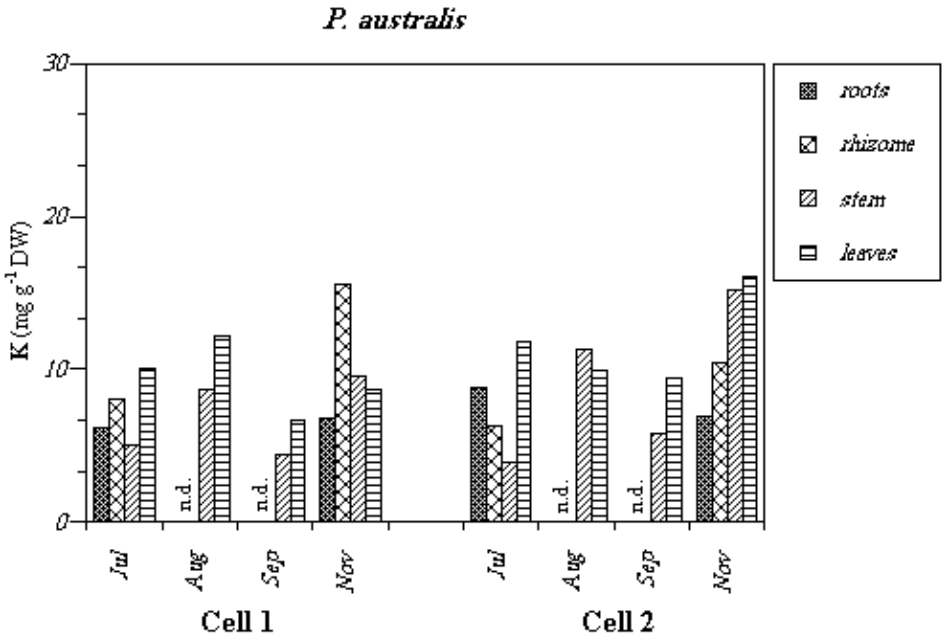


Figure 3.19 Potassium (K^+) (expressed as mg g^{-1} DW) content in aboveground (stems and leaves) and belowground (roots and rhizome) parts of* P. australis *in Cell1 and Cell2, for the different sampling time, (n.d.: no data).

Plants of *S. lacustris* growing in Cell2 shown a slightly higher potassium than plants in Cell1. Mean K values in roots were 7.1 mg g^{-1} DW in Cell1 and 11.7 mg g^{-1} DW in Cell2, whereas in aboveground parts K contents were 15.0 and 18.2 mg g^{-1} DW in Cell1 and Cell2, respectively (Figure 3.20).

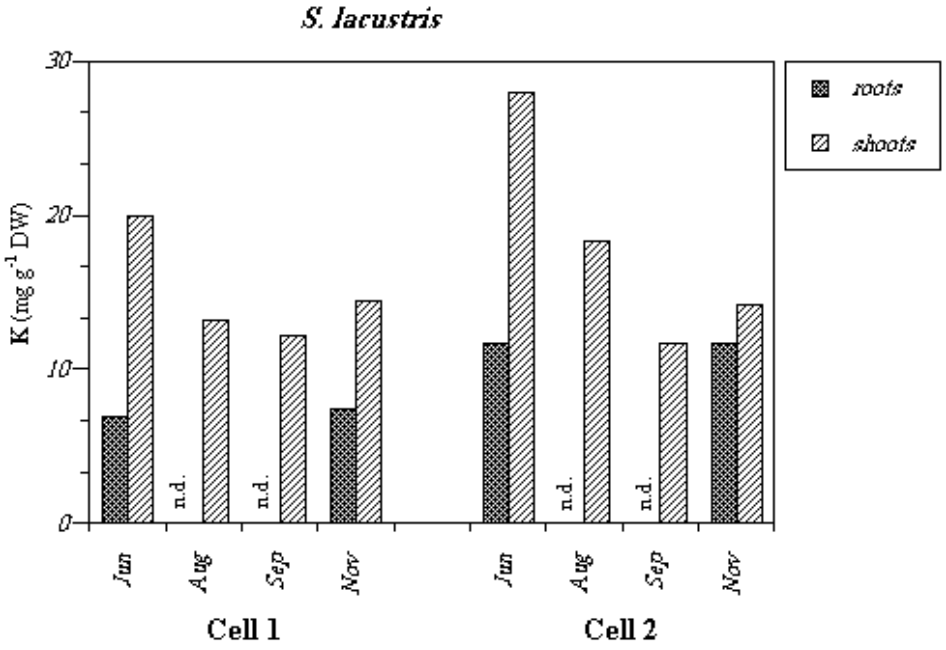


Figure 3.20 Potassium (K^+) (expressed as mg g^{-1} DW) content in aboveground (shoots) and belowground (roots) parts of* S. lacustris *in Cell1 and Cell2, for the different sampling time, (n.d.: no data).

Phosphorus content in *T. latifolia* in belowground parts was lower than in the aerial parts, except in November in Cell2, where root-rhizome P content resulted higher than in shoots tissues (Figure 3.21). Moreover, belowground parts in both Cells accumulated more phosphorus in November with respect to June (Figure 3.21). Shoots P concentration shown a positive trend until September, followed by a decrement in November. Maximum values were 1.1 mg g^{-1} DW in Cell1 and 1.2 mg g^{-1} DW, whereas minimum value was 0.5 mg g^{-1} DW in both Cells.

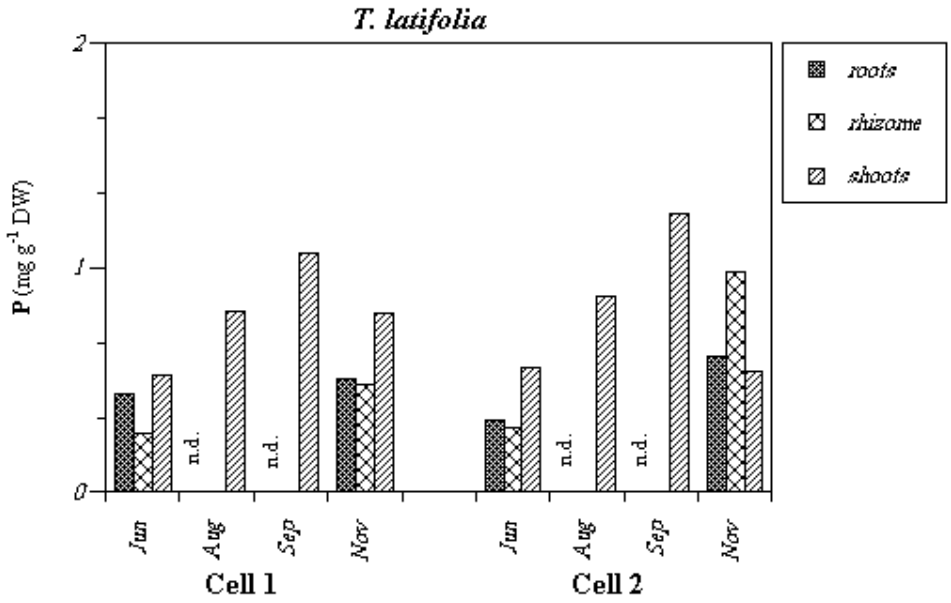


Figure 3.21 Phosphorus (P) (expressed as mg g^{-1} DW) content in aboveground (shoots) and belowground (roots and rhizome) parts of* T. latifolia *in Cell1 and Cell2, for the different sampling time, (n.d.: no data).

Phosphorous content in *P. australis* shown a positive trend during the sampling time, both in above- than belowground parts, with higher accumulation in leaves than in the others tissues (Figure 3.22).

In roots of Cell1 plants the mean values of P content were 0.39 mg g^{-1} DW and in rhizome 0.36 mg g^{-1} DW whereas in Cell2 the mean P levels were 0.41 mg g^{-1} DW and 0.35 mg g^{-1} DW in roots and rhizome, respectively. Aboveground parts accumulated more phosphorus, with mean values in stem of 0.41 mg g^{-1} DW in Cell1 and 0.60 mg g^{-1} DW in Cell2, whereas of 0.82 and 1.15 mg g^{-1} DW in leaves in Cell1 and Cell2, respectively.

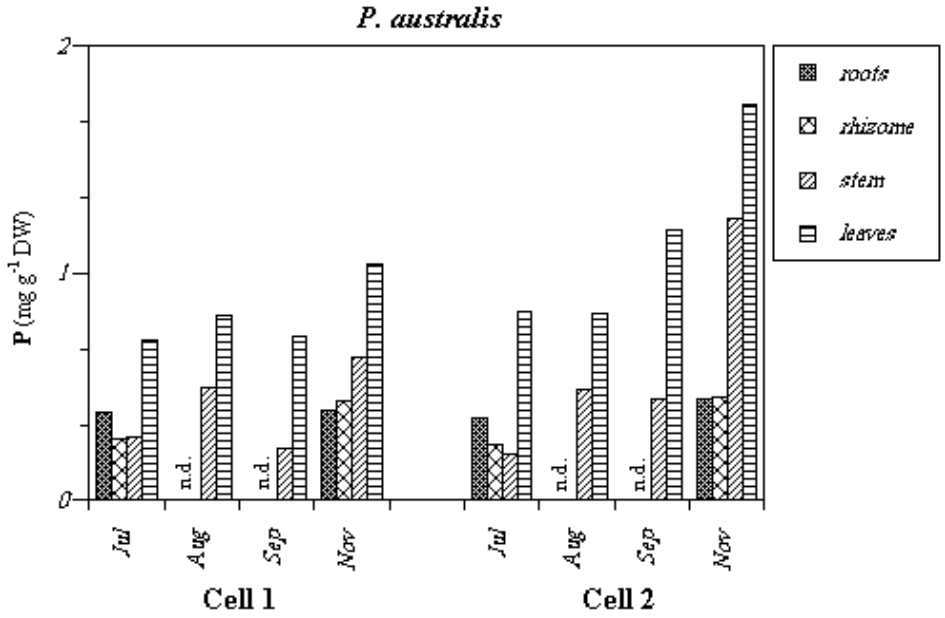


Figure 3.22 Phosphorus (P) (expressed as mg g^{-1} DW) content in aboveground (stem and leaves) and belowground (roots and rhizome) parts of* P. australis *in Cell1 and Cell2, for the different sampling time, (n.d.: no data).

In *S. lacustris* tissues phosphorus content was relative constant during the whole period, nevertheless a decreasing trend in shoots concentration from July to November can be observed in the plants of both Cells, corresponding to a slightly increased in P content in roots in November (Figure 3.23). Relative higher concentration in aboveground tissues with respect to belowground parts was observed, with mean values in roots of 0.34 mg g^{-1} DW in Cell1 and 0.50 mg g^{-1} DW in Cell2, whereas in shoots of 0.76 and 0.86 mg g^{-1} DW in Cell1 and Cell2, respectively.

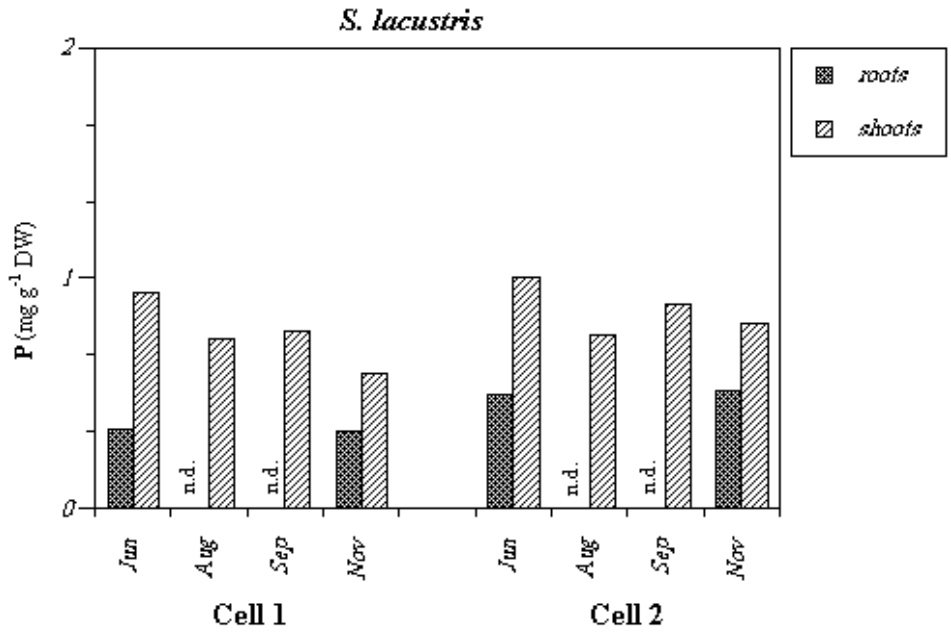


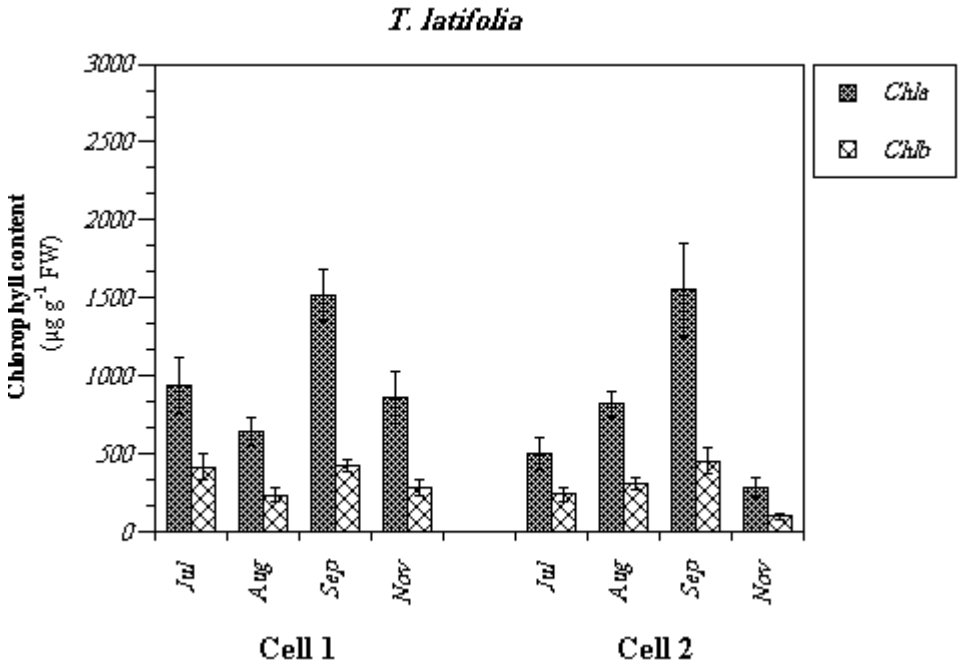
Figure 3.23 Phosphorus (P) (expressed as mg g^{-1} DW) content in aboveground (stem and leaves) and belowground (roots and rhizome) parts of* S. lacustris *in Cell1 and Cell2, for the different sampling time, (n.d.: no data).

3.4.3.4 Photosynthetic pigments

A significant variability in chlorophyll *a* content in leaves of *T. Latifolia* was observed during the period (Figure 3.24), with a decrease from July to August in plants growing in Cell1 ($p<0.01$) and an increase in Cell2 plants ($p<0.01$) followed by a decrement from September to November ($p<0.001$). The peak in Chl *a* was measured in September (1713 and 1873 μg g^{-1} FW in Cell1 plants and Cell2 plants, respectively).

The chlorophyll *b* content was, as expected, significantly lower than chlorophyll *a* content and it followed a trend similar to Chl *a* during the sampling period in both Cells. Maximum value occurs in July in Cell1 (556 μg g^{-1} FW) and in September in Cell2 (387 μg g^{-1} FW).

The Chl *a* and Chl *b* contents in *T. latifolia* leaves were significantly different between Cell1 and Cell2 in July and November ($p<0.001$) with a minimum value in November in plants of Cell2 (216 μg g^{-1} FW). In addition, for Chl *b*, there was a significant difference between Cell1 and Cell2, although less marked ($p<0.05$), in August.



Chl *a*

	P1-t1	P2-t1	P1-t2	P2-t2	P1-t3	P2-t3	P1-t4	P2-t4
P1-t1	***	**		***		n.s.		
P2-t1			**		***		n.s.	
P1-t2			n.s.	***		*		
P2-t2					***		***	
P1-t3					n.s.	***		
P2-t3							***	
P1-t4							***	
P2-t4								

Chl *b*

	P1-t1	P2-t1	P1-t2	P2-t2	P1-t3	P2-t3	P1-t4	P2-t4
P1-t1	***	***		n.s.		**		
P2-t1			n.s.		***		**	
P1-t2			*	***		n.s.		
P2-t2					***		***	
P1-t3					n.s.	**		
P2-t3							***	
P1-t4							***	
P2-t4								

Figure 3.24 Chlorophyll* a *(Chl* a*) and Chlorophyll* b *(Chl* b*) contents in leaves of* T. latifolia *for the sampling period at Cell1 and Cell2 (mean±SD, n=6).
***Statistical analyses (Fisher's LSD) * p<0.05; **p<0.01; ***p<0.001 for Chl* a *and Chl* b.**

Carotenoid content in leaves of *T. latifolia* (Figure 3.25), reached a maximum value in September in Cell2 (624 µg g^{-1} FW) and a minimum value in November in Cell2 (84 µg g^{-1} FW). Significant difference between Cell1 and Cell2 plants was observed in July and November ($p<0.001$).

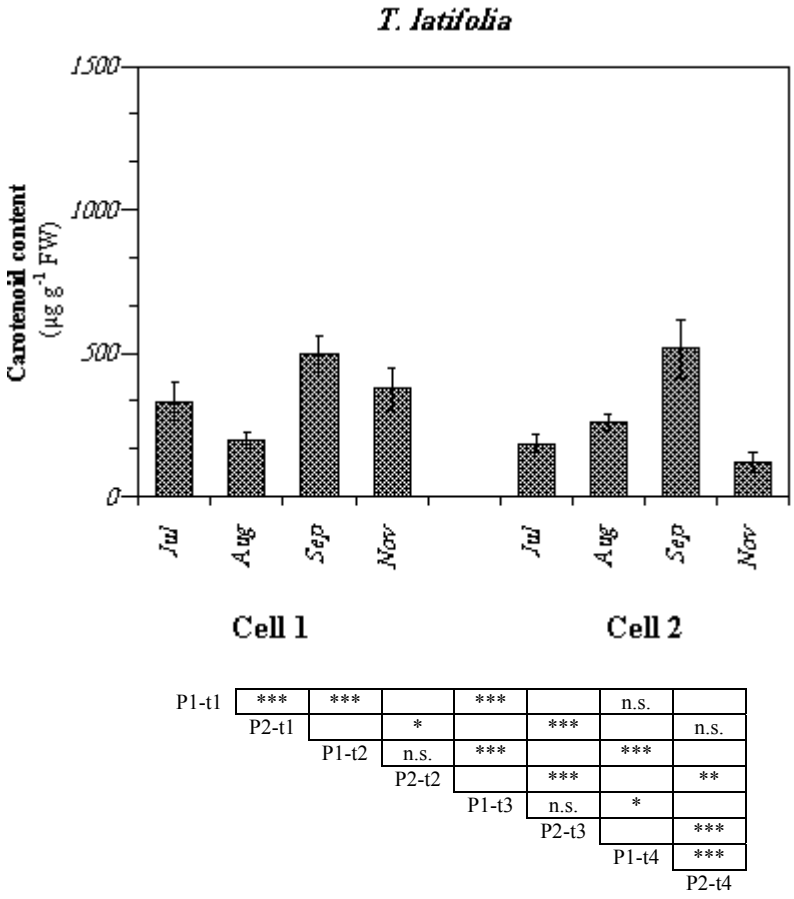
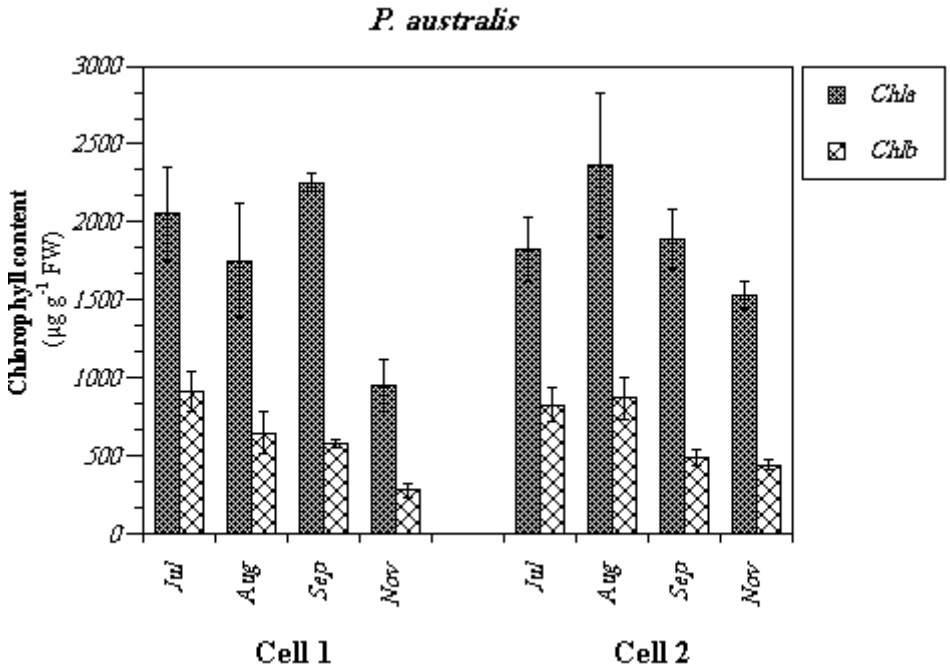


Figure 3.25 Carotenoid contents in leaves of* T. latifolia *for the sampling period at Cell1 and Cell2 (mean±SD, n=6).
Statistical analyses (Fisher's LSD) *p<0.05; **p<0.01; ***p<0.001.

A general decrease in chlorophyll *a* content in *P. australis* located in Cell1 was recorded during the sampling period, interrupted, in September by a significant higher ($p<0.001$) Chl *a* value (Figure 3.26).

In Cell2, Chl *a* increased significantly from July to August ($p<0.01$), with a maximum peak in August (2957 µg g^{-1} FW), followed by a decrement until reaching the minimum value (1421 µg g^{-1} FW) in November. A trend similar was observed for chlorophyll *b* content, with the minimum value reached in November (231 µg g^{-1} FW) and a maximum value in July (1108 µg g^{-1} FW) in Cell1.

The Chl *a* and *b* contents were significantly different from Cell1 and Cell2 in August ($p<0.01$) and November ($p<0.05$).



Chl *a*

	P1-t1	P2-t1	P1-t2	P2-t2	P1-t3	P2-t3	P1-t4	P2-t4
P1-t1		n.s.	n.s.		n.s.		***	
P2-t1				**		n.s.		n.s.
P1-t2				**	*		***	
P2-t2						*		***
P1-t3						n.s.	***	
P2-t3								n.s.
P1-t4								*
P2-t4								

Chl *b*

	P1-t1	P2-t1	P1-t2	P2-t2	P1-t3	P2-t3	P1-t4	P2-t4
P1-t1		n.s.	n.s.		n.s.		***	
P2-t1				**		n.s.		n.s.
P1-t2				**	*		***	
P2-t2						*		***
P1-t3						n.s.	***	
P2-t3								n.s.
P1-t4								*
P2-t4								

***Figure 3.26 Chlorophyll* a *(Chl* a*) and Chlorophyll* b *(Chl* b*) contents in leaves of* P. australis *for the sampling period at Cell1 and Cell2 (mean±SD, n=6) Statistical analyses (Fisher's LSD) * p<0.05; **p<0.01; ***p<0.001 for Chl* a *and Chl* b.**

In Cell1, *P. australis* leaves carotenoid content shown a variability during the time, with a significant decrement from July to August ($p<0.05$), a significant increment in September ($p<0.001$) and a decrement in November ($p<0.001$), whereas in Cell2 plants the carotenoid content was relatively stable throughout the sampling period (Figure 3.27). The maximum value (989 µg g^{-1} FW) was reached in Cell1 plants in September, while the minimum (385 µg g^{-1} FW) in August.

The carotenoid content was significantly different from Cell1 and Cell2 in August ($p<0.01$) and November ($p<0.05$).

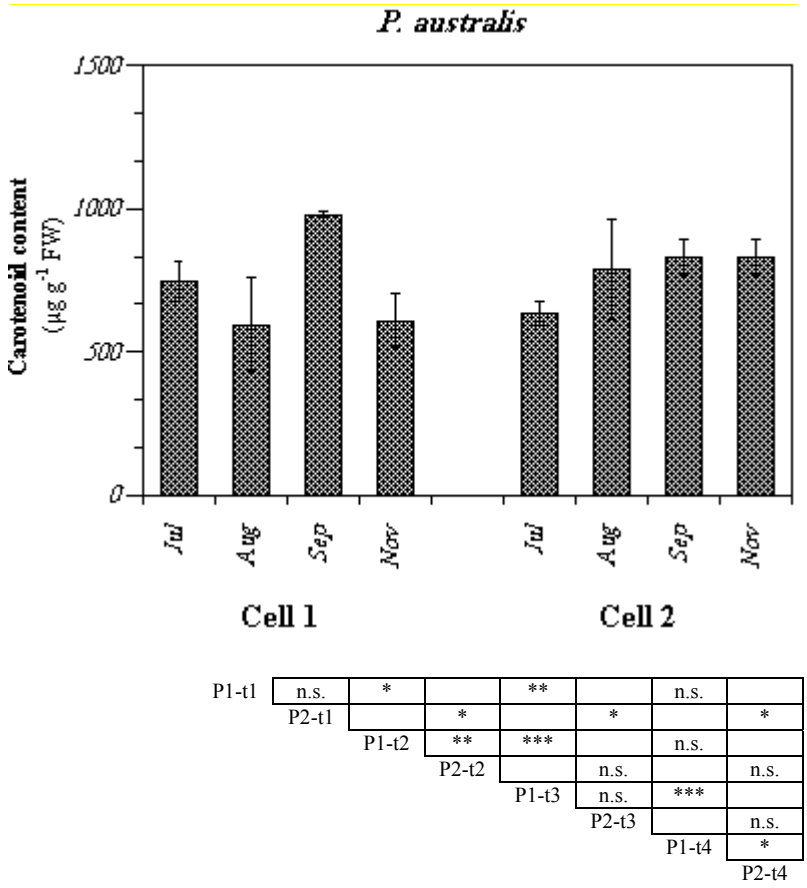
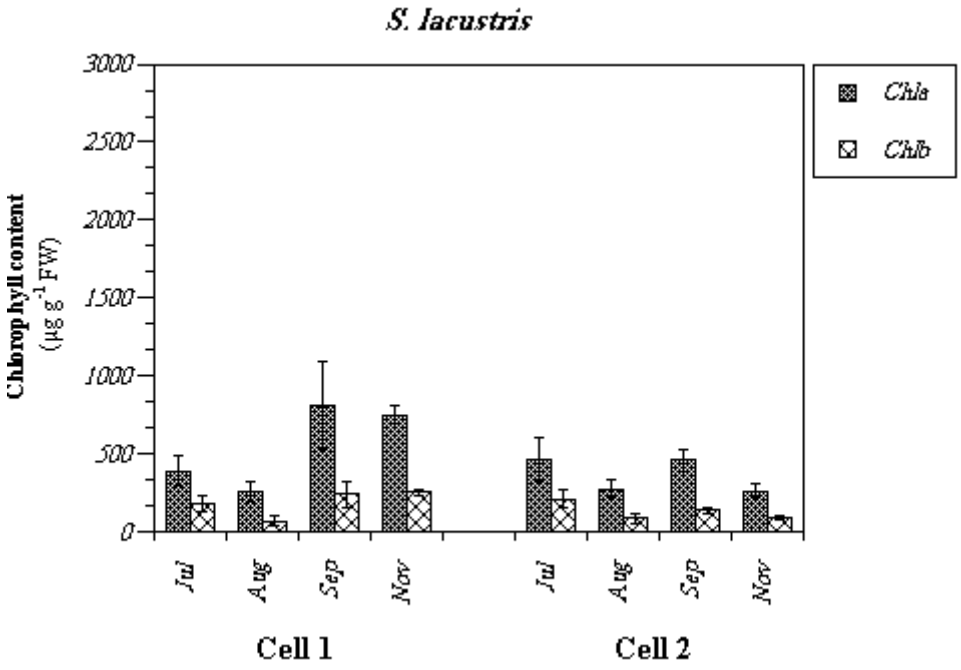


Figure 3.27 Carotenoid contents in leaves of* P. australis *for the sampling period at Cell1 and Cell2 (mean±SD, n=6).
Statistical analyses (Fisher's LSD) * p<0.05; **p<0.01; ***p<0.001.

Chlorophyll *a* content in leaves of *S. lacustris* in Cell1 and Cell2 differed significantly in July and August ($p<0.05$). Maximum value (1055 µg g^{-1} FW) and minimum value (199 µg g^{-1} FW) were reached in Cell1 plants in September and August, respectively (Figure 3.28).

The chlorophyll *b* content was significantly lower than chlorophyll *a* content and it followed a trend similar to Chl *a* during the sampling period. Maximum value occurred in September in Cell1 plants (309 µg g^{-1} FW) and in July in Cell2 plants (104 µg g^{-1} FW).

The chl *a* and *b* contents were significantly different from Cell1 and Cell2 in November ($p<0.05$).



Chl *a*

	P1-t1	P2-t1	P1-t2	P2-t2	P1-t3	P2-t3	P1-t4	P2-t4
P1-t1	n.s.	*		*		*		
P2-t1			*		n.s.		*	
P1-t2	n.s.	*			*		*	
P2-t2		*				*		n.s.
P1-t3	n.s.	n.s.	*				n.s.	
P2-t3				*	n.s.			*
P1-t4			*		n.s.			*
P2-t4		n.s.		*		*	*	

Chl *b*

	P1-t1	P2-t1	P1-t2	P2-t2	P1-t3	P2-t3	P1-t4	P2-t4
P1-t1	n.s.	**		n.s.		*		
P2-t1			**		n.s.		*	
P1-t2	n.s.	**			*		*	
P2-t2		*				*		n.s.
P1-t3	n.s.	n.s.	*				n.s.	
P2-t3				*	n.s.			*
P1-t4			*		n.s.			*
P2-t4		n.s.		*		*	*	

Figure 3.28 Chlorophyll* a *(Chl* a*) and Chlorophyll* b *(Chl* b*) contents in leaves of* S. lacustris *for the sampling period at Cell1 and Cell2 (mean±SD, n=6).
***Statistical analyses (Kruskal-Wallis test) * p<0.05; **p<0.01; ***p<0.001 for Chl* a *and Chl* b.**

Carotenoid content in *S. lacustris* leaves was significantly different between Cell1 and Cell2 plants in November, with the maximum value (309 µg g^{-1} FW) recorded in September in Cell1 (Figure 3.29), and the minimum value (68 µg g^{-1} FW) in August in Cell1.

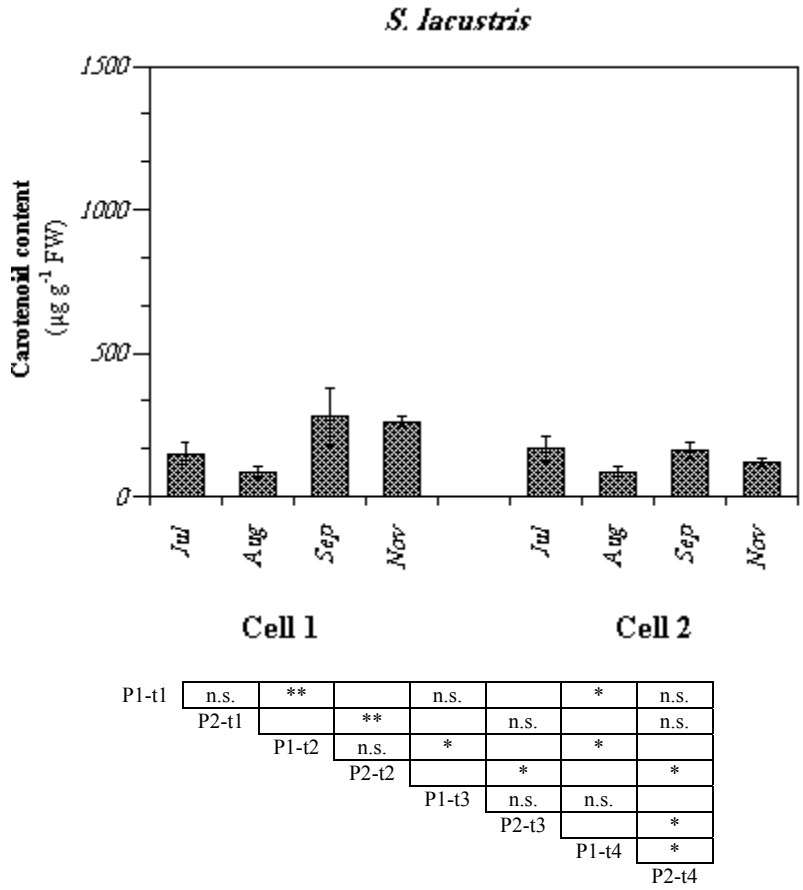


Figure 3.29 Carotenoid contents in leaves of* S. lacustris *for the sampling period at Cell1 and Cell2 (mean±SD, n=6).
Statistical analyses (Kruskal-Wallis test) * p<0.05; **p<0.01; ***p<0.001.

3.4.3.5 Metals content

T. latifolia, *P. australis* and *S lacustris* tissues were investigated for heavy metal content. Analyses were carried out for arsenic, cadmium, chromium, mercury, copper, lead and zinc, but detection was not possible for Hg because the concentration was below the detection limit (1 µg L^{-1}). Table 3.26, Table 3.27 and Table 3.28 show the element concentrations in different plant parts.

The general picture is that roots accumulated greater concentration of metals than shoots, stems and leaves, in all the species considered. For *T. latifolia* samples, the order of accumulation for all the metals was roots>rhizomes>shoots, although less marked for copper and zinc (Table 3.26). The same behaviour was found in *S. lacustris*, with higher accumulation in roots than in shoots (Table 3.28). For *P.*

australis samples, the difference in metal accumulation was not so marked (Table 3.27).

In *P. australis*, the data from this study indicate that stems contained lower Cr, Cu and Zn concentration than leaves and the amount depended greatly on the metals analysed rather than sampling time.

Table 3.26 Heavy metal concentration (µg g^{-1} DW) in the aerial part and root/rhizome of* T. latifolia, *at the two locations (Cell1 and Cell2), for the sampling period, n.d.: no data; < d.l.: below detection limit (As: 6 µg L^{-1}, Cd: 1 µg L^{-1}, Cr: 2 µg L^{-1}, Pb: 9 µg L^{-1}); Hg was always below the detection limit (1 µg L^{-1}) and is not included.

T. latifolia		Cell1			Cell2		
		root	*rhizome*	*shoot*	*root*	*rhizome*	*shoot*
As	**Jul**	90.67	34.58	<d.l..	35.90	10.42	<d.l.
	Aug	n.d.	n.d.	<d.l.	n.d.	n.d.	<d.l.
	Sep	n.d.	n.d.	<d.l.	n.d.	n.d.	<d.l.
	Nov	92.30	11.24	<d.l.	44.63	9.72	<d.l.
Cd	**Jul**	0.31	<d.l.	<d.l.	0.82	<d.l.	<d.l.
	Aug	n.d.	n.d.	<d.l.	n.d.	n.d.	<d.l.
	Sep	n.d.	n.d.	<d.l.	n.d.	n.d.	<d.l.
	Nov	<d.l.	<d.l.	<d.l.	0.48	<d.l.	<d.l.
Cr	**Jul**	4.70	1.25	0.33	3.35	3.23	<d.l.
	Aug	n.d.	n.d.	<d.l.	n.d.	n.d.	<d.l.
	Sep	n.d.	n.d.	0.40	n.d.	n.d.	0,24
	Nov	9.79	1.28	0.41	10.09	1.66	0,66
Cu	**Jul**	11.51	2.35	3.02	14.93	3.97	2.38
	Aug	n.d.	n.d.	1.88	n.d.	n.d.	1.97
	Sep	n.d.	n.d.	1.83	n.d.	n.d.	2.25
	Nov	10.11	1.28	2.16	16.28	1.98	1.73
Pb	**Jul**	9.79	1.02	<d.l.	12.16	2.81	<d.l.
	Aug	n.d.	n.d.	<d.l.	n.d.	n.d.	<d.l.
	Sep	n.d.	n.d.	<d.l.	n.d.	n.d.	<d.l.
	Nov	8.35	<d.l.	<d.l.	16.28	1.50	<d.l.
Zn	**Jul**	91.80	28.27	28.65	64.58	26.43	13.92
	Aug	n.d.	n.d.	14.77	n.d.	n.d.	17.05
	Sep	n.d.	n.d.	13,32	n.d.	n.d.	15.04
	Nov	61.78	14.95	20.43	76.04	20.50	15.81

As and Zn were the two most concentrate heavy metals in the roots of all plants, ranging from 28.7 to 181.9 µg g^{-1} DW and from 48.5 to 105.7, respectively. Cu and Pb were accumulated more than Cr, whereas Cd shown lowest levels in all plant species.

Differences between Cell1 and Cell2 were found, in particular for *T. latifolia*, higher levels of Cu and Pb were measured in Cell2 than in Cell1, whereas opposite trends were found for As and Zn. In *P. australis* and *S. lacustris* Pb, Zn, Cr and Cu concentrations were higher in Cell2 than in Cell1, whereas lower As and Zn levels were found in Cell2 than in Cell1.

Table 3.27 Heavy metal concentration (µg g^{-1} DW) in the aerial part and root/rhizome of* P. australis *at the two locations (Cell1 and Cell2) for the sampling period, n.d.: no data; < d.l.: below detection limit (As: 6 µg L^{-1}, Cd: 1 µg L^{-1}, Cr: 2 µg L^{-1}, Pb: 9 µg L^{-1}); Hg was always below the detection limit (1 µg L^{-1}) and is not included.

P. australis		Cell1				Cell2			
		root	*rhizome*	*stem*	*leave*	*root*	*rhizome*	*stem*	*leave*
As	**Jul**	92.11	6.27	<d.l.	0.74	28.70	1.47	<d.l.	<d.l.
	Aug	n.d.	n.d.	<d.l.	<d.l.	n.d.	n.d.	<d.l.	<d.l.
	Sep	n.d.	n.d.	<d.l.	<d.l.	n.d.	n.d.	<d.l.	<d.l.
	Nov	64.85	3.11	<d.l.	<d.l.	181.86	1.64	<d.l.	<d.l.
Cd	**Jul**	0.16	<d.l.	<d.l.	<d.l.	0.66	<d.l.	<d.l.	<d.l.
	Aug	n.d.	n.d.	<d.l.	<d.l.	n.d.	n.d.	<d.l.	<d.l.
	Sep	n.d.	n.d.	<d.l.	<d.l.	n.d.	n.d.	<d.l.	<d.l.
	Nov	0.23	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Cr	**Jul**	5.89	1.06	<d.l.	<d.l.	14.80	0.24	<d.l.	0.24
	Aug	n.d.	n.d.	0.25	0.31	n.d.	n.d.	0.24	0.33
	Sep	n.d.	n.d.	0.32	0.90	n.d.	n.d.	0.25	0.33
	Nov	4.82	0.33	0.25	0.65	11.32	1.15	0.33	0.72
Cu	**Jul**	12.19	2.12	1.23	3.37	29.03	3.26	1.32	3.37
	Aug	n.d.	n.d.	0.99	2.44	n.d.	n.d.	2.12	4.51
	Sep	n.d.	n.d.	0.97	3.09	n.d.	n.d.	1.41	3.68
	Nov	12.44	1.39	1.32	2.51	22.10	2.71	3.06	5.91
Pb	**Jul**	8.80	<d.l.	<d.l.	<d.l.	13.90	<d.l.	<d.l.	<d.l.
	Aug	n.d.	n.d.	<d.l.	<d.l.	n.d.	n.d.	<d.l.	<d.l.
	Sep	n.d.	n.d.	<d.l.	<d.l.	n.d.	n.d.	<d.l.	<d.l.
	Nov	7.85	<d.l.	<d.l.	<d.l.	17.32	<d.l.	<d.l.	<d.l.
Zn	**Jul**	85.77	25.81	49.25	10.57	94.78	21.38	23.28	9.83
	Aug	n.d.	n.d.	27.65	16.40	n.d.	n.d.	30.01	12.51
	Sep	n.d.	n.d.	22.83	24.22	n.d.	n.d.	13.02	16.50
	Nov	68.47	13.48	19.91	48.23	105.75	25.62	31.91	44.98

Table 3.28 Heavy metal concentration (µg g^{-1} DW) in the aerial part and root of* S. lacustris, *at the two locations (Cell1 and Cell2) for the sampling period, n.d.: no data; < d.l.: below detection limit (As: 6 µg L^{-1}, Cd: 1 µg L^{-1}, Cr: 2 µg L^{-1}, Pb: 9 µg L^{-1}); Hg was always below the detection limit (1 µg L^{-1}) and is not included.

S. lacustris		Cell1		Cell2	
		root	*shoot*	*root*	*shoot*
As	**Jul**	116.55	<d.l.	32.01	<d.l.
	Aug	n.d.	0.99	n.d.	<d.l.
	Sep	n.d.	<d.l.	n.d.	<d.l.
	Nov	75.25	<d.l.	56.09	<d.l.
Cd	**Jul**	<d.l.	<d.l.	0.90	<d.l.
	Aug	n.d.	<d.l.	n.d.	<d.l.
	Sep	n.d.	<d.l.	n.d.	<d.l.
	Nov	0.16	<d.l.	0.57	<d.l.
Cr	**Jul**	3.64	<d.l.	6.14	<d.l.
	Aug	n.d.	<d.l.	n.d.	<d.l.
	Sep	n.d.	<d.l.	n.d.	<d.l.
	Nov	7.05	0.56	10.32	0.66
Cu	**Jul**	8.78	2.55	17.11	2.82
	Aug	n.d.	1.82	n.d.	2.24
	Sep	n.d.	1.91	n.d.	1.97
	Nov	11.07	2.07	14.66	2.39
Pb	**Jul**	7.79	<d.l.	10.40	<d.l.
	Aug	n.d.	<d.l.	n.d.	<d.l.
	Sep	n.d.	<d.l.	n.d.	<d.l.
	Nov	7.54	<d.l.	13.10	<d.l.
Zn	**Jul**	48.50	13.29	70.93	10.28
	Aug	n.d.	11.84	n.d.	13.97
	Sep	n.d.	9.58	n.d.	8.93
	Nov	52.75	12.83	86.76	15.38

3.4.3.6 Discussion

The growth in biomass of *P. australis* in Cell1 and Cell2 followed a normal seasonal pattern, although not so marked, with maximum mean fresh weight in August. This result is in accordance with the *P. australis* growth cycle observed by Hardej & Ozimek (2002). On the contrary, differences in growth dynamics between Cell1 and Cell2 plants have been observed, for both *T. latifolia* and *S. lacustris*. Maximum fresh weight value was reached in August for both species in Cell2, whereas in November and in September for *T. latifolia* and *S. lacustris*, respectively in Cell1.

Considering nutrient accumulation in *T. latifolia*, nitrate and TKN translocation from aerial part to belowground part at the end of the growing season resulted more evident in Cell2 plants than in Cell1 plants. This result seems to confirm the differences in growth dynamic found between Cells and it indicates that plants growing in Cell2 entered in the quiescent stage earlier than plants in Cell1. Opposite behaviour occurred for *P. australis*, with earlier shoot-root translocation in Cell1 than Cell2. No differences in N translocation between sampling site were observed in plants of *S. lacustris*.

Many macrophytes possess the ability to translocate nutrients from above-ground parts to their roots and rhizomes prior to fall senescence, and to use these stored materials to foster growth during the early part of the following growing season (Garver et al., 1988).

Plant physiological and biological processes, such as photosynthesis, show an immediate response to changes in the environmental conditions, e.g. temperature, soil characteristics, nutrients availability, etc. (Lessmann et al., 2001; Lippert et al., 2001). Therefore, we used foliar pigment contents to understand the general status of the plants growing in the pilot wetland, like a signal of the local environmental conditions. The pigment contents in leaves of *P. australis*, *T. latifolia* and *S. lacustris* did not vary significantly between the two Cells in the seasonal course. Chlorophyll contents in *P. australis* were higher than in the other species with values slightly higher compared to other study (Bragato et al., 2006). Moreover, in the present study we found a correlation between nitrate leaves concentration and Chl *a* and *b* contents, as previously found by Lipper et al. (2001).

Arsenic, cadmium, chromium, mercury, lead, zinc and copper were the heavy metals considered in this study for a number of reasons; arsenic, cadmium, chromium, mercury, lead are toxic metals not required by plants, whereas zinc and copper are micronutrients essential for plants to survive, and they are supplied mostly from the soil. In particular, zinc is involved in the activation of several enzymes in the plant and is required for the synthesis on indoleacetic acid, a plant growth regulator.

Copper is usually absorbed by plants in very small quantities. The uptake process appears to be an active process and it is adversely affected by high Zn concentrations.

Copper is not highly mobile in plants but some Cu can be translocate from older to newer leaves (Hochmuth et al., 2004). Chromium can be both beneficial and toxic to animals and humans depending on its oxidation state and concentration (Zayed et al., 1998). The presence of cadmium above trace levels in the environment is an indicator of contamination, and lead is a common pollutant from road runoff. Zinc is a common metal present in variable amounts, and if found in appreciable amounts can be an indicator of industrial pollution while copper is also an indicator of industrial contamination of urban streams.

Aquatic plants are known to accumulate metals from contaminated water and substrate (Peverly et al. 1995, Rai et al., 1995; Cardwell et al., 2002).

In general, in this study, roots revealed greater metal concentrations than leaves, while stems had the lowest concentrations. This results are in agreement with the reports of Peverly et al., (1995), Cheng et al., (2002), Stoltz & Greger, (2002), Weis & Weis, (2004) that found higher concentration of different metals in below-sediment tissues of different wetland macrophytes. In a review provided by Zayed et al. (1998), *T. latifolia* is shown to be an accumulator of Cd, Cu, Ni, and Pb, while *Schoenoplectus sp*. also accumulates Cr. Moreover, Dunbabin & Browmer (1992) reported that metal concentration (Pb, Zn, Cd, Cu) in *T. latifolia* were consistently in the order roots>rhizome>non-green leaves>green leaves, so with the greater portion of the metal taken up by the plants retained in the roots.

The source of most element nutrition for rooted aquatic macrophytes is the sediments within which plants are rooted. Following root uptake elements are transported to above-sediments tissues. Nevertheless, element availability for uptake by biota depends on different factors, such as sediment texture, composition, pH, redox potential and organic content (Jackson, 1998).

Considering *P. australis*, mean concentration of Cu, Zn, Pb and Cr detected in roots, and shoots was comparable to the values obtained by Baldantoni et al., (2004) and to the value of Cr, Cu and Zn found by Bragato et al., (2006).

3.5 Conclusions

The pilot-scale wetland involved in this study shown a considerable potential for removing BOD_5, TSS and nutrients. Previous studies demonstrated that natural and constructed wetlands can remove significant amounts of suspended solids, organic matter, nitrogen, phosphorus, trace elements, and microorganisms contained in effluents and wastewater (Kadlec & Knight, 1996; Lin et al., 2002). Aquatic macrophytes in CWs can have a positive influence on nutrient and other substances removal, not only because the plants take up nutrients, but also because they serve as a substrate for microbial biofilms (Gersberg et al., 1986).

The design chosen in this study may have a positive effect improving the removal efficiency. Deep zones are often incorporated into constructed wetlands in order to distribute the flow laterally, provide refugia for fish and other wildlife in dry weather, increase wetland volume and thereby residence time, provide quiescent areas to enhance the settling of suspended solids, contribute to the passive aeration of the water column, and furnish anaerobic environments for denitrifying bacteria (Hammer & Knight, 1994; Lightbody et al., 2007).

The integration of chemical and biological analyses allowed an overall assessment of water quality in the system. Toxicity bioassays are suggested by Italian Legislation as a rapid and cost-effective screening tool to evaluate water pollution responding to the bioavailable fraction of toxicants and thus providing ecologically relevant information. They are widely use in the assessment of the toxicity of effluent and/or wastewater discharges in the environment and receiving waters (Janssen & Persoone, 1993; Rosen & Lennox, 2001; Mansour & Sidky, 2003; Pehlivanoglu & Sedlak, 2004; Hernando et al., 2005). This chemical-biological approach was particularly effective in demonstrating no toxicity in the effluents of the pilot constructed wetland.

Plant metal accumulation, particularly in roots, was correlated with sediment metal concentration. As shown in other studies (Cardwell et al., 2002) heavy metals were poorly translocated inside the plants. The evidence of higher growth of plants in Cell2, mainly characterised by a higher heavy metal sediment concentrations and clayey soil, is maybe due to the elevated content of organic matter in the substrate.

The greater amount of all heavy metals in the clay sediment (Cell2), compared to the sandy sediment (Cell1), was probably due to the metal bounding of the clay micelle (Stumm & Morgan, 1981). Consequently, plants grown in Cell2 shown a more evident accumulation in plant tissues of all the metals considered concomitantly with a higher plant growth.

The availability of trace metals to aquatic plants is complex and is dependent on physico-chemical factors associated with the metal properties and the substrate in which plants grow (Sparling & Lowe, 1998; Mays & Edwards, 2001).

The difference in textures determined in the sediments from Cell1 – mainly sandy – and Cell2 – mainly clayey – in particular between subcells planted with *S. lacustris* (S1 and S2), links to a higher content of organic matter, TKN and metals in Cell2. As evidenced in several studies, variations in the composition of bottom sediments may strongly influence the growth and distribution of plants in wetlands (Barko & Smart, 1983). The principal influence of sediment upon the distribution of rooted aquatic plants may be due to its physical texture rather than to its chemical composition.

For that reasons, the differences in growth dynamics evidenced in this study are probably due to the differences soil texture among the subcells.

Zn and Cu are essential elements to all plants and their concentration in any of the plants considered appeared to be independent of soil concentration or water chemistry and regulated by plant organism via physiological mechanism. This result was confirmed by others studies performed in wetland sites with different substrates (Sparling & Lowe, 1998).

In conclusion, for the three plant species analyzed, *T. latifolia*, *P. aus*tralis and *S. lacustris*, soil factors seemed to have an overriding effect on metal concentration in tissues, except for zinc and copper, and in growth dynamic.

Data from this study shown that at the two Cells investigated, metals concentration in sediments were generally lower than in the roots of resident plants for all metals. Due to the very low concentration of metals in water compared to the ones observed in the sediments, no correlation analysis was possible to assess the translocation between the two matrices. For the same reason, it was not possible to evaluate the

translocation between plant tissues and water, as metals in plant tissues were mainly correlated with concentration in sediments.

Plant accumulation and decay, plus strong retention of metals by inorganic and organic soil components, are the most probable mechanisms of accumulation of metal ions near the soil surface (Dunbabin & Bowmer, 1992; Mays & Edwards, 2001). The plant metal uptake and translocation in the field may be affected by interactions between root-soil particles, root-bacteria and/or root-mycorrhiza (Burd et al., 2000; Khan et al., 2000; Stoltz & Greger, 2002).

In this study, we found that the combined information obtained from aquatic macrophytes and sediments can help in describing the translocation patterns of nutrients and metals in the wetland systems.

4 CASE STUDY: HYDRAULIC PERFORMANCE

4.1 Introduction

It is well recognized that flow distribution to, and hydraulics within, wetlands affect the contaminant removal efficiency (Kadlec, 1994, 2000; Kadlec & Knight, 1996; Persson et al., 1999; Dal Cin & Persson, 2000).
Several studies, most of them performed with tracer, demonstrated that the internal hydraulic performance in wetland systems, in addition to a combination of other factors such as mass loading rates (Kadlec & Knight, 1996), inflow pollutant speciation (Kadlec, 2003), and specific removal processes (Dierberg et al., 2002), is a major factor in determining treatment effectiveness (Dierberg et al., 2005).

4.1.1 The hydraulic efficiency of wetlands

Constructed Wetlands (CWs) are increasingly used for the removal of pollutants from rivers and lake, municipal and industrial wastewater, urban runoff in different part of the world since the 1950s (Gerke et al., 2001). However, those systems may fail to meet environmental guidelines because they are not well designed (Thackston et al., 1987; Persson et al, 1999; Kadlec, 2000; Goulet et al., 2001; Persson, 2005). As reported by Persson et al. (1999) many wetland management problems can be attributed to poor hydrodynamic characteristics within the wetland systems.

In particular, features that can affect the hydrodynamic characteristics within a wetland system are:

- Shape of the wetland (Wörman & Kronnäs, 2005);
- Hydraulic characteristics of the inlet and outlet structures (Persson et al., 1999; Persson, 2000; Suliman et al., 2006);
- Wetland bottom topography that can increase friction against the bottom (Kjellin et al., 2007);
- Vegetation type, density and spatial distribution (Dal Cin & Persson, 2000;

Serra et al., 2004; Kjellin et al., 2007);

- Mixing (Lightbody et al., 2007).

In particular, Persson et al., (1999) investigated the effects of wetland shape on the hydraulic efficiency and found that wetlands with large values of length/weight ratio (L/W) produced a higher efficiency. Moreover, when island or a subsurface berm was placed in front of the inlet, the flow distribution of the incoming water resulted improved. Furthermore, a curved pond or an island placed near the side does not lead to lower hydraulic performance, and the locations of inlets and outlets have a considerable impact on the hydraulic performance.

In wetland systems macrophytes are often the major component and several studies have shown their importance in water-pollution control (Nichols, 1983; Weisner et al, 1994; Kadlec & Knight, 1996;) as well the modification of hydrodynamics, turbulence and sediment and dust dispersion by plant canopies (Leonard & Luther, 1995; Worcester, 1995; Nepf et al., 1997; Nepf & Koch, 1999; Fonseca & Koehl, 2006). They play an important role in natural and physical processes such as filtration, stabilization of sediments and provision of increased surface for biofilm growth. In the same time, the spatial distribution of the vegetation within the wetland affects significantly the hydraulic characteristics of the system (Jenkins & Greenway, 2005).

Typically, wetlands comprise a variety of vegetation types including those that are emergent, floating leaved attached, free floating and submerged (Greenway, 2004). The complex hydraulic characteristics produced by the different types of vegetation found in a wetland are due in part to the "flexible nature" of the vegetation (Jenkins & Greenway, 2005). This flexibility means that during high flows, the vegetation will tend to bend and flatten, thus reducing the apparent roughness. Under low flow conditions the vegetation undergoes relatively little flexibility. Kadlec (1990) has shown that the drag on an individual stem of emergent vegetation can be used to describe the shear stress produced by the vegetation in the wetland.

Heterogeneous velocity fields can also result from variation in vegetation densities over depth, patches of dense and sparse vegetation, and deeper and less vegetated channels (Kadlec, 1994, 2000; Kadlec & Knight, 1996; Werner & Kadlec, 1996;

Dierberg et al., 2005; Wörman & Kronnäs, 2005; Lightbody & Nepf, 2006). In many wetlands, it has been observed the phenomenon known as short-circuiting (Thackston et al., 1987). In these cases, channels develop preferential flow paths with low resistance that allow a large portion of the water entering the wetland to travel directly to the outlet in much less time than the hydraulic residence time (Dal Cin & Persson, 2000; Dierberg et al., 2005; Lightbody et al., 2007). When this phenomenon occurred, a significant reduction in water quality improvement provided by wetlands has been observed (Kadlec & Knight, 1996; Economopoulou & Tsihrintzis, 2004; Dieberg et al., 2005).

4.1.2 Hydraulic retention time

In constructed free-water surface wetland two idealized flow patterns can occurred, plug-flow and mixed flow. To describe the real flow conditions existing in the real system (reactor) it is important to consider the deviation between the two ideal flow patterns: Plug-Flow Reactors (PFR) and Continuous Stirred Tank Reactors (CSTR – the name is derived from the chemical engineering processes) (Kadlec et al., 1993).

The ideal residence pattern in most treatment basins would be plug-flow, but this is impossible to achieve in practice. Under ideal plug-flow conditions, all of the water that enters the wetland stays together as a single plug as it flows through and exits the system. The time that this plug of water stays in the system is referred to as the hydraulic retention time (HRT). Water moving through the wetland system travels at the same velocity, and all the water reaches the exit at exactly the same nominal (theoretical) hydraulic residence time (t_n). A longer hydraulic retention time allows for more of the treatment processes to be completed. The hydraulic retention time, under ideal plug-flow conditions can be defined by Eq. 4.1.

Eq. 4.1
$$t_n = \frac{V}{Q}$$

Where:

t_n = nominal (or theoretical) hydraulic retention time (day);
V = wetland volume (m^3);

Q = flow rate through wetland (m^3 day^{-1}).

Perfect plug-flow is never obtained because some velocity heterogeneity is always present, which results in dispersion (Wörman & Kronnäs, 2005).

In real wetland systems, the water does not stay together as a single plug as it flows through the system. All of the features listed above (Par. 4.1.1) play a role in the distribution of water flow through the wetland system and they affect the hydraulic retention time of water within the system (Jenkins & Greenway, 2005).

In addition, dispersion caused by unsteady flow rate, wind, inlet and outlet effects, and shear stresses at the sides and bottom cause some parcels of water to exit earlier than the nominal residence time (t_n) and some to exit later. The deviation from plug-flow can create "dead zones" within the basins, in which velocities towards the outlet are considerably less than average (Thackston, 1987).

These zones are relatively ineffective in treating pollutants flowing through the wetland because relatively small amounts of influent enter these zones (Jenkins & Greenway, 2005).

Velocity heterogeneity, and short-circuiting in particular, can lead to reduced water quality improvement in wetlands (Wörman & Kronnäs, 2005).

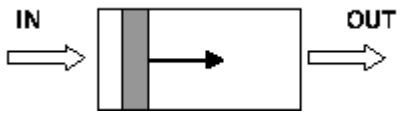
As previously marked, plug or continuously stirred flow conditions never occur in natural systems and the flow in the basin is generally a combination of plug-flow and a number of Continuously Stirred Tanks Reactors (CSTRs) (Werner & Kadlec, 1996). The analytical tools for analyzing non-ideal flows are contained in residence time distribution (RTD) theory (Levenspiel, 1972; Kadlec, 1994). RTD aids in describing the manner and extent of deviation from ideal flow. It can be generated from tracer study to better understand the macroscopic mixing and hydrodynamics in the system (Werner & Kadlec, 1996).

The extreme possibilities between an "ideal" pattern of flow and complete mixing can be resumed as:

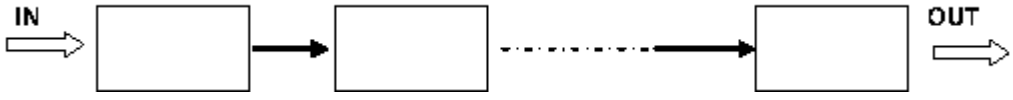
⇒ **single continuous stirred tank reactor (CSTR)** (N = 1): it results in a tank, or basin in which the inflow is immediately and completely mixed with the existing contents (Wong et al., 2002).

If completely mixed, all parcels have equal probability of leaving the wetland at a given moment. This behaviour results in a pollutant hydraulic residence time distribution (RTD) represented by an exponential function (Figure 4.1).

⇒ **plug-flow reactor (PFR)** ($N = \infty$) (Kadlec & Knight, 1996). A **plug-flow** condition is the result of the ∞ number of CSTRs series. The concentration versus time distribution takes the form of a positively skewed distribution function. The extent to which flow conditions depart from an idealised plug-flow condition is reflected in the spread of the distribution (Persson et al., 1999). The plug flow is considered the optimal flow: all the fluid elements reside around the nominal residence time (which is defined by the ratio between the volume and the flow). Under ideal plug-flow conditions, all the water that enters the wetland stays together as a single plug as it flows through and exits the system (Jenkins & Greenway, 2005). The degree of plug-flow is a measure of the amount of mixing and it is correlated with advection-dispersion processes. The plug-flow degree is indicated by the number of stirred tanks (N), used in a tank-in-series model.



⇒ the **tanks-in-series model (TIS)** is based in the CSTR model (Continuously Stirr Tank Reactors) (Kadlec, 1994). In this model the wetland is partitioned into a number (N) of equally sized CSTRs reactors tanks and the concentration of a certain pollutant leaving each tanks is equal to the uniform internal concentration (Kadlec & Knight, 1996).



Perfect plug-flow is never obtained because some velocity heterogeneity is always present (Figure 4.1).

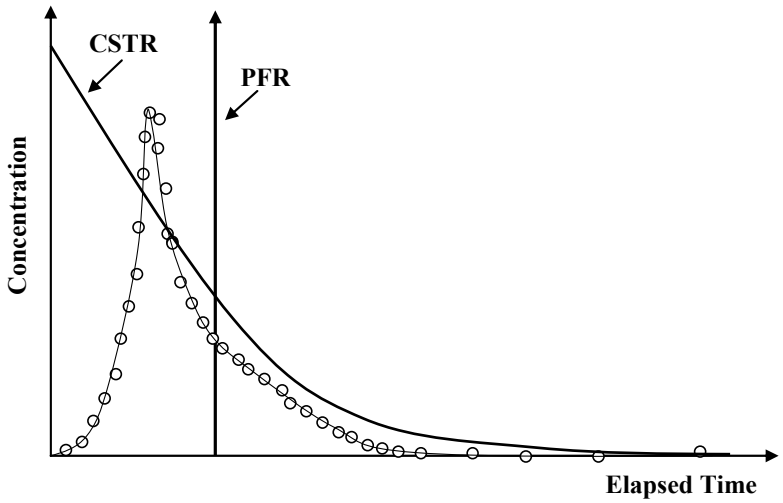


Figure 4.1 Tracer response and representations of ideal well-mixed (CSTR) and plug-flow (PFR) conditions.

4.1.3 Tracer test

Several studies have been performed to analyse and model the hydraulic behaviour in wetlands systems, both in field and in laboratory (Torres et al., 1997; Nameche & Vasel 1998; Adamsson et al., 1999). The internal flow behaviour in wetlands and ponds can be studied experimentally by stimulus-response techniques, using a tracer to detect possible anomalies in the flow – dead areas, short circuiting, etc. – and their relation with the phenomena which influence the wetland hydrodynamics. Moreover, it is widely recognized that the removal efficiency of a free surface CW depends on flow pattern, often described in terms of the residence time distribution (RTD), or the equivalent number (N) of tanks in series (TIS) (Kadlec & Knight, 1996; Persson et al., 1999; Kadlec, 2000).

An important consideration in completing a successful tracer study is the choice of the tracer chemical. Tracer selection for hydrologic (volumetric flow rate, velocity, seepage) and hydraulic (HRT, dispersion, residence-time distribution) studies in water bodies depends on a number of considerations (Dierberg & DeBusk, 2005).

These include:

- Solubility;
- Background concentration;
- Analytical detection;
- Chemical and biological inertness;
- Toxicity;
- Materials handling;
- Costs.

Although all of these factors are important in selecting a tracer, the extent to which a tracer is conservative is frequently considered one of the most essential properties in returning reliable data for analysis (Werner & Kadlec 2000; Lin et al. 2003; Dierberg & DeBusk, 2005).

A non-reactive, soluble material is required for tracer studies. The tracer is required to move with the water, and it is necessary that the tracer does not react with or adsorb to any ecosystem component, such as soils, sediments, litter or vegetation. Comparative wetland studies (Netter & Bischofsberger, 1990; Dierberg & DeBusk, 2005) demonstrated that bromine, lithium, and fluorescein dyes are all approximately equivalent for this purpose. Lithium was demonstrated to do not adsorb to wetland soils and sediments associated with wetland and it was widely used in several tracer studies (Kadlec, 1994; King et al., 1997; Dal Cin & Persson, 2000).

Other aspects important to investigate a tracer candidate prior to conducting the tracer test are the determination of necessary tracer mass, initial sample-collection time, and subsequent sample collection frequency (Field, 2003).

In a wetland, the residence time will be influenced primary by two factors: hydrology (the temporal distribution of the inflows) and hydraulics (the flow patterns that develop in the basin) (Walker, 1998).

The importance of the flow patterns and the movement of inflows through the basin have been previously noted by a number of researchers (Kadlec, 1993; Walker, 1998; Persson, 2000).

Flow patterns, and the effect on residence time, in wetlands basins have been widely investigated. For example, Thackstone et al. (1987), Kadlec et al. (1993), Schmid et al., (2004), Serra et al., (2004) have been used to determine the residence time distribution (RTD) for basins in the laboratory and field. In many cases, the wetland was under steady flow conditions and the determination of RTD was achieved by releasing a slug of dye or tracing material, and measuring its concentration at the outlet. More recent work by Werner & Kadlec (1996) has further developed the method so that dye tracing experiments can be used to determine meaningful RTDs during non-steady flow conditions (Thackstone et al., 1987; Kadlec et al., 1993; Schmid et al., 2004; Serra et al., 2004).

Tracer tests are useful tools and are used to establish hydraulic parameters, which may have a large effect on wetland performance (Kadlec & Knight, 1996). If a tracer impulse is instantaneously added to the inlet of a wetland, the form of the outlet pulse for the TIS model is given by the retention time distribution (RTD), which is the distribution function of hydraulic residence time (Persson, 2000). The RTD reflects the degree in which the residential time varies and can be quantified by tracer field study or through numerical simulation. Transit time distribution and mixing processes are quantified by the RTD model.

A typical tracer response is shown in Figure 4.2. The tracer response curve is characterised by a relatively steep rising limb, followed by a flatter receding limb.

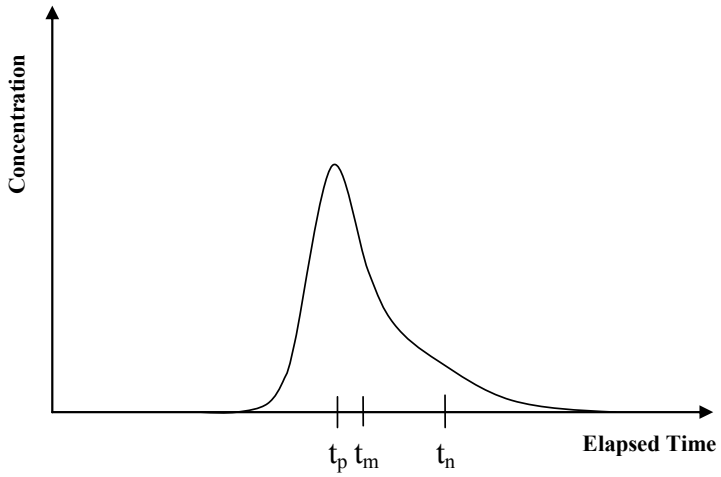


Figure 4.2 Typical tracer response curve.

Velocity profiles in the wetland can lead to a distribution of residence times (Figure 4.2). The shortest time is experienced by the water moving at the maximum velocity in the profile, which would normally be in the surface layer of the micro-channels. The longest times is experienced by water that moves near drag-inducing surfaces, such as the wetland bottom. This type of 'mixing' scales to the distance down the wetland, and to the average speed of the water (Werner & Kadlec, 2000).

Kadlec & Knight (1996), Persson et al. (1999) and Persson (2000) shown that the dimensionless variance of the tracer response curve, provides information on the amount of dispersion and mixing present within the system.

4.1.4 Hydraulic parameters

The tracer response curve can be studied in order to understand the hydrodynamics of the system (e.g. the amount of mixing (*N*), measure of short circuiting, effective volume, measure of hydraulic efficiency). Those measures require a quantification of the range of detention time within a wetland to allow calculation of the mean and standard deviation of the tracer response (Persson et al., 1999).

For an impulse input of tracer into a steadily flowing system, the function *f(t)* is (Eq. 4.2):

Eq. 4.2

$$f(t) = \frac{QC(t)}{\int_0^\infty QC(t)dt} = \frac{C(t)}{\int_0^\infty C(t)dt}$$

where:

C(t) = exit tracer concentration
Q = water flow rate

An important expression is the variance (θ^2), that is the square of the spread of the distribution, or a measure of the dispersive processes (Eq. 4.3):

Eq. 4.3

$$\sigma^2 = \frac{\int_0^\infty (t_m - t)^2 f(t)dt}{\int_0^\infty f(t)dt}$$

A plug-flow condition will induce a RTD with a variance equalling 0, that means no dispersion.

The RTD defines the key parameters that characterize the actual detention time.

The mean residence time, t_m, which is the average time that a tracer particle spends in the water system, is defined as the centroid of the RTD (Eq. 4.4):

Eq. 4.4

$$t_m = \frac{\int_0^\infty t f(t) dt}{\int_0^\infty f(t) dt}$$

By comparing the theoretical or nominal (t_n) and observed or mean (t_m) HRT, a unique hydraulic behaviour can be remarked:

- ⇒ $\boxed{t_n > t_m}$: Inflow crosses the reed bed without reacting; this is an indicator of short-circuiting. Fluid follows a preferential path and the first peak is seen at an earlier stage of the curve than in the theoretical curve, which peaks at a later stage.
- ⇒ $\boxed{t_n < t_m}$: Fluid stagnates in the reactor and does not participate in reactions. This phenomenon is due to the presence of dead or stagnant zones.

The dead zones, as explained before, are not part of the volume through which water flows, and thus, the effective wetland volume ratio (e) is less than the total volume, with mean residence time (t_m) less than the nominal residence time (t_n). The relationship between t_m and t_n is derived by Thackstone et al., (1987) as (Eq. 4.5):

Eq. 4.5

$$e = \frac{t_m}{t_n} = \frac{V_{effettive}}{V_{total}}$$

Where:

$V_{effective}$ = total volume minus the dead zones,
V_{total} = total volume of the water system.

The number of cells (N) in the tank-in-series model, is equal to Eq. 4.6 (Kadlec & Knight, 1996):

Eq. 4.6

$$N = \frac{t_m^2}{\sigma^2}$$

Another way to describe hydraulic efficiency (λ) and to have a general measure of hydrodynamic conditions in pond and wetlands is done by Persson et al., (1999) (Eq. 4.7):

Eq. 4.7
$$\lambda = e\left(1 - \frac{1}{N}\right) = \frac{t_p}{t_n}$$

This expression (Eq. 4.7) relates the number of CSTRs (N) to the hydraulic efficiency (λ). This parameter has a range of 0 to 1.

4.1.5 Model description

The increasing application of treatment wetlands coupled with increasingly strict water quality standards has been an incentive for the development of better design tools. Several studies are focused to elaborate design models in order to improve the quality effluent and understand the key parameter affecting efficiency in wetlands and ponds (Willems et al., 1997; Gerke et al., 2001; Rousseau et al., 2004; Marsili-Libelli & Checchi, 2005; Zimmels et al., 2009).

As previously remarked, flow characterisation in CW is extremely important and hydraulic modelling has evolved from the initial plug-flow and tank-in-series assumptions (Kadlec & Knight, 1996) to more elaborate schemes based on the approximation of two-dimensional dispersed flow (Langergraber, 2003; Marsili-Libelli & Checchi, 2005).

Nevertheless, wetlands are often treated as continuously stirred tank reactors or plug flow reactors in the literature (Kadlec & Knight, 1996; Gerke et al., 2001). These models assume constant flow of water.

Nitrate (or nutrients in general) removal data can be represented by a first-order model (Spieles & Mitsch, 2000; Carleton et al., 2001):

- K-C* model (Eq. 4.8):

Eq. 4.8
$$\ln\left(\frac{C_{out} - C^*}{C_{in} - C^*}\right) = -\frac{K}{q}$$

where:

K = area-based first-order TN rate constant (m/yr);

q = hydraulic loading rate (m/yr);

C_{out} = outlet TN concentration (mg L^{-1});

C_{in} = inlet TN concentration (mg L^{-1});

C^* = background wetland TN concentration (mg L^{-1}).

This is a first order rate expression, for an unidirectional, non-infiltrating, constant flow wetland (steady state and plug-flow assumptions).

Another approach is based on:

- Tanks-in-series (TIS) model (Kadlec, 2005) (Eq. 4.9):

Eq. 4.9

$$\frac{(C_{out} - C^*)}{(C_{in} - C^*)} = \left(1 + \frac{K}{Nq}\right)^{-N}$$

where:

K = first-order uptake rate constant (m yr^{-1});

q = hydraulic loading rate (m yr^{-1});

C_{out} = outlet TN concentration (g m^{-3});

C_{in} = inlet TN concentration (g m^{-3});

C^* = background wetland TN concentration (g m^{-3});

N = hydraulic efficiency parameter.

Nitrate removal data are best represented by the tanks-in-series model (TIS) (first order model). It is commonly used for modelling of pollutant removal in ponds and wetlands (Kadlec & Knight, 1996). It represents a series of continuously stirred tank reactors where a substance is removed in each tank according to first order kinetics. The number of tanks, N, represents the degree of mixing (Persson & Wittgren, 2003).

The N value can be presumed from previous studies, or can be determined using tracer experiments.

Temperature effects upon denitrification are presumed to be described by a modified Arrhenius temperature relation (Kadlec, 2005) (

Eq. 4.10):

Eq. 4.10 $$K_{aT} = K_{20}\theta^{(T-20)}$$

Where:

K_{20} = first order uptake rate constant at 20°C (m yr^{-1});
T = water temperature (°C):
Θ = temperature factor.

The higher the value of the model parameter *N*, the better the performance of the treatment wetland.

Weisner et al., (1994) and Kadlec, 2005 reached the conclusion that fully vegetated marshes with either emergent and submerged communities seem to be more efficacy for nitrate reduction. Moreover, an alternating banded pattern perpendicular to flow would additionally provide hydraulic benefits.

4.2 Aim of the research

The aim of this study is to describe the effects of wetland vegetations and different inlets in the hydraulic performance in 18 experimental wetlands and to understand flow properties.

To meet the objectives set forth in this study, a series of tracer studies were performed to determine the hydraulic behaviour of the wetlands under the same operational and environmental conditions, but characterised to different vegetations, (mixed, emergent and submerged) and the presence or absence with a narrow barrier in the inlets.

4.3 Material and methods

4.3.1 Site description

This study was performed in pilot scale wetlands in Plönninge, near Halmstad, Sweden (Figure 4.3).

The system was constructed in 2002 and consists of 18 wetlands with similar shape, an area of about 12 m^2 at the bottom and 40 m^2 at the ground surface and a slide slope at about 1:1. The incoming water was discharged into each wetland through an inlet pipe at one short side. Each wetland had an outlet at the opposite short side (Figure 4.4; Table 4.1).

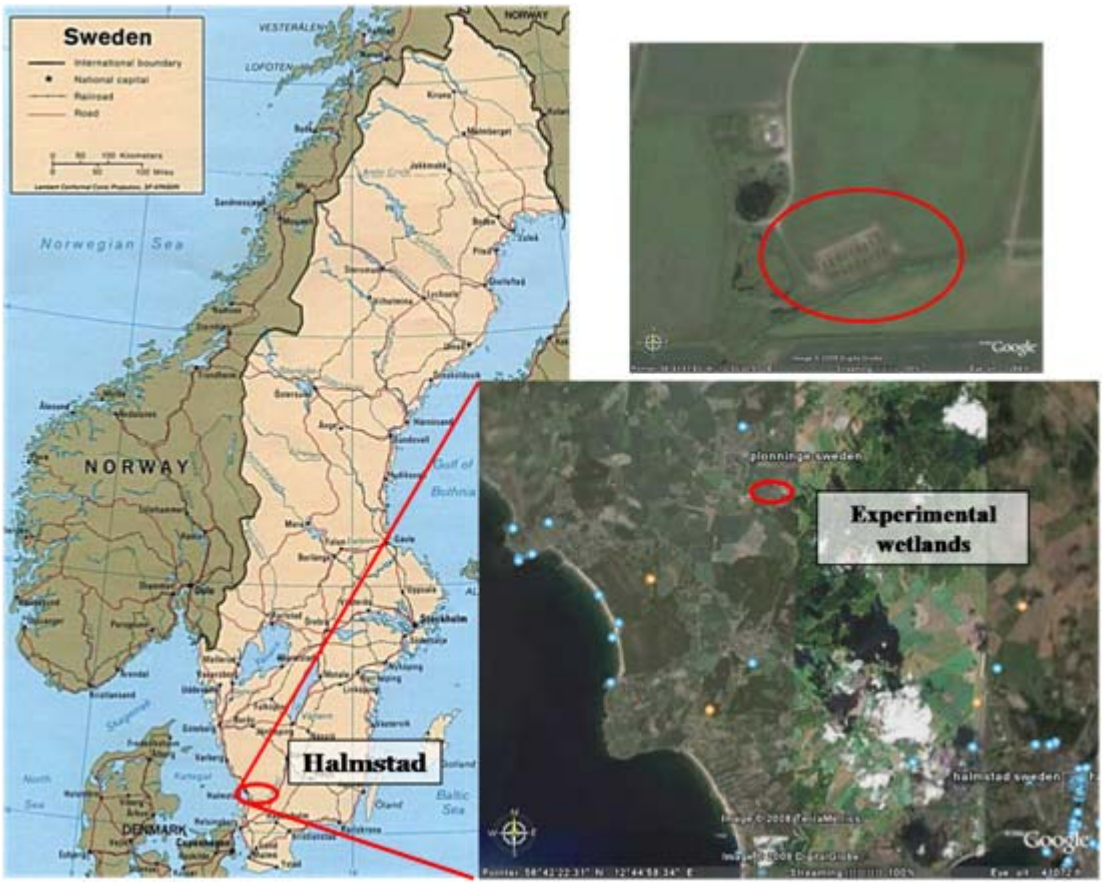


Figure 4.3 Location of the experimental wetlands in Plönninge – Sweden.

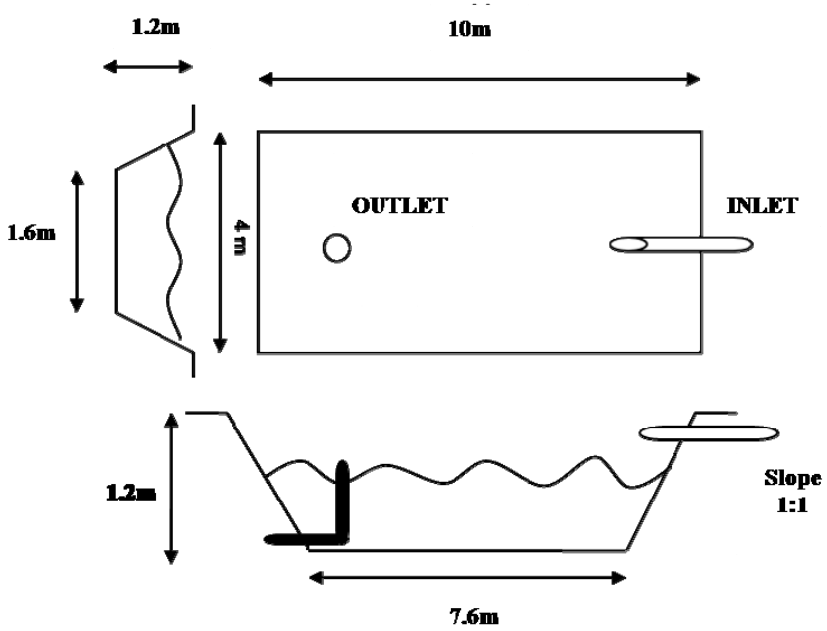


Figure 4.4 Schematic drawing of the Plönninge water surface constructed wetland system.

The wetland basins were planted with the two different vegetation types (emergent and submersed) during May 2003, while one third of the basins were left unplanted in order to achieve freely developing vegetation (control) (Figure 4.5). In the basins with emergent vegetation, *Phragmites australis* (Trin.), *Glyceria maxima* (Hartm.) and *Phalaris arundinacea* (L.) were established. The basins with submersed vegetation were planted and dominated by *Elodea canadensis* (Rich.), *Myriophyllum alterniflorum* (DC.) and *Ceratophyllum demersum* (L.). From 2004 to 2006, the remaining basins were gradually colonized by algae and higher plants, which were dominated by *Alopecurus geniculatus* (L.), *Agrostis gigantea* (Roth.) and *Typha latifolia* (L.).

This resulted in three different vegetation states dominated either by dense emergent vegetation, by submerged vegetation with a carpet of filamentous green algae, or by a more mixed vegetation (some emergent and floating-leaved macrophytes and some open water with filamentous green algae), during this study. In addition, a barrier in front of the inlet pipes in half of the wetlands (three of each vegetation state) were installed to facilitate spreading of water within the wetlands (Figure 4.5).

Temperature and total nitrogen concentration were measured from 2003 to 2008.

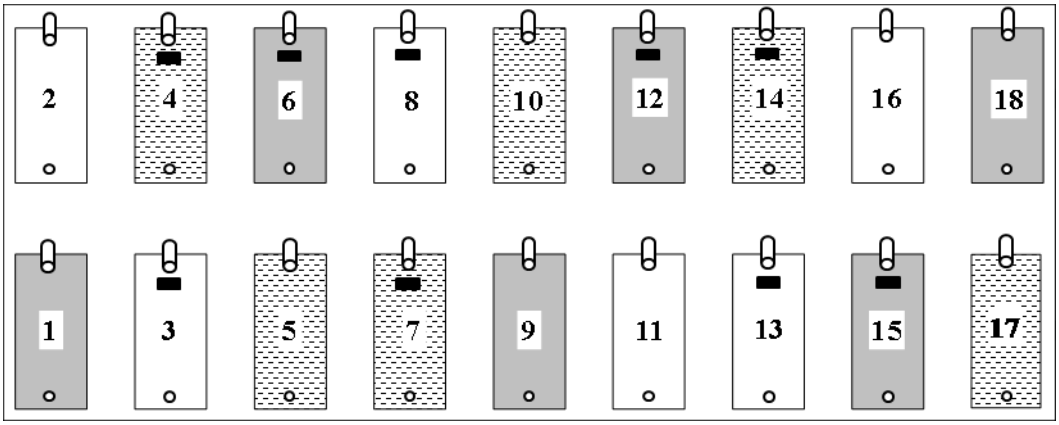


Figure 4.5 Schematic drawing of the Plönninge free water surface constructed wetland systems. Six wetlands (white) considered as control, six wetlands (interrupted lines) with submerged vegetation and six (grey) with emergentd vegetation. Black line indicates barrier in the inlet.

Some hydraulic and morphological characteristics of the 18 wetlands in Plönninge are listed in Table 4.1.

Table 4.1 Hydraulic and morphological characteristics of the Plönninge wetland system

Area (m^2)	29.44
Volume (m^3)	16.3
Length (m)	9.2
Width (m)	3.2
Depth (m)	0.8
Flow (L min^{-1})	2.3 - 2.8
Retention time (day)	4.2 - 4.4
Hydraulic loading rate (m day^{-1})	0.14

4.3.2 Experimental design

The tracer experiment was carried out in September – October 2008 in all the 18 experimental wetlands in Sweden. Before performing tracer experiment, water flows were adjusted using gate valves fitted on the inlet pipe of each wetland to obtain theoretical residence times (t_n) of about 4 days. The water level in the wetlands can be regulated by adjusting the outlet pipes. The water depth in the deep section of the wetlands was set to 0.8 m during this study. This corresponds to a mean depth of

0.55 m, and a water surface area of 29 m^2 (Table 4.1). As previously explained, during this study, a narrow barrier was installed in front of the inlet pipes in half of the wetlands (three of each vegetation state).

In this study, lithium chloride was chosen as a tracer because it behaves more conservatively than other dyes (Dieberg & DeBusk, 2005).

The tracer solution was previously prepared in a bucket with a lid by adding 300 g of lithium chloride (LiCl) in 9 L of water, in order to mix and solve the LiCl and to avoid amounts of tracer elements settling at the bottom of the wetlands. After that, the tracer was poured in the three wells with a volume approximately of 500 L, from where water flows in the 18^{th} wetlands. To calculate the amount of lithium entering in each wetland, the flows measured during the first day of test had been considered (Table 4.2).

4.3.3 Laboratory analysis

Water samples were collected by putting a clean polyethylene bottle (50 mL volume) below the water surface adjacent to each outlet of the 18 wetlands. The first sample was taken after few minute the addition of lithium (time-0) and the second one after 19^{th} hours. The sampling frequency was every 6 hours for the first 56 hours, after 12, 27, 48, 72 hours until the end of the experiment (last sample after 226 hours the addiction).

The flow was measured manually in the inlets and in the outlets during each sampling time.

The sample were acidified with concentrated HNO_3 until they reach pH<2 *in situ*.

Unfiltered lithium concentration was analyzed with an atomic spectrophotometer (SPECTRA 100), by direct intensity measurements at a wavelength of 670.8 nm.

Samples analyses were performed at Wetland Research Centre, Halmstad University, Sweden.

4.3.4 Statistical analysis

Statistical analysis was computed using standard statistical packages (STATISTICA® for Windows). A statistical comparison of means was done with analysis of variance (ANOVA) followed by Post-Hoc test ($p<0.05$).

4.4 Results and discussion

4.4.1 Flow characteristics

During the test (300-350 h), the flow rate in the 18 cells was relatively constant, varied between 2.3 and 2.8 L min^{-1}.

In wetlands, Reynolds number (dimensionless) indicates the ratio of inertial to viscous forces on the flow and indicates the presence of laminar or turbulent flow conditions in the system. The Reynolds number (Re) is defined as (Eq. 4.11):

Eq. 4.11
$$\mathrm{Re} = \frac{V\ h}{\nu}$$

Where:
V = velocity (m s^{-1});
h = water depth (m);
ν = kinematic viscosity of water (m^2 s^{-1}).

The transition from laminar to turbulent flow is considered to occur at 2000 to 2300, with Re<2000 corresponding to laminar flow and Re>2300 being turbulent (Kadlec & Knight, 1996). Oldham & Sturman (2001) indicated that in wetlands densely vegetated low Reynolds number occurs, with value of ~ 100.

In our study, Reynolds number varied from 12 to 15, depending to the water flow, without significant differences between wetlands, indicating that laminar flow occurred in the basins.

4.4.2 Tracer mass recovery

The lithium chloride mass balance was calculated by comparing the added mass to the tracer mass recovered at the effluent of each cells.

Table 4.2 shows the cumulative mass recovery at the exit of cells. The lithium mass balance was checked, by comparing the added lithium to the total lithium found in the exit flow. The total mass of lithium exiting a wetland during a test is given by Eq. 4.12.

Eq. 4.12

$$M_0 = \int_0^{t_f} Q_e(t) C(t) dt$$

Where:

$C(t)$ = exit tracer concentration (mg m^{-3});
t_f = total time span of the outflow pulse (h);
Q_e = volumetric inflow rate of water (m^3 h^{-1}).

A comparison of the recovered tracer mass and the amount added to the 18 wetlands demonstrated a mean recovery close to 100%, with maximum value of 112% and minimum value of 77%. Mean recovery in the control and submerged wetlands with and without barrier and in emergent wetlands with barrier was similar, ranged from 82% to 112%, whereas in emergent wetlands without barrier the recovery was lower, with mean value of 78%.

The high recovery for Li^+, greater than 100%, in the wetlands 8 and 12 – emergent vegetation, with barrier in the inlet – indicated a slight overestimation, possibly due in part to the less frequency Li^+ sampling in the ‘‘tail’’ region of the profile.

Slightly lower recovery in the wetlands 1, 9 and 18 – emergent vegetation, with no barrier in the inlet – can be attributed to the effect of the incoming water temperature that resulted colder than the water in the basins. The differences in temperature, and consequently the higher density of the water entering in the wetland with respect to the water present in the wetland, in conjunction to the high emergent vegetation density and the absence of flow distribution provided by the inlet barrier, could determine a relatively low recovery of tracer, partially trapped in the bottom of the

basins. In addition, some inaccuracies in the inflow measurements may have contributed to reduce mass recovery.

Table 4.2 Lithium balances of 18 wetlands, planted with mixed (C), emergent (E) or submerged vegetation (S), with or without barrier in Plönninge (Sweden) pilot systems.

Number of wetland	Vegetation	Sampling point	Li (g)	Mass recovery (%)
3	C	IN - with barrier	9.1	100
		OUT	8.4	92
8	C	IN - with barrier	7.1	100
		OUT	7.9	112
13	C	IN - with barrier	8.0	100
		OUT	6.8	85
2	C	IN - without barrier	8.1	100
		OUT	7.8	97
11	C	IN - without barrier	8.3	100
		OUT	8.0	97
16	C	IN - without barrier	7.2	100
		OUT	6.7	92
6	E	IN - with barrier	7.8	100
		OUT	7.9	101
12	E	IN - with barrier	10.1	100
		OUT	11.3	110
15	E	IN - with barrier	7.4	100
		OUT	6.1	82
1	E	IN - without barrier	9.4	100
		OUT	7.2	77
9	E	IN - without barrier	7.2	100
		OUT	5.7	79
18	E	IN - without barrier	9.0	100
		OUT	7.0	78
4	S	IN - with barrier	7.9	100
		OUT	8.1	102
7	S	IN - with barrier	8.0	100
		OUT	7.4	92
14	S	IN - with barrier	9.4	100
		OUT	7.9	84
5	S	IN - without barrier	6.9	100
		OUT	5.7	83
10	S	IN - without barrier	8.4	100
		OUT	8.4	100
17	S	IN - without barrier	8.1	100
		OUT	7.6	94

4.4.3 Tracer response curves

The hydraulic residence time (HRT) distributions obtained by tracer tests are represented in Figure 4.6, Figure 4.7 and Figure 4.8. The tracer responses consisted in the typical response curve with bell shaped with a long tail.

The final baselines were not well defined and did not return to starting zero concentration of Li tracer. Based on that, the tails were determined as an exponentially decreasing function until reach the time corresponding to 3 t_n (Kadlec & Knight, 1996).

The mean residence time in the ponds at the time of the experiment was about 3.4 days and no differences were found among types of wetlands (Table 4.3). From Table 4.1 and Table 4.3, it is possible to see that the nominal residence time calculated by Eq. 4.1 (volume/flow rate through wetland) was about 4 days because all water in the wetland actively participated in a complete plug flow (the maximum possible residence time). Indeed, the ascending limb of the tracer curve peaked before to the nominal residence time, t_n.

As expected, dye concentration-time profiles (Figure 4.6, Figure 4.7 and Figure 4.8) shown a dispersion and asymmetry, as expected for flow through a vegetate systems, with "dead zones" which are inaccessible to the main flow.

Similarity in the shape tracer curve among control and submerged wetlands was observed (Figure 4.6, Figure 4.7), with curve characterised by elongated tail on the right. In contrast, emergent wetland shape curves were steeper with respect to control and submerged wetlands (Figure 4.8).

Observing the breakthrough curves, in some wetlands, in particular in emergent ones, a second peak, less marked than the main one, was present, indicating that slightly channelling was occurring.

CONTROL WETLANDS

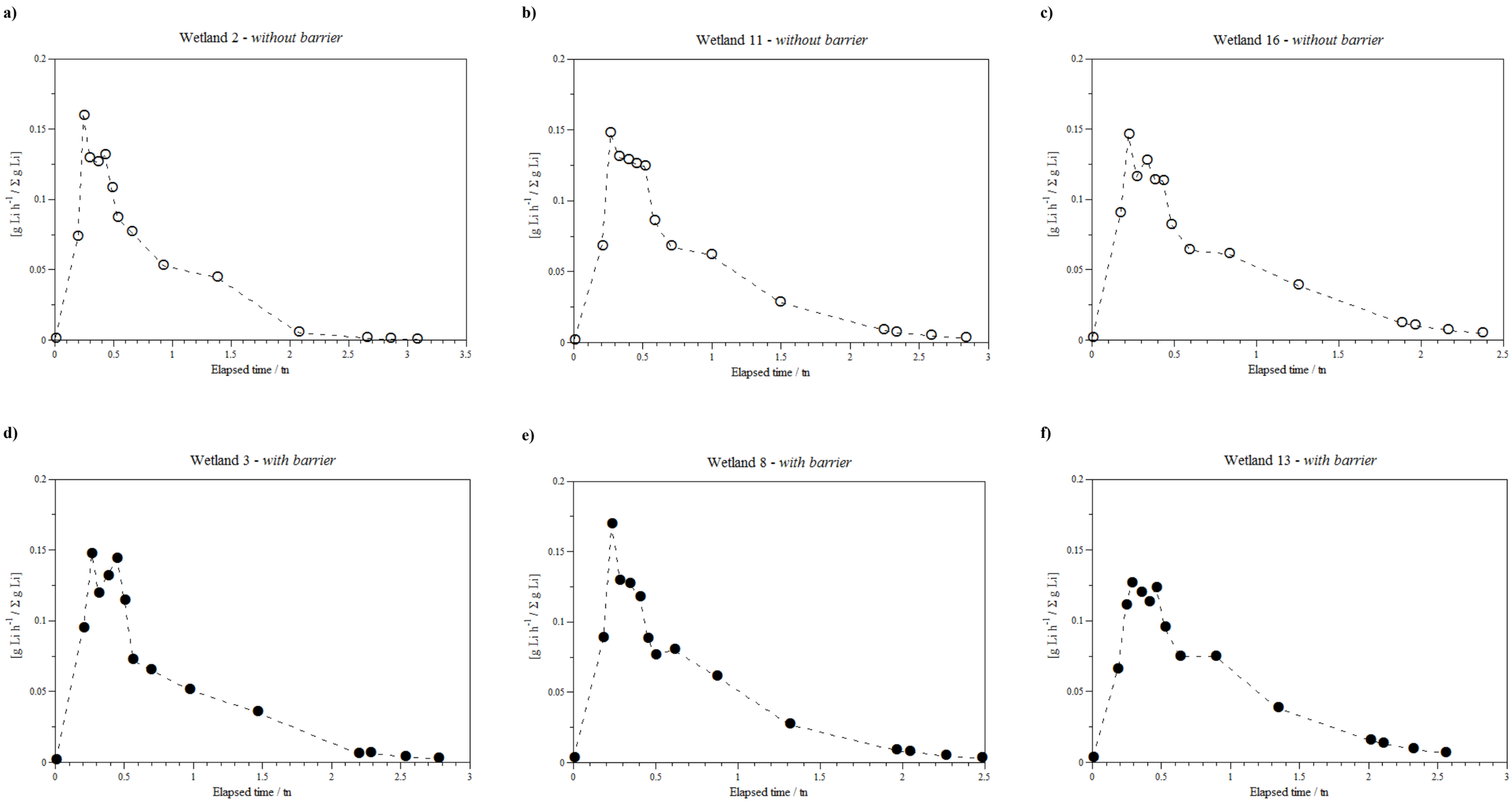


Figure 4.6 Normalized residence time distribution curves for control free water surface constructed wetlands without (a, b, c) and with (d, e, f) barrier in the inlet. The x-axis shows time normalized by the nominal residence time (t_n). The y-axis shows tracer mass at each time (g L^{-1}) normalized by the total mass (g L^{-1}).

SUBMERGED WETLANDS

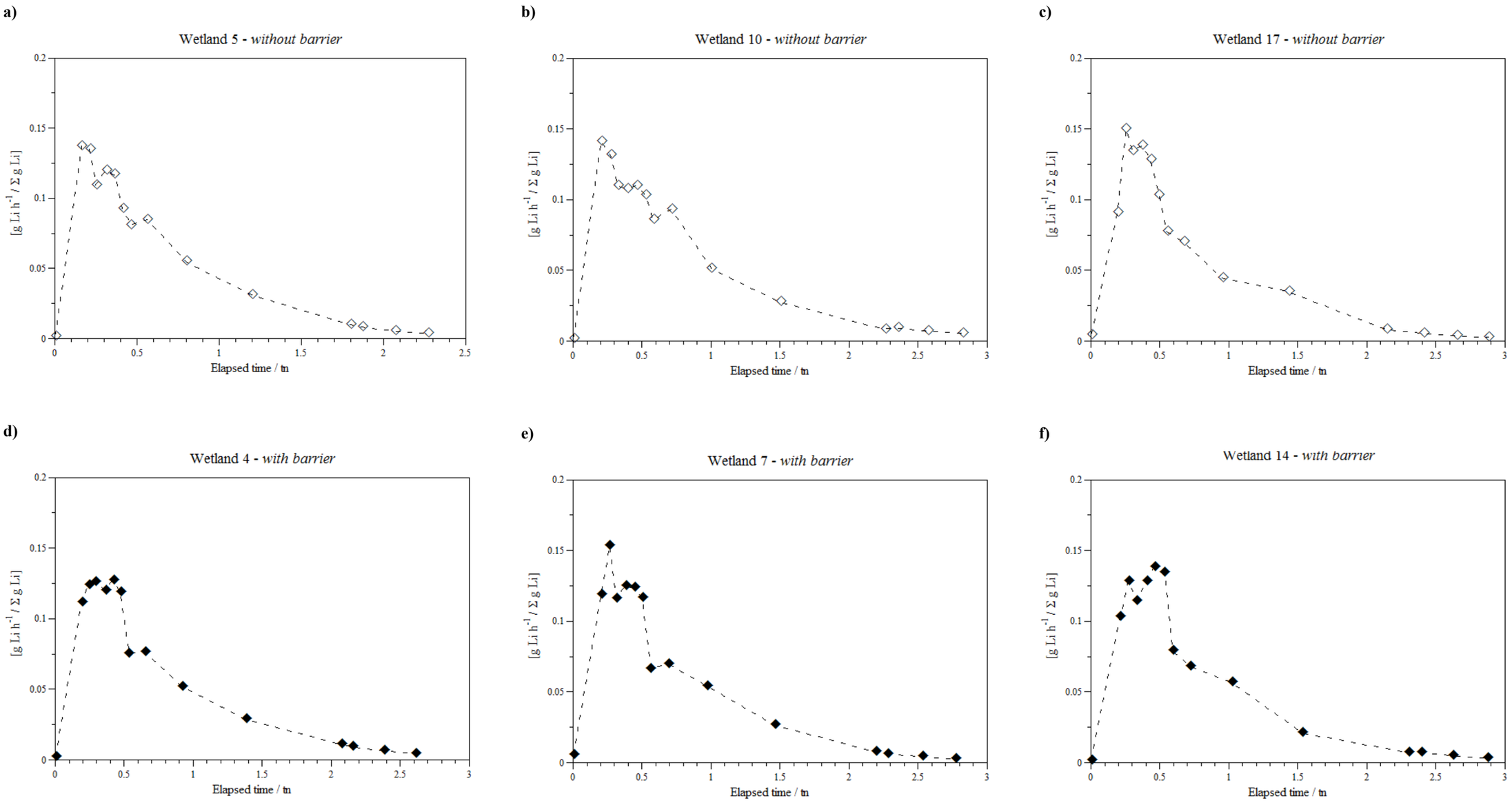


Figure 4.7 Normalized residence time distribution curves for submerged free water surface constructed wetlands without (a, b, c) and with (d, e, f) barrier in the inlet. The x-axis shows time normalized by the nominal residence time (t_n). The y-axis shows tracer mass at each time (g L^{-1}) normalized by the total mass (g L^{-1}).

EMERGENT WETLANDS

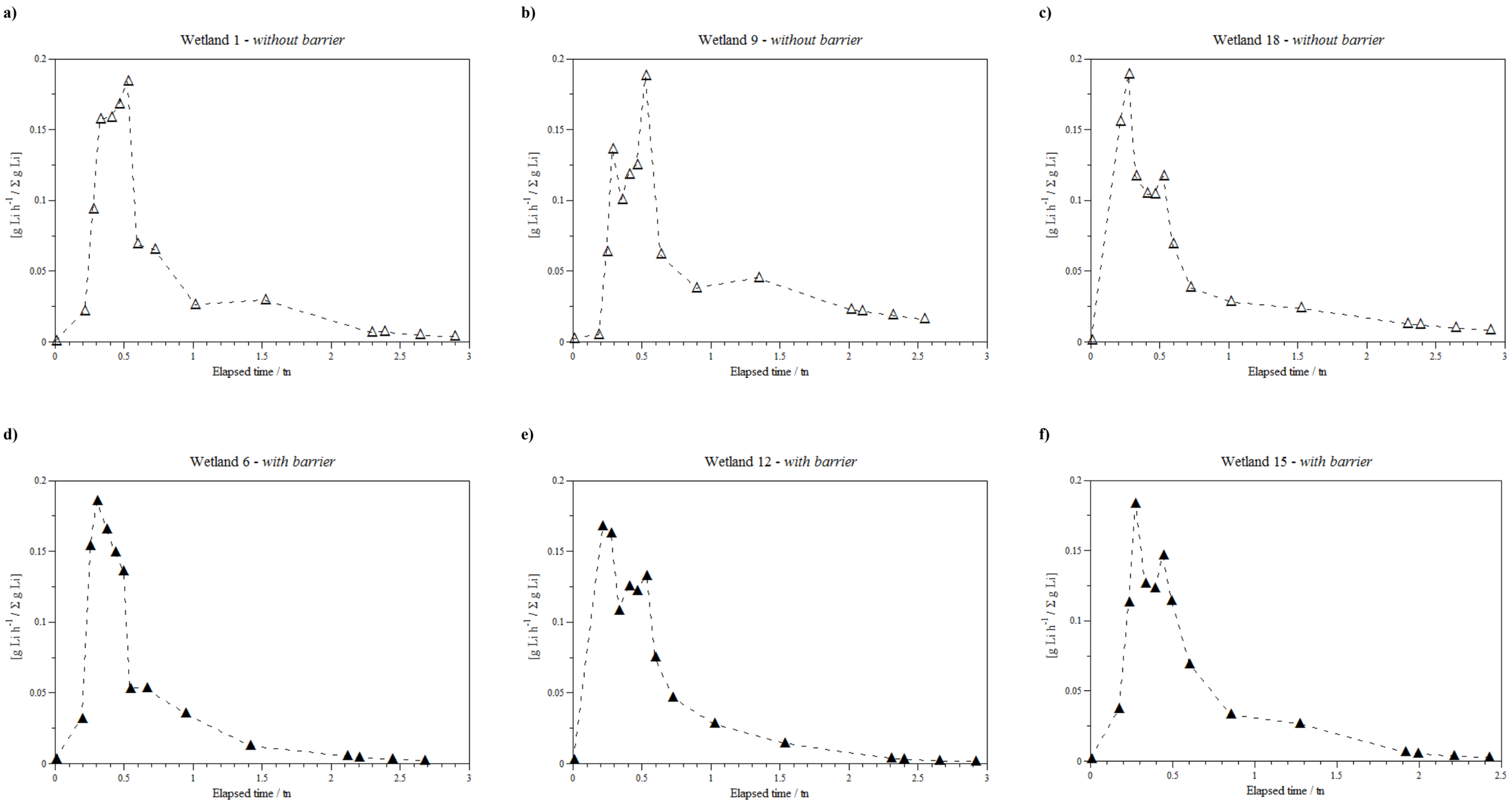


Figure 4.8 Normalized residence time distribution curves for emerged free water surface constructed wetlands without (a, b, c) and with (d, e, f) barrier in the inlet. The x-axis shows time normalized by the nominal residence time (t_n). The y-axis shows tracer mass at each time (g L^{-1}) normalized by the total mass (g L^{-1}).

4.4.4 Wetland hydraulic characteristics

The results calculated through the tracer study performed in the 18 pilot wetlands in Plönninge are summarised in Table 4.3 and in Table II. 1.

Table 4.3 Results of the tracer study during September-October 2008 in 18 wetlands in Plönninge (Sweden) pilot systems: 6 controls (C), 6 planted with emergent vegetation (E) and 6 with submerged vegetation (S), with (b.) or without barrier (no b.) in the inlet. Tracer recovery (%), nominal hydraulic retention time (t_n), mean hydraulic retention time (t_m), peak time (t_p), effective volume ratio (e), hydraulic efficiency (λ), and number of tanks n the CSTR model (N) are shown (mean±SD, n=3).

Vegetation	Inlet	Mass recovery (%)	t_n (day)	t_m (day)	t_p (day)	*e*	*λ*	*N*
C	b.	96.6±13.9	4.4±0.3	3.5±0.3	1.1±0.1	0.79±0.07	0.26±0.03	2.0±0.1
	no b.	95.3±2.5	4.4±0.4	3.5±0.2	1.1±0.0	0.79±0.03	0.24±0.02	2.1±0.2
S	b.	92.9±9.0	4.1 ±0.2	3.2±0.2	1.3±0.4	0.78±0.02	0.38±0.11	1.8±0.1
	no b.	92.0±8.7	4.4±0.5	3.3±0.1	1.1±0.1	0.76±0.07	0.21±0.05	1.8±0.1
E	b.	97.8±14.6	4.3±0.4	2.9±0.4	1.1±0.1	0.66±0.03	0.28±0.02	1.7±0.2
	no b.	77.9±1.0	4.1±0.3	3.8±0.9	1.9±0.7	0.89±0.09	0.44±0.14	1.6±0.3

The mean residence time, calculated using Eq. 4.4, ranged from 3.2 to 3.9 days in control wetlands, from 2.8 to 4.9 days in emergent wetlands and from 3.1 to 3.4 days in submerged wetlands (Table II. 1).

Variability of the effective volume ratio, *e*, as indicated in Table 4.4, was mostly due to the interaction between the inlet factor (presence or absence of the barrier) and vegetation type, which explained 47% of observed variation. Moreover, inlet factor explained 18% of the variation. In particular, considering the different types of vegetations, in emergent cells, *e* value was significantly higher in wetland without barrier ($p<0.05$), whereas not significant differences were observed in control and submerged wetlands, with and without barrier. Comparing cells with different vegetation and with barrier in the inlet, *e* value was significantly higher in control wetlands with respect to emergent wetlands ($p<0.05$), with mean values of 0.79 and 0.66 in control and emergent wetlands, respectively. Not significant differences were found between wetlands without barrier.

Table 4.4 Factors influencing hydraulic parameters (effective volume ratio, e, hydraulic efficiency, λ, and number of tanks in the CSTR model, N) considering the three vegetation types, control, emergent and submerged.

Parameter	Factor	DF	% variation	*p*-value
e	Vegetation	2	1.6	**n.s.**
	Inlet	1	17.7	**p<0.05**
	Vegetation*Inlet	2	47.2	**p<0.01**
	Residual	12	33.6	
λ	Vegetation	2	18.5	**n.s.**
	Inlet	1	0.2	**n.s.**
	Vegetation*Inlet	2	43.5	**p<0.05**
	Residual	12	37.9	
N	Vegetation	2	49.5	**p<0.05**
	Inlet	1	0.0	**n.s**
	Vegetation*Inlet	2	3.6	**n.s**
	Residual	12	47.0	

For each parameter, factors (vegetation – control, emergent and submerged – inlet types – with and without barrier - and the interaction between them), degree of freedom, variation percentage associated at each factors and p-value resulting from Two-factor ANOVA have been considered.

Concerning hydraulic efficiency, λ, the interaction between factor vegetation types and inlet (presence or absence of barrier) (Table 4.4) was significant, explaining 43.5% of the variation in λ value in the wetlands. In particular, hydraulic efficiency in emergent wetlands without barrier was significantly higher in respect with submerged wetlands without barrier ($p<0.05$). Mean values were 0.44 and 0.21 in emergent and submerged wetlands without barrier respectively.

Vegetation factor was significant ($p<0.05$) for number of tanks, *N*, explaining 49.5% of the variability, while inlet factor and the interaction between factors have no significant effect on *N* (Table 4.4). Comparing the different types of vegetation (removing inlet factor from the statistical analyses), a significant difference was found between control and emergent wetlands, with mean values of 2.0 and 1.7 in control and emergent, respectively ($p<0.01$). The value of N in this study ranged from 1.8 to 2.4 in control wetlands, from 1.7 to 1.9 in submerged wetlands and from 1.3 to 1.9 in emergent wetlands (Table II. 1).

Considering the results obtained from the statistical analyses based on three vegetation types and two inlet types, no significant differences in the hydraulic parameters between control and submerged wetlands have been found, whereas

significant differences were reported between emergent vs. control and emergent vs. submerged vegetation. This could be supported by the shape of the curve obtained by the tracer tests (Figure 4.6, Figure 4.7) as well as from the pictures in Annex II (Figure II. 1, Figure II. 2). Since that result, we could consider control and submerged wetlands belong to the same group, indicated as mixed vegetation in order to better describe hydraulic conditions in the study system.

Mean values of the different hydraulic parameters on mixed and emergent vegetation are summarized in Table 4.5.

Table 4.5 Results of the tracer study during September-October 2008 in 18 wetlands in Plönninge (Sweden) pilot system: 12 with mixed vegetation (M), 6 planted with emergent vegetation (E), with (b.) or without barrier (no b.) in the inlet. Tracer recovery %, nominal hydraulic retention time, t_n, mean hydraulic retention time, t_m, peak time, t_p, effective volume ratio, e, hydraulic efficiency, λ, and number of tanks n the CSTR model, N are shown (mean±SD, n=6 for mixed cells, n=3 for emergent cells).

Vegetation	Inlet	Mass recovery (%)	t_n (day)	t_m (day)	t_p (day)	*e*	λ	*N*
M	b.	94.7±10.7	4.3±0.3	3.2±0.2	1.3±0.4	0.78±0.05	0.32±0.10	1.9±0.1
	no b.	93.7±6.0	4.4±0.4	3.4±0.2	1.0V0.1	0.77±0.05	0.23±0.04	1.9±0.2
E	b.	97.8±14.6	4.3±0.4	2.9±0.4	1.2±0.1	0.66±0.03	0.28±0.02	1.7±0.2
	no b.	77.9±1.0	4.1±0.3	3.8±0.9	1.8±0.7	0.89±0.09	0.44±0.14	1.6±0.3

In this case, variability of effective volume ratio, *e*, between the wetlands was mostly due to the inlet factor and to the interaction between inlet and vegetation factor, which explained 32% and 39% of the observed variation respectively (Table 4.6).

Value of *e* in emergent vegetation with barrier was significantly lower than emergent wetland without barrier ($p<0.05$). Comparing wetlands with barrier, *e* calculated in emergent vegetation was significantly lower than mixed vegetation ($p<0.01$). Moreover, in the case of the absence of barrier in the inlet, *e* in emergent vegetation was significantly higher than mixed ($p<0.05$).

Mean value of *e* in emergent vegetation was 0.66 and 0.89 with and without barrier respectively, whereas was 0.78 and 077 in mixed vegetation wetlands with and without barrier, respectively.

Table 4.6 Factors influencing hydraulic parameters, effective volume ratio, e, hydraulic efficiency, λ, and number of tanks in the CSTR model, N, considering the two vegetation types, mixed and emergent.

Parameter	Factor	DF	% variation	*p*-value
e	Vegetation	1	0.0	**n.s.**
	Inlet	1	32.2	**p<0.01**
	Vegetation*Inlet	1	38.8	**p<0.001**
	Residual	14	29.1	
λ	Vegetation	1	15.4	**n.s.**
	Inlet	1	2.1	**n.s.**
	Vegetation*Inlet	1	32.7	**p<0.01**
	Residual	14	49.7	
N	Vegetation	1	29.8	**p<0.05**
	Inlet	1	0.1	**n.s**
	Vegetation*Inlet	1	1.1	**n.s**
	Residual	14	69.0	

For each parameter, factors (vegetation – mixed and emergent– inlet types – with and without barrier - and the interaction between them), degree of freedom, variation percentage associated at each factors and p-value resulting from Two-factor ANOVA have been considered.

Considering hydraulic efficiency, λ, among wetlands, interaction between the factor inlet (presence or absence of the barrier) and vegetation (mixed or emergent vegetation), as shown in Table 4.6 was significantly, explaining 33% of the variation. No significant differences were found between inlet types (on different inlet types separately), nevertheless hydraulic efficiency in mixed vegetation with barrier was slightly higher than in wetlands without barrier. Comparing wetlands without barrier, λ in mixed vegetation was significantly lower than in emergent ($p<0.01$). No differences were found among wetlands with barrier in the inlet.

Mean value of hydraulic efficiency in emergent vegetation was 0.28 and 0.44 in wetlands with and without barrier, whereas was 0.32 and 0.23 in mixed vegetation with and without value.

Number of tanks, *N*, among wetlands was affected only by the factor vegetation ($p<0.05$), which explained 30% of the variability (Table 4.6). Therefore, without considered the inlet factor, *N* value in mixed vegetation was significantly higher than in emergent wetlands ($p<0.05$). In mixed vegetation mean *N* value was 1.9, whereas in wetlands characterised by emergent vegetation was 1.7.

Considering statistical analyses performed on data of wetlands without barrier, tracer mass recovery (%) in emergent vegetation was statistically lower than mixed one ($p<0.01$). Mean values in wetlands without barrier were 78% in emergent and 94% in mixed vegetation.

As previously explained, lower recovery in emergent vegetation could be due to trapping of the tracer in wetlands bottom. This result can influence hydraulic parameters calculation and, consequently, has to be taken into account in the discussion of the systems.

4.4.5 Discussion

From data obtained in this study it seems that no significant differences in hydraulic condition were found among control and submerged wetlands. The similarities in the general vegetation distribution (Figure II. 1, Figure II. 2 and Figure II. 3) and the shape of the tracer response curves (Figure 4.6, Figure 4.7 and Figure 4.8) of those wetlands confirmed this result.

Narrow barrier placed in the inlet seems to have a positive effect in hydraulic conditions, as evidenced by higher value of hydraulic efficiency and effective volume ratio in wetlands with mixed vegetation and with barrier. Hence, as described by Persson, et al. (1999), changing the inlet configuration can significantly affect the hydraulic performance in wetlands. In particular, the presence of subsurface berm or barrier has positive influences in the hydraulic in the wetland, decreasing mixing and short-circuiting.

Considering emergent vegetation wetlands, it seems that hydraulics was improved by the absence of the barrier in the inlet. As previously discussed a comparison among emergent wetlands with and without barrier resulted difficult, because of the low tracer recovery measured in wetlands without barrier could have affected the calculation of the hydraulic parameters. In the other hand, this result can be considered as a useful tool to better understand tracer behaviour and dependence of water temperature and density.

The low recovery was observed only in wetlands without barrier, and that can demonstrate that tracer entering the wetland was not well distributed and was trapped in the bottom.

The derived values of the number of CSTRs in series (N) for the 18 wetlands were not affected by inlet type, but vegetation types significantly influenced them. In both vegetation wetlands, low value of N occurred, because some degree of bypassing (short-circuiting) is inevitable, as evidenced by $1<N<5$ (Kadlec, 2005). Nevertheless, mixed vegetation wetlands (control and submerged wetlands) explained lower degree of dispersion than emergent ones. That fact can be explained considering the variation in vegetation densities and distribution occurred in wetlands. In mixed vegetation wetlands, plants occupied the whole volume of the basins and they were distributed homogeneously, on the contrary, emergent wetlands were more densely distributed. In emergent wetlands, the vegetation was more densely distributed compared to mixed wetlands.

Moreover, effective volume ratio between the two different types of vegetation with barrier in the inlet was significantly higher in mixed vegetation than in emergent one. This indicated the presence, more marked in emergent wetlands, of some dead-zones and that part of the volume was not interested by the water flows. This resulted in effective volume basin lower than the total volume and the mean residence time lower than the nominal residence time. Water exchange with stagnant zones and dispersion are two different ways of representing additional mixing mechanisms present in wetlands. These mechanisms are highly related, because transverse exchange of water parcels between low- and high-velocity zones can occurred and can increase the deviation from an ideal plug-flow.

As observed in several studies, the fact that the observed residence times were lower than the nominal residence time, could be partly a result of dead-volumes of water caused by channelling of the flow (Wörman & Kronnäs, 2005). Formation of channelling in the flow pattern can be seen also as multiple-peaks in breakthrough curves mainly occurred in emergent than mixed wetlands. In particular, nominal residence time was 27% longer in the case of emergent vegetation, whereas it was 21% and 23% longer in control and submerged wetlands respectively.

4.5 Conclusions

This study shown that vegetation types and distribution significantly affect the flow conditions. In particular, in the presence of mixed vegetation the hydraulic efficiency was improved, with lower degree of dead-zones and short-circuiting. Moreover, in mixed vegetation wetlands the flow was closer to plug-flow condition, which is the ideal optimal flow to achieve in wetlands.

In addition, the comparison of the 18 wetlands shown that the inlet type influenced the hydraulic performances, although with less marked effects with respect to vegetation types, with no significant differences between wetland types.

As shown in others studies, salt tracer experiments are a convenient and widespread method for use in the flow characterization on constructed wetlands (King et al., 1997; Dal Cin & Persson, 2000; Dierberg & DeBusk, 2005). In this study, lithium chloride tracer was used to obtain indications of hydraulic efficiency in the 18 free-water-surface pilot wetlands in Sweden. Salt tracers are the most commonly used tracers for several reasons, in particular because they are inert, typically not hazardous and inexpensive.

However, the results concerning emergent wetlands without barrier were compromised by density effects as shown by the low mass recovery occurred in this case. Furthermore, because of salt tracer injections and the formation of density layers into wetland ponds with emergent vegetation, density stratification can occur, as evidenced by Schmid et al. (2004).

This study verified that vegetation types and density in wetlands can affect hydraulic performance, enhancing short-circuiting and dead-zones. It also indicates that something more is needed to better explain hydraulic behavior, in order to improve removal efficiency.

Further research will be helpful to refine the methodology approach of the lithium chloride in this wetland system, to ensure a better application of this tracer.

5 CONCLUSIONS

Constructed wetland systems are widely used in removing nutrients and heavy metals by many different processes, including plant uptake and accumulation.

In this study, we considered two important functions of wetland systems: removal efficiency and hydraulic performance. These two aspects are strictly connected because a principal controlling factor of pollutants removal is water movement patterns in the wetland. Mixing and flow of the water parcels and the residence time that each one resides in a wetland determine the extent in space and time of pollutants removal reactions. Based on that, wetland design may play an important role for nutrient and metal retention, as well as for enhancing biodiversity and recreational values in constructed wetland systems.

The removal efficiency of TSS, nutrients and heavy metals obtained in the pilot wetland in Fusina was high and confirmed the capacity and effectiveness of wetland systems and aquatic plants in reduction of those elements. No significant differences in removal were found between parallel cells, although a variation in plant growth dynamic between Cell1 and Cell2 was evidenced, probably due to the differences in sediment texture and composition between the two Cells.

The application of a battery of toxicity tests, as suggested by Italian legislation, is a very useful tool to detect synergic or antagonistic effects of substances in the wastewater and to assess effluents acceptability (Janssen & Persoone, 1993; USEPA, 2002; Hernando et al., 2005; D. Leg. N°152, 2006). In this study, the toxicity assays applied, microbial test, with Microtox®, algae test, with *Pseudokirchneriella subcapitata*, and test with the aquatic invertebrate *Daphnia magna*, allowed indicating no toxicity in water flows through the wetland system. Partial stimulation of algal growth in the effluents was observed, as expected in wetlands environment in which nutrients are generally available (Sbrilli et al., 2003).

Among the different plant species growing in the pilot constructed wetland of Fusina, we selected for monitoring the three main emergent macrophytes, *Typha latifolia*,

Phragmites australis and *Schoenoplectus lacustris*. From our results, *Phragmites* shown higher accumulation of both nutrients and heavy metals than the other plant species analysed. *Phragmites* is the macrophyte most widely used in phytoremediation due to the endemic distribution, rusticity and high biomass production. However, all the plant species considered were able to concentrate nutrients and heavy metals in their tissues. In particular, at the end of the growing season the translocation of those elements to the belowground parts was found. Such knowledge of the period of maximum nutrients and heavy metals content in the aboveground plant tissues may help in programming the harvesting practices in order to maximize removal of these elements from the system.

The study of hydraulic performance in 18 pilot wetlands in Sweden highlighted the great importance and the influence of type and distribution of vegetation on the water flow patterns. In particular, wetlands characterised by mixed vegetation type shown a higher hydraulic performance and flow patterns closer to plug-flow conditions than wetlands with dense emergent vegetation. Furthermore, the results obtained by placing a narrow barrier at the inlet indicated the fundamental role played by design configuration in enhancing the flow spreading in the wetland systems.

Another important result of this study was related to the tracer methodology approach: the fact that low lithium mass recovery was measured in emergent wetlands without barrier needs to be better investigated, in order to understand the relationship between Li density stratification and water temperature.

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ANNEX I

⇒ CASE STUDY: Removal Efficiency: Pilot wetland system in Fusina, Venice.

a) Cell1 – *T. latifolia*

b) Cell2 – *T. latifolia*

c) Cell1 – *P. australis*

d) Cell2 – *P. australis*

e) Cell1 – *S. lacustris*

f) Cell2 – *S. lacustris*

Figure I. 1 Fusina pilot wetland: subcells planted with* T. latifolia *(a-Cell1 and b-Cell2),* P.australis *(c-Cell1 and d-Cell2) and* S. lacustris *(e-Cell1 and f-Cell2). Pictures taken on September 2008.

Table I. 1 Meteo data, air temperature (°C), relative humidity (%), rain (mm), pressure (mbar) and solar radiation (W m^2, MJ m^2) measured in July in Station N°23 by Rete Ente Zona Industriale di Porto Marghera.

July 2008 Station N° 23 - CED Rete Ente Zona								
	AIR TEMPERATURE (°C)			RELATIVE HUMIDITY	RAIN	PRESSURE	SOLAR RADIATION	
day	average	MIN	MAX	average %	mm	average mbar	global W/m2	global MJ/m2
1	23.9	18.8	27.4	73	4.4	1014	179.2	0.65
2	24.9	21.3	27.7	74	0.0	1011	273.4	0.98
3	24.8	20.7	27.3	78	0.0	1009	247.2	0.89
4	26.1	20.8	29.7	71	0.0	1007	280.5	1.01
5	23.0	19.4	25.7	76	0.0	1009	240.5	0.87
6	22.8	18.8	25.7	85	31.4	1009	216.3	0.78
7	22.6	18.7	26.7	80	1.2	1006	255.7	0.92
8*	20.1	18.0	23.3	73	0.0	1009	265.1	0.95
9*	21.5	16.7	24.4	67	0.0	1013	286.7	1.03
10	23.1	18.7	25.3	75	0.0	1013	278.7	1.00
11*	24.0	20.7	25.7	79	0.0	1011	268.2	0.97
12	24.0	20.2	26.5	82	0.0	1008	250.1	0.90
13	23.1	20.0	28.9	76	0.6	1005	237.8	0.86
14	20.2	17.1	24.9	78	5.6	1011	180.5	0.65
15	21.7	16.1	25.0	71	0.0	1021	284.6	1.03
16	22.3	18.0	24.2	72	0.0	1018	289.6	1.04
17	22.5	19.4	25.1	79	0.6	1010	202.2	0.73
18	19.0	16.0	21.6	82	8.2	1010	130.2	0.47
19	22.2	18.1	25.0	73	0.0	1013	287.6	1.04
20	22.7	20.4	24.7	83	3.2	1010	220.8	0.79
21	20.3	18.1	21.9	74	0.2	1010	191.8	0.69
22	19.3	15.7	21.6	61	0.0	1014	301.0	1.08
23	20.7	15.5	23.1	68	0.0	1013	293.0	1.05
24	23.1	18.1	26.8	63	0.0	1010	275.4	0.99
25	23.0	17.7	26.0	73	0.0	1007	261.1	0.94
26	23.8	22.0	26.9	76	2.8	1008	182.5	0.66
27	24.3	20.5	27.1	72	0.0	1011	268.7	0.97
28	25.3	21.5	28.4	67	0.0	1012	270.8	0.97
29*	25.5	21.3	28.6	72	0.0	1014	254.7	0.92
30	25.8	22.4	27.9	72	0.0	1015	259.4	0.93
31	25.4	21.3	29.0	73	0.0	1014	231.7	0.83
min/max		**15.5**	**29.7**		**31.4**			
average	**22.9**	**19.1**	**25.9**	**74**	**1.9**	**1011**	**247.3**	**0.89**
total					**58.2**			

Sampling day

Table I. 2 Meteo data, air temperature (°C), relative humidity (%), rain (mm), pressure (mbar) and solar radiation (W m^2, MJ m^2) measured in August in Station N°23 by Rete Ente Zona Industriale di Porto Marghera.

AUGUST 2008 Station N° 23 - CED Rete Ente Zona								
	AIR TEMPERATURE (°C)			RELATIVE HUMIDITY	RAIN	PRESSURE	SOLAR RADIATION	
day	average	MIN	MAX	average %	mm	average mbar	global W m^2	global MJ m^2
1	25.9	22.2	28.9	73	0.0	1013	237.2	0.85
2	25.9	21.6	29.6	69	0.0	1013	255.4	0.92
3	26.0	21.9	28.6	75	0.0	1013	256.9	0.92
4	26.4	23.7	28.7	82	0.0	1009	237.4	0.85
5*	26.3	22.2	29.6	80	0.0	1009	205.5	0.74
6	25.0	22.3	26.7	67	0.0	1011	243.5	0.88
7	25.2	21.1	28.0	73	0.0	1009	249.5	0.90
8	23.6	17.0	26.8	83	3.4	1004	210.9	0.76
9	21.8	16.3	25.2	62	0.0	1009	256.7	0.93
10	22.5	18.5	24.4	70	0.0	1011	245.1	0.88
11	23.4	19.6	25.5	75	0.0	1007	256.6	0.92
12	23.9	20.4	26.5	84	0.0	1006	182.1	0.66
13	24.0	21.2	26.8	78	0.0	1009	212.7	0.77
14	24.2	20.0	28.0	76	7.4	1010	248.2	0.89
15	21.7	17.0	26.6	87	5.4	1002	146.2	0.53
16	18.9	14.0	22.6	73	26.2	1005	236.2	0.85
17	19.2	16.8	22.0	76	0.0	1010	153.7	0.55
18	20.8	16.4	23.7	77	0.0	1015	248.7	0.90
19	22.1	19.0	23.9	84	0.0	1013	232.6	0.84
20	23.3	20.1	25.5	78	0.2	1011	233.3	0.84
21	23.5	19.7	26.4	73	0.0	1014	239.3	0.86
22	23.6	19.6	26.6	76	0.0	1011	237.2	0.85
23	21.5	17.6	26.5	82	0.0	1006	159.5	0.57
24	19.7	15.6	22.3	65	1.0	1010	253.2	0.91
25	20.5	16.3	23.7	64	0.0	1014	237.7	0.86
26	22.0	16.9	25.8	65	0.0	1018	238.7	0.86
27	23.2	20.2	26.1	63	0.0	1017	236.3	0.85
28	23.4	20.1	26.5	67	0.0	1014	225.8	0.81
29	23.8	20.7	26.7	70	0.0	1013	215.1	0.77
30	22.9	19.1	25.8	80	0.0	1013	210.2	0.76
31	22.2	19.8	24.1	78	0.0	1015	203.7	0.73
min/max		**14.0**	**29.6**		**26.2**			
average	**23.1**	**19.3**	**26.1**	**74**	**1.4**	**1011**	**226.0**	**0.81**
total					**43.6**			

Sampling day

Table I. 3 Meteo data, air temperature (°C), relative humidity (%), rain (mm), pressure (mbar) and solar radiation (W m^2, MJ m^2) measured in September in Station N°23 by Rete Ente Zona Industriale di Porto Marghera.

SEPTEMBER 2008 Station N° 23 - CED Rete Ente Zona								
	AIR TEMPERATURE (°C)			RELATIVE HUMIDITY	RAIN	PRESSURE	SOLAR RADIATION	
day	average	MIN	MAX	average %	mm	average mbar	global W/m2	global MJ/m2
1	22.0	19.1	24.5	74	0.0	1014	183.8	0.66
2	21.6	19.0	24.1	85	0.0	1013	164.1	0.59
3	22.1	19.0	24.6	87	0.8	1010	188.3	0.68
4	22.4	18.2	26.3	81	0.8	1009	202.9	0.73
5	22.2	18.5	25.1	86	0.0	1010	192.5	0.69
6	23.3	20.4	27.5	86	0.0	1009	185.3	0.67
7	22.9	19.2	28.2	80	0.0	1008	164.2	0.59
8*	21.0	16.0	24.6	72	0.0	1013	212.0	0.76
9	21.7	17.8	24.7	72	0.0	1017	208.4	0.75
10	22.4	18.5	25.8	75	0.0	1015	181.8	0.65
11	23.2	19.2	25.8	78	0.0	1010	168.1	0.61
12	21.0	17.5	25.0	90	3.4	1005	135.2	0.49
13	15.8	11.6	20.4	97	57.6	1005	36.5	0.13
14	13.0	11.1	16.5	80	5.2	1009	104.2	0.38
15	13.4	10.9	15.9	69	0.2	1009	121.2	0.44
16	15.3	11.1	19.0	76	0.0	1010	183.4	0.66
17	15.1	11.8	17.0	76	0.0	1015	175.3	0.63
18	14.8	12.1	17.2	76	0.0	1018	128.9	0.46
19	15.1	13.0	17.6	70	0.0	1017	101.7	0.37
20	14.7	11.8	17.3	66	0.0	1019	182.8	0.66
21	13.9	12.5	15.0	76	0.0	1016	29.1	0.10
22	13.8	9.1	17.5	74	0.0	1013	184.2	0.66
23*	13.7	11.1	16.1	74	0.0	1013	155.0	0.56
24	14.0	10.5	17.0	70	0.2	1015	182.3	0.66
25	14.4	12.1	17.2	69	0.0	1017	170.4	0.61
26	14.1	11.5	16.1	74	0.0	1022	88.3	0.32
27	14.3	12.4	17.3	62	0.0	1024	144.9	0.52
28	14.5	12.5	17.1	59	0.0	1022	162.2	0.58
29	13.8	8.7	16.6	68	0.2	1018	170.8	0.61
30	13.3	10.0	16.4	85	0.0	1014	82.7	0.30
31								
min/max		**8.7**	**28.2**		**57.6**			
average	**17.4**	**14.2**	**20.5**	**76**	**2.3**	**1014**	**153.0**	**0.55**
total					**68.4**			

Sampling day

Table I. 4 Meteo data, air temperature (°C), relative humidity (%), rain (mm), pressure (mbar) and solar radiation (W m^2, MJ m^2) measured in October in Station N°23 by Rete Ente Zona Industriale di Porto Marghera.

OCTOBER 2008 Station N° 23 - CED Rete Ente Zona								
	AIR TEMPERATURE (°C)			RELATIVE HUMIDITY	RAIN	PRESSURE	SOLAR RADIATION	
day	average	MIN	MAX	average %	mm	average mbar	global W/m2	global MJ/m2
1	14.8	12.4	17.4	87	0.2	1010	100.1	0.36
2	14.8	11.6	17.7	90	0.0	1007	76.3	0.27
3	16.6	10.8	21.3	71	0.0	1003	119.1	0.43
4	12.6	8.9	16.1	64	0.0	1010	139.6	0.50
5	12.5	7.0	17.7	70	0.0	1016	162.7	0.59
6	13.5	7.9	18.0	77	0.0	1018	152.4	0.55
7	14.5	10.7	17.6	86	0.0	1018	92.8	0.33
8	15.5	12.9	17.7	89	0.0	1017	106.2	0.38
9	15.5	11.8	18.5	88	0.0	1023	103.8	0.37
10	16.7	12.0	20.3	80	0.0	1029	139.3	0.50
11	16.8	13.4	20.0	77	0.0	1030	140.1	0.50
12	15.8	11.3	19.8	86	0.0	1027	129.9	0.47
13*	17.5	12.5	23.8	78	0.0	1021	126.8	0.46
14	17.5	13.6	21.5	75	0.0	1018	128.0	0.46
15	17.5	14.7	20.9	81	0.0	1017	120.0	0.43
16	15.3	13.1	16.9	94	0.0	1013	37.6	0.14
17	14.1	11.2	17.8	82	0.0	1011	70.7	0.25
18	12.6	10.2	15.8	60	0.2	1019	100.4	0.36
19	12.7	8.9	17.6	74	0.0	1023	88.0	0.32
20	13.1	8.6	16.3	86	0.0	1022	116.8	0.42
21	12.8	11.7	14.1	96	0.0	1018	28.7	0.10
22	14.3	12.2	17.0	87	0.0	1016	88.0	0.32
23	14.9	11.8	18.8	81	0.0	1020	93.0	0.33
24	13.8	10.8	16.6	72	0.0	1022	103.6	0.37
25	14.1	11.4	17.7	72	0.0	1025	61.2	0.22
26	12.4	9.2	15.9	83	0.0	1023	110.6	0.40
27	11.6	8.4	13.6	86	0.0	1014	49.3	0.18
28	13.9	11.7	16.6	91	5.4	1006	49.0	0.18
29	15.9	14.3	18.2	94	12.6	999	40.1	0.14
30	13.3	11.4	16.3	72	6.0	998	95.8	0.34
31	10.6	8.6	13.4	97	8.2	1009	19.8	0.07
min/max		**7.0**	**23.8**		**12.6**			
average	**14.4**	**11.1**	**17.8**	**81**	**1.1**	**1016**	**96.5**	**0.35**
total					**32.6**			

Sampling day

Table I. 5 Meteo data, air temperature (°C), relative humidity (%), rain (mm), pressure (mbar) and solar radiation (W m^2, MJ m^2) measured in November in Station N°23 by Rete Ente Zona Industriale di Porto Marghera.

NOVEMBER 2008 Station N° 23 - CED Rete Ente Zona								
	AIR TEMPERATURE (°C)			RELATIVE HUMIDITY	RAIN	PRESSURE	SOLAR RADIATION	
day	average	MIN	MAX	average %	mm	average mbar	global W/m2	global MJ/m2
1	13.6	11.8	15.9	92	10.0	1009	75.1	0.27
2	10.6	8.7	12.9	97	0.6	1017	55.2	0.20
3*	13.9	10.9	16.1	92	0.2	1016	49.1	0.18
4	14.0	13.5	14.4	95	19.8	1016	16.9	0.06
5	15.0	13.0	18.0	91	0.2	1015	102.7	0.37
6	13.2	11.4	15.3	91	22.8	1013	75.0	0.27
7	12.9	10.2	15.4	88	0.0	1014	59.4	0.21
8	11.5	9.1	13.7	92	0.0	1019	58.1	0.21
9	10.5	8.5	13.1	96	0.0	1027	71.8	0.26
10	8.6	7.9	9.2	98	0.2	1027	24.5	0.09
11	8.4	7.8	9.3	96	1.2	1021	15.4	0.06
12	9.8	8.8	11.0	97	8.4	1017	28.7	0.10
13	9.5	8.7	10.7	98	31.0	1014	13.1	0.05
14	11.0	8.5	12.6	78	0.6	1020	73.1	0.26
15	10.8	8.7	13.2	71	0.0	1023	91.3	0.33
16	9.9	4.6	15.7	80	0.0	1021	91.6	0.33
17*	10.5	8.0	13.7	78	0.0	1020	65.5	0.24
18	6.4	4.9	8.4	67	0.0	1017	58.6	0.21
19	5.7	1.7	10.3	78	0.0	1016	84.4	0.30
20	5.0	1.8	8.0	89	0.0	1015	63.0	0.23
21	4.9	3.4	6.6	90	0.0	999	41.5	0.15
22	6.9	3.4	8.9	37	0.0	997	67.8	0.24
23	3.1	-0.7	7.6	65	0.0	1004	89.3	0.32
24	0.5	-1.3	2.4	92	3.8	996	16.7	0.06
25	2.0	-1.6	6.2	90	1.8	1003	26.6	0.10
26	4.4	1.6	6.9	63	0.0	1020	83.1	0.30
27	3.0	0.0	6.8	71	0.0	1024	78.9	0.28
28	2.5	0.0	4.7	88	10.8	1008	11.5	0.04
29	4.7	3.5	6.0	96	3.2	999	24.4	0.09
30	7.3	5.1	11.1	95	20.8	1003	23.4	0.08
31								
min/max		**-1.6**	**18.0**		**31.0**			
average	**8.3**	**6.1**	**10.8**	**85**	**4.5**	**1014**	**54.5**	**0.20**
total					**135.4**			

Sampling day

Table I. 6 Concentration of As, Cd, Cr, Cu, Hg, Pb and Zn (µg g^{-1} DW) in soil-sediment collected from the study site (Fusina pilot wetland) at locations Cell1 and Cell2 planted with* T. latifolia *throughout the experimental period.

	Month	Layer (cm)	As	Cd	Cr	Cu	Hg	Pb	Zn
Cell1	Jul	0-5	3.35	<d.l.	5.71	3.44	<d.l.	1.97	24.40
		5-20	3.75	<d.l.	5.47	3.14	<d.l.	1.93	22.80
	Aug	0-5	3.26	<d.l.	5.23	1.28	<d.l.	3.26	23.88
		5-20	4.96	<d.l.	5.67	3.34	<d.l.	2.33	24.10
	Sep	0-5	3.24	<d.l.	5.30	1.08	<d.l.	3.73	25.61
		5-20	4.44	<d.l.	5.45	2.73	<d.l.	2.73	22.71
	Nov	0-5	3.24	<d.l.	5.57	0.71	<d.l.	3.95	25.50
		5-20	3.78	<d.l.	5.52	3.37	<d.l.	2.04	25.03
Cell2	Jun	0-5	5.19	0.20	8.88	7.48	<d.l.	6.28	40.89
		5-20	4.44	<d.l.	6.26	3.53	<d.l.	2.22	24.72
	Aug	0-5	4.86	0.30	10.32	9.62	<d.l.	11.31	57.63
		5-20	5.16	0.20	9.22	7.24	<d.l.	4.86	41.06
	Sep	0-5	5.93	0.19	8.94	6.32	<d.l.	8.75	47.73
		5-20	5.10	<d.l.	7.14	4.18	<d.l.	3.06	29.98
	Nov	0-5	9.81	0.49	16.50	17.86	<d.l.	17.67	94.66
		5-20	6.24	0.20	9.27	8.16	<d.l.	5.34	44.92

Table I. 7 Concentration of As, Cd, Cr, Cu, Hg, Pb and Zn (µg g^{-1} DW) in soil-sediment collected from the study site (Fusina pilot wetland) at locations Cell1 and Cell2 planted with* P. australis *throughout the experimental period.

	Month	Layer (cm)	As	Cd	Cr	Cu	Hg	Pb	Zn
Cell1	Jul	0-5	4.56	<d.l.	5.75	3.07	<d.l.	1.98	22.71
		5-20	4.41	<d.l.	5.71	2.91	<d.l.	1.90	22.04
	Aug	0-5	4.54	<d.l.	4.54	0.30	<d.l.	3.13	22.20
		5-20	4.67	<d.l.	5.17	2.69	<d.l.	2.29	21.38
	Sep	0-5	3.91	<d.l.	5.22	0.40	<d.l.	3.31	24.19
		5-20	5.36	<d.l.	5.96	2.78	<d.l.	1.59	22.84
	Nov	0-5	4.08	<d.l.	5.18	0.50	<d.l.	3.58	24.88
		5-20	4.64	<d.l.	5.45	2.72	<d.l.	2.32	21.79
Cell2	Jun	0-5	13.49	0.81	23.35	26.27	<d.l.	15.80	126.81
		5-20	4.80	0.20	8.30	6.90	<d.l.	4.00	40.02
	Aug	0-5	7.93	0.40	16.97	19.08	<d.l.	18.88	94.80
		5-20	8.77	0.50	14.81	14.41	<d.l.	8.57	78.60
	Sep	0-5	8.94	0.51	17.57	20.71	<d.l.	19.29	104.18
		5-20	9.55	0.41	15.29	14.68	<d.l.	7.80	80.17
	Nov	0-5	9.08	0.59	16.12	18.07	<d.l.	17.68	113.69
		5-20	6.47	0.29	8.63	8.33	<d.l.	5.49	50.39

Table I. 8 Concentration of As, Cd, Cr, Cu, Hg, Pb and Zn (µg g^{-1} DW) in soil-sediment collected from the study site (Fusina pilot wetland) at locations Cell1 and Cell2 planted with* S. lacustris *throughout the experimental period.

	Month	Layer (cm)	As	Cd	Cr	Cu	Hg	Pb	Zn
Cell1	Jul	0-5	4.74	<d.l.	7.16	5.15	<d.l.	3.63	30.47
		5-20	3.75	<d.l.	5.82	2.86	<d.l.	1.77	22.28
	Aug	0-5	3.94	<d.l.	5.76	1.62	<d.l.	3.94	27.81
		5-20	3.83	<d.l.	5.85	2.82	<d.l.	1.71	24.41
	Sep	0-5	3.05	<d.l.	5.43	1.05	<d.l.	3.34	25.55
		5-20	4.65	<d.l.	5.86	2.73	<d.l.	2.53	23.94
	Nov	0-5	4.11	<d.l.	5.62	1.00	<d.l.	4.11	27.98
		5-20	4.32	<d.l.	5.24	2.88	<d.l.	2.06	21.78
Cell2	Jun	0-5	8.04	0.50	16.87	18.16	<d.l.	12.21	88.13
		5-20	13.37	0.81	22.29	26.55	<d.l.	15.10	127.66
	Aug	0-5	10.72	0.68	18.62	23.01	<d.l.	18.33	120.98
		5-20	14.45	1.20	22.88	30.10	<d.l.	16.15	163.15
	Sep	0-5	9.16	0.70	16.01	18.83	<d.l.	17.22	114.10
		5-20	11.99	0.61	18.70	19.91	<d.l.	12.29	112.27
	Nov	0-5	10.98	0.79	17.90	21.56	<d.l.	19.19	132.54
		5-20	7.74	0.60	13.27	14.77	<d.l.	9.75	87.34

Table I. 9 Seasonal trend of shoot lenght (cm) of* T. latifolia, P. australis, S. lacustris *in the study site (Fusina pilot wetland) at location Cell 1 and Cell2 (mean±SD, n=5).

Month	Plant species	Cell 1	Cell 2
Jul	*T. latifolia*	144.6±10.7	156.4±6.6
	P. australis	175.6±22.6	219.2±20.0
	S. lacustris	96.6±13.8	108.2±7.5
Aug	*T. latifolia*	142.2±12.6	151.4±9.5
	P. australis	181,8±13,1	218.0±29.6
	S. lacustris	103.4±11.4	111.5±10.7
Sep	*T. latifolia*	132.5±14.8	149.9±11.5
	P. australis	189.8±21,0	224.6±19.7
	S. lacustris	100.3±12.0	107.0±9.2
Nov	*T. latifolia*	133.2±8.3	150.0±11.0
	P. australis	183.4±19,3	217.0±26.1
	S. lacustris	104.0±14.1	110.5±10.8

ANNEX II

⇒ CASE STUDY: Hydraulic Performance: Pilot wetland system in Plönninge, Sweden.

Table II. 1 Results of the tracer study during September-October 2008 in the 18 in Plönninge (Sweden) pilot system, planted with mixed (C), emergent (E) or submerged vegetation (S), with (b.) or without barrier (no b.). Tracer recovery (%), nominal hydraulic retention time (t_n), mean hydraulic retention time (t_m), peak time (t_{peak}), effective volume ratio (e), hydraulic efficiency (λ), and number of tanks n the CSTR model (N) are shown.

Wetland	Vegetation	Inlet	t_n (day)	t_m (day)	t_p (day)	e	λ	N
3	C	b.	4.1	3.2	1.1	0.79	0.26	2.1
8	C	b.	4.6	3.3	1.1	0.72	0.23	1.8
13	C	b.	4.5	3.9	1.3	0.87	0.28	2.1
2	C	no b.	4.3	3.4	1.1	0.77	0.24	2.4
11	C	no b.	4.0	3.3	1.1	0.83	0.26	2.1
16	C	no b.	4.8	3.7	1.1	0.77	0.22	1.9
6	E	b.	4.3	2.8	1.3	0.67	0.30	1.7
12	E	b.	3.9	2.5	1.1	0.63	0.27	1.5
15	E	b.	4.7	3.2	1.3	0.69	0.27	1.9
1	E	no b.	3.9	3.3	2.1	0.84	0.52	1.9
9	E	no b.	4.5	4.9	2.3	1.00	0.52	1.7
18	E	no b.	3.9	3.3	1.1	0.85	0.27	1.3
4	S	b.	4.3	3.4	1.8	0.78	0.42	1.7
7	S	b.	4.1	3.1	1.1	0.76	0.26	1.9
14	S	b.	3.9	3.1	1.8	0.79	0.46	1.9
5	S	no b.	5.0	3.4	0.8	0.67	0.16	1.7
10	S	no b.	4.0	3.2	0.8	0.81	0.20	1.8
17	S	no b.	4.2	3.3	1.1	0.79	0.25	1.9

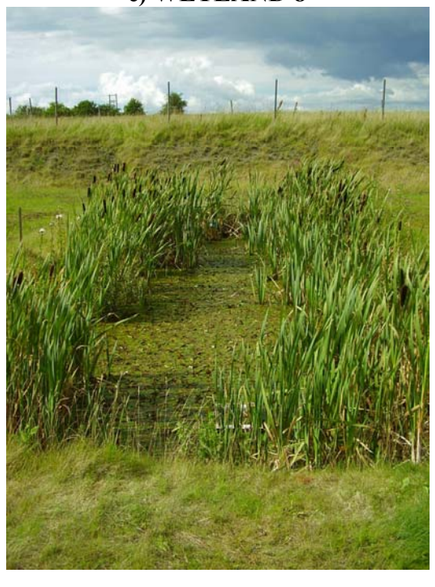
a) WETLAND 2

b) WETLAND 11

c) WETLAND 16

d) WETLAND 3

e) WETLAND 8



f) WETLAND 13

Figure II. 1 Plönninge (Sweden) pilot system: control wetlands without barrier (a, b, c) and with barrier (d, e, f). Pictures taken on June 2008.

a) WETLAND 5

b) WETLAND 10

c) WETLAND 17

d) WETLAND 4

e) WETLAND 7

f) WETLAND 14

Figure II. 2 Plönninge (Sweden) pilot system: submerged wetlands without barrier (a, b, c) and with barrier (d, e, f). Pictures taken on June 2008.

a) WETLAND 1

b) WETLAND 9

c) WETLAND 18

d) WETLAND 6

e) WETLAND 12

f) WETLAND 15

Figure II. 3 Plönninge (Sweden) pilot system: emergent wetlands without barrier (a, b, c) and with barrier (d, e, f). Pictures taken on June 2008.