Influence of system type, loading regimes and helophyte species on inorganic sulfur transformations in constructed wetlands

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Rania A. B. Saad

Died not, he who left knowledge behind

Into the darkness may he go not, he who left light behind

And forgotten shall be not, he who left remembrance behind

To the soul of Dr. Peter Kuschk

"And We verily gave knowledge unto David and Solomon, and they said: Praise be to Allah, Who hath preferred us above many of His believing slaves!" [Holly Quran 27:15]

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Summary

Constructed wetlands (CWs) are valuable systems for the treatment of various types of contaminated wastewater. They offer numerous advantages over conventional wastewater treatment technologies, which make them an attractive alternative especially when economic savings are targeted or compulsory. Nonetheless, our incomplete understanding of the processes that contribute to pollutants removal or transformations is one of the main limitations towards optimizing their performance and consequently encouraging the broader application of CWs worldwide.

Sulfur is a ubiquitous constituent of numerous types of wastewater, but its cycling processes in CWs are so far not sufficiently investigated. This is in part because its commonly occurring compounds such as sulfate have no stringent requirements for disposal in the environment. Nonetheless, sulfur cycling affects and interacts with carbon, nitrogen and metal removal processes and hence the overall performance of CWs. Since the effect of sulfur cycling on carbon removal has been in most cases of a desired nature, the main focus of this research was set on the sulfur processes and their influence on nitrogen removal in CWs.

Applying different system types (e.g. horizontal subsurface flow, HSSF; vertical flow, VF designs) was found to clearly influence the subsurface oxygen availability and consequently the redox potential levels inside CWs. Redox potential was in turn the main indicator for which sulfur transformations occur in a CW. In vertical, aerated vertical and aerated horizontal subsurface flow CWs, redox potential remained at high levels and the oxidative half of sulfur cycle processes took place, as the reduced sulfur compounds from influent wastewater were oxidized to sulfate, and no sulfate reduction activity was detected. Conversely, the horizontal subsurface flow CWs which were not mechanically aerated reflected significantly lower redox potential levels, and the net of the sulfur processes was on the reductive half of the cycle, as the concentrations of reduced compounds increased at outflow compared to influent levels. In these systems, ammonium-nitrogen removal was as well significantly lower than removal from the vertical, aerated vertical and aerated horizontal CWs, which was attributed to both lower oxygen availability and inhibition of ammonium oxidizing microorganisms by the detected high sulfide concentrations. Sulfur processes were thus found to have no significance for pollutant turnover in CWs with elevated redox potential, as organic carbon and nitrogen removal processes processed regardless of sulfur cycling. However, a stoichiometric balance revealed that sulfate was an important electron acceptor for organic carbon removal in CWs with low redox potential.

Sulfur processes were then investigated in detail in unaerated HSSF CWs. Fostered by low redox potential levels the available sulfate was utilized as a terminal electron acceptor for the oxidation of organic matter. In addition, the dynamic of sulfur compounds along the flow path of the wastewater through the CWs reflected simultaneous reduction and oxidation of sulfate, with the concentrations of the intermediately-oxidized sulfur compounds higher at planted than unplanted CWs, reflecting the role of plants in the provision of aerobic condition inside the wetlands. The preliminary interpretation of stable isotope abundance patterns of different reduced and oxidized sulfur compounds reflect the activity of sulfate reducing and sulfide

oxidizing prokaryotes (SRPs and SOPs). In addition, methanogenesis -a competing process to sulfate reduction- was found to be of significance in these HSSF systems.

Moreover, finding that significant sulfur removal took place in HSSF CWs raised the interest to investigate the fate of the removed sulfur. Firstly assumed to be mainly trapped in the soil matrix as precipitates (as sulfate salts, metal sulfides or in organic compounds as carbon-bound sulfur), it was found that sulfur did not actually accumulate in the soil matrix of the HSSF CW. Therefore it was assumed that a significant amount of the sulfur that was removed from the water column was eventually lost to the atmosphere as hydrogen sulfide via volatilization or phytovolatilization processes.

In addition, the helophytes applied in CWs were found to have direct influence on pollutant transformations, including sulfur turnover. Amongst 4 investigated helophytes, some were found more beneficial for pollutant removal than others, as the different helophytes deposit different amounts of organic compounds and release different amounts of oxygen through their roots. The influence of helophytes was more pronounced in systems that received low loads of organic contaminants, since there the rhizodeposits presented a major source of organic carbon. This effect was less visible in the high loaded systems in which organic carbon was not limited. Relatedly, not only the soluble organic rhizodeposits were found to be contributing to pollutant transformations in CWs, but as well insoluble compounds such as dead root material were found to contribute. In addition, the role of oxygen release was found very important, as rooted zones of 3 investigated helophytes had significant differences in terms of redox potential and transformation processes, thus based on which pollutant transformations are targeted, the depth of CWs can be linked to the depth of the selected helophyte. Furthermore, the significant differences found amongst different helophyte species suggest that species selection should be awarded higher importance.

For future development of CWs and for their performance optimization, differences among helophyte species should be further investigated, especially for regional interests. Better understanding of rhizodeposition and radial oxygen loss patterns is of high value. In addition, sulfur cycle process pathways should be identified. The contribution of sulfur cycling to carbon removal should be quantified, and measures to mitigate the detrimental effects on nitrogen removal should be provided for the designing engineers.

Zusammenfassung

Pflanzenkläranlagen (PfKA) sind wertvolle Abwasserreinigungssysteme. Sie wurden für die Behandlung verschiedener Abwasserströme erfolgreich angewendet, da sie zahlreiche Vorteile gegenüber herkömmlichen Abwasserbehandlungstechnologien bieten. Die Anwendung von PfKA ist vor allem sinnvoll, wenn wirtschaftliche Einsparungen bei der Abwasserbehandlung erwünscht oder obligatorisch sind. Um die Leistung der PfKA zu optimieren und dadurch ihre welweite Anwendung zu fördern, benötigen wir ein besseres Verständnis der Schadstoffabbau- und -transformationsprozesse.

Schwefel ist ein allgegenwärtiger Bestandteil zahlreicher Arten von Abwasser, aber seine Kreislaufprozesse in PfKA sind bisher nicht ausreichend untersucht, zum Teil weil seine häufig vorkommenden Verbindungen wie Sulfat keine hohen Anforderungen für die Entsorgung in die Umwelt haben. Dennoch sind die Wechselwirkungen zwischen den Schwefelkreislaufprozessen und den Kohlenstoff-, Stickstoff- und Metallentfernungsverfahren bedeutend für die allgemeine Leistung von PfKA. Da die Wirkung von Schwefelkreislaufprozessen auf der Entfernung organischer Kohlenstoffverbindungen in den meisten Fällen erwünscht ist, wurde der Schwerpunkt dieser Forschung auf den Schwefelkreislaufprozessen und deren Einfluss auf die Stickstoffentfernung in PfKA gesetzt.

Die Untersuchung verschiedener technologischen Varianten von PfKA (z.B. Horizontal und Vertikal durschströmte Bodenfilter) hat herausgestellt, dass das Systemdesign die Sauerstoffverfügbarkeit (und damit das Redoxpotenzial) innerhalb PfKA deutlich beeinflußt. Redoxpotential wurde als Indikator für die Art der Schwefeltransformationen gefunden. In untersuchten Vertikal-, belüfteten Vertikal- und belüfteten Horizontalfiltern lag ein hohes Redoxpotential vor und die oxidative Hälfte der Schwefelkreislauf fand statt. Keine Oxidation von reduzierten Schwefelverbindungen bzw. keine Sulfatreduktionaktivität konnten in diesen Systemen festgestellt werden. Im Gegensatz blieb das Redoxpotential in den untersuchten Horizontalfiltern auf niedrigem Niveau. Das hat steuernd auf die reduktiven Prozessen wie dissimilatorische Sulfatreduktion gewirkt. Das war in den erhöhten Konzentrationen reduzierter Schwefelverbindungen im Ablauf im Vergleich zu deren Zulaufkonzentrationen manifestiert. In den Horizontalfiltern war Ammoniumentfernung auch deutlich geringer als dessen Niveau in den Vertikal-, belüfteten Vertikal- und belüfteten Horizontalfiltern. Die Begründungen dafür waren im Wesentlichen die niedrige Sauerstoffverfügbarkeit und die erhöhte Sulfidkonzentrationen, die auf die Ammonium-oxidierenden Mikroorganismen gleichzeitig eine Hemmung sowie eine Konkurrenz über verfügbaren Sauerstoff ausüben. Daher beeinflussten die Schwefelkreislaufprozesse die Schadstoffabbauprozesse hauptsächlich in PfKA mit niedrigem Redoxpotenzial (z.B. hat eine stöchiometrische Berechnung ergeben, dass Sulfat eine wichtige Rolle in der Entfernnung organischer Kohlenstoffverbindungen in solchen PfKA spielte).

Eine detaillierte Untersuchung der anorganischen Schwefeltransformationen in den unbelüfteten Horizontalfiltern wurde danach durchgeführt. Aufgrund des niedrigen Redoxpotenzials, das eine limitierte Verfügbarkeit von Elektronenakzeptoren wie Sauerstoff und Nitrat implizierte, wurde Sulfat als grundsätzlicher terminaler Elektronenakzeptor für die Oxidation organischer

Kohlenstoffverbindungen verwendet. Außerdem wurde der gleichzeitige Verlauf der Sulfatreduktion, Sulfidoxidation und Elementarschwefeloxidation festgestellt. Dabei waren die Konzentrationen intermediär-oxidierter Schwefelverbindungen höher im bepflanzten gegenüber unpflantzen Horizontalfilter, was die Rolle der Helophyten in der Förderung aerober Bedingungen in PfKA nachweist. Das Isotopenverhältnis unterschiedlicher reduzierter und oxidierter Schwefelverbindungen deutete auf die Aktivität von Sulfat-reduzierenden und Sulfid-oxidierenden Prokaryoten (SRPs und SOPs) hin.

Die Erkenntnis, dass eine erhebliche Schwefelentfernung in der unbelüfteten Horizontalfiltern stattfand, weckte das Interesse, das Schicksal des entfernten Schwefels zu untersuchen. Zunächst wurde angenommen, dass er hauptsächlich in der Bodenmatrix gespeichert wird (als Sulfatsalze, Metallsulfide oder in organischen Verbindungen). Jedoch wurde bei der Überprüfung des Bodens eines Horizontalfilters keine Akkumulation des Schwefeles gefunden. Daher wird vermutet, dass erhebliche Mengen des Schwefels, der aus der Wassersäule entfernt wurde, schließlich in die Atmosphäre als Schwefelwasserstoff verflüchtet wird.

Darüber hinaus wurde herausgefunden, dass die in PfKA angewendeten Helophyten auf Schadstofftransformationen -einschließlich Schwefeltransformationen- einen direkten Einfluss haben. Unter vier untersuchten Helophyten erwiesen einige vorteilhafter zur Schadstoffentfernung als andere, da die verschiedenen Helophyten unterschiedliche Mengen an organischen Verbindungen und unterschiedliche Mengen an Sauerstoff über ihre Wurzeln abgeben. Der Einfluss von Helophyten war bei Systemen, die geringe Belastungen von organischen Kohlenstoffverbindungen erhalten, stärker ausgeprägt, da dort die Rhizodepositionsprodukte eine wichtige Quelle von organischem Kohlenstoff präsentierten. Dieser Effekt war weniger sichtbar in den mit organischem Kohlenstoff hochbelasteten Systemen. Weiterhin wurde festgestellt, dass nicht nur die löslichen sondern auch die unlöslichen organischen Rhizodepositionsprodukten wie z.B. abgestorbene Wurzelstoffe zu Schadstofftransformation und -abbau in PfKA beitragen. Zusätzlich erwies sich die Rolle der Sauerstofffreisetzung von Helophyten als sehr wichtig , da die durchwurzelten Zonen in untersuchten PfKA, die mit Monokulturen von drei Helophyten gepflanzt waren, sich signifikant unterschieden von nichtdurchwurzelten Zonen bezüglich des Redoxpotentials und der Transformations-Prozesse. Folglich kann die Wurzeltiefe der ausgewählten Helophyten für die Bautiefe der PfKA entscheidend sein. Zudem deuten die signifikanten Unterschiede zwischen den verschiedenen Helophytenspezies an, dass der Auswahl von Helophyten eine höhere Beachtung verliehen werden sollte.

Für die zukünftigen Entwicklungen von PfKA und für die Optimierung ihrer Leistung sollten die Unterschiede zwischen den Helophyten weiter untersucht werden, insbesondere im Hinblick auf regionale Gegebenheiten. Ein besseres Verständnis der Rhizodeposition und des radialen Sauerstoffverlustes wäre von hohem Wert. Zudem sollten die mikrobiellen Abbauwege des Schwefelverbindungen identifiziert werden. Der Beitrag des Schwefelkreislaufs zur Kohlenstoffentfernung sollte quantifiziert werden und Maßnahmen gegen die schädlichen Wechsel- und Auswirkungen auf die Stickstoffentfernung sollten Entwicklungsingenieuren der PfKA zur Verfügung gestellt werden.

Résumé

Les installations de filtres plantés (FPs) pour l'épuration de plusieurs flux d'eau usées ont remporté un franc succès. Les FPs offrent de nombreux avantages sur les technologies conventionnelles de traitement des eaux usées, ce qui en fait une bonne alternative surtout lorsqu'une réduction des coûts est souhaitée ou obligatoire. Pour optimiser cependant leur performance et pour finalement encourager leur application dans le monde entier, nous devons mieux comprendre les processus de transformation et d'élimination des polluants.

Le soufre est un constituant commun de nombreux types d'eaux usées, mais ses transformations dans les FPs n'ont jusqu'à présent pas été suffisamment étudiées, en partie parce qu'il est souvent présent dans les eaux usées sous la forme de sulfate et qu'il peut ainsi être rejeté dans l'environnement sans être soumis à des contraintes élevées. Pourtant, les transformations du soufre affectent et interagissent avec les processus d'élimination du carbone, de l'azote et des métaux et influencent donc la performance globale des FPs. Puisque les effets de la transformation du soufre sur l'élimination du carbone sont dans la plupart des cas les bienvenus, l'objectif principal de cette recherche est d'étudier le cycle du soufre et son influence sur l'élimination de l'azote dans les FPs.

L'étude des différents types de FPs (par exemple à écoulement horizontal, FPH, à écoulement vertical, FPV) a révélé que le design des FPs influence clairement la disponibilité souterraine de l'oxygène et par conséquent le niveau du potentiel redox à l'intérieur des FPs. Le potentiel redox a été identifié comme indicateur principal des transformations de soufre à l'œuvre dans un FP. Dans les systèmes verticaux, verticaux aérés et horizontaux aérés étudiés, le potentiel redox est resté à des niveaux élevés et des processus oxydants du cycle de soufre ont eu lieu (les composés de soufre réduits ont été oxydés en sulfate, et aucune activité de réduction de sulfate n'a été détectée). A l'inverse, les FPH non aérés ont conservé un niveau bas de potentiel redox et des procédés de réduction de soufre se sont produits (les concentrations de composés de soufre réduits ont augmenté à la sortie des FPH par rapport à leur niveau d'entrée). Dans ces systèmes, l'enlèvement de l'azote ammoniacal était ainsi significativement plus faible comparé au reste des systèmes étudiés (les FPs verticaux, verticaux aérés et horizontaux aérés). Cela s'explique essentiellement par la moindre disponibilité souterraine de l'oxygène et par l'inhibition des microorganismes responsables de l'oxydation d'ammonium causée par les fortes concentrations en sulfure dans les FPH non aérés. C'est ce qui explique que le cycle du soufre influence surtout l'élimination des polluants dans les FPs à faible potentiel redox. Un calcul stœchiométrique a cependant révélé que le sulfate est un accepteur d'électrons important dans l'élimination du carbone organique dans les FPs à bas niveaux de potentiel redox.

Une étude détaillée des transformations anorganiques du soufre a été ensuite menée dans des FPH non aérées. Dans ces systèmes, le sulfate disponible a été utilisé comme accepteur terminal d'électrons pour l'oxydation de la matière organique. En outre, la dynamique des composés de soufre le long de la voie d'écoulement des eaux usées à travers les FPH a reflété simultanément une réduction du sulfate, une oxydation du sulfure et du soufre élémentaire. En plus, les concentrations des composés soufrés intermédiairement oxydés étaient supérieures en présence d'hélophytes comparé à un système de contrôle non planté, ce qui reflète le rôle important joué

par les hélophytes dans l'augmentation des conditions aérobies dans les FPs. L'interprétation préliminaire des compositions isotopiques des différents composés soufrés réduits et oxydés reflète l'activité des procaryotes sulfato-réducteurs et sulfo-oxydants. Par ailleurs, il a été trouvé que la méthanogénèse -un procédé concurrent à réduction de sulfate- jouait un rôle important dans les FPH.

Le constat qu'une élimination considérable du soufre a eu lieu dans les FPH nous a incité à étudier le destin du soufre enlevé. Dans un premier temps, nous avons supposé qu'il avait été surtout accumulé dans le massif filtrant (sous la forme de sels de sulfate, de sulfures métalliques ou de composés organiques tels que le soufre lié au carbone). Contrairement à cette supposition, aucune accumulation de souffre dans le massif filtrant n'a été trouvée. Par conséquent, nous en avons supposé que les quantités importantes de soufre éliminées de la colonne d'eau ont principalement disparu dans l'atmosphère sous la forme de sulfure d'hydrogène par des procédés de volatilisation ou de phytovolatilisation.

En outre, nous avons trouvé que les hélophytes utilisés dans les FPs ont une influence directe sur la transformation des polluants, comme dans celle du soufre. Parmi les 4 hélophytes étudiées, certaines se sont révélées plus efficaces que d'autres dans l'élimination des polluants puisque, selon le type, elles déposent des quantités différentes de composés organiques et libèrent des quantités différentes d'oxygène à travers leurs racines. L'influence de l'hélophyte a été plus prononcée dans les systèmes qui ont reçu de faibles charges de contaminants organiques, puisque dans ce cas les rhizodépôts présentent une source importante en carbone organique. Cet effet était moins perceptible dans les systèmes fortement chargés en carbone organique. Il a été constaté ensuite que non seulement les rhizodépôts organiques solubles, mais aussi les rhizodépôts insolubles comme les racines mortes contribuent à la transformation et à la décomposition des polluants dans les FPs. De plus, la capacité des hélophytes à libérer de l'oxygène s'est avérée jouer un rôle très important. Dans les systèmes plantés en monoculture avec les 3 hélophytes étudiés, les zones qui présentaient un bon enracinement se distinguaient de façon significative en termes de potentiel redox et de processus de transformation des polluants des zones où il n'y avait pas de racines. La profondeur des racines de l'hélophyte sélectionnée s'avéra ainsi déterminante pour la profondeur des FPs. En conclusion, les différences significatives présentées par les différentes espèces poussent à accorder une plus grande importance à la sélection du type d'hélophyte.

Les différences entre les hélophytes devraient continuer à être étudiées de façon plus approfondie afin de développer les FPs et d'augmenter leur performance, par rapport en particulier aux données régionales. Nous estimons qu'une meilleure compréhension de la rhizodéposition et des taux de perte d'oxygène radiaux serait d'une grande valeur. De plus, les procédés microbiens impliqués dans les transformations du soufre devraient être identifiés. La contribution du turnover du soufre dans l'élimination du carbone doit être quantifiée et des mesures visant à atténuer les effets néfastes des transformations de soufre sur l'élimination de l'azote doivent être mises à la disposition des ingénieurs chargés de développer les FPs.

Contents

List of acronyms

AMD	Acid mine drainage
ANOVA	Analysis of variance
AVS	Acid volatile sulfide
BMBF	German Federal Ministry of Education and Research
BOD_5	Biochemical oxygen demand
COD	Chemical oxygen demand
CRS	Chromium reducible sulfur
CSO	Combined sewer overflow
CSTR	Continuously stirred tank reactor
CW	Constructed wetland
DOC	Dissolved organic carbon
DSR	Dissimilatory sulfate reduction
E_h	Redox potential
EPA	Environmental protection agency of the United States
ET	Evapotranspiration
EWaTT	Ecological Water Treatment Technologies group of the UFZ
FWS	Free water surface
FHRM	Floating hydroponic root mat
GC	Gas chromatography
GDP	Gross domestic product
GW	Ground water
HLR	Hydraulic loading rate
HRT	Hydraulic retention time
HS^-	Hydrogen sulfide anion
HSSF	Horizontal subsurface flow
IPSWaT	International Postgraduate Studies in Water Technologies
MCB	Monochlorobenzene
MPA	Methane producing archaea
MSL	Mean sea level
nHRT	Nominal hydraulic retention time
O&M	Operation and maintenance
OLR	Organic loading rate
PE	Person equivalent
RMF	Root mat filter
ROL	Radial oxygen loss
RTD	Residence time distribution
SDG	Sustainable development goals of the UN
SOP	Sulfide and sulfur oxidizing prokaryotes
SRP	Sulfate reducing prokaryotes
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
TS	Total sulfur
TSS	Total suspended solids
UASB	Up-flow anaerobic sludge blanket
UBZ	Environmental and Biotechnology Centre of the UFZ

UFZ	Helmholtz Centre for Environmental Research
UN	United Nations
UNDP	United Nations Development Programme
UNEP	United Nations Environmental Programme
UV	Ultraviolet
VF	Vertical flow
WHO	World Health Organization
WSP	Waste stabilization pond
WWTP	Wastewater treatment plant

1. Introduction

1.1 Constructed wetlands: an alternative wastewater treatment technology

1.1.1 Wastewater treatment as prerequisite to sustainable development of rural and urban settlements

Many of the human activities as fundamental as personal hygiene and as sophisticated as industrial endeavours produce wastewater. The produced wastewaters end up in the environment, mixing with and adding contamination to freshwater or saltwater. The pollution loads transferred to the environment may include organic carbon, nutrients and pathogens and a cocktail of industrial chemicals and micro-pollutants, dependant on the type of wastewater (Henze 2008). The receiving environments have some self-cleansing capacities. However, such capacities are overburdened if the received contamination load is high and constant (Mbuligwe and Kaseva 2005).

The general consequences of pollution of the receiving waters are oxygen depletion, eutrophication, toxicity to flora and fauna, and contamination with pathogens; eventually causing serious environmental threats on water resources suitability for uptake and consumption by humans and on biodiversity. In addition, humans may suffer serious disease outbreaks upon contact with or consumption of these waters, or even without contact if these waters serve as habitats for disease vectors. Great savings can be achieved by providing adequate wastewater treatment systems in the places where they are absent but needed, as the economic burden of diseases can be mitigated. The World Health Organization (WHO) has defined this economic burden to include not only the cost of disease treatment but also the costs at the microeconomic level of households, firms or government (such as the impact of ill-health on a household's income or a firm's profits) and the costs at the macroeconomic level (such as the aggregate impact of a disease on a country's current and future gross domestic product).

This necessitates wastewater treatment, especially in populous locations, in order to protect receiving environments and hence human health. Unfortunately, wastewater treatment imposes considerable costs that are not affordable everywhere on earth. High-tech wastewater treatment technologies such as activated sludge systems are not only expensive to construct, operate and maintain; but also they require highly educated personnel to run them. Such personnel are not always available, especially in remote rural settlements. This calls for preference of applying semi-natural systems that are easier to operate and maintain and as well cheaper to construct, such as waste stabilization ponds (WSPs) and constructed wetlands (CWs).

1.1.2 Definitions and types of CWs

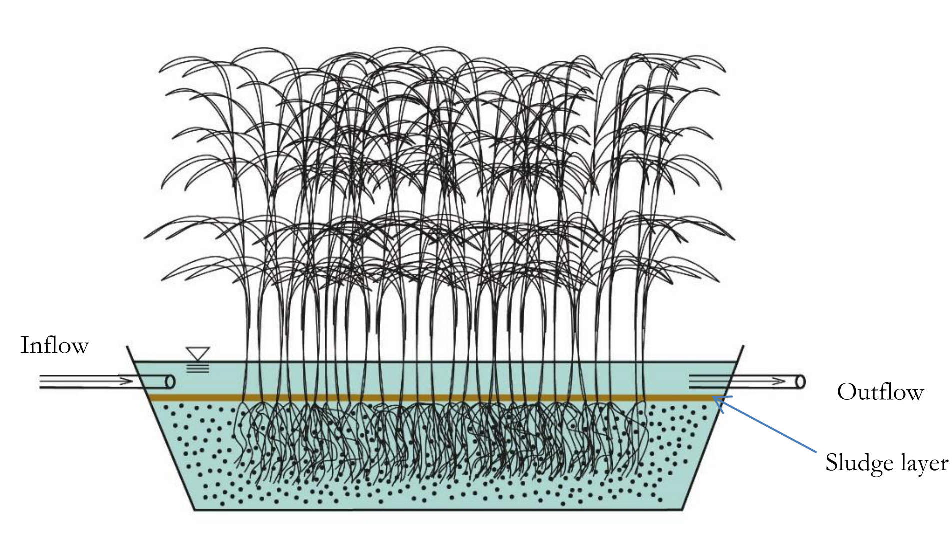
CWs are engineered systems that have been intentionally created in non-wetland sites to treat wastewater or stormwater (Hammer 1996). They have been developed to emphasize specific characteristics of natural wetland ecosystems, where high rate of pollutant transformation occur due to the existence of higher biological activity than most ecosystems. Natural wetlands are land areas that are wet during part or all of the year, due to their location in the landscape, as they are frequently transitional between uplands (terrestrial systems) and continuously flooded (aquatic) systems. Due to being wet for long periods, they do not host plant species that cannot grow in saturated soils. However, some plants species can survive such saturated conditions (e.g. helophytes). Many of the common pollutants that occur in conventional wastewaters can be transformed in CWs into by-products or nutrients that are less or not harmful to the environment. Moreover, CWs are of the least expensive systems to operate and maintain, mainly because they rely on a resilient ecosystem using naturally available energies (e.g. sun, wind) and processes (of microbes and plants) to treat wastewater (Kadlec and Wallace 2008). Due to these advantages and others, the application of CWs has increased worldwide in the past four decades (Vymazal 2009). Faulwetter et al. (2009) reported that CWs have been successfully used to remove a large spectrum of contaminants, originating from almost all sources of contamination. These include, but are not limited to: domestic wastewater; contaminated groundwater; agricultural runoff; animal wastewater; industrial wastewater (e.g. acid mine drainage, AMD), landfill leachate, urban stormwater, *etc.*

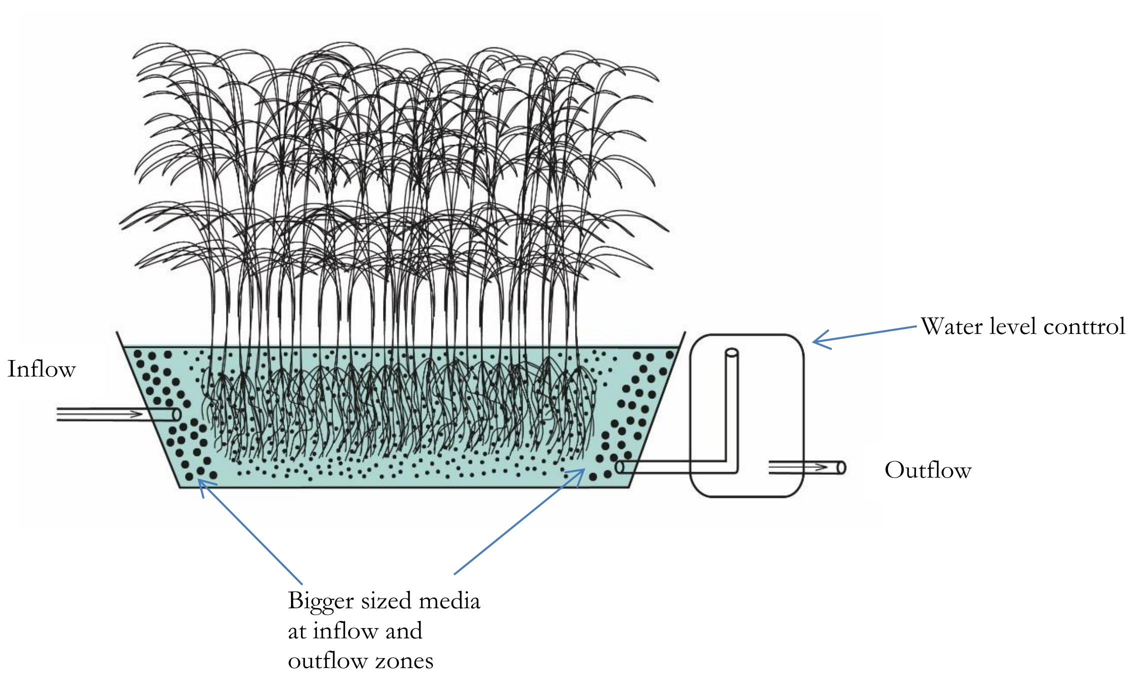
There are numerous types of conventional and novel CWs that are currently being applied. Conventional CWs can be classified in terms of hydraulic design or in terms of plant immerse conditions. Regarding the plant conditions, there exist: free floating plants; floating leaved plants; emergent plants and submerged plants systems. In terms of hydraulic modes, CWs with emergent plants can be further classified into surface flow and subsurface flow wetlands (Vymazal 2007). In general, the common types of CWs include:

- Free water surface (FWS) CWs: they have areas of open water (i.e. exposed water surface) and are similar in appearance to natural marshes. Because of the potential for human exposure to pathogens and to eradicate an disease vector (e.g. to eliminate the possibility of mosquitos breeding), FWS CWs are rarely used for secondary treatment (Kadlec and Wallace 2008);
- Subsurface flow CWs: can be subdivided to horizontal subsurface flow (HSSF) and vertical flow (VF) wetlands, depending on the mode of operation. In addition, the VF CWs can be further sub-divided into up-flow or down-flow, depending on applied flow direction. HSSF CWs are more expensive than FWS CWs and VF CWs are more expensive than both (Kadlec and Wallace 2008, Vymazal 2007);
- French systems: mainly two-stage VF CWs with no requirement for pretreatment of the wastewater, allowing for easier sludge management than the systems that require wastewater pre-treatment (Molle et al. 2005);
- Hybrid systems: a combination of different types of CWs. Such systems are generally employed in order to achieve higher pollutant removal efficiency. Currently, most hybrid systems utilize combinations of HSSF and VF CW cells (Kadlec and Wallace 2008).

Moreover, the novel CW technologies include:

- Intensified systems: (e.g. with some degree of added mechanical aeration, mainly when saving in land area is needed or when higher effluent quality is required);
- Soil-free systems: (e.g. hydroponic systems such as floating hydronic root mats (FHRM), root mat filters (RMF), *etc.*);
- Reciprocating or tidal flow CWs: where the wastewater is applied to the CWs in a tidal mode (via sequential filling and draining of wastewater) for obtaining better subsurface oxygen availability (Nivala et al. 2013a, Sun et al. 1999).





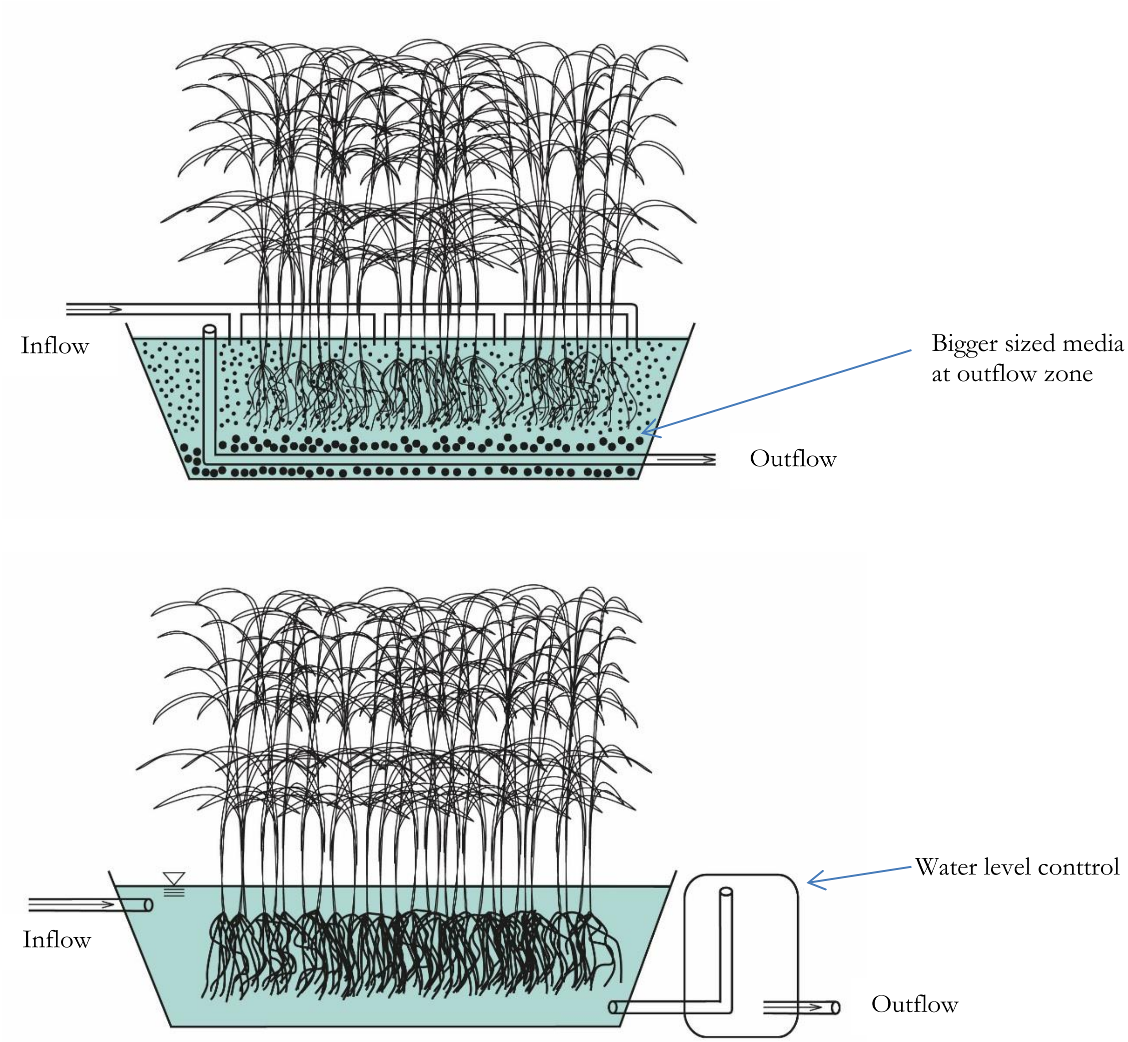


Figure 1.1-1 Schematic of some common types of CWs (from top to bottom): FWS CW, HSSF CW, VF CW and FHRM CW. Adapted from Vymazal (2007) and Kadlec and Wallace (2008).

1.1.3 Comparison with conventional wastewater treatment systems

CWs have numerous advantages and some disadvantages compared to conventional wastewater treatment systems. Compared to high-tech systems such as activated sludge systems, the main two disadvantages are the size requirement and the removal performance of some pollutants. So far, CWs are extensive systems that require large land areas (Kadlec and Wallace 2008). In addition, the performance related to some contaminants such as phosphorus (P) may not comply with guidelines for discharge in the environment. The aforementioned disadvantages and others can be rectified by taking some suitable measures. For instance, applying a specific helophyte species or a combination of different species in hybrid CW systems can lead to significant savings on costs. In addition, if land is expensive or limitedly available, adding artificial aeration or anaerobic pretreatment can lead to significant savings in land requirement.

On the other hand, the advantages include lower costs of construction and operation, less complexity of operation and maintenance (O&M), lesser to no requirement for highly-specialized personnel, higher aesthetic value and wildlife incorporation. Furthermore, CWs are scale-flexible, which offers great advantage over high-tech alternatives. For example, CWs can be suitably applied at household level or for small or large settlements; without imposing severe economies of scale. Therefore, CWs are suitable as decentralized wastewater treatment technology. In addition, CWs offer higher flexibility to tolerate wastewater quantity fluctuations (alternating periods of draught and flooding). Thus they can be better suited for stormwater run-off and/or combined sewer overflow (CSOs) treatment. This is valuable for planning and designing wastewater treatment systems under uncertainties, e.g. under different climate change scenarios.

It is worth mentioning that CWs are not *per se* a substitute of conventional systems, but can as well be used coupled to them as a tertiary treatment.

1.1.4 Pretreatment Requirements for CWs

CWs are naturally prone to clogging, due to the fact that the water to be treated has to pass through the media (sand and/or gravel), and the plant root area. Therefore, a pretreatment is required, as CWs can mainly perform secondary or tertiary (advanced) treatment. The pretreatment steps that are commonly performed include: screening, primary sedimentation and grit removal. The main aim of such pretreatment techniques is the removal of large objects (screening) and the reduction of total suspended solids (TSS, sedimentation and grit removal) to reduce the risk of clogging and to improve the hydraulic conductivity of the systems (Kadlec and Wallace, 2008).

The different types of contaminated streams may require different types of pretreatment. For the case of domestic wastewater, septic tanks and Imhof tanks are most commonly utilized as pretreatment technology to provide settling and to allow some processes such as fermentation to take place (Álvarez et al. 2008). In addition, different infrequently utilized technologies can be integrated as pretreatment. For instance, Álvarez et al. (2008) have utilized anaerobic digesters as pretreatment for domestic sewage, and found that such high rate anaerobic systems provide high TSS removal, that resulted in delaying the clogging in the gravel beds of the CWs that were used as secondary treatment. In addition, they found that the organic matter removal that took place in the anaerobic digesters has resulted in reduction by 30% - 60% in the CW area requirement. Taking into consideration the possibility of energy recovery from anaerobic treatment technologies such as high rate digesters and up-flow anaerobic sludge blanket (UASB) reactors, it is likely that cost savings can be obtained if such technologies are selected rather than septic/Imhof tanks.

In the view of the above, it is evident that the pretreatment step can be better exploited, not only to improve the treatment efficiency of the CWs and eventually reduce the pollution loads to be disposed to the environment, but also to achieve considerable cost savings.

1.1.5 Wastewater treatment processes in CWs

Kadlec and Wallace (2008) have classified the processes that contribute to pollutant removal in CWs into: microbially-mediated processes, chemical networks, volatilization, sorption, sedimentation, photo-degradation, plant uptake, vertical diffusion in soil and sediments, transpiration flux, accretion and seasonal cycles. Our understanding of all these processes and how they interact with one another is rather insufficient (Stottmeister et al. 2003, Wiessner et al. 2010, Wu et al. 2013). Moreover, the existence of a large number of factors that affect the occurrence and rates of these processes make the task of understanding them a big challenge.

The pollutants of interest for removal are mostly some of the carbon (C), nitrogen (N), P and sulfur (S) compounds; metals and pathogenic germs. It has been recognized that the microbial activity is the primary precursor for the removal of majority of pollutants in CWs (Reddy and D'angelo 1994). Microbial pollutant transformation and/or removal in CWs are directly tied to C, N and S cycles (Faulwetter et al. 2009). Moreover, CWs were found to perform pathogen removal at rates higher than those of most conventional wastewater treatment systems (Kadlec and Wallace 2008, Wu et al. 2016).

It is known that microbial respiration processes and the subsequent pollutant removal depend on the prevailing redox conditions and the associated electron acceptors in the CW system. Higher redox potential is associated with an oxidized environment, which promotes aerobic respiration pathways. Adversely, lower redox states indicate reduced conditions that induce anaerobic respiration pathways. It is found that in the different types of CWs, different redox potential conditions prevail, which in turn allow for different types of biochemical processes to take place (Faulwetter et al. 2009).

The helophyte plant root zone (named the rhizosphere by Hiltner and Störmer (1903)) hosts conditions of high redox potential states due to the oxygen that plants supply to their roots to survive the saturated soil conditions, which is partially released by the roots. These oxic conditions allow processes such as microbial nitrification and biochemical oxygen demand (BOD) removal to take place. Furthermore, within the same CW, macro- and micro-gradients exist, due to the existence of different areas within the CW that have variable redox potential conditions, from very high to very low redox potential levels (Stottmeister et al. 2003). Thus, CWs harbor anoxic zones in which denitrification, sulfate reduction and methane production can take place.

Kadlec and Wallace (2008) have illustrated some of the factors that influence the treatment efficiency in CWs. These factors include: temperature and season, water losses and gains, interactions with solids, system start-up, and system hydraulics. Furthermore, (García et al. 2004b) have investigated the effects of the medium size (sand/gravel) and the aspect ratio on the hydraulic behavior and subsequently on the removal efficiency of HSSF CWs, and found that the effect of the aspect ratio on the hydraulic efficiency is more important, which points out that not only the direct factors but also some indirect factors affect the treatment performance of CWs.

1.2 Sulfur cycle processes in CWs

Large quantities of sulfur from natural and industrial sources end up in the atmosphere and eventually return to earth in the form of acid rain (containing sulfuric acid). This atmospheric sulfur represents one of sulfur sources in CWs. In addition, a main source is the sulfur in the drinking water which ends up as wastewater constituent. This can be a considerable sulfur input, as the standards for sulfate in drinking water are not very stringent (e.g. 240 mg/L in Germany (TrinkwV 2001), 250 mg/L in United States (US EPA)). In addition, sulfur can be included in the chemicals that are contaminating the wastewater intended for treatment (Kadlec and Wallace 2008). In general, sulfate enters CWs via the atmosphere or the wastewater; and sulfides are either produced in the sewer, the settling tanks (if any), or within the CW itself. Additionally, other intermediately oxidized sulfur compounds also exist in CWs as a result of biotic and abiotic sulfur cycle processes (Vymazal and Kröpfelová 2008).

Sulfur removal is generally not a treatment goal as it can be safely released to the environment in its oxidized forms. However, inorganic sulfur transformations represent important processes that not only contribute to the removal of organic carbon and metals but also interact with the C and N cycles and the P removal processes in CWs (Faulwetter et al. 2009). The main biotic and abiotic sulfur cycle processes that take place in subsurface flow CWs are shown in Figure 1.2-1 and processes most relevant to this research are explained in detail in sections 1.2.1 to 1.2.4.

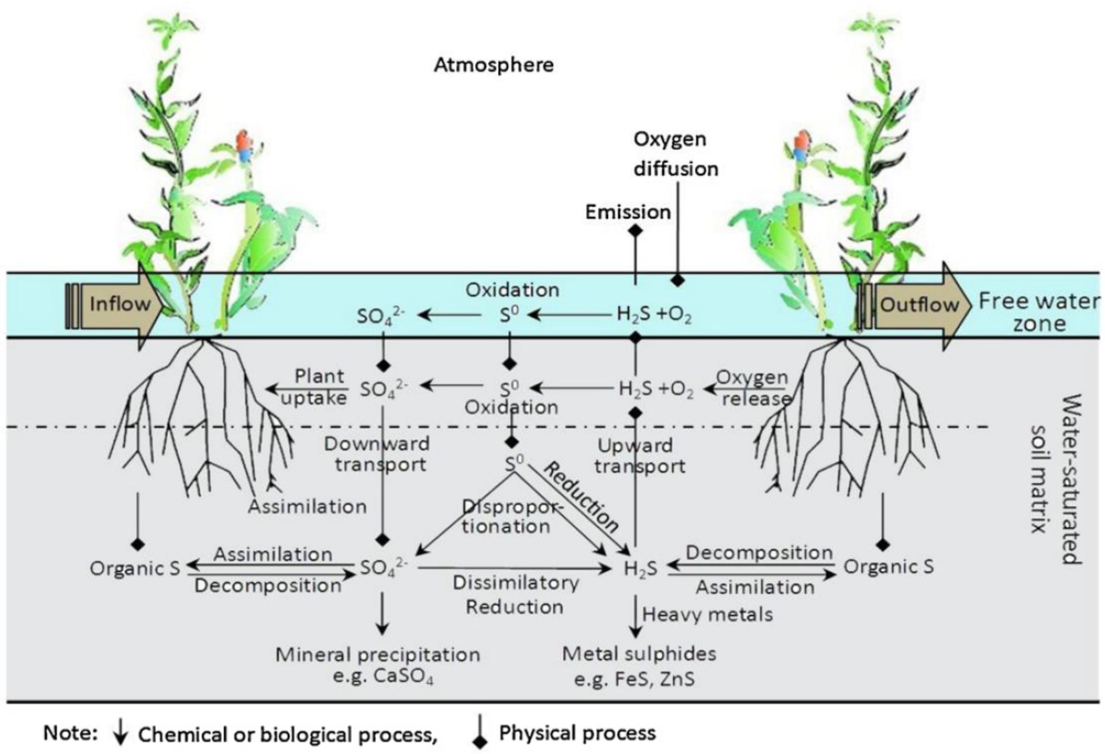


Figure 1.2-1 Main known sulfur transformation processes in surface-flow CWs. Source: Wu et al. (2013). Reprinted with author's permission.

1.2.1 Assimilatory sulfate reduction

The uptake of sulfur is essential for growth of all living organisms, including CWs' plants and microorganisms. The organisms need sulfur in reduced forms to be incorporated in sulfur-containing amino acids which are then incorporated into proteins. Since sulfate is the most abundant form of sulfur in nature and sulfide is not always readily present, most organisms can take up sulfate as sole source of sulfur and then transform it intracellularly to sulfide in the process of assimilatory sulfate reduction (Killham 1994, Takahashi et al. 2011, Vymazal and Kröpfelová 2008, Zehnder and Zinder 1980).

Dijkshoorn and Van Wijk (1967) have indicated that as 80% of the organic forms of S and N present in plants are employed in the synthesis of proteins, the ratio of organic-S to organic-N in the plant should lie close to that in its proteins (ratios found between 0.025 – 0.032). It was documented that both the uptake and requirements of S by plants vary between species, between the cultivars of the same species as well as according to the growth stage. Values from 0.1% to 1.5% of S content in plants were reported from various locations around the world and even higher values (up to 3%) were reported for halophytes or crops growing in saline soils (Duke and Reisenauer 1986, Jones and Martin 1964). In one of the investigations embedded in this thesis, S content of plant tissue was found to be roughly 0.6% and 0.3% for two investigated helophytes (section 3.3.3).

The assimilatory sulfate reduction accounts for limited amount of sulfate removal, since living organisms need sulfur at low levels. In addition, upon decomposition of decayed plants and microorganisms, the organic sulfur is transformed to hydrogen sulfide and released again to the water column (Figure 1.2-1). Despite the importance of assimilatory sulfate reduction, its effect's magnitude from the view point of sulfur cycling in CWs is considered minimal especially in sulfur rich wastewaters. As such, this process is not further discussed.

1.2.2 Dissimilatory sulfate, sulfur, thiosulfate and sulfite reduction

Inorganic sulfur cycling may vary considerably within the same CW, depending on the various conditions that prevail in different zones of CWs (Scholz and Lee 2005). In the anaerobic zones of CWs, sulfate can be utilized by sulfate reducing prokaryotes (SRPs) as a terminal electron acceptor heterotrophically for the oxidation of organic matter or autotrophically using H_2 as electron donor in the process of dissimilatory sulfate reduction (DSR). Including sulfate reducing bacteria and sulfate reducing archaea; SRPs are of the most ubiquitous microorganisms in the planet and they produce H_2S as end product of DSR process. The heterotrophic SRPs include (as examples): *Desulfovibrio* and *Desulfobacter* species, and the spore-forming genus *Desulfotomaculum* (Faulwetter et al. 2009, Rabus et al. 2006, Sturman et al. 2008, Vymazal and Kröpfelová 2008). The heterotrophic and autotrophic DSR are represented by the following reactions:

$$\mathbf{2CH_2O + SO_4^{2-} + H^+ \rightarrow 2CO_2 + HS^- + 2H_2O} \qquad 1.2\text{-}1$$

$$\mathbf{SO_4^{2-} + 4\,H_2 \rightarrow S^{2-} + 4H_2O} \qquad 1.2\text{-}2$$

DSR is an eight-electron step process with a number of intermediates. However, SRPs usually don't excrete the intermediate oxidation states of sulfur but only the end product sulfide (Rabus et al. 2006). The existence of intermediately-oxidized sulfur species such as elemental sulfur, thiosulfate, sulfite and numerous others in CWs are thus not the result of DSR. Rather, it is a result of the oxidation processes indicated in section 1.2.3, as the oxidation processes proceed step-wise and can lead to complete sulfur oxidation to sulfate or one of the intermediately-oxidized forms, depending on the availability of electron acceptors (Robertson and Kuenen 2006). Once formed, the intermediately-oxidized sulfur compounds can be used by specific group of prokaryotes in dissimilatory reduction processes with sulfide as end sulfur product. The biologically-mediated sulfur transformations are shown in Table 1.2-1 below.

The group of prokaryotes that perform dissimilatory sulfur reduction include obligate sulfur reducers such as the genus *Desulfuromonas acetoxidans*. Moreover, the sulfate reducing bacteria, a portion of SRPs, were found to be able of performing dissimilatory sulfur reduction. In addition, many SRPs are capable of utilizing sulfite or thiosulfate instead of sulfate, which are then reduced to sulfide in the processes of dissimilatory sulfite or thiosulfate reduction (Rabus et al. 2006).

1.2.3 Microbially-mediated sulfide, sulfur, thiosulfate and sulfite oxidation

The chemolithotrophic sulfide oxidizing prokaryotes (SOPs) include some functional groups such as the colourless sulfur bacteria (e.g. *Desulfovibrio sulfodismutans* which was found to be facultative SRP/SOP, the filamentous *Beggiatoa*, *etc*), the photosynthetic (phototrophic) green and purple bacteria and other groups (Pfennig 1977, Robertson and Kuenen 2006). These authors also demonstrated some of the possible reactions used by the colorless SOPs to gain energy for growth including the following:

$$\mathbf{H_2S + 2O_2 \rightarrow H_2SO_4} \qquad 1.2\text{-}3$$

$$\mathbf{3H_2S + 3O_2 \rightarrow 2S^0 + H_2SO_4 + 2H_2O} \qquad 1.2\text{-}4$$

$$\mathbf{2S^0 + 3O_2 + 2H_2O \rightarrow 2H_2SO_4} \qquad 1.2\text{-}5$$

$$\mathbf{Na_2S_2O_3 + 2O_2 + H_2O \rightarrow Na_2SO_4 + H_2SO_4} \qquad 1.2\text{-}6$$

1.2.4 Abiotic oxidation processes

Alongside the microbially mediated oxidation processes, spontaneous chemical oxidation can take place upon the availability of appropriate electron acceptors (Sturman et al. 2008) following the sequence:

$$\mathbf{SH^- \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}} \qquad 1.2\text{-}7$$

Table 1.2-1 Main biologically-mediated sulfur transformations in CWs

Process	Organisms	Initial form of sulfur	End form of sulfur	In the presence of
Assimilatory sulfate reduction	All living organisms	SO_4^{2-}	Organic sulfur	
Mineralization / decomposition		Organic sulfur	H_2S, HS^-	
Dissimilatory sulfate reduction	Sulfate reducing prokaryotes (SRPs)	SO_4^{2-}	H_2S, HS^-	Organic matter or H_2
Sulfide oxidation	Sulfide oxidation prokaryotes (SOPs)	H_2S	S^0 $S_2O_3^{2-}$ SO_3^{2-} SO_4^{2-}	O_2 and/or NO_3^-
Anaerobic sulfide oxidation	Phototrophic bacteria	H_2S	S^0 $S_2O_3^{2-}$ SO_3^{2-} SO_4^{2-}	Sun light
Sulfur oxidation	Sulfur oxidizing prokaryotes	S^0	$S_2O_3^{2-}$ SO_3^{2-} SO_4^{2-}	O_2 and/or NO_3^-
Anaerobic sulfur oxidation	Phototrophic bacteria	S^0	$S_2O_3^{2-}$ SO_3^{2-} SO_4^{2-}	Sun light
Thiosulfate oxidation	Thiosulfate oxidizing prokaryotes	$S_2O_3^{2-}$	SO_3^{2-} SO_4^{2-}	O_2 and/or NO_3^-
Anaerobic thiosulfate oxidation	Phototrophic bacteria	$S_2O_3^{2-}$	SO_3^{2-} SO_4^{2-}	Sun light
Sulfite oxidation	Sulfite oxidizing prokaryotes	SO_3^{2-}	SO_4^{2-}	O_2 and/or NO_3^-
Anaerobic sulfite oxidation	Phototrophic bacteria	SO_3^{2-}	SO_4^{2-}	Sun light
Dissimilatory sulfur reduction	Sulfur reducing prokaryotes	S^0	H_2S, HS^-	Organic matter or H_2
Dissimilatory thiosulfate reduction	Thiosulfate reducing prokaryotes	$S_2O_3^{2-}$	H_2S, HS^-	Organic matter or H_2
Dissimilatory sulfite reduction	Sulfite reducing prokaryotes	SO_3^{2-}	H_2S, HS^-	Organic matter or H_2

Adapted from Rabus et al. (2006), Robertson and Kuenen (2006) and the therein cited references.

1.2.5 Inorganic sulfur disproportionation

The processes of inorganic sulfur disproportionation are a type of fermentation processes that occur at moderate temperatures (0–80 ◦C). They are microbiologically catalyzed chemolithotrophic processes in which compounds like elemental sulfur, thiosulfate, and sulfite serve as both electron donor and acceptor, and generate hydrogen sulfide and sulfate. The processes are performed by certain types of sulfate reducing bacteria (Bak and Cypionka 1987, Finster 2008). Bak and Pfennig (1987) reported disproportionation of thiosulfate and sulfite to sulfate and sulfide as follows:

$$\mathbf{S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+} \qquad 1.2\text{-}8$$

$$\mathbf{4SO_3^{2-} + H^+ \rightarrow 3SO_4^{2-} + HS^-} \qquad 1.2\text{-}9$$

Krämer and Cypionka (1989) found out that the disproportionation of sulfite and thiosulfate are rather common among sulfate reducers, though only few strains couple this metabolism to growth. In addition, Canfield and Thamdrup (1994) have reported the disproportionation of elemental sulfur to sulfate and sulfide as follows:

$$\mathbf{4S^0 + 6OH^- \rightarrow 3S^{2-} + 2H_2O + SO_4^{2-} + 2H^+} \qquad 1.2\text{-}10$$

Jørgensen (1990a) and Jørgensen (1990b) has investigated the significance of the disproportionation process for the global sulfur cycle and classified thiosulfate disproportionation as key process in the transformations of intermediately-oxidized sulfur compounds in both freshwater and marine environments. Unfortunately, there is no available information on the significance of sulfur disproportionation in CWs. In general, the effect of the disproportionation processes is difficult to assess and cannot be distinguished from the other inorganic sulfur reduction and oxidation processes within the framework of this research. Hence these processes are not further discussed.

1.2.6 Implications of sulfur cycling on organic carbon and nutrients removal in CWs

Wiessner et al. (2010) evaluated the DSR in laboratory scale CWs and found that the addition of carbon (C; about 120 mg/L total organic carbon, TOC) has immediately triggered the transformation of about 90% of incoming sulfate. This indicates the importance of C availability for both occurrence and intensity of DSR in CWs. C sources in CWs include being a wastewater constituent, but in the cases of limited C in the inflowing stream, microorganisms such as denitrifiers and SRPs take carbon from local sources such as litter and dead plant material, or from the rhizodeposits (Stottmeister et al. 2003). Hence, DSR can be main process contributing to organic carbon removal in CWs, especially when oxygen and nitrate fluxes are below the stoichiometric requirements (Baptista et al. 2003, Faulwetter et al. 2009, Sturman et al. 2008). That is normally at redox potential (E_h) ranges from -200 to -100 mV (Reddy and D'angelo 1994). DSR is documented to account for as much as 50% of organic carbon removal in marine systems (Jørgensen 1982) and for up to 100% of organic carbon in terms of chemical oxygen demand (COD) in horizontal flow reed beds treating urban wastewater (Garcı□a et al. 2004a). Contradictorily, Wiessner et al. (2008b) have reported a clear correlation between the availability of reduced sulfur compounds and the decreasing organic C and TN removal efficiencies and plant viability in laboratory scale CWs. This implies that DSR has both beneficial and deleterious effects on C removal in CWs. The extent to which one of these two effects overcomes the other may depend in many factors, such as the concentrations of sulfate, organic C and oxygen in the wetlands. However an assessment of the net effects of sulfur cycling on C removal in CWs is not explicitly provided in the literature.

In addition, DSR interacts both negatively and positively with TN removal in CWs. On one hand, the produced sulfide is inhibitory to the nitrifying communities even at concentrations as low as 0.5 mg L^{-1} (Æsøy et al. 1998). In the absence of sufficient concentrations of oxygen, nitrate or metals; the produced H_2S may volatilize or accumulate in the water column, depending on the pH (as it can be found as well in its ionized forms HS^- and S^{2-}, Figure 1.2-2). In addition, depending on the existence of oxygen and/or nitrate, sulfide can be oxidized as indicated in sections 1.2.3 and 1.2.4. Hence, in addition to its inhibitory effects, sulfide works as oxygen scavenger and competes with the nitrifiers and other aerobic microorganisms for the available oxygen. Moreover, at relatively elevated concentrations between 10 – 50 mg L^{-1}, sulfide is reported to be inhibitory to helophytes' growth. The mechanisms of inhibition for different plants were investigated by some researchers, and were found to include blockages of the gas pathways in the roots, and impacts on the photosynthetic capacity of the leaf (Armstrong et al. 1996, Chambers 1997, Chambers et al. 1998, Tretiach and Baruffo 2001). Hence, the associated decrease of helophyte activity is decreasing their uptake rates of nitrogen. On the other hand, sulfide presence may play a positive role for TN removal as it serves as electron donor for autotrophic denitrification (Moraes et al. 2012).

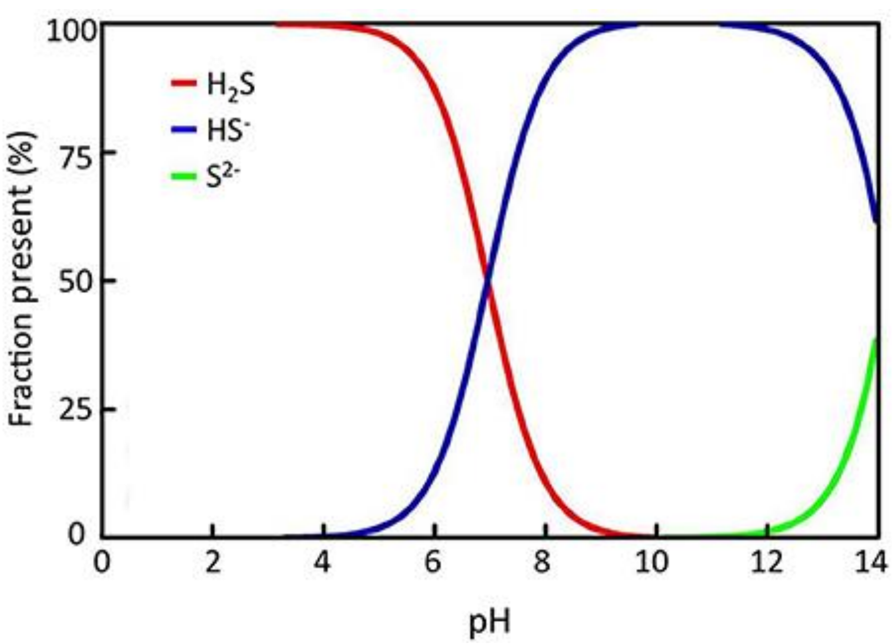


Figure 1.2-2 Sulfide solubility chart showing the relative fractions of different sulfide species at different pH levels. Source: Holmer and Hasler-Sheetal (2014).

The efficiency decrease of total phosphorous (TP) removal in CWs associated with sulfur cycling is explained in two main ways. Firstly, sulfide toxicity decreases the growth of both plants and microorganisms and subsequently the associated assimilatory P uptake; and secondly, sulfide metal precipitation competes with P precipitation for the available binding capacity. The combined effect of decreased TN and TP removal from CWs due to sulfur cycling and the subsequent release of these nutrients into receiving water bodies is often referred to as sulfur-induced eutrophication (Kadlec and Wallace 2008, Lamers et al. 1998).

1.2.7 Other implications of sulfur cycling in CWs

The aforementioned toxicity of sulfide to helophytes does not only affect nutrients removal via plant uptake but also it alters other CWs' treatment processes, since the helophytes represent an important component of CWs and play several roles in these systems as illustrated in section 1.3. Hence, the consequences of decreased helophyte efficiencies may end up in decreased CW system's performance if the roles of helophytes (e.g. oxygen release, organic rhizodeposition or pollutant uptake) were accounted for in a specific treatment system (e.g. in systems where organic rhizodeposits are main electron donor for denitrification).

In addition, metal-sulfide precipitation is considered as one of the important mechanisms of metal removal in CWs (Dvorak et al. 1992, Stein et al. 2007). Such mechanism is highly valuable, especially in the remediation of AMD, where both sulfate and metals are of the targeted contaminants.

Another major drawback of sulfur cycling and sulfide production in CWs is its volatilization to the environment. Solubility of H_2S is pH and temperature dependent. H_2S has an unpleasant odor and is toxic to human beings and animals (Beauchamp et al. 1984). The significance of the emission of H_2S from CWs at local and global scales is unknown and need to be investigated (Wu et al. 2013).

Furthermore, DSR does not occur in isolation but in concert with other microbial reactions including fermentation and methanogenesis (Sturman et al. 2008). It is well known that methane producing archaea (MPA) and SRPs perform their metabolism in relatively similar conditions in terms of redox potential, and they compete for the same electron donors (acetate or molecular hydrogen) (Widdel 1988, Zinder 1993). It is also documented that methane is emitted from CWs (Johansson et al. 2004). The implication of the competition between the DSR and methanogenesis processes on CH_4 emission from CWs is not sufficiently studied, but it is expected to be of a positive nature from environmental view point in the cases where anaerobic conditions cannot be mitigated and both processes occur. In other words, if DSR occurrence results in a net decrease of CH_4 production and subsequent emission from CWs, the net greenhouse effect is reduced. The expected net decrease of produced CH_4 in scenarios where DSR occurs or not is not merely due to the competition between the SRPs and MPA but also due to toxicity of sulfide to MPA (Harada et al. 1994, Maillacheruvu et al. 1993).

1.3 Roles of helophytes in CWs

The different roles helophytes play in wastewater treatment represent one of the vital questions in constructed wetlands (CWs) research. The most important benefits from helophytes in CWs were firstly thought to be the physical functions they provide, such as bed surface stabilization, conditions for physical filtration, clogging prevention, frost insulation and provision of surface for attached microbial growth (Brix 1994). However, other benefits exist in addition to these physical effects, including the direct uptake of some contaminants, helophyte-mediated oxygen transfer to their rhizosphere and the provision of root exudates (Brix 1997, Stottmeister et al. 2003). Moreover, microbial transformations are of the most important processes that contribute to pollutant removal in CWs (Faulwetter et al. 2009, Kadlec and Wallace 2008, Reddy and D'angelo 1994), yet the roles helophytes play in supporting the microbial communities are not sufficiently understood.

Helophytes or wetland plants in general are morphologically adapted to growing in water-saturated sediments due to having internal air spaces that serve for convectively transporting oxygen from above-ground to submerged plant tissues such as roots and rhizomes. The oxygen channeled through the gas spaces is depleted by two processes: the respiration of roots and rhizomes, and the radial oxygen loss (ROL). The latter is used by plants to protect themselves against soluble phytotoxins (such as some ferrous and manganous compounds and H_2S) and by the rhizosphere microorganisms (Armstrong and Wright 1975, Brix and Orr 1992, Colmer 2003, Končalová 1990). The ROL results as well in oxygenating the rhizosphere and hence changing the redox potential of the anoxic zones, which allows biotic and abiotic oxidation processes to take place (Kuschk et al. 1999). Several types of CWs helophytes were found to tolerate relatively high concentrations of sulfide. Wiessner et al. (2008a) have investigated the mechanisms of sulfide detoxification by plants. They have examined the reoxidation of sulfides in the rhizosphere of laboratory scale CWs, as a detoxification strategy in the rhizosphere.

The available information on ROL rates from different helophytes is very limited, but published data shows big variability among helophyte species. In addition, ROL from a single species was found to vary with factors such as distance from the apex and redox potential in the rhizosphere. Sorrell and Armstrong (1994) reported the difficulties on measuring ROL and recommended incorporating high vigilance in experimental approaches and rejecting the published findings if the experimental methods are flawed. Despite the limited information, it is generally accepted that ROL rates from helophytes are significant and of relevance to CWs treatment processes (Armstrong et al. 2000, Wießner et al. 2002).

In addition, rhizodeposition is one of the main contributions of helophytes to CWs processes. The rhizodeposits can constitute an important component of the total carbon balance in CWs, especially where organic carbon is limited in the contaminated waters intended for treatment. Firstly introduced by Whipps and Lynch (1985), the term rhizodeposition includes all organic materials originating from the roots. It is divided into five components, depending on their nature and method of formation: exudates (water soluble compounds, e.g. sugars and amino acids); secretions (e.g. low molecular weight compounds which are released metabolically); mucilages (polysaccharides); mucigel; and lysates (compounds released by autolysis of old cells). These five components were classified by Cheng and Gershenson (2007) into two main groups: water soluble exudates, including the first component; and water insoluble materials, comprising the last four components. Initially, the soluble portion of rhizodeposits, i.e. the soluble exudates, caught more attention since it encompasses substrate directly available to microorganisms. In recent years, an increased interest has been dedicated also to the insoluble organic materials produced by roots, which represent a potential substrate for rhizosphere microorganisms (Newman et al. 1985, Whipps 2001).

Investigations on crop plants have shown that rhizodeposition differs between various plant species. In addition, the compounds released by an individual plant can vary significantly in quality and quantity over time and space (van Veen et al. 2007). Wetland plants are expected to vary in their rhizodeposition regimes analogously, yet precise information on the total extent as well as species-variations in rhizodeposition from helophytes is hard to obtain experimentally. So far, occasionally large differences in pollutant removal efficiencies of CWs planted with different species were noted (Brisson and Chazarenc 2009). Zhai et al. (2013) have found that the rhizodeposits represent a valuable organic C source for heterotrophic denitrification and found significant differences in the magnitude of rhizodeposits between two tested helophytes. Hence, additional information is needed to better understand the differences in rhizodeposition capacities and as well to identify the species with high vs. low capacities.

In addition to rhizodeposition, helophytes influence the carbon balance in CWs via litterfall. In general, helophytes tissues (living leaf and stem material) fall as litter at variable rates depending on the survival strategy of individual species. This litter then undergoes decomposition on the wetland surface at various rates depending on the physical and chemical composition of material as well as environmental conditions at the site of decomposition (Kadlec and Wallace 2008, Vymazal 1995).

Furthermore, helophytes contribute to treatment processes in CWs by taking up some of the pollutants and nutrients from the wastewater. Apart from hyperaccumulators which are outside the focus of this research, helophytes are reported to take up nutrients such as phosphorus and nitrogen, sulfur and metals (Dubois 1994, Dykyjová 1978, Kuschk et al. 1999). The uptake of nitrogen and phosphorus is considered a sustainable removal mechanism only if the plants are harvested, as in case of no harvest the eventual decay and decomposition of plant tissues will lead to returning part of the taken up nutrient. In general, the effect size of the uptake processes depend on many factors such as the helophyte species, the growth stage (uptake rates at early growth season are higher than at the end of season) and the concentrations of the taken up compounds in the wastewater (Kadlec and Knight 1996, Vymazal 2007).

1.4 Research objectives and motivation

The ongoing increase in world's population associated with increased rates of urbanization call for the application of sustainable wastewater treatment technologies. As of today, about one third of the world's population has no access to proper sanitation (www.un.org). It is clear that this one third of population is mainly situated in developing countries in Africa, Asia and South America. An integral part of any intended sanitation provision is the collection and treatment of the produced wastewater, especially in big urban settlements. For future, application of CWs for wastewater treatment worldwide can be broadened if these systems are introduced to communities that are currently not applying any form of wastewater treatment and if the advantages of applying CWs are highlighted. Before this is achieved, better understanding of the processes that contribute to pollutant removal and subsequent provision of design standards are required.

The processes that take place in the different segments of CWs, mediated by the plants, microorganisms, soil and wastewater constituents have a high degree of complexity. Our understanding of these processes and how they interact with one another is rather insufficient (Stottmeister et al. 2003). Some of the less researched processes in CWs are the sulfur cycle processes. The overall objective of this work is to assess inorganic sulfur cycling in CWs via evaluating the dynamics of sulfur compounds in different CW technology types and at different organic carbon loading conditions. The importance of sulfur cycling in CWs is viewed based on its occurrence and magnitude as well as on its interactions with organic carbon and nitrogen removal processes.

Firstly, a black box approach was selected to assess the inorganic sulfur turnover in different designs of CW technologies. For this investigation, the systems were receiving high TOC loads. Subsequently, the CW technology type that was found to reflect high rates of sulfur turnover, namely the unaerated HSSF type, was investigated in more detail. For this, a grey box approach was followed firstly, to monitor the dynamics of reduced and oxidized sulfur compound within the CWs; secondly, to differentiate the processes as biotic or abiotic via analysing stable isotope abundance signatures; and thirdly, to quantify the overall processes based on load calculations and the intermediate processes based on isotope fractionation studies.

In addition, a main objective was to assess the helophyte species role as provider of organic carbon and its influence on sulfur and nitrogen cycling in both low- and highly-loaded CWs. Four different helophyte species were tested in three different experiments, each comparing two or three species. Capacities of helophytes for depositing organic compounds were assessed indirectly, as direct quantification of rhizodeposits was not feasible within the framework of this research.

Last, the fate of inorganic sulfur that was removed from the water column of HSSF CW receiving contaminated groundwater with high sulfate content and low load of organic carbon was investigated. This was done as well to better understand the possible consequences of sulfur removal from CW systems to the environment by evaluating to which extent the sulfur removed from the water column is trapped in the soil compartment and to which extent it is lost to the atmosphere.

2. Thesis Layout

This thesis comprises 5 chapters. Chapter 1 provides a broad background about CWs and their use for wastewater treatment, the main pollutant transformation processes in CWs with special focus on inorganic sulfur transformations and their interactions with organic carbon and inorganic nitrogen transformations, and the overall objectives of this research work.

To achieve the stated objectives, 6 sets of experiments were conducted and the output is presented in 0chapter 3. Each experiment set is presented independently, and the specific objectives of each set are stated in the corresponding section. Firstly, the consequence of applying different designs of passive and intensified (with mechanical aeration) VF and HSSF CWs on the overall inorganic sulfur transformations is presented in section 3.1. In addition, a detailed investigation of the dynamics of inorganic sulfur processes within the technology type that was found to show highest sulfur turnover (the unaerated HSSF CWs) was illustrated in section 3.2.

The following 3 sections were dedicated to understand helophyte interspecies differences and the consequences of these differences on pollutant transformations in CWs planted with a specific helophyte. Section 0 reflects the differences found between two helophyte species in FHRM CWs run under low organic carbon loading. The sulfate and nitrate reduction estimations were applied to indirectly estimate the rhizodeposition capacity of each of the two helophytes. In section 0, dead root material was collected from the same two helophyte species and were incubated anaerobically in the dark in the presence of sulfate as sole electron acceptor to quantify the contribution of dead root matter as an insoluble portion of rhizodeposits to the pools of bioavailable organic carbon which was estimated as the theoretical stoichiometric equivalent of DOC to match the noted sulfate concentration decrease. The last experiment to deal with helophyte interspecies differences is presented in section 0. In this experiment, 3 helophytes were compared related to the treatment performance and inorganic sulfur turnover in intermittently-fed HSSF CWs planted with them.

In numerous occasions within and outside this research framework, sulfur removal was documented from CWs, especially HSSF CWs. The main inorganic sulfur transformation processes were found to be sulfate reduction, sulfide and sulfur oxidation, precipitation and volatilization (Vymazal and Kröpfelová 2008). The CW soil matrix (for the soil-based CWs) is expected to be one of the segments of the CW where the sulfur which is removed from the water column may end. It is thus important to understand the fate of removed sulfur. For this, pools of inorganic sulfur in a HSSF CW were investigated in the pore-water and soil matrix and the findings are shown in section 3.6.

Results from each experiment are discussed in the corresponding section. In addition, chapter 3.6 offers a general outlook of the findings and the relevance of research for increased application of CWs for wastewater treatment in future, as well as some recommendations for future research.

3. Research Output

3.1 Inorganic sulfur turnover in dependence on system type in pilot-scale constructed wetlands receiving domestic wastewater with high organic load

3.1.1 Introduction

As stated in section 1.1.2 there are different CW technologies that are currently applied. The design of each technology (e.g. HSSF or VF) has direct influence on the environmental conditions inside the CW, such as subsurface oxygen availability. As a consequence, within each type of CWs different microbial consortia may be at action leading to different pollutant transformations and thus different quality of the CW's effluent. Such differences between CW technologies were noted for TOC, TN and *Escherichia coli* removal (Button et al. 2015, Headley et al. 2013, Nivala et al. 2013b). However, a comparison of the influence of system design on sulfur cycling has not been conducted in the past.

The aim of this experiment was hence to characterize the changes in inorganic sulfur pools (between oxidized and reduced compounds) associated with the treatment of domestic sewage in different CWs designs. 7 different technologies represented in 14 individual CWs (unplanted and planted with *Phragmites australis* pairs) of pilot-scale CWs were evaluated for one year in a black box approach (sampling of inflow/outflow sulfur compounds; no internal sampling).

3.1.2 Material and methods

Description of the pilot-scale CWs

This investigation was conducted at the Ecotechnology Research Facility which is situated in the village of Langenreichenbach in Saxony, Germany (51° 29' 00" N, 12° 54' 00" E and 96 m above mean sea level (MSL)). The facility contains 15 individual pilot scale subsurface flow CWs, representing 8 different design variants (vertical, horizontal and reciprocating flow systems) (Figure 3.1-1). The designs vary in terms of flow direction, media type, degree of media saturation, loading regime and aeration scheme. All systems receive pre-treated wastewater from the adjacent wastewater treatment plant (WWTP). The pretreatment is achieved via sedimentation tank, from which the wastewater is directed into two commercial-size septic tank filters (Zoeller filters, screen size 0.8 mm) and therefrom to the systems (Nivala et al. 2013a). The main characteristics of influent wastewater entering the CWs are summarized in Table 3.1-1. The main design and operational parameters of the 14 investigated CWs are shown in Table 3.1-2.

Table 3.1-1 Mean influent wastewater characteristics

	*BOD_5 (mg L^{-1})	*TN (mg L^{-1})	*E_h (mV)	$SO_4^{2-}-S$ (mg L^{-1})	S^{2-} (mg L^{-1})	S^0 (mg L^{-1})	$S_2O_3^{2-}-S$ (mg L^{-1})
Average	240	72	-148	53.6	8.6	9.2	13.8
Standard deviation	74	16	78	13.3	3.6	3.5	3.7
Count (n)	65	66	66	19	19	19	6

*Data from Nivala et al. (2013a). Data of sulfur compounds from 2012-2013.

Figure 3.1-1 The pilot scale CW systems in Langenreichenbach: pairs of unplanted and planted with *Phragmites australis* subsurface flow CWs. Photo: André Künzelmann (UFZ); courtesy Dr. Jaime Nivala.

Table 3.1-2 Design and operational characteristics of the investigated CWs

System	System Type	Saturation Status	Main Media Type	Main Media Depth [m]	Dosing Interval [h]	Surface Area $[m^{-2}]$	Hydraulic Loading Rate [L m^{-2} d^{-1}]	▲BOD_5 Loading rate [g m^{-2} d^{-1}]
H50, *H50p	HSSF	Saturated	Medium gravel	0.50	0.5	5.64	36	9.8 ± 4.2
H25, *H25p	HSSF	Saturated	Medium gravel	0.25	0.5	5.64	18	4.9 ± 2.1
HA, *HAp	HSSF+ aeration	Saturated	Medium gravel	1.00	0.5	5.64	130	37.0 ± 15.8
VA, *VAp	VF+ aeration	■Saturated	Medium gravel	0.85	1.0	6.2	95	27.1 ± 11.8
VG, *VGp	VF	Unsaturated	Fine gravel	0.85	1.0	6.2	95	27.1 ± 11.8
VS1, *VS1p	VF	Unsaturated	Coarse Sand	0.85	1.0	6.2	95	No data
VS2, *VS2p	VF	Unsaturated	Coarse Sand	0.85	2.0	6.2	95	No data

Adapted from Nivala et al. (2013a). *p refers to the planted bed of each pair. ■The intensified systems were operated saturated to benefit from the applied aeration.
▲Average ± standard deviation (values from 2012-2013, n = 23)

The H50 pair represents horizontal flow planted/control beds with a saturated gravel depth of approximately 50 cm. This design was provided since it is commonly applied and since it can be operated passively (e.g. without energy inputs). The H50 systems have dimensions length × width of 4.7 × 1.2 m and are operated at a hydraulic loading rate of approximately 36 $\mathrm{mm\ d^{-1}}$ (resulting in a nominal hydraulic retention time (nHRT) of approximately 5.5 days). The saturated HSSF beds with 25 cm depth (H25 pair) were designed to test the effect of depth on CWs' performance. They have equivalent length × width dimensions to H50 pair but receive half of their hydraulic loading, resulting in preserving the nHRT. In addition to the HSSF systems, four unsaturated vertical downflow systems were applied (VS1 and VS2 pairs) with coarse sand as the main filter media. Each bed is 2.75 × 2.4 m length × width and contains an outlet shaft of 0.5 × 0.8 m (the outlet shaft is located within the bed). The outlet shaft is subtracted from the total surface area of the bed, for an effective area of 6.2 $\mathrm{m^2}$ per bed. The VS1 pair is dosed once every hour, whereas VS2 pair is dosed once every two hours. The systems receive a hydraulic loading rate of approximately 95 $\mathrm{mm\ d^{-1}}$. A third unsaturated vertical downflow pair (VG) was applied with dimensions/loading identical to the other two vertical pairs but with gravel as filter media, to allow for performance comparison against sand-based systems. Furthermore, two pairs of intensified (with mechanical aeration) systems were also provided. The VA pair (A in VA denotes aeration) was characterized with saturated vertical downflow and had the same dimension/loading as the other vertical flow systems; the saturated HA pair had similar length × width dimensions to its HSSF passive counterparts but a greater depth (1 m) and a higher loading (130 $\mathrm{mm\ d^{-1}}$) (Nivala et al. 2013a).

Sampling and analytic procedures

The systems were sampled at inflow and outflow from especially prepared valves at about two weeks' intervals, from the start of April 2012 to the start of April 2013. The operation of the HA system (unplanted horizontal aerated bed) was changed in July 2012 to incorporating a windmill as aerator. From July 2012 there was then no longer continuous mechanical aeration for this bed. The results from the period of July 2012 – April 2013 were evaluated separately for this bed, and the difference between the two methods of aeration was evaluated with statistical tests.

The redox potential, pH, sulfide ($\mathrm{S^{2-}}$), nitrite ($\mathrm{NO_2^- - N}$) and nitrate ($\mathrm{NO_3^- - N}$) were measured at the on-site laboratory of the research facility directly after taking the samples, as these parameters change rapidly. The samples were kept in cooling boxes protected from light until they were transported to the laboratory at the end of each sampling day. Most physical-chemical parameters were analyzed on the day of sampling. For the parameters that were analyzed afterwards (e. g. $\mathrm{SO_4^{2-} - S}$), samples were kept frozen at -20°C in the dark. Redox potential was measured using a SenTix ORP electrode (WTW, Weilheim, Germany), and temperature was measured with a temperature sensor (PT 1000, Pre-Sens, Regensburg, Germany). The pH value was measured with a SenTix41 electrode with pH 537 Microprocessor (WTW, Weilheim, Germany). Settled unfiltered samples were used for the analysis of sulfide, sulfite, thiosulfate and elemental sulfur. Photometric estimation of sulfide concentrations was performed using Test kit LCW053 (0.1 - 2 mg/L) (HACH LANGE, Germany). Concentrations of sulfite and thiosulfate were analyzed after derivatisation with monobromobimane and determined by HPLC (Beckman, USA) using fluorescence detector RF 551 (Shimadzu, Japan) and columns Li-Chrospher 60, RP Select B (250-4) according to Rethmeier et al. (1997). Elemental sulfur was measured according to Rethmeier et al. (1997) by extracting samples with chloroform and analyzing by HPLC (Beckman, USA) using a Li-Chrospher 100, RP 18 column (5 µm, Merck, Germany) equipped with a UV-detector at 263 nm. For the analysis of the remainder of anions and cations, filtered samples were used. Filtration was carried out with a 5 µm-syringe filter (Ministart NML, Sartorius) to remove bigger particles. Sulfate concentrations were measured photometrically as the turbidity of $BaSO_4$ solution at 880 nm (HACH LANGE, Germany). Concentrations of ammonium, nitrite and nitrate were quantified photometrically using the respective test kit (Merck, Germany). TN values were analyzed by a multi N/C® TOC/TN analyzer (Analytik Jena, Jena, Germany). Methane samples were taken separately in 10 ml headspace vials to which 100 µL of sodium azide (6.5 g/100 ml) was added with a micro-syringe to inhibit microbial activity. Methane samples were preserved at 4°C until analysis with gas chromatography (HSS-GC, DANI HSS 86.50). BOD_5 concentrations were measured via incubation of the samples for 5 days at 20°C using OxiTop® automated system (WTW, Weilheim, Germany). Allylthiourea was added to hinder the oxidation of ammonium nitrogen. However, the BOD values measured do not represent carbonaceous BOD_5 ($cBOD_5$) since nothing was done to account for example for the oxygen demand of the reduced sulfur compounds.

Calculations

The influent and effluent pollutant loads (g m^{-2} d^{-1}) were calculated from the equation:

$$\mathbf{Load_{in/out}} = \frac{(\mathbf{V_{in/out}} * \mathbf{C_{in/out}})}{\mathbf{A}} \qquad 3.1\text{-}1$$

Where:

V_{in} and V_{out} : the volumes of wastewater that entered or exited a CW system in $L\ d^{-1}$.
C_{in} and C_{out} : influent and effluent pollutant concentration in g/L;
A: is the surface area of the CW in m^{-2}.

The load removed from a specific system was calculated as:

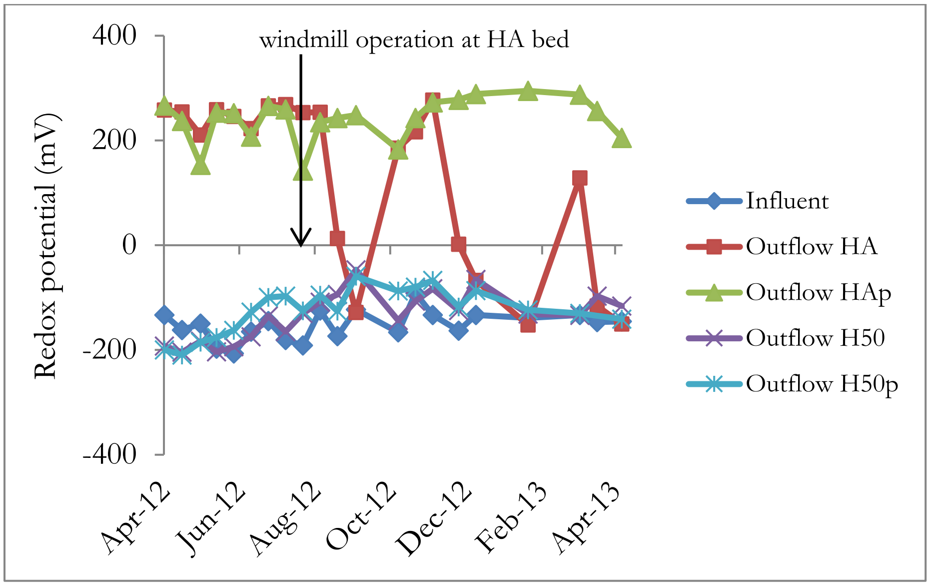
$$\mathbf{Load_{removed}} = \mathbf{Load_{in}} - \mathbf{Load_{out}} \qquad 3.1\text{-}2$$

Statistical procedures

All the statistical analyses were conducted using the R statistical computing environment (R-Core-Team 2013). A statistically significant difference was defined at 95% confidence (p value < 0.05). For comparing the means of any parameter from the different CW technologies, Student's t-test (paired) was performed after testing the normality of the distribution (using Shapiro test) and the homoscedasticity of variances (using Fisher's F test). When the parameters had non-linear distribution, the Wilcoxon's rank test was used.

3.1.3 Results

The redox potential values for some selected beds are illustrated below.



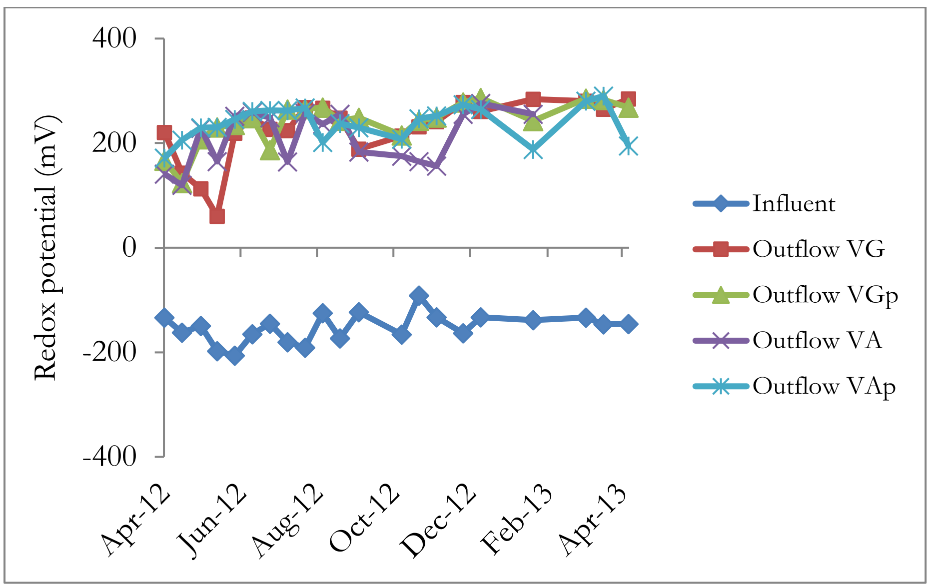


Figure 3.1-2 Redox potential (E_h) at the inflow and outflow of some of the HSSF and VF CWs

The redox potential was predominantly in the negative range at inflow and outflow of the HSSF unaerated systems H25 pair (data not shown) and H50 pair throughout the experimental period; and for the HA bed occasionally (after applying the windmill for aeration in July 2012). At these systems, redox potential at outflow was not significantly different from that at inflow ($p > 0.05$). Conversely, the redox potential was permanently in the positive range at outflow of the unsaturated and the saturated-aerated VF beds (VG, VS1, VS2 and VA pairs) and the HAp bed throughout the sampling period. The HA bed hosted elevated positive redox potential levels when it was mechanically aerated (till July 2012). Redox potential data is obtained in collaboration with Dr. Jaime Nivala (UBZ).

The removal of some pollutants was calculated using Table 3.1-2 equation and shown in Table 3.1-3.

Table 3.1-3 Load removal of some selected parameters

System	▲BOD_5 $g\ O_2\ m^{-2}\ d^{-1}$ (%)	▲$NH_4^+ - N$ $g\ N\ m^{-2}\ d^{-1}$ (%)	▲TN $g\ N\ m^{-2}\ d^{-1}$ (%)	■$SO_4^{2-} - S$ $g\ S\ m^{-2}\ d^{-1}$ (%)
H50	7.6 ± 2.7 (75 ± 10)	0	0.41 ± 0.38 (12 ± 12)	0.71 ± 0.25 (46 ± 19)
H50p	8.4 ± 2.6 (82 ± 8)	0.37 ± 0.46 (12 ± 20)	0.76 ± 0.45 (25 ± 14)	0.53 ± 0.11 (37 ± 8)
H25	3.8 ± 1.3 (76 ± 11)	0	0.2 ± 0.18 (12 ± 12)	0.23 ± 0.07 (35 ± 15)
H25p	4.4 ± 1.3 (86 ± 9)	0.47 ± 0.32 (35 ± 23)	0.64 ± 0.33 (43 ± 22)	0.27 ± 0.12 (39 ± 15)
HA	*33.4 ± 4.4 (98 ± 2) **28.3 ± 10.6 (77 ± 16)	*9.2 ± 1.9 (97 ± 6) **1.0 ± 2.6 (9 ± 23)	*4.5 ± 1.4 (44 ± 9) **1.7 ± 1.7 (14 ± 14)	*0 **3.32 ± 1.1 (55 ± 20)
HAp	38.1 ± 7.8 (99 ± 1)	9.1 ± 2.7 (97 ± 14)	4.1 ± 5.7 (35 ± 52)	0
VA	28.3 ± 7 (99 ± 1)	6.7 ± 1.9 (93 ± 9)	5.8 ± 2.0 (68 ± 15)	0
VAp	27.3 ± 7.1 (99 ± 1)	6.9 ± 1.9 (99 ± 1)	4.8 ± 1.7 (57 ± 15)	0
VG	26.3 ± 7.4 (95 ± 3)	5.5 ± 2.4 (76 ± 17)	2.6 ± 1.3 (34 ± 11)	0
VGp	26 ± 7.3 (95 ± 3)	6.1 ± 2.2 (85 ± 13)	2.9 ± 1.2 (34 ± 11)	0
VS2	7.9 ± 2.7 (98 ± 3)	1.75 ± 0.96 (81 ± 20)	0.67 ± 0.29 (27 ± 10)	0
VS2p	7.5 ± 2.7 (97 ± 3)	1.78 ± 0.96 (83 ± 14)	0.66 ± 0.32 (27 ± 12)	0

In means ± standard deviations; count (n) is ≤ 23 (total number of sampling events was 23, however samples were not collectable from all systems at all sampling dates due to either O&M measures at some beds or due to unanalysed parameters at some dates). Data from the VS1 pair is not included due to incomplete information for load calculation (flow data is not available). *Data from HA bed till July (mechanical aeration). **Data from HA bed after applying the windmill for aeration. ▲Data obtained in collaboration with Dr. Jaime Nivala (UBZ). ■Sulfate data with 0 values means no removal was noticed. In general, some calculated removal rates at these cases were even negative in value (reflecting that the sampling method needs to be refined).

Related to concentration removal in VS1 pair, it behaves similar to the other VF systems.

As shown in Table 3.1-3, higher BOD_5 and higher $NH_4^+ - N$ removal took place in the VF and the aerated HSSF beds (HAp and HA during the mechanical aeration) and was significantly different from removal in the remainder of systems and HA in windmill application period. However, the higher $NH_4^+ - N$ removal did not automatically lead to high TN removal, as mainly nitrification took place in these systems but there was insufficient denitrification ($NO_3^- - N$ up to 80 mg/L and limited concentrations of $NO_2^- - N$ were measured at outflow of these systems). In addition, there was no $SO_4^{2-} - S$ removal in these systems. Reversely, the reduced sulfur compounds (S^{2-}, S^0, $SO_3^{2-} - S$ and $S_2O_3^{2-} - S$) which were detected in the influent wastewater were all below detection at the outflow of these systems, hinting on sulfide and sulfur oxidation processes within the beds.

$SO_4^{2-} - S$ removal took place exclusively in the unaerated HSSF CWs and HA bed during windmill aeration. The main removal mechanism of sulfate was estimated to be DSR. Significant concentrations of sulfide (Figure 3.1-3) were measured in the outflow of these systems (sampling of these systems was started in October 2012, as they were not available for sampling beforehand due to occupancy by another research group). Data from H50 pair during the non-sampled period was analyzed by Carranza-Diaz et al. (2014) and reflected as well considerable sulfate removal activity in this pair. The intermediately-oxidized sulfur compounds were as well only detectable in the outflow of these systems. In general, $S_2O_3^{2-} - S$ up to 18 mg/L, $SO_3^{2-} - S$ predominantly below 0.5 mg/L and S^0 up to 19 mg/L were detected at outflow of these systems. Data are summarized in Table 3.1-4.

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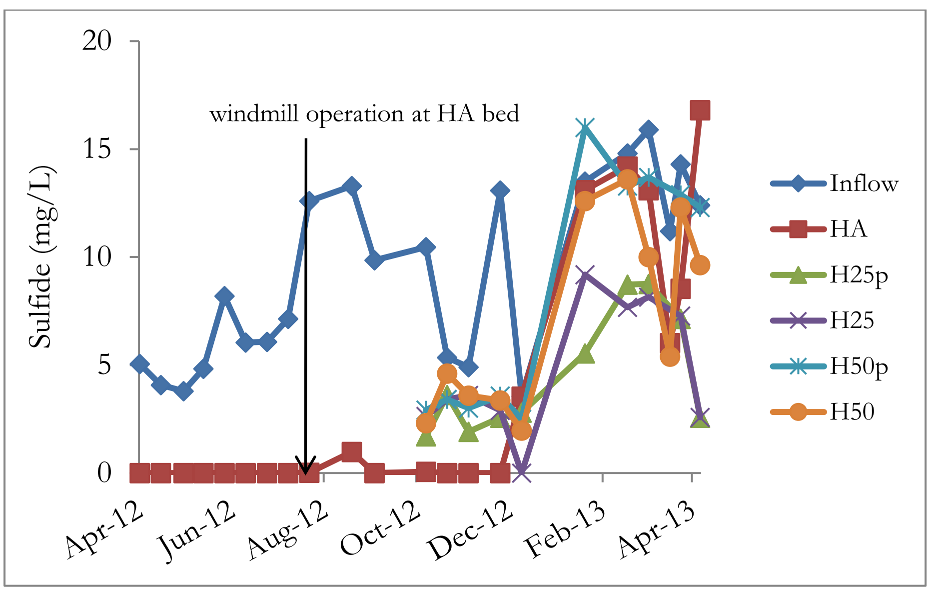


Figure 3.1-3 Concentrations of sulfide in the systems (where detected)

Table 3.1-4 Intermediately-oxidized sulfur compounds at outflow of the systems where they were produced

System	S^0 mg/L	$S_2O_3^{2-}-S$ mg/L
H50	9.7 ± 3.9	6.5 ± 1.4
H50p	8.3 ± 4.8	11.8 ± 5.2
H25	9.1 ± 2.4	-
H25p	8.9 ± 4.7	-
HA	8.1 ± 4.6	-

Means ± standard deviations. Data from 2012-2013. (n ≤ 13 for S^0 and ≤ 6 for $S_2O_3^{2-}-S$). $S_2O_3^{2-}-S$ was not analysed for the HA and H25 pairs.

In addition, methane production was only detectable in the unaerated HSSF systems and the HA bed after applying windmill for aeration.

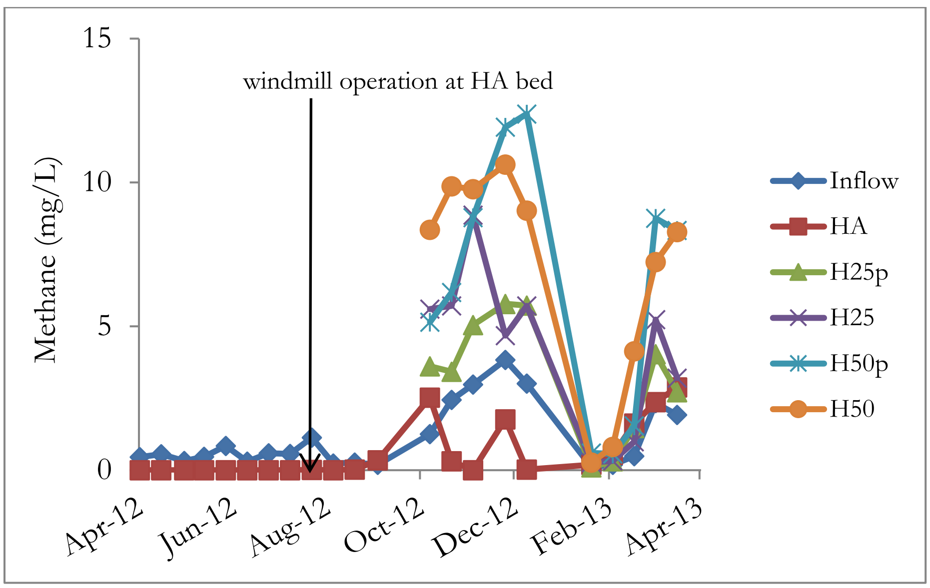


Figure 3.1-4 Concentrations of methane in the systems (where detected)

The concentrations of Fe^{2+} were monitored throughout the sampling period. Inflow concentrations of 2.2 ± 0.6 mg/L (mean ± standard deviation; n = 23) were mostly utilized within the beds and outflow concentrations of all systems were predominantly < 0.5 mg/L, with no statically significant differences between the different system types.

3.1.4 Discussion

System type and its hydraulic design have direct influence on subsurface oxygen availability in CWs. Nivala et al. (2013b) noted that the oxygen demand of the wastewater is generally higher than the available oxygen within CWs, especially in the herein investigated systems as the influent wastewater had high organic carbon and considerable TN loads. Hence they assumed that their calculated oxygen consumption rates can as well represent the total oxygen availability. They reported oxygen consumption rates between 0.5 and 12.9 $\mathrm{g\ m^{-2}\ d^{-1}}$ for unaerated HSSF CWs, between 7.9 and 58.6 $\mathrm{g\ m^{-2}\ d^{-1}}$ for VF systems and between 10.9 and 87.5 $\mathrm{g\ m^{-2}\ d^{-1}}$ for both aerated HSSF (before windmill was applied) and aerated VF beds. This trend was as well reflected on the redox potential levels in the different systems. Apart from incorporating artificial aeration, the VF hydraulic design leads to higher subsurface oxygen availability merely due to the mode of applying the wastewater; however it is more expensive and requires more know-how to operate. It is worth emphasizing that the unaerated VF systems here were operated with unsaturated media, and hence the findings cannot be extrapolated to saturated unaerated VF systems, in which some anaerobic niches may exist. Noticeably, the application of mechanical aeration had led to higher oxygen availability regardless of the hydraulic design.

This difference in subsurface oxygen levels has led to opposite trends in treatment processes within the different system types. Whilst $\mathrm{BOD_5}$ removal in the VF and the aerated VF and HSSF systems was attributed mainly to aerobic respiration, DSR was estimated to be an important route of the measured > 75 % $\mathrm{BOD_5}$ removal in the unaerated HSSF systems. In addition, TN removal in all systems was not complete, however different nitrogen species were found at outflow. Whilst $\mathrm{NH_4^+ - N}$ was the main form of nitrogen in the unaerated HSSF systems, $\mathrm{NO_3^- - N}$ was the main form in the outflow of the VF and the aerated VF and HSSF systems. In similar manner, the sulfur processes responded to oxygen availability. The limited sulfur cycling in systems with higher oxygen availability (and $\mathrm{E_h}$ = +100 to +300 mV) was expected. Net sulfide production was noticed in the unaerated HSSF CWs. This agrees with the fact that DSR almost always occurs at lower $\mathrm{E_h}$ ranges.

The positive influence of helophyte existence is reflected only in the unaerated HSSF CWs related to $\mathrm{BOD_5}$ and $\mathrm{NH_4^+ - N}$ removal and was not noticeable in the rest of the systems, mainly because the prevalent aerobic conditions in these systems had masked it. It was also noticed how the performance of HA has changed when the mechanical aeration was stopped and the windmill aeration was applied as shown in Figure 3.1-2 to Figure 3.1-4. Shortly after this operational change, the bed manifested a shift from stable to fluctuating performance. This is expected as the wind speed changes with time, and hence the amount of energy input available to provide aeration changes as well. This is why the performance of HA bed was very variable after applying the windmill. In general, it safe to say that this system performed as artificially aerated system only with mechanical aeration. However, with the windmill operation, the performance was comparable to unaerated HSSF systems. This is not to totally exclude the windmill function, but rather its effect was hard to notice given that the high hydraulic loading of the system remained the same. It is thus recommended to test the effect of windmill in the shallower H50 and H25 pairs, or to reduce the hydraulic loading of the HA bed to improve the aeration influence.

The noted considerable methane production was also only in the unaerated HSSFs and HA after windmill operation. As known, MPA compete with SRPs for some electron donors under similar environmental conditions. Had sulfate been not available in the inflow, DSR would not have taken place in these systems and even higher amounts of methane would have been produced. Since methane capturing for energy recovery from CW beds is not feasible, the produced methane volatilizes or phytovolatilizes to the atmosphere after reaching saturation in the pore-water, and hence the occurrence of DSR and the associated lower methane levels reduced the net greenhouse effect of HSSF CWs.

In few occasions the calculate load removal of sulfate was slightly negative. Since it is not possible that the systems produced sulfur, it was attributed to the sampling method applied. The systems were sampled at inflow/outflow at the same time, so the water that collected at outflow sample was not from the same quota that was collected at inflow valve. In addition, knowledge about the residence time distribution (RTD), the possible occurrence of short circuiting, the implication of evapotranspiration patterns and the general flow behavior of CWs is poorly understood. Therefore it is recommended to augment the research related to hydraulic behavior of different types of CWs to improve our understanding, and as well to discuss better sampling approaches.

Based on the above-mentioned, unaerated HSSF CWs seem to be less advantageous in terms of subsurface oxygen availability and in terms of hosting DSR and methane production processes. In addition, these systems were allocated less HLR implying higher area requirement to treat the same amount of flow in comparison to VF and aerated systems. However, it is noted that complete TN removal in VF CWs alone cannot be achieved. Hence the best approach if the goal was to achieve efficient performance with CWs as secondary treatment is to apply zigzag designs with aerated-unaerated segments or to apply hybrid VF-HSSF systems.

3.1.5 Conclusion

- In general, we can conclude that the design parameters in terms of flow direction and aeration scheme affect directly the subsurface oxygen levels, which in term dictate the degree of the sulfur cycle processes in the system. Therefore, of all the parameters that influence the occurrence and degree of DSR (e.g. availability of electron donor, temperature, etc.), the availability or absence of competitive electron acceptors is the most influencing factor;

- In the VF systems and the horizontal aerated planted pair (HAp and HA before windmill application), redox potential was at high levels in both warm and cold seasons, and there was no noticeable change between inflow and outflow loads of sulfate. Reduced sulfur species were not detectable in the outflow of these systems throughout the period of observation, reflecting efficient oxidation of these compounds to sulfate within these beds;

- The scenario is completely opposite in the unaerated HSSF systems where DSR rates of 35 - 46 % were measured. A closer look is needed in these systems to see the variation of the sulfur cycle processes in depth and length profiles.

3.2 Dynamics and stable isotope abundance patterns of inorganic sulfur pools in pilot-scale horizontal subsurface flow constructed wetlands

3.2.1 Introduction

The inorganic sulfur cycle in horizontal subsurface flow (HSSF) CWs is dynamic, and shifts in sulfur pools from oxidized to reduced forms were observed, which indicated the simultaneous sulfate reduction and sulfide reoxidation in these systems (Wiessner et al. 2008b, Wu et al. 2011). It is necessary to better understand the overall processes, and the magnitude and implications of the intermediate cycling of inorganic sulfur.

The analysis of natural abundances of the sulfur isotopes is a useful technique to identify whether the sulfur transformations are biologically or chemically mediated and as well to give a quantitative description of some of the important processes. For instance, the preference of SRPs of the lighter over heavier sulfur isotope of sulfate leads to an enrichment of the lighter isotope in the produced sulfide and to an enrichment of the heavier sulfur isotope in the remaining sulfate. The magnitude of the enrichment of heavy sulfur isotope in dissolved sulfate can provide an evidence of if DSR process was the main process contributing to sulfate removal or if other processes (e.g. uptake, adsorption, desorption, etc.) were more important. Reported isotopic fractionation values from DSR process such as by Canfield (2001), Kaplan and Rittenberg (1964) and Canfield and Teske (1996) can be used as reference values. In addition, Knöller et al. (2008) have gone further and tried benefit from the isotope data to quantify the DSR process and to interpret from the obtained enrichment factors the interference with DSR with other sulfur transformation processes such as sulfide and sulfur reoxidation processes.

In this experiment, the H50 pair (planted with *Phragmites australis* and unplanted HSSF CWs with 50 cm depth; one of the system pairs investigated in section 3.1) was investigated in a grey box approach. Firstly, dynamics of reduced and oxidized sulfur compounds within the planted and unplanted beds were monitored; secondly, the stable isotope abundance signatures of the different sulfur compounds were analysed. This will allow quantifying the overall processes based on load calculations and the intermediate processes based on isotope fractionation studies.

3.2.2 Material and methods

The pilot-scale CWs

The pilot-scale CWs are part of the Ecotechnology Research Facility which is described in section 3.1.2. A schematic representation of the planted bed is shown in Figure 3.2-1Figure 3.2-1 Schematic representation of H50p bed. Source: Nivala et al. (2013a). below.

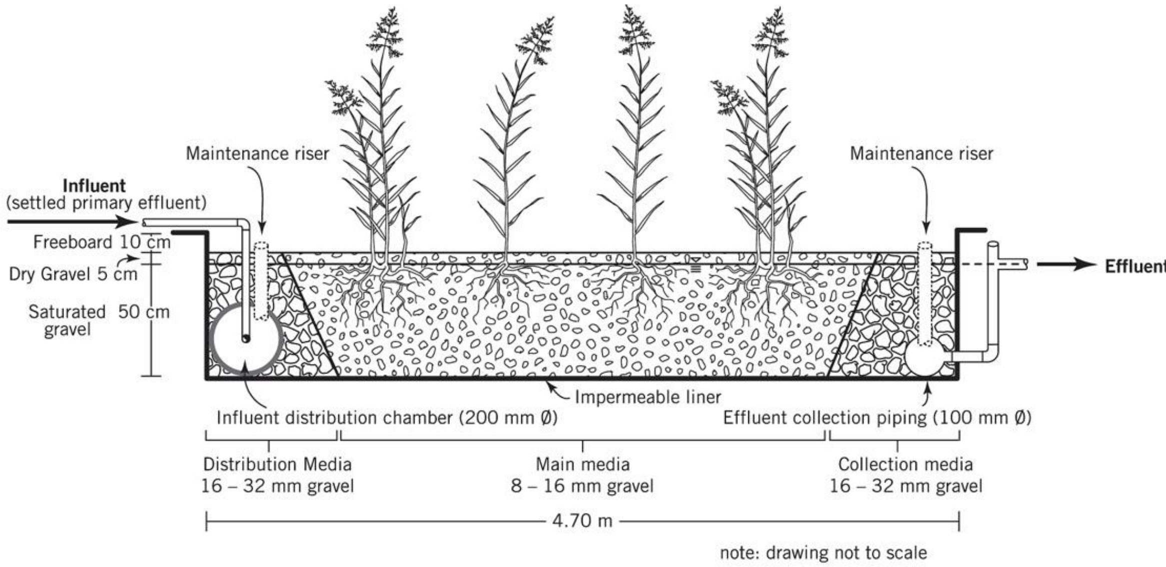


Figure 3.2-1 Schematic representation of H50p bed. Source: Nivala et al. (2013a). Reprinted with author's permission.

Sampling procedure

The regular sampling was conducted every 2-3 weeks (total number of sampling events from April-November 2013 is 12). For the estimation of the physical-chemical parameters, pore-water samples were collected from the pair of CWs at distances 0.6, 1.2, 2.4, 3.5 and 4.7 m from the inflow and at two depths of 0.15 and 0.4 m. The sample at distance 4.7 m and depth 0.4 m was defined as outflow. Pore-water was pumped at a slow rate using a peristaltic pump through stainless steel lances of 3.5 mm inner diameter which were inserted in each sampling point. Inflow sampling and sample taking and preservation for physical-chemical analyses are identical to methods described in section 3.1.2.

Water samples for the isotope investigation were taken by adding the water samples to Zn-Acetate solution (final concentration in the sample is 5% Zn-Acetate) to remove sulfides from the water phase. Isotope samples were stored without headspace at 8 °C until analysis. These samples are taken at four sampling events in May, July, September and November.

Water balance of the pair of CWs

The water loss due to evapotranspiration (ET) was predominantly higher in the planted bed than the evaporation losses from the unplanted bed. The water balance during the sampling period is represented in Figure 3.2-2 below.

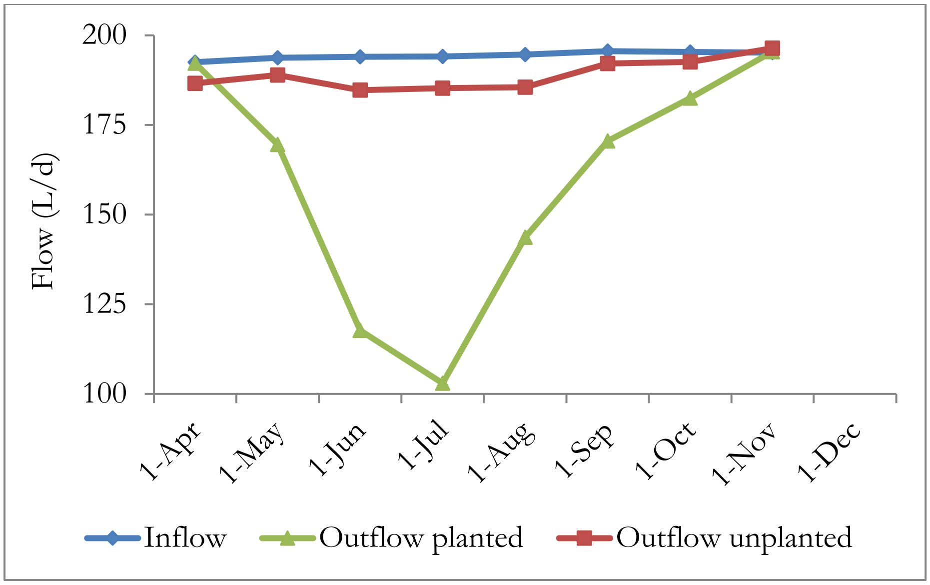


Figure 3.2-2 Water balance of the H50 pair

Analytic procedures

Analysis of physical-chemical parameters was conducted as indicated in section 3.1.2.

Sulfur isotope analysis was determined according to Knöller and Schubert (2010). The Zn-Acetate which was added to the water samples lead to precipitating the sulfides as ZnS. The precipitated ZnS was then removed by filtration (0.45 µm). The material collected on the filters was then placed in a distillation apparatus, 6M HCl was added and the released hydrogen sulfide was stripped with N_2 gas and then trapped as ZnS in Zn-Acetate solution. This first step determines the acid volatile sulfide (AVS). The AVS traps were then removed from the distillation setup and a second set of vials containing Zn-Acetate solution was placed for performing the second distillation step to retrieve the chromium reducible sulfur (CRS). In this step, chromium (II) chloride ($CrCl_2$) was added to the distillation and the formed H_2S was stripped with N_2 and trapped as ZnS. The precipitated ZnS (AVS or CRS) was converted afterwards to Ag_2S by addition of 0.1 M $AgNO_3$ solution. The dissolved sulfate was recovered from the filtrate of the samples through the addition of $BaCl_2$ and subsequent precipitation as $BaSO_4$ at 70 °C after adjusting the pH of the solution to 3.0.

Sulfur isotopic compositions were measured after conversion of $BaSO_4$ (or Ag_2S) to SO_2 using an elemental analyzer (continuous flow flash combustion technique) coupled with an isotope ratio mass spectrometer (delta S, ThermoFinnigan, Bremen, Germany). Sulfur isotope measurements were performed with an analytical error better than ± 0.3‰ and results are reported in delta notation ($\delta^{34}S$) as part per thousand (‰) deviations relative to the Vienna Cañon Diablo Troilite (VCDT) standard, according to the following equation:

$$\delta\ (‰) = [(\mathbf{R}_{\mathbf{sample}} - \mathbf{R}_{\mathbf{standard}}) / \mathbf{R}_{\mathbf{standard}}] * 1000 \qquad 3.2\text{-}1$$

Where: R is the ratio of the heavy to light isotopes (e.g. $^{34}S/^{32}S$ or $^{18}O/^{16}O$).

Analysis of oxygen isotopes of the $BaSO_4$ was performed by high temperature pyrolysis at 1450 °C in a TC/EA connected to a delta plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany). The analytical error is better than ± 0.5‰. Results of oxygen isotope measurements are expressed in delta notation ($\delta^{18}O$) as part per thousand (‰) deviations relative to Vienna Standard Mean Ocean Water (VSMOW). For normalizing the $\delta^{34}S$ data, the IAEA-distributed reference materials NBS 127 ($BaSO_4$) and IAEA-S1 (Ag_2S) were used. The normalization of oxygen isotope data of sulfate was carried out using the reference material NBS 127.

Calculations

The pollutant load calculations based on monthly-averaged data of influent and effluent flow rates indicated in Figure 3.2-2 of the planted and unplanted beds are as explained in section 3.1.2.

Statistical procedure

The statistical procedure is as indicated in section 3.1.2. Data was visualized using ggplot2 (Wickham 2009) and MS Excel 2010. Boxplots show median (horizontal lines), 25th and 75th percentiles (bottom and top of each box), the 'whiskers' show the data range (max. and min. values). Points that are more than 1.5 times the interquartile range above the third quartile and more than 1.5 times the interquartile range below the first quartile are defined as outliers and plotted individually.

3.2.3 Results

The design and operational characteristics of the H50 pair are described in Table 3.1-2.

Redox potential and pH

The redox potential of the influent wastewater was measured at one sampling event at -145 mV (thus in the range of the values in Table 3.1-1 of -148 ± 78 mV, in means and standard deviations of 66 measurements). Redox potential levels of the pore-water inside the beds are shown in Figure 3.2-3 below.

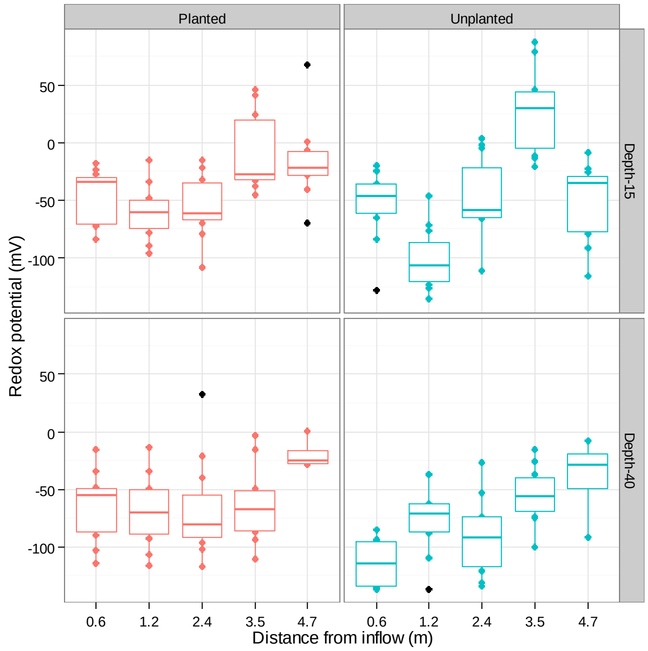


Figure 3.2-3 Pore-water redox potential in the H50 pair during the sampling period (April-November 2013). (n $\leq$ 12).

The pH of the inflow during the sampling period was 7.3 ± 0.2. No significant pH differences were reflected in both beds, as the pore-water maintained mostly a neutral pH range. The outflow pH of the planted bed was 6.9 ± 0.4 and of the unplanted bed was 7.3 ± 0.3 (all pH values in means ±standard deviations, n = 12).

Dynamics of inorganic sulfur compounds

The influent wastewater contained sulfide that was produced in the sewers or in the septic tank. This influent concentration decreased along the flow path at depth 15 cm (Figure 3.2-4). At depth 40 cm, sulfide concentrations were generally higher than at depth 15 cm and were not less than influent concentration except for the outflow. In general, differences between the planted and unplanted beds were not statistically significant ($p > 0.05$). A statically significant difference between the planted and unplanted beds was only identified at distance 3.5 m from inflow and depth 15 cm.

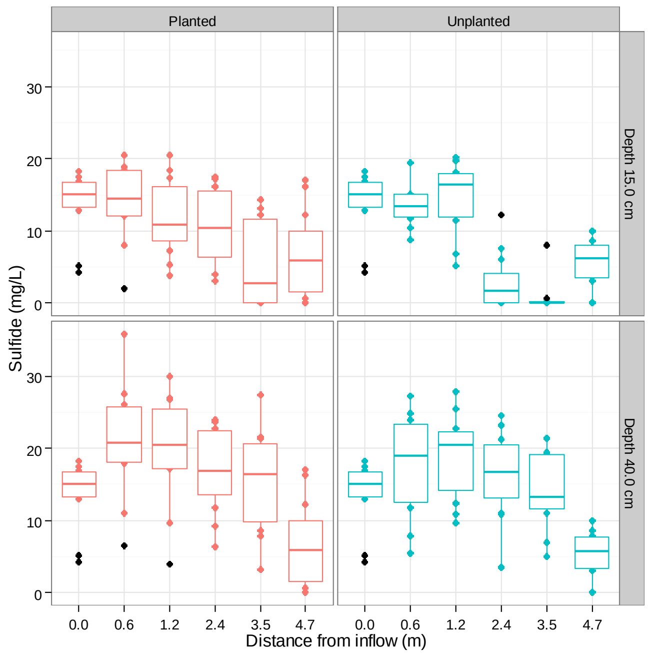


Figure 3.2-4 Sulfide concentrations at inflow and pore-water

The sulfate was predominantly removed in both systems and reflected seasonal trends. During the warm period (end of May to start of October; framed by the box in Figure 3.2-5 A), the planted CW had higher sulfate removal than the unplanted bed. During the rest of the sampled period, the unplanted CW considerably outperformed the planted one in sulfate removal. In addition, in some events, outflow loads of sulfate exceeded the inflow loads.

The other investigated sulfur species except sulfite (i.e. elemental sulfur and thiosulfate) were as well present at influent and showed either net production or net removal. Figure 3.2-5 B, C and D show the loads of sulfide, sulfite and thiosulfate; respectively. Whilst sulfite was purely produced in the systems (influent load is zero); sulfide and thiosulfate showed predominantly net removal in both CWs. Seasonal trends of sulfite and thiosulfate were not noticed due to shorter period of analyses. The sulfur dynamics reflecting the overall (net) processes are shown in

Table 3.2-1 for the planted and in Table 3.2-2 for the unplanted CW.

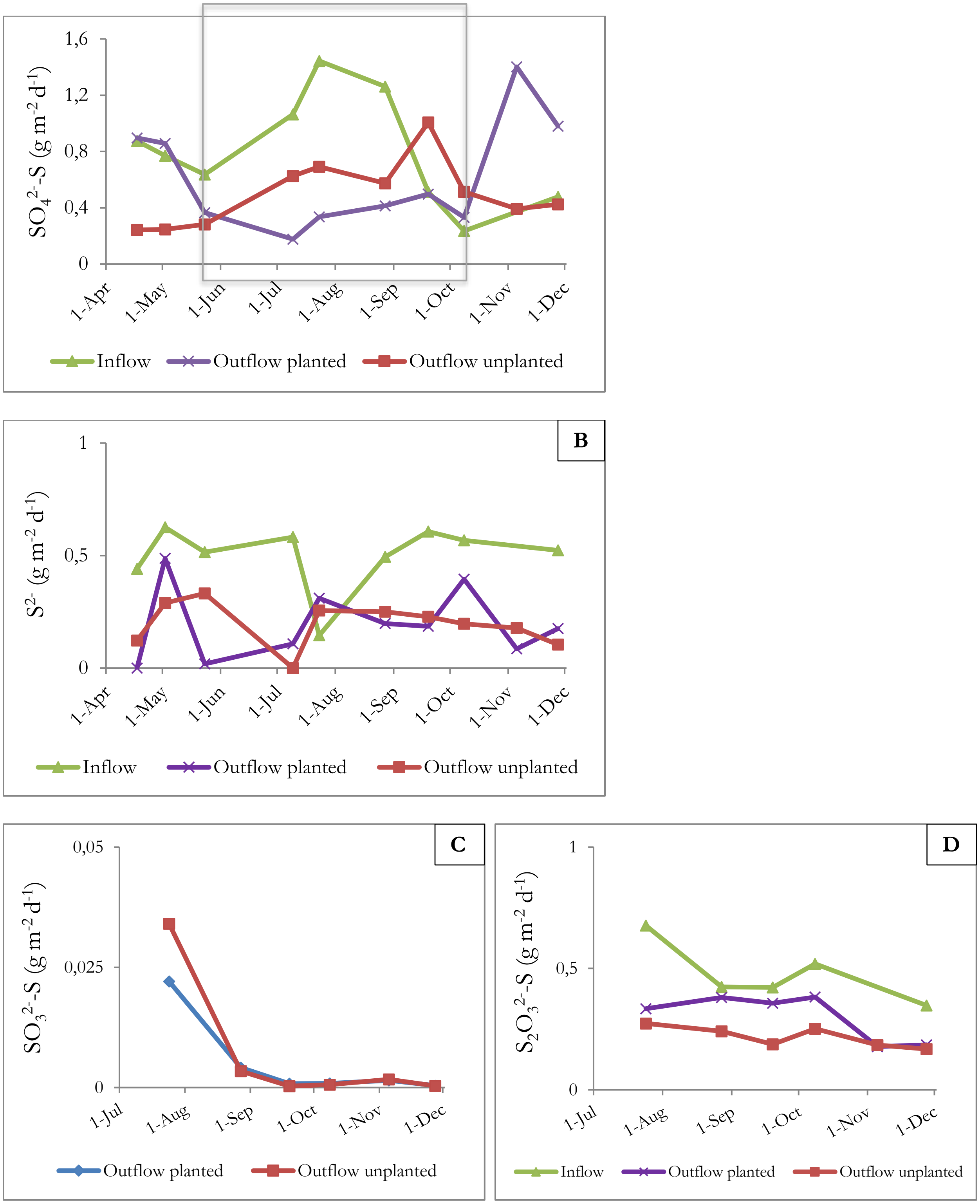


Figure 3.2-5 Area-specific loads of sulfate-S (A), sulfide (B), sulfite-S (C) and thiosulfate-S (D)

Table 3.2-1 Sulfur balance in the planted CW (in g S m^{-2} d^{-1})

Date	17-Apr	2-May	23-May	9-Jul	23-Jul	27-Aug	19-Sep	8-Oct	5-Nov	27-Nov
$SO_4^{2-}-S$ in	0.875	0.770	0.635	1.064	1.444	1.261	0.514	0.234		0.477
$SO_4^{2-}-S$ out	0.897	0.857	0.366	0.176	0.336	0.413	0.497	0.331	1.402	0.980
$SO_4^{2-}-S$ removed	-0.022	-0.087	0.269	0.888	1.108	0.848	0.017	-0.097		-0.503
S^{2-} in	0.440	0.625	0.515	0.582	0.145	0.494	0.607	0.568		0.523
S^{2-} out	0.000	0.487	0.018	0.108	0.310	0.197	0.185	0.395	0.085	0.176
S^{2-} removed	0.440	0.138	0.497	0.474	-0.165	0.296	0.422	0.173	-0.085	0.347
*$SO_3^{2-}-S$ produced					0.022	0.004	0.001	0.001	0.001	0.000
$S_2O_3^{2-}-S$ in					0.676	0.423	0.423	0.518		0.348
$S_2O_3^{2-}-S$ out					0.335	0.381	0.357	0.382	0.178	0.186
$S_2O_3^{2-}-S$ removed					0.341	0.042	0.066	0.136		0.162
**S^0 in	0.274	0.511	0.222	0.774			0.360			
S^0 out	0.076									
S^0 removed	0.198									
All-in	1.589	1.906	1.373	2.419	2.265	2.178	1.903	1.320		1.348
All-out	0.318	0.732	0.299	0.734	1.358	1.157	1.549	1.292	0.656	0.786
▲Difference in balance	1.271	1.174	1.074	1.685	0.907	1.021	0.355	0.029		0.562

*Sulfite at inflow was zero; outflow sulfite was noted: produced;

**Elemental sulfur data are only recorded occasionally as the measurements faced multiple interruptions due to device failure and data was considered unreliable;

▲Difference in balance does not represent the removed sulfur due to missing data; however it gives an indication of it.

The highlighted cells refer to a sampling event wherein the influent sulfate was considerably lower than its usual values, possibly due to a major rain event.

Table 3.2-2 Sulfur balance in the unplanted CW (in g S m^{-2} d^{-1})

Date	17-Apr	2-May	23-May	9-Jul	23-Jul	27-Aug	19-Sep	8-Oct	5-Nov	27-Nov
$SO_4^{2-}-S$ in	0.875	0.770	0.635	1.064	1.445	1.264	0.513	0.234		0.477
$SO_4^{2-}-S$ out	0.242	0.245	0.281	0.626	0.690	0.574	1.006	0.514	0.392	0.424
$SO_4^{2-}-S$ removed	0.632	0.524	0.355	0.438	0.754	0.690	-0.493	-0.280		0.053
S^{2-} in	0.440	0.625	0.515	0.582	0.145	0.495	0.606	0.567		0.522
S^{2-} out	0.122	0.289	0.332	0.000	0.256	0.250	0.228	0.196	0.177	0.104
S^{2-} removed	0.318	0.336	0.184	0.582	-0.111	0.245	0.378	0.370	-0.177	0.418
*$SO_3^{2-}-S$ produced					0.034	0.003	0.000	0.001	0.002	0.000
$S_2O_3^{2-}-S$ in					0.677	0.424	0.422	0.519		0.348
$S_2O_3^{2-}-S$ out					0.274	0.241	0.188	0.252	0.184	0.168
$S_2O_3^{2-}-S$ removed					0.403	0.183	0.235	0.267		0.180
**S^0 in	0.274	0.511	0.222	0.774			0.360			
S^0 out	0.078									
S^0 removed	0.195									
All-in	1.589	1.905	1.373	2.420	2.266	2.183	1.901	1.320		1.347
All-out	0.443	0.534	0.612	0.626	1.254	1.068	1.422	0.963	0.755	0.696
▲Difference in balance	1.146	1.372	0.760	1.794	1.012	1.115	0.479	0.357		0.650

*Sulfite at inflow was zero; outflow sulfite was noted: produced;

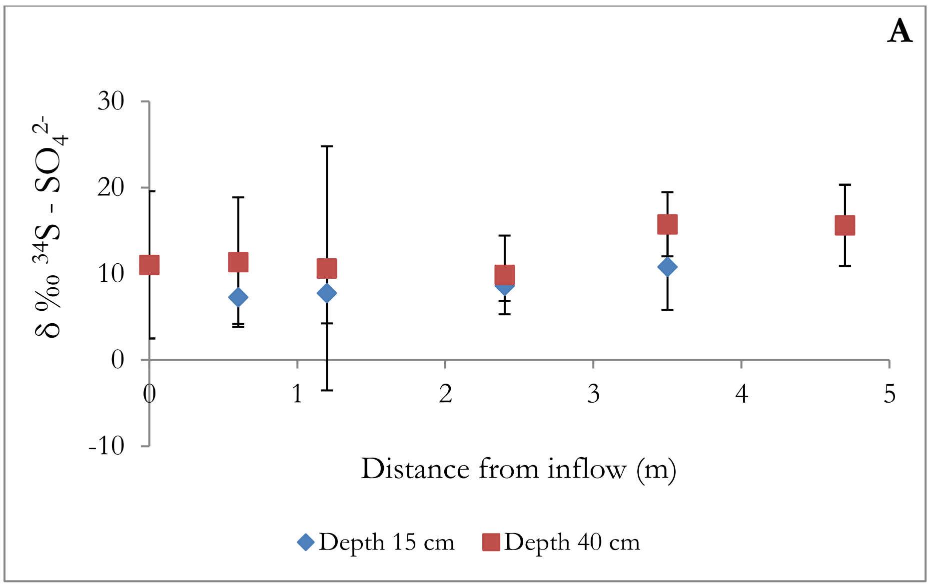
**Elemental sulfur data are only recorded occasionally as the measurements faced multiple interruptions due to device failure and data was considered unreliable;

▲Difference in balance does not represent the removed sulfur due to missing data; however it gives an indication of it.

The highlighted cells refer to a sampling event wherein the influent sulfate was considerably lower than its usual values, possibly due to a major rain event.

Isotope patterns and process characterization

The isotope results serve firstly to identify the type of processes and secondly to give quantitative description of the intermediate processes. Figure 3.2-6 shows the isotope patterns for $^{34}S-SO_4^{2-}$. Data from 4 sampling events reflect variation, however a general trend of enrichment of heavy isotope is observed (from δ $^{34}S-SO_4^{2-}$ at inflow of 11.0 ± 8.5 ‰ to 15.6 ± 4.7 ‰ at outflow of the planted bed and 23.0 ± 2.6 at outflow of the unplanted bed; in means ± standard deviations, Figure 3.2-6). This enrichment was statistically significant only for the unplanted bed ($p < 0.05$). The observed values of enrichment in heavy isotope reflect that sulfate removal processes were mostly microbially driven (Chambers and Trudinger 1979, Kaplan and Rittenberg 1964, Knöller and Schubert 2010).



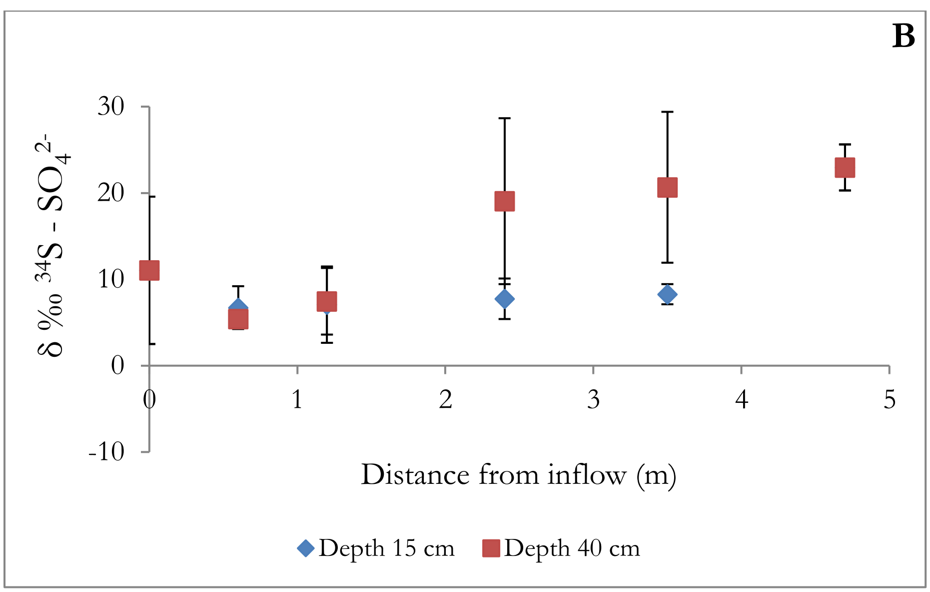


Figure 3.2-6 δ $^{34}S-SO_4^{2-}$ (‰) A: planted CW and B: unplanted CW. Dots represent mean values and error bars represent standard deviations (n = 4).

The higher enrichment of the heavy sulfur isotope of sulfate in the unplanted compared to the planted bed reflects that other microbial sulfur processes than DSR were higher in the planted than the unplanted bed (i.e. sulfate was not only a substrate but also a product of microbial processes, such as reoxidation of reduced and intermediately oxidized sulfur compounds to sulfate, which leads to counteracting isotope fractionations). Precise calculations of fractionation and enrichment factors will reveal if the oxidation processes are superimposed to DSR process (especially in the cold period in the planted system where sulfate showed net production rather than removal, Figure 3.2-5 A). In addition, enrichment of the heavier sulfur isotope of sulfate at depth 40 cm compared to depth 15 cm in both beds reflect higher influence of DSR process at the deeper depth compared to the upper layer of CWs. However, due to high variability in the data, this finding is not conclusive.

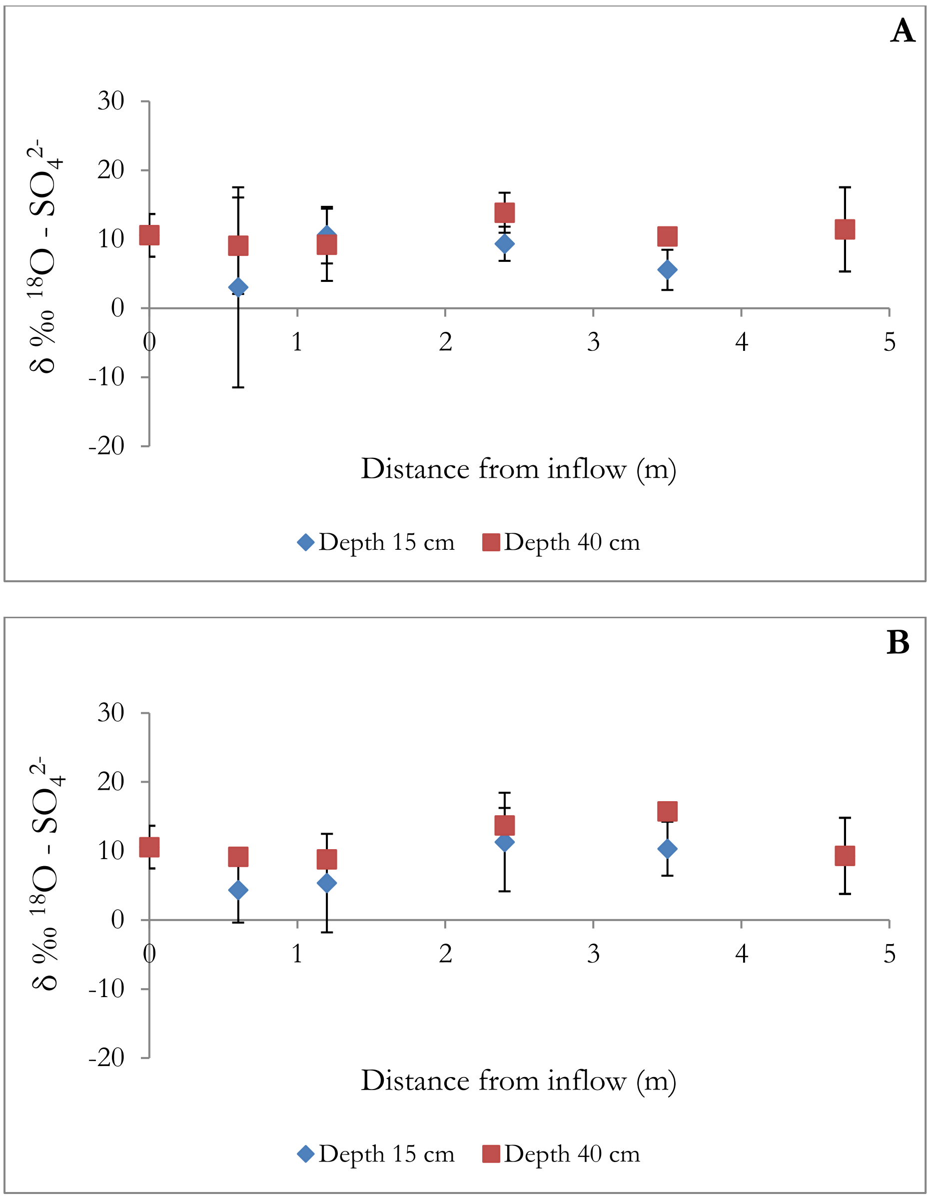


Figure 3.2-7 $\delta\,^{18}O - SO_4^{2-}$ **(‰) A: planted CW and B: unplanted CW.** Dots represent mean values and error bars represent standard deviations (n = 3).

The $^{18}O - SO_4^{2-}$ data reflect high variations (Figure 3.2-7). However, a general trend of higher enrichment in the heavier isotope at 40 cm depth compared to 15 cm depth reflect again the higher DSR activity in the deeper layers of both CWs.

The ^{34}S -AVS data are shown below. ^{34}S-CRS data did not allow for pattern identification due to insufficiency of samples to enable analyses for most sampling events.

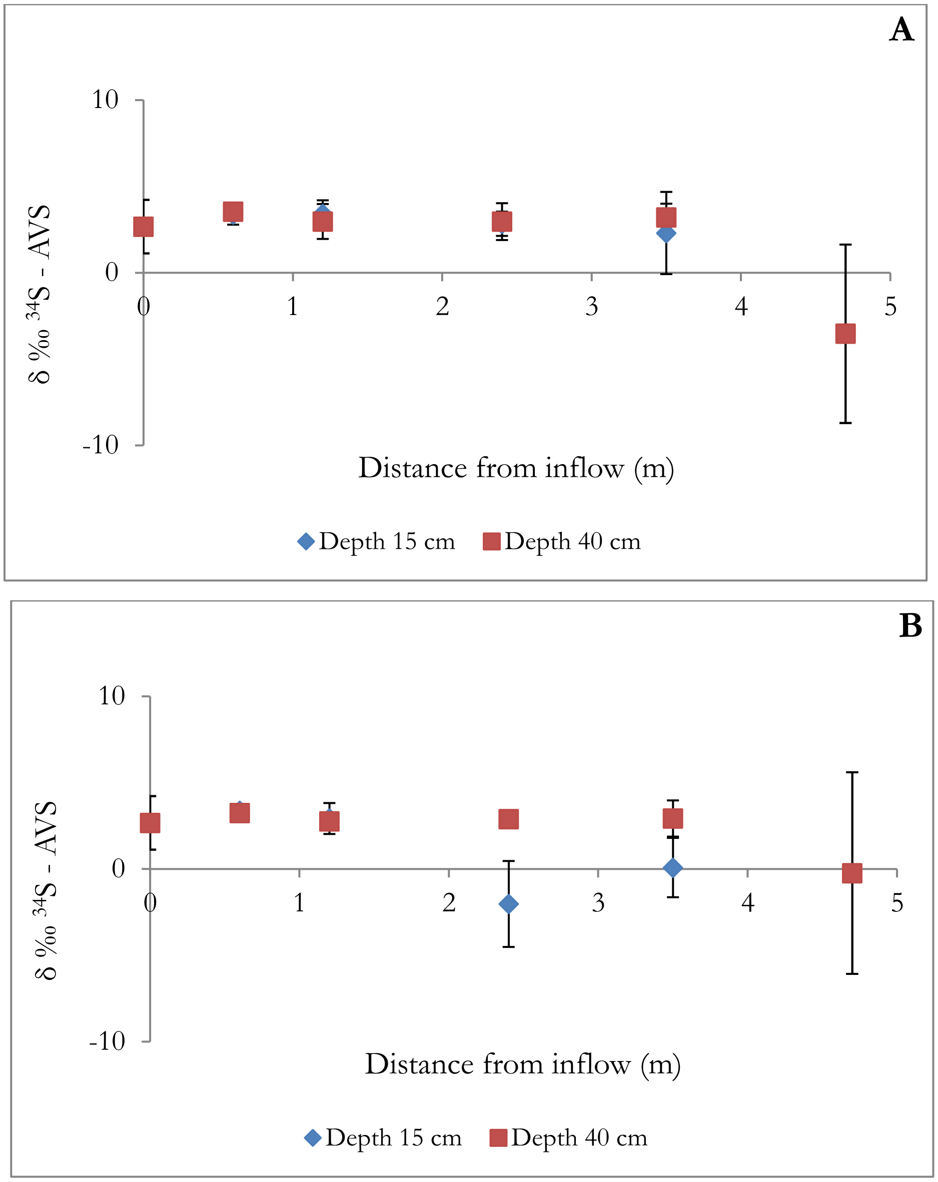


Figure 3.2-8 δ ^{34}S-AVS (‰) A: planted CW and B: unplanted CW. Dots represent mean values and error bars represent standard deviations (n = 4).

3.2.4 Discussion

As discussed in section 3.1.4 the sampling method (taking the inflow/outflow samples at the same time) and the limited understanding of the flow conditions inside the CWs may lead to underestimation of the CW's performance. It is evident that the flow conditions inside HSSF CWs are closer to a plug flow regime than a continuously stirred tank reactor (CSTR) regime, but no ideal plug flow behavior can be assumed. Detailed investigation of flow conditions in HSSF CWs is therefore essential to estimate adequately the performance of these systems. In addition, Figure 3.2-2 reflects the significant differences in the water balance between the planted and unplanted beds. The high water losses in the planted bed have implications on its hydraulic flow conditions which need to be further investigated. In addition, if the CWs were to be planted with a different helophyte species than *P. australis*, this would have led to different water balance scenarios and hence different magnitude of influence on the CW's flow conditions, as was established for some helophytes in sections 3.3.2 and 3.5.3.

The dynamics of sulfur compounds reflect the influence of *P. australis* existence on sulfur cycling. In the warmer season (from ~ end May to ~ beginning of October) the planted bed outperformed the unplanted bed in sulfate removal. It appears that this helophyte during the warmer season (also plant's growth season) had a bigger influence on SRPs leading to higher DSR. On the other hand, the planted bed had lower thiosulfate removal than the unplanted bed throughout the sampling period. It is a plausible assumption that this lower thiosulfate removal is an indicator of higher SOP activity in the planted bed. The additional supply of oxygen by *P. australis* was apparently contributing to sulfide and sulfur oxidation processes, of which thiosulfate is a common product, especially when there is no abundance of oxygen (as is the case in both beds) to allow complete oxidation to sulfate. Therefore, the measured outflow concentrations of thiosulfate represent the net of both thiosulfate reduction and sulfide and sulfur oxidation. Hence, the measured contribution of DSR and thiosulfate reduction to organic carbon removal may not be estimated alone from the stoichiometric DOC equivalents of removed sulfate; since the DOC flux was not measured and since the intermediate processes were not considered and were not possible to calculate with the given information.

Investigating the natural abundance of sulfur stable isotopes is a very useful tool to shed the light on the nature of the sulfur cycle processes. Here, it was possible to identify DSR process. However, additional calculations are needed to identify and if possible to quantify some of the intermediate sulfur processes.

In addition, it was visible that the lower depth of both planted and unplanted beds had higher net sulfate reduction. Headley et al. (2005) indicated that the majority of plant roots occur within the top 20 cm of the soil, in agreement with findings in section 3.6.3. Hence, the deeper layer of the planted bed had a lower influence of the helophyte than the upper layer.

3.2.5 Preliminary conclusion

- Sulfur transformations in HSSSF CWs reflect both spatial and seasonal trends. Depth-wise comparison reveals lower redox potential and higher sulfide concentrations in the lower depths of both planted and unplanted beds;

- Observed values of $^{34}S - SO_4^{2-}$ enrichment in both beds indicate the occurrence of DSR. The values indicate that the net sulfate reduction activity was higher in the unplanted compared to the planted bed. At the same time, the planted bed had higher net sulfate removal during the warm period. This indicates that net sulfide generation is more influenced by other processes (such as sulfide and sulfur oxidation processes) in the planted than in the unplanted bed;

- Additional calculations are needed to retrieve the full benefit from the analyses of the stable isotope abundance data of the two beds.

3.3 Role of plants in nitrogen and sulfur transformations in floating hydroponic root mats: a comparison of two helophytes

3.3.1 Introduction

This investigation aims at elucidating differences in the rhizodeposition capacities among helophytes via monitoring the pollutant transformations that may depend on organic carbon availability. Specifically, the turnover of inorganic nitrogen and sulfur compounds was monitored and the stoichiometric requirement of organic carbon for the processes of denitrification and dissimilatory sulfate reduction (DSR) was estimated. A special type of CWs, floating hydroponic root mats (FHRMs), was selected. FHRMs represent a variant of CWs in which helophytes grow as floating mats on the water surface, thus are not rooted in any media such as soil or sediment. They may be described as a hybrid between a pond and a wetland, as they share aspects of both systems (Chen et al. 2016, Headley and Tanner 2008). The FHRMs allow better root development than soil-based systems as roots do not need to compete for space with the substratum and they allow a maximum contact between the roots and the contaminated water. This guarantees obtaining the highest benefit from the plants and their intensified root structures. FHRMs are being used for storm water management, treatment of combined sewer overflows (CSOs), and numerous other types of contaminated waters (Chen et al. 2014, Headley and Tanner 2008, Seeger et al. 2013). Smith and Kalin (2001) applied FHRMs for the treatment of acid mine drainage (AMD) and found numerous benefits in selecting them over conventional soil-based CW systems for AMD treatment as well as for suspended solids elimination.

Two helophytes, *Phragmites australis* (common reed) and *Juncus effusus* (common or soft rush), were selected in this study since they fit the following criteria: they are commonly applied in CWs; they are frequently investigated; and they have different morphologies of both above-ground and below-ground components. FHRMs of these two species were set in a greenhouse, fed with synthetic wastewater loaded with low organic carbon and sampled weekly within a six-month period at inflow, outflow and internal points. The following questions were posed: firstly, how do the nitrogen and sulfur transformations vary; secondly, how does the organic carbon input from the two helophytes vary (estimated from the observed nitrogen and sulfur transformations); and thirdly, what does the observation imply for the selection of a helophyte for a given treatment process?

3.3.2 Material and Methods

Experimental setup

Mature plants of *P. australis* or *J. effusus* were grown in duplicate in pilot-scale FHRM CWs inside a greenhouse in which the internal environment was not controlled. The 4 CW systems were made of metal containers with dimensions length × width × depth of: 100 × 15 × 35 cm, respectively. The water level was adjusted to 27 cm via an overflow control (Figure 3.3-1). The systems were operated as FHRMs at the start of the experiment. However, the vigorous plant growth that took place within the experimental timeframe resulted in the roots of both species filling the whole submerged depth of the wetlands and eventually transforming the systems into dense root mat filters (RMFs) rather than FHRMs. For the purpose of presenting the output of this experiment, the systems will be referred to as FHRMs since it could not be precisely decided when the transformation into non-floating RMFs took place.

In order to insure a uniform distribution of the wastewater and to promote a plug-flow regime, sieves of perforated stainless steel were placed 3 cm in front of the inflow and outflow of each system to create plant-free zones. The plants were allowed to acclimatize in the containers for 6 months with tap water and nutrients and for further two and a half months with the synthetic wastewater prior the start of the sampling campaign. The synthetic wastewater was prepared essentially as described before (Wiessner et al. 2010) but omitting organic compounds. The resulting wastewater composition was (in mg/L, dissolved in deionized water): 118.0 NH_4Cl (corresponding to 30.9 $NH_4^+ - N$; the actual measured $NH_4^+ - N$ concentration at the inflow tank was 26 ± 7, the variation was attributed to occasional non-ideal mixing of the inflow water); 36.7 $K_2HPO_4.3H_2O$ (corresponding to 5.0 $PO_4^{3-} - P$); 7.0 NaCl; 3.4 $MgCl_2.6H_2O$; 4.0 $CaCl_2.2H_2O$; 221.8 Na_2SO_4 (corresponding to 50.0 $SO_4^{2-} - S$) and a trace mineral solution after Kuschk (1991). Although no organic carbon compounds were added to the feed wastewater, limited concentrations of dissolved organic carbon (DOC) were detected in the feed tanks. This was recognized as resulting from impurities in the chemicals used for the preparation of the wastewater and partially coming from the plastic material of the tanks.

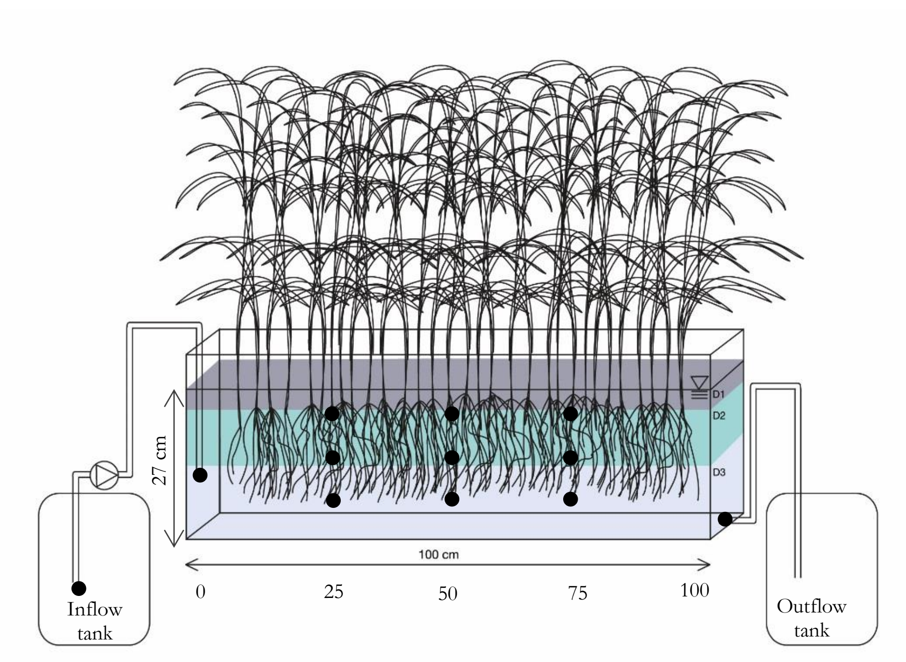


Figure 3.3-1 Schematic representation of the pilot-scale FHRMs and the different sampling points (at three depths: D1, 5 cm; D2, 12.5 cm; and D3, 22 cm from the water surface). For the *P. australis* systems, all the indicated points were sampled, whereas for the *J. effusus* systems the internal points were sampled only at depth 12.5 cm.

A buffer solution was added to the synthetic wastewater (420 mg/L $NaHCO_3$, corresponding to 5 mM HCO_3^-) to neutralize the acidification associated with nitrification which was observed in FHRMs of *J. effusus* in a previous experiment (data not shown) and estimated to affect the integrity of the plants' physiological status. The synthetic wastewater was freshly prepared twice a week and purged with a high-flow of oxygen-free N_2 gas for 15 minutes. The inflow tanks were kept closed at all times to minimize air intrusion. In addition, the inflow tanks were thoroughly washed every two weeks to limit microbial growth inside the tanks to hinder possible nitrification of the feed solution prior to entering the root mats.

The sampling of the well-established FHRMs started in June 2013 and was concluded early December 2013. The pore-volume was estimated at the start of April 2013 for all 4 systems and was found to be 25 ± 3 L for each wetland. The inflow rate was maintained between April and June at 5 L/d (hydraulic load of 33.3 L m^{-2} d^{-1}), corresponding to a nominal hydraulic retention time (nHRT) of about 5 days. As the season progressed, the water loss from the *P. australis* systems was so high that it led to zero-discharge; hence the flow rate was increased gradually in July and August to up to 8 L/d (53.3 L m^{-2} d^{-1}). This flow regime was applied to all 4 systems to assure equivalence of the operating conditions, even though the *J. effusus* root mats were not at zero-discharge at any time. The flow rate was then gradually reduced as the water loss from the *P. australis* plants decreased towards the end of the year (Figure 3.3-2).

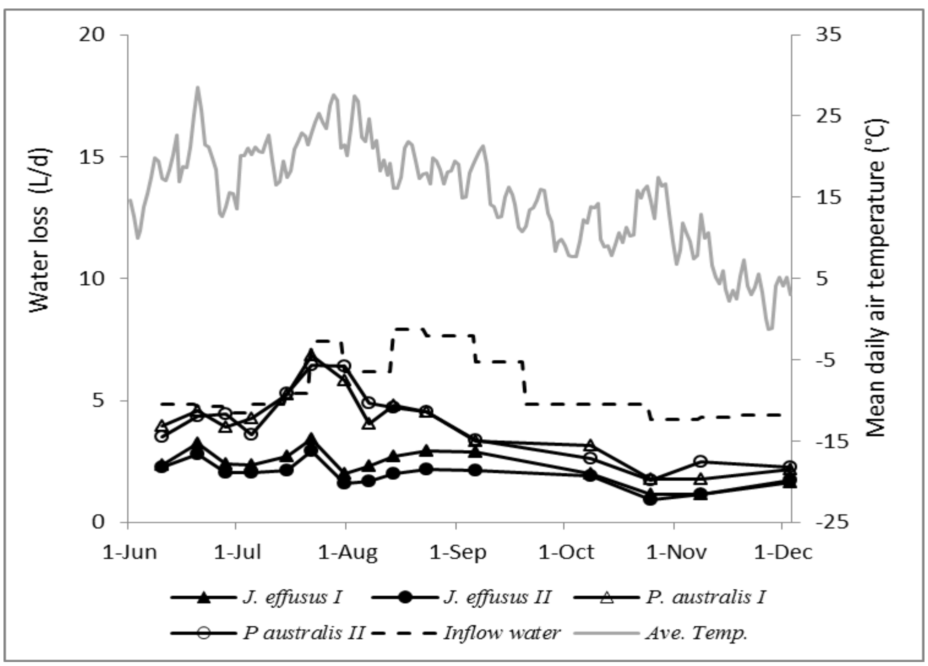


Figure 3.3-2 Mean daily air temperatures and water loss (ΔV) of the 4 FHRM units during June thru December 2013. The inflow volume is depicted as dashed line.

Sampling and analysis

Sampling was carried out on a weekly basis. For the quantification of the physical-chemical parameters, pore-water samples were collected from the 4 systems at distances 0, 25, 50, 75 and 100 cm from inflow and at depth 12.5 cm from the water surface. For the *P. australis* units, additional two depths of 5 and 22 cm were sampled (Figure 3.3-1) to elaborate on the depth profiles of the reduced sulfur compounds. Pore-water was withdrawn using a peristaltic pump at a slow rate through stainless steel lances of 3.5 mm inner diameter which were inserted in each sampling point. The sample at 0 cm was defined as inflow of each system, but the feed tanks were sampled as well to monitor any possible nitrification inside the tanks. The sample at 100 cm was defined as outflow.

Physical-chemical parameters were preserved and analyzed as indicated in section 3.1.2. DOC was analyzed with the same TOC/TN analyzer after additional filtration with 0.45 μm-syringe filter (Ministart NML, Sartorius). The above- and below-ground plant biomass was harvested at the end of the experiment and dried at 105°C to a constant weight after Ahern et al. (2004). Above-ground biomass was defined as the above-water biomass and the below-ground biomass represented the root biomass. For *P. australis*, the leaves were separated from the shoots prior to drying, and the dry biomass of leaves and shoots was estimated separately. Average shoot height was estimated for the harvested shoots by grouping the shoots from each $^1/_4$ root mat filter in three groups: tallest, medium-high, and shortest; and selecting 20 shoots (5 shoots from each $^1/_4$ mat area) from the medium-high group for measurement. The below-ground biomass of *P. australis* was collected by taking core samples using stainless steel cylinders with 3.0 cm inner diameter. For the *J. effusus* roots, rectangular sections were defined and the plants were collected relevant to these sections. Subsequently, roots were separated manually from the shoots (due to soft root structure, it was not possible to take core samples with the steel cylinders). The N and S content of the different plant components were estimated after grinding and sieving the dry biomass (< 200 μm) using the elemental analyzer for C, H, N and S (EA-CHNS).

Calculations

Rhizodeposits and DOC in general are a complex mixture of organic compounds, and it is unknown which particular compounds are used as electron donors and in which quantities. To estimate the minimum amount of the organic rhizodeposits required for complete denitrification and dissimilatory sulfate reduction, we used an average redox state of carbon (C_{ox}) of zero as it is close or identical to the average C_{ox} in biomass and DOC. Thus, the nitrate-depended oxidation of DOC can be expressed as:

$$\mathbf{10\,CH_2O + 8\,NO_3^- \rightarrow 4\,N_2 + 10\,HCO_3^- + 4\,H_2O + 2\,H^+} \qquad 3.3\text{-}1$$

in which 0.37 mg $NO_3^- - N$ are reduced by oxidation of 1 mg DOC as CH_2O. The theoretical DOC required for sulfate removal via DSR was calculated according to the equation:

$$\mathbf{2\,CH_2O + SO_4^{2-} \rightarrow H_2S + 2\,HCO_3^-} \qquad 3.3\text{-}2$$

in which 0.53 mg $SO_4^{2-} - S$ are reduced by oxidation of 1 mg DOC as CH_2O.

The water loss (ΔV) due to evapotranspiration from the 4 wetlands during a defined period was calculated from the water balance equation:

$$\Delta \mathbf{V} = \frac{(\mathbf{V_{in}} - \mathbf{V_{out}})}{\mathbf{T}} \qquad 3.3\text{-}3$$

Where:

ΔV: water loss by evapotranspiration in L/d
V_{in} and V_{out} : influent and effluent water volumes in L
T: the time period in d

The influent and effluent pollutant loads ($g\ m^{-2}\ d^{-1}$) were calculated from the equation:

$$\mathbf{Load_{in/out}} = \frac{(\mathbf{V_{in/out}} * \mathbf{C_{in/out}})}{\mathbf{T} * \mathbf{A}} \qquad 3.3\text{-}4$$

Where:

C: pollutant concentration in g/L
A: is the surface area of the wetland in m^{-2}

Statistical data analysis

Statistical analysis was conducted on the parameters measured at depth 12.5 cm, with each root mat having 5 sampling points along the flow path (Figure 3.3-1). Since the mixing inside each system did not exemplify an ideal plug-flow regime (plug-flow regime was assumed although considerable back-mixing took place), spatial pseudoreplication was assumed in the data. To remove pseudoreplication, the means from the 5 sampling points of each CW were used to represent the data from each sampling date (except for redox potential). Temporal pseudoreplication was not accounted for since seasonal variation is assumed to diminish it. The statistical tests and data visualization were conducted as described in sections 3.1.2 and 3.2.2.

3.3.3 Results

Physical-chemical parameters

The water loss due to evapotranspiration was permanently higher in the *P. australis* root mats than in the *J. effusus* systems (Figure 3.3-2). The water loss changed with the development of the season and was highest in July and August, with a maximum of 6.7 L/d (~ 45 $L\ m^{-2}\ d^{-1}$) from *P. australis* FHRMs in July (constituting 90-100% of the inflow water). The seasonal trend of water loss reflected not only the ambient temperatures but also the noted physiological status of plants as *P. australis* plants started to change to yellowish leaf color in mid-September. The transpiration correlated positively with the above-ground dry biomass which was determined at the end of the experiment. The *P. australis* mats were superior in size and had significantly more above-ground and more below-ground dry biomass than those of *J. effusus* (Table 3.3-1). The *P. australis* plants had also larger average shoot height than the *J. effusus* plants (197 ± 5 cm vs. 96 ± 34 cm).

Table 3.3-1 Dry biomass, N and S content of different components of plants

Component / species	Dry biomass at end g/m^2	N content at end $g\ N/m^2$	S content at end $g\ S/m^2$	**N uptake $g\ N\ m^{-2}\ d^{-1}$	**S uptake $g\ S\ m^{-2}\ d^{-1}$
Shoots of *P. australis*	5064 ± 391	34 ± 6	22 ± 5.4	0.065 ± 0.011	0.050 ± 0.035
Shoots of *J. effusus*	2349 ± 414	37 ± 7.6	7.5 ± 1.1	0.071 ± 0.015	0.014 ± 0.002
Roots of *P. australis*	1068 ± 114	1.24 ± 0.21	0.28 ± 0.05	0.024 ± 0.004	0.005 ± 0.001
Roots of *J. effusus*	84 ± 5.3	0.11 ± 0.013	0.016 ± 0.005	0.002	0.0003
Leaves of *P. australis*	1601 ± 78	22.4 ± 1.8	2.4 ± 0.4	0.043 ± 0.003	0.046 ± 0.008
Total of *P. australis*	7734 ± 355	69 ± 5.6	49 ± 9.9	0.132 ± 0.010	0.094 ± 0.019
Total of *J. effusus*	2432 ± 409	38 ± 7.5	7.7 ± 1	0.073 ± 0.014	0.015 ± 0.002

Means and standard deviations from plant matter from 2 FHRM CWs of each species.

** Calculated uptake of N and S with assumptions described later in this section.

Furthermore, the FHRMs of the two plant species displayed substantial differences of the pore-water redox potential (Figure 3.3-3). The redox potential was exclusively in the positive range in the *J. effusus* systems and did not vary highly from that of the feed wastewater. The *P. australis* systems however showed a decreasing trend of the redox potential along the flow path. Despite apparently distinct trends, the difference of redox potential was statistically significant only at distance 75 cm from inflow where it was higher in the *J. effusus* CWs (mean = +351 mV) than in the *P. australis* pair (mean = +3 mV; *p* value: < 0.05).

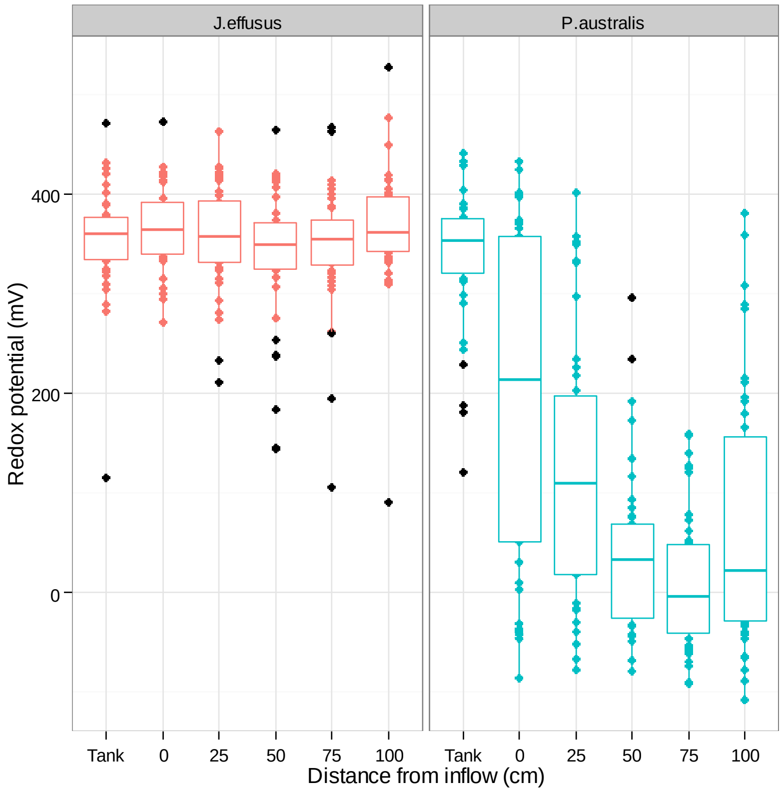


Figure 3.3-3 Redox potential, E_h (mV), at different distances from inflow and at depth 12.5 cm [n = 44].

The pH values showed a slight drop from 7.7 ± 0.4 at the inflow to 6.6 ± 0.3 at the outflow of all systems. Changing the plant species and the different observed processes within the systems did not lead to notable differences in pH. This was attributed to the added buffer in the wastewater.

Nitrogen turnover

In general, nitrogen transformations showed opposite trends in FHRM of the two plant species, in agreement with the redox potential levels in each respective pair. The influent TN loads of 1.15 ± 0.4 g m^{-2} d^{-1} was in the form of $NH_4^+ - N$. Ammonium removal was essentially complete in both FHRM types (Figure 3.3-4 A); however TN removal was complete only in the *P. australis* systems whereas *J. effusus* mats removed on average only 23% of TN. The two *J. effusus* units showed effective nitrification ($NO_3^- - N$ detected corresponded to 77% of $NH_4^+ - N$ removed, $NO_2^- - N$ remained below detection). In contrast, nitrate and nitrite were permanently below detection in *P. australis* CWs (Figure 3.3-4 B). The accumulation of nitrate in the *J. effusus*

CWs suggests incomplete denitrification, which was attributed to insufficiency of electron donors. The removal of TN in all systems was attributed mainly to coupled nitrification-denitrification, plant uptake, volatilization and microbial assimilation. The N content of the different above- and below-ground components of the plant matter that was harvested at the end of the experiment is shown in Table 3.3-1. The calculated uptake of N from the different plants assuming that 50% of the harvested plant matter was grown during the 8.5 months (260 days) of feeding with the synthetic wastewater is provided in Table 3.3-1. This assumption is supported by the noted significant growth during this period.

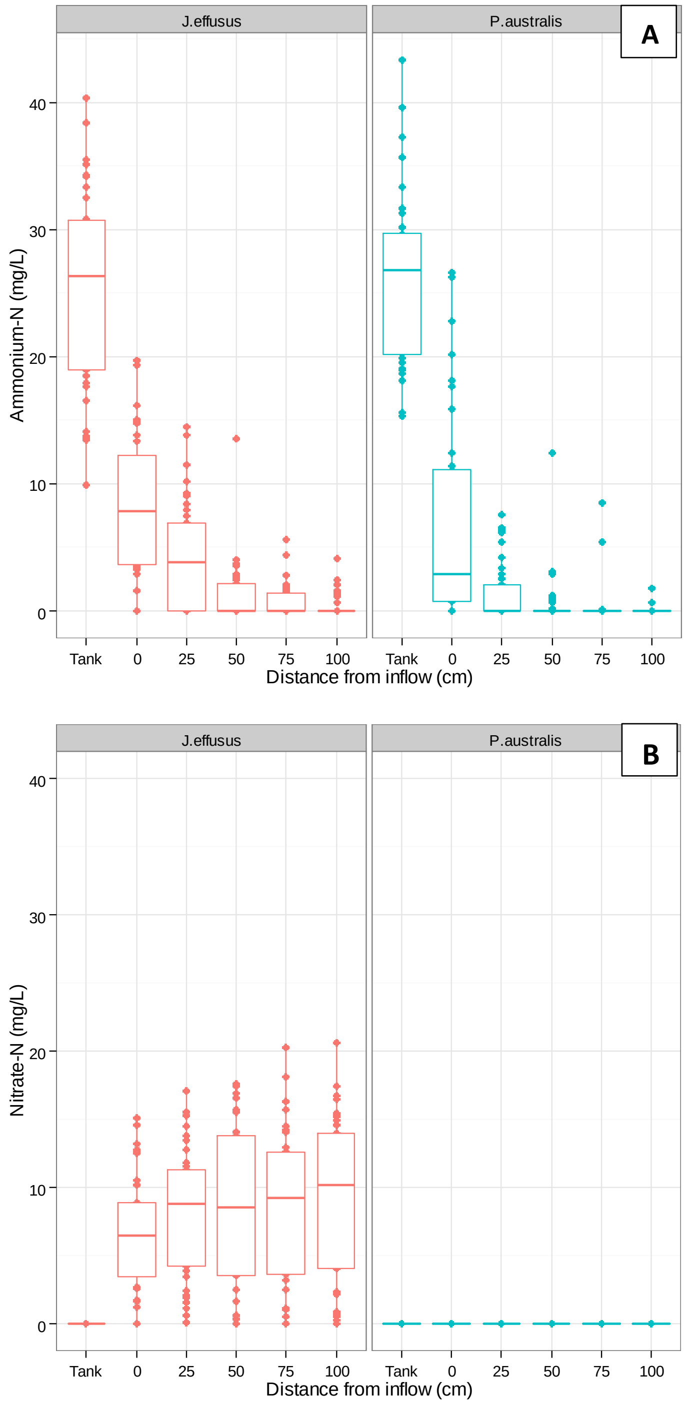


Figure 3.3-4 A: ammonium-N concentrations; and B: nitrate-N concentrations [n = 36] at different distances from inflow and at depth 12.5 cm.

Sulfur turnover

Striking differences were noticed in the inorganic sulfur turnover processes between the two pairs of root mats. The influent S loads of 2.0 ± 0.7 g m^{-2} d^{-1} was in the form of $SO_4^{2-} - S$. The *P. australis* systems showed effective inorganic sulfur transformations (DSR and sulfide oxidation processes). Reduced and intermediately oxidized sulfur compounds were produced and enriched in their pore-water. Sulfide concentrations exceeded 30 mg/L in some cases and were generally much higher in the second half of the wetland. This is in agreement with the redox potential that is much lower at distances 50 and 75 cm than at distance 25 cm from inflow. Elemental sulfur was detected occasionally at concentrations up to 1 mg/L, and ≤ 30%, ≤ 10% and ≤ 1% of the influent $SO_4^{2-} - S$ concentration were detected as S^{2-}, $S_2O_3^{2-} - S$ and $SO_3^{2-} - S$, respectively. These reduced and intermediately oxidized inorganic sulfur compounds showed depth profiles as they were at significantly lower concentrations at depth 5.0 cm as compared to depths 12.5 and 22.0 cm (Figure 3.3-5). These compounds did not account for a significant portion of the removed $SO_4^{2-} - S$ of 1.1 ± 0.45 g m^{-2} d^{-1} as their effluent loads represented only 4% of removed $SO_4^{2-} - S$.

In contrast, the analyzed reduced and intermediately oxidized sulfur compounds (sulfide, elemental sulfur, sulfite and thiosulfate) were not detectable in the *J. effusus* systems throughout the experimental period. However, limited $SO_4^{2-} - S$ removal of 0.37 ± 0.29 g m^{-2} d^{-1} (on average 18% of influent $SO_4^{2-} - S$) was noticed in these systems. Plant content of sulfur at the end of the experiment and the estimated plant uptake during the experiment (calculated with the previously stated assumptions) are shown in Table 3.3-1.

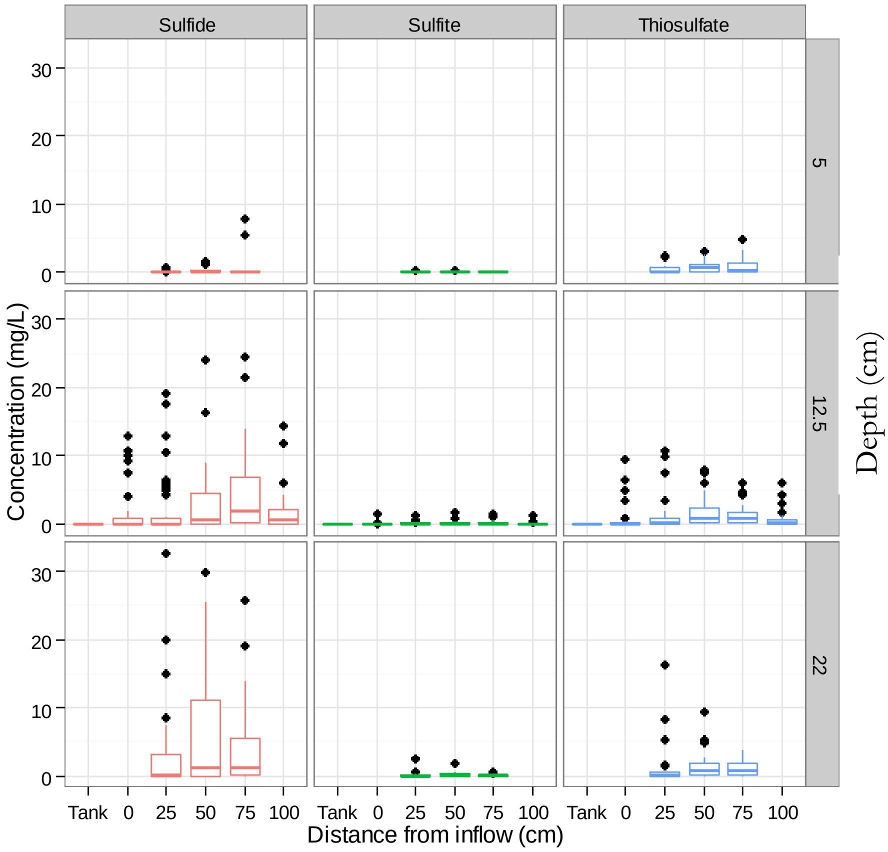


Figure 3.3-5 Concentrations of reduced and intermediately oxidized sulfur compounds at different distances from inflow and at 3 depths in *P. australis* FHRM CWs [sulfide: n=48; sulfite-S and thiosulfate-S: n=26].

Organic carbon availability

The DOC concentrations in the pore-water along the entire flow paths were about the same in the systems planted with *P. australis* (mean = 11.8 mg/L) and in the ones planted with *J. effusus* CWs (mean = 10.7 mg/L). The difference was not statistically significant (p value > 0.05; n=40). Concentrations in most samples were below 20 mg/L in all 4 units.

The limited DOC from the influent wastewater (mean = 12.4 mg/L; corresponding to mean loading of 0.48 g m^{-2} d^{-1}) did not meet the stoichiometric requirements of the observed nitrogen and sulfur transformations. After deducting the estimated average plant N uptake (shown in Table 3.3-1) and assuming mainly nitrification-denitrification pathway for the remainder of observed TN removal, the stoichiometric DOC requirement for denitrification was calculated in Table 3.3-2. Analogously, the stoichiometric requirement for DSR was calculated after deducting the plant uptake of sulfur and assuming mainly DSR pathway for the remainder of the observed $SO_4^{2-} - S$ removal.

Table 3.3-2 Calculated stoichiometric DOC requirement

Helophyte species	Mean DOC required for denitrification $g\ m^{-2}\ d^{-1}$	Mean DOC required for DSR $g\ m^{-2}\ d^{-1}$	Total mean DOC requirement $g\ m^{-2}\ d^{-1}$	DOC provided by influent wastewater* %
P. australis	2.6	1.9	4.5	11
J. effusus	0.5	0.7	1.2	41

* As percentage of the total DOC requirements for denitrification and DSR

Thus, the estimated total DOC consumption for N and S turnover in the systems of *P. australis* was about 4 times higher than for *J. effusus*. After deducting the amount provided by the influent wastewater, the remainder of the DOC requirements of the noted N and S processes was estimated to come from the rhizodeposits and was found to be 6 times higher for *P. australis* than for *J. effusus* systems. Hence, provision of bioavailable organic rhizodeposits for denitrification and DSR by *P. australis* was about 6 times higher than by *J. effusus* for the same CW area.

Methanogenesis

The methane concentrations were monitored, as the process of methanogenesis competes with DSR for the available electron donors. Methane was detected at low concentrations (below 0.1 mg/L) in the *P. australis* CWs, but was always below the analytical detection limit of 5 µg/L in the *J. effusus* CWs. This in turn agrees with the measured redox potential in the 4 systems, as methanogenesis typically occurs only at redox potentials below -100 mV (Faulwetter et al. 2009).

3.3.4 Discussion

The results show that changing the applied helophyte species can have significant influence on nitrogen and sulfur turnover processes that take place within FHRM CWs and subsequently on their overall performance. The transformations of nitrogen and sulfur are assessed based on both analyzed parameters and the estimation of most likely transformation pathways within the given conditions. For instance, there was no direct proof that nitrification occurred in the *P. australis* systems, since its products (nitrate and nitrite) were not detected. It was however assumed that nitrification took place at the rhizoplane of *P. australis*, where oxygen can be available. The produced nitrite/nitrate was presumably consumed either in the process of denitrification, exploiting the available DOC/rhizodeposits as electron donors or via plant uptake. The plant uptake of nitrogen is estimated to be of importance in all investigated systems, since the two helophytes are known to use both $NH_4^+ - N$ and $NO_3^- - N$ as nitrogen source (Tylova-Munzarova et al. 2005, Yao et al. 2011). The documented values of helophytes uptake of nitrogen from vertical and horizontal subsurface flow systems vary significantly due to several factors such as nitrogen concentrations, plant growth rate and the existing nitrogen content in the plant tissues (Langergraber 2005, Vymazal 2007), and may not represent the uptake in FHRM systems. The hereby calculated uptake values from the measured values of N content in the plant tissue at the end of the experiment are meant to provide approximation to allow an estimation of denitrification.

Furthermore, the non-detection of sulfide in the *J. effusus* systems is consistent with the high redox potential of their pore-water, since DSR is expected to take place at a redox potential below -100 mV (Faulwetter et al. 2009). This however does not exclude the possibility that some DSR occurred in anoxic niches of the *J. effusus* systems, and that subsequent reoxidation of sulfide to sulfate took place (Wiessner et al. 2008b). In addition, in the presence of metals, sulfide can form a precipitate and be immobilized inside the CWs. Since metals were present only at very limited concentrations to cover the micro-nutrient requirements of the plants and microorganisms, this process is assumed to have no significance to the overall sulfur balance in the present experiment. Nevertheless, some black precipitate was observed on the root-surface of *P. australis* which was harvested at the end of the experiment (Figure 3.3-6). On the other hand, the volatilization and phytovolatilization of H_2S can be significant (Kadlec and Wallace 2008).

Figure 3.3-6 Blackish precipitate noticed on the root mats of *P. australis*. Such precipitate was not observable in the *J. effusus* root mats.

The considerable differences in N and S transformations between the FHRM CWs of the two investigated helophytes and the great differences in the estimated stoichiometric DOC requirements to support them (which must have come from the plants) reflect that there can be extreme differences amongst helophytes in their rhizodeposition regimes. This agrees with the fact that different helophytes are expected to deposit various spectra of organic compounds and in different quantities. Michaletz et al. (2014) found that the size of woody plants influences their net primary productivity, i.e. the amount of biomass that plants produce. In addition, carbon allocation to the roots is coupled to photosynthetic activity as the below-ground photosynthate can be released into soil as rhizodeposits (Holland et al. 1996, Kuzyakov and Cheng 2001). Since *P. australis* is morphologically superior to *J. effusus* in terms of size (here more than 2 times larger in above-ground and more than 12 times larger in below-ground components), it can be assumed that the *P. australis* had higher net primary productivity than the *J. effusus* and subsequently higher amount of organic carbon allocated to the roots and eventually partially lost from the roots. Yet the size aspect alone is insufficient to provide understanding of the differences in rhizodeposition between the plant species and direct quantification approaches are needed. Zhai et al. (2013) quantified the fluxes of soluble root exudates from *P. australis* to be significantly higher than that from *J. effusus*. They presented average values for DOC exudation rates of 9 ± 0.9 and 4.3 ± 0.4 μg per g of root dry mass and hour for *P. australis* and *J. effusus*, respectively. If these values were to be representative of the rhizodeposition from the *P. australis* and *J. effusus* plants investigated here, then the *P. australis* root mats must have had significantly higher organic rhizodeposition due to both higher rates per g root mass and higher root biomass. Their method however did not account for the insoluble rhizodeposition components such as dead root matter, which may constitute an additional source of bioavailable organic carbon for the pollutant removal processes in CWs. Dead root amounts are directly linked to root longevity and root turnover rates. Lai et al. (2011) tested the longevity of roots from selected wetland plants and found significant differences amongst their tested species. This indicates that the amount of dead root production per unit time differs as well between the different helophytes and consequently the organic carbon that can be obtained from this component of rhizodeposition will vary.

The estimated values of DOC from organic rhizodeposition in this investigation are still an underestimation of the total quantity, since other processes such as aerobic respiration that transform organic substrates were not considered. Aerobic respiration processes are energetically more favorable for heterotrophic microorganisms; therefore typically proceed preferable to denitrification and DSR. Oxygen for aerobic respiration may come from raw wastewater, surface reaeration as well as helophytes' root-mediated radial oxygen loss (ROL). ROL was found to vary between plant species and is altered by the existing redox potential in the rhizosphere (Wiessner et al. 2002b). These authors found a maximum release rate from *J. effusus* of 0.5 mg O_2/h plant at a redox potential level of ~ -200 mV. On the other hand, Brix et al. (1996) have estimated the net flux of oxygen from *P. australis* to its below-ground tissues and sediment to be up to 5.7 L m^{-2} d^{-1}. As each study was performed under a specific set of experimental conditions, it is not possible to compare ROL from the two investigated plants or to assume which species was channeling more oxygen through its roots. Hence, additional knowledge regarding rhizodeposition and ROL regimes of some important helophytes is needed to provide better understanding of the helophytes capacities.

It appears possible to optimize the design of CWs by selecting a more suitable species for an intended treatment objective, basing the selection on the additional understanding of the helophytes capacities. For instance, application of *J. effusus* CWs can be advantageous when the wastewaters intended for treatment are not limited in organic carbon and/or when the removal of carbon is required, since this helophyte releases considerable oxygen (Wiessner et al. 2002b) that can boost the oxidation of organic matter and at the same time does not release enormous quantities of organic rhizodeposits that may pose extra treatment requirement. On the other hand, *P. australis* can be a recommended helophyte for treatment of contaminated waters when organic carbon is limited but desired (e.g. if the treatment goal is to stimulate nitrogen removal via coupled nitrification-denitrification or to promote sulfate reduction; or for the treatment of acid mine drainage). This would provide economic savings as well as environmental benefits since the current practice in these cases is to add external organic carbon to stimulate the required processes (Henze 1991). In addition, both helophytes can be applied in hybrid CW systems. The better understanding of the performance of these helophytes and the processes they promote may help selecting the optimum species to be applied at each stage of a hybrid CW system. Yet choosing the optimal helophyte for a stated treatment objective may not always be straightforward as some other aspects have to be considered. For example, the implications of the associated high rates of water loss due to evapotranspiration from *P. australis* must be taken into consideration, especially for wastewater treatment with CWs in hot climatic regions if water reclamation and reuse become of interest. In addition, adaptability of a certain plant species to new environments must be considered.

This investigation comprised some limitations that should be considered in future endeavors. Firstly, the short time span of the sampling campaign led to missing information about cold season, thus the findings represent only the plants' growing season. Secondly, plant uptake of nutrients is important to quantify. Since variations can be great, the best way is to quantify the plant matter nutrient content at the start and the end of the experiment, alongside with the precise quantification of plants' weight development. Thirdly, the findings represent FHRM and RMF and should not be incautiously extrapolated to other conventional soil-based CW systems.

3.3.5 Conclusion

- The FHRM CWs of two species *J. effusus* and *P. australis* showed significantly different inorganic nitrogen and sulfur transformations as they had different environmental conditions in terms of redox potential. The main cause of the noted differences in redox potential levels between the FHRM CWs of two species was estimated to be the difference in rhizodeposition capacities between the two helophytes and the subsequent higher demand on electron acceptors such as oxygen in the *P. australis* systems, where the amount of rhizodeposits was higher;

- The aerobic process of nitrification was more apparent in the *J. effusus* systems in agreement with the prevalent high redox potential levels, whilst the anaerobic process of dissimilatory sulfate reduction was rather noticeable in the *P. australis* systems where the redox potential levels were reduced;

- The ammonium removal was high in FHRMs of the two investigated species; however the TN removal was almost complete only in *P. australis* systems while it amounted to a mean value of 23 % in the *J. effusus* systems, as 77% of influent ammonium-N has accumulated as nitrate-N in these CWs. Insufficiency of electron donors or competition by oxygen as more energetically favorable electron acceptor in the *J. effusus* systems apparently hinder the complete denitrification of the generated nitrate;

- The sulfate removal rates were 3 times higher in the *P. australis* as compared to the *J. effusus* FHRM CWs. DSR was estimated to be the main process of sulfate removal and was proved by the detected sulfide in the *P. australis* CWs. In addition, simultaneous occurrence of DSR and sulfide oxidation processes was proved in the *P. australis* CWs due to the coexistence of sulfide, elemental sulfur, sulfite, thiosulfate and sulfate;

- The limited influent DOC loads were not sufficient to explain the noticed TN removal and DSR activity in all systems; hence plants' organic rhizodeposits were estimated to have provided an additional source of electron donors. Higher organic rhizodeposition activity from *P. australis* as compared to that of *J. effusus* was estimated to have led to the differences in the observed N and S transformations;

- Changing the applied helophyte did change the rates of N and S removing processes. It is thus very important to consider the selection of the optimum helophyte species when designing CWs. To help guide helophyte species selection, better understanding of rhizodeposition regimes (of both soluble and insoluble components) from the most frequently applied helophytes is a prerequisite.

3.4 Role of insoluble rhizodeposits from dead roots of *Juncus effusus* and *Phragmites australis* in sulfate removal from wastewater

3.4.1 Introduction

Zhai et al. (2013) have quantified the soluble component of rhizodeposits by measuring DOC fluxes exuded by roots of these two helophytes. In addition, it was found in section 0 that these two helophytes provided considerable amounts of organic rhizodeposits that were utilized in processes such as DSR and denitrification. It was then clear that the soluble rhizodeposit components were of importance, but the magnitude of insoluble rhizodeposits could not be separately estimated in the previous experiment and hence it remained masked. Here, it was intended to unmask the role insoluble components, such as dead roots, by excluding the exudation activity of living roots. The aim of this study was thus to indirectly quantify the bioavailable insoluble organic rhizodeposits from the dead roots of the two helophytes to elaborate on their role as supplier of electron donor for various contaminant removal processes in CWs.

It was hypothesized that analogous to soluble exudates, different plant species provide different spectra of insoluble organic rhizodeposits and in different amounts, and with different degrees of bioavailability for CWs microbial consortia.

3.4.2 Material and Methods

Experimental setup

The roots of *P. australis* and *J. effusus* were cut from the living plants, washed briefly with deionized water and directly incubated anaerobically for 10 days in continuously stirred anoxic synthetic wastewater (in duplicate reactors of each plant species). The roots were taken as a whole root system (containing all portions of root systems such as fine roots, roots and rhizomes). The dry mass of the roots incubated in each reactor was estimated only at the end of the incubation and shown in Figure 3.4-4. The immediate and artificial release of exudates from roots related to the injuries was measured as DOC at time zero (after 1.5 hours of incubation). To each reactor was added 2.5 L of synthetic wastewater prepared with deionized water after (Wiessner et al. 2010) with trace mineral solution (Kuschk 1991), adjusted by omission of organic carbon and doubling the sulfate concentration. The final wastewater composition was as described in section 3.3.2, except for $SO_4^{2-} - S$ concentration which was 100 mg/L. The reactors were kept anoxic via vigorous purging with O_2-free N_2 at the start and for half an hour every 2 days to assure air exclusion and to hinder the accumulation of the toxic sulfides that would decrease the microbial activity (sulfide in itself was measured during the incubation only as indicator, as some of it escaped the reactors during the purging with N_2, and as the main purpose was to investigate the sulfate concentration reduction; water loss was zero). The reactors were sealed against any air exchange with the external environment, except at the times of purging with N_2, where a small opening was set and the pressurized N_2 was assumingly creating gas flow only from the reactors to the outside, and not the opposite (Figure 3.4-1). The reactors were kept in a greenhouse where the day-night regime and the temperature were constantly regulated as: daytime from 6-21 h at 22°C and with artificial light turned on, nighttime from 21-6h at 16°C and no artificial light (therefore, to exclude algal activity, the reactors were sealed against light intrusion via painting the glass with special black paint and additionally wrapping around all sides with aluminum foil).

From the experimental setup it was assumed that the only available electron acceptors for microbial communities inside the reactor were mostly SO_4^{2-} for the process of DSR as well as small amounts of fumarate, protons, and carbon dioxide.

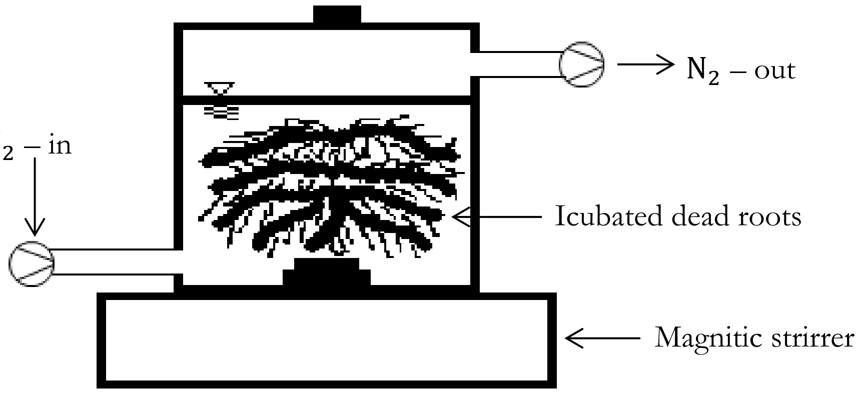


Figure 3.4-1 Schematic representation of the incubation reactors.

Sampling and analytic procedures

Sampling was conducted at time 0 (day 1), and then at day 2, 3, and 10 respectively. Samples were analyzed only for DOC (analyzed as described in section 3.3.2), $SO_4^{2-} - S$ and S^{2-} (analyzed as described in section 3.1.2). Methane and acetate were not analyzed.

Calculations of the theoretical DOC consumed in DSR as well as drying of root biomass at the end of the incubation are described in section 3.3.2.

3.4.3 Results

Incubated roots of *P. australis* had significantly lower concentrations of sulfate at the end of the experiment, as compared to the incubated roots of *J. effusus* (results shown for the start and end of the incubation, Figure 3.4-2). Consistently, the incubated roots of *P. australis* had significantly higher sulfide concentrations. In the *J. effusus* reactors, sulfide was detectable only at day 10, whereas in the *P. australis* systems, sulfide was already detected from day 1, and at much higher concentrations (Figure 3.4-3).

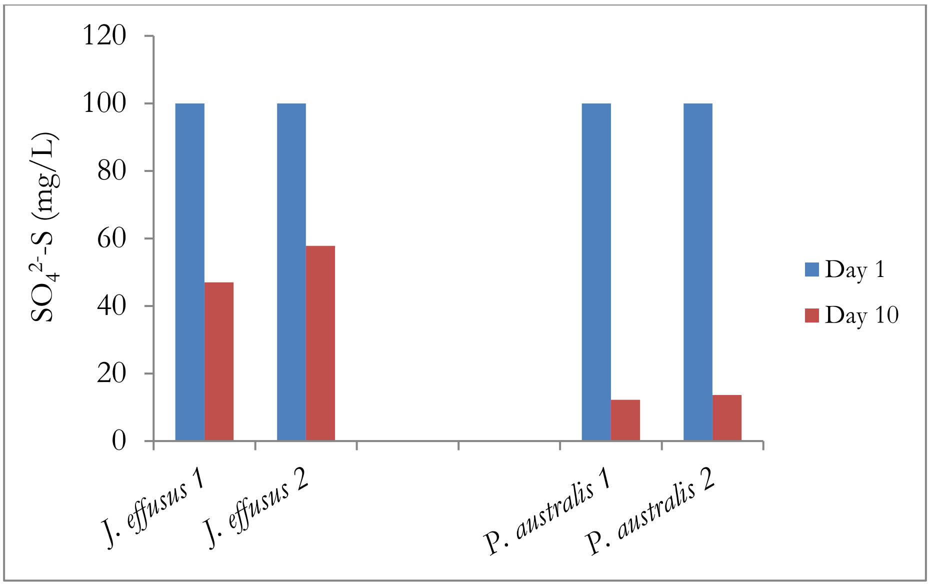


Figure 3.4-2 $SO_4^{2-} - S$ concentrations in the incubation reactors

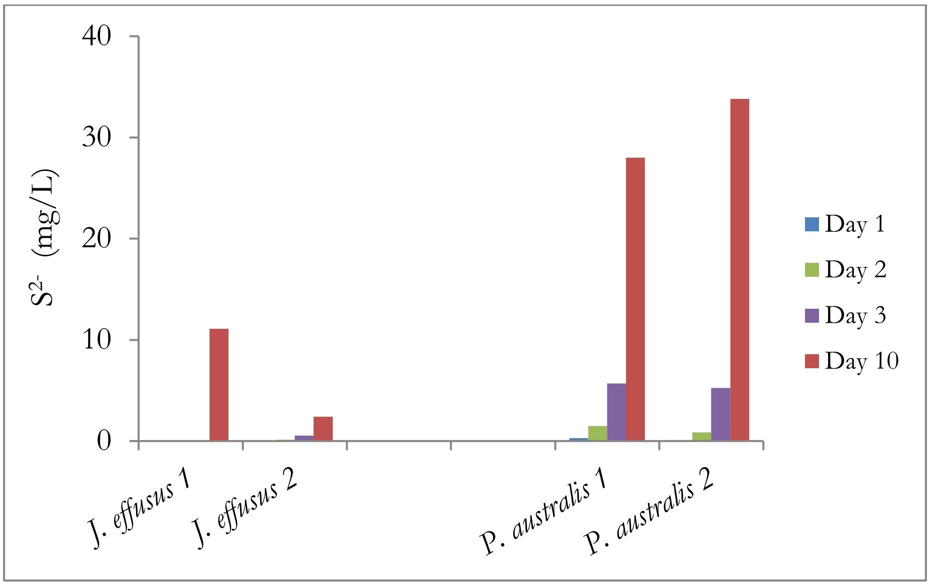


Figure 3.4-3 S^{2-}concentrations in the incubation reactors

The measured concentrations of DOC were not considerably different between the two species, and were predominantly < 10 mg/L, even at time 0 (it was expected to see higher DOC concentration at start due to injuries, but probably the effect of root injuries was minimal or it was instant and was lost during root washing). The dry mass of the roots in the different reactors (Figure 3.4-4) was used to normalize the decrease in sulfate concentration for each plant species. It was found that the incubations with dead roots of *P. australis* have decreased (or removed from the water phase) considerably higher $SO_4^{2-} - S$ concentrations than the incubations with dead roots of *J. effusus* per g dry biomass in 10 days. The measured $SO_4^{2-} - S$ concentration decrease and the calculated DOC equivalent of it (assuming only DSR took place and neglecting other sulfate consuming processes such as the microbial assimilatory sulfate reduction; and other DOC consuming processes such as fumarate respiration, acetogenesis and methanogenesis) are summarized in Table 3.4-1.

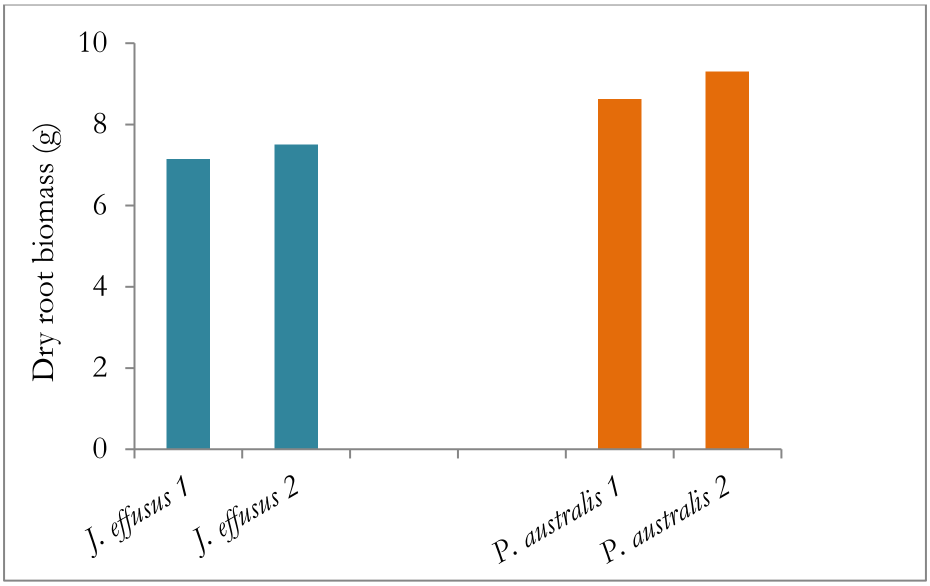


Figure 3.4-4 Dry root biomass from the 4 reactors at the end of the incubation in 2.5 L artificial wastewater

Table 3.4-1 Measured $SO_4^{2-} - S$ and calculated DOC concentrations

Incubations with roots of plant species	Measured $SO_4^{2-} - S$ concentration decrease mg/g dry root biomass (in 10 days)	DOC stoichiometric equivalent for the noted $SO_4^{2-} - S$ concentration decrease mg/g dry root biomass (in 10 days)
P. australis	29.2 ± 1.9	55.1 ± 3.6
J. effusus	19.6 ± 3.8	36.9 ± 7.2

In means ± standard deviations from 2 incubation reactors of each plant species.

3.4.4 Discussion

Rhizodeposits include 4 portions of insoluble material as described in section 1.3, some of which are linked to living plants activity. Rasmussen (2011) claimed that the term rhizodeposits should include only materials related to living roots, but due to practical difficulty has allowed the inclusion of turnover of root hairs, fine roots and minor root fragments while excluding turnover of decaying roots and larger parts of the root system. It was however considered in this research work that all dead root materials from any portion of the root systems, and regardless of if the roots are dead or alive, are encompassed in the term rhizodeposition. In addition, in this experiment, only the insoluble dead roots material was incubated and was found to be utilizable by SRPs.

Regrettably, it is not clear if these insoluble compounds are made available to the bacteria, archaea or fungi (many fungi can degrade pectin and cellulose, which are constituents of plant cell wall, whilst only very few bacteria can (Newman et al. 1985, Rouatt et al. 1960)). The extreme complexity and interactions of the different microbial communities in the rhizosphere, combined with the complexity of the different components of the insoluble rhizodeposits and their respective degree of biodegradability, add up and make it very difficult to provide sufficient understanding (if any quantification) of the process of rhizodeposition from different helophytes without having to perform elaborate analyses. In general, it is safe to say that processes such as hydrolysis and fermentation of the insoluble organic matter prior to its utilization by SRPs were performed by the anaerobic consortia living on the roots. It is worth mentioning that the incubation reactors were not inoculated with any special microbial groups, and hence the microbial activity in this aspect is illustrative to that in the CWs from which the roots were taken.

The non-accumulation of organic matter in the water (COD remained at low concentrations) reflected that the bioavailable organic matter was directly utilized by the SRPs on the dead root surface and did not diffuse to the water. The findings here represent the available organic carbon that microorganisms can extract from complete dead root systems (including fine roots, bigger roots and rhizomes). Thus it cannot be extrapolated to real constructed wetlands systems, where dead root material comes mainly from fine root hairs with rhizomes having very long lifespan. For instance, Čížková and Lukavská (1999) found the age of some *P. australis* populations to span several years.

It appears that the *P. australi*s roots provide much more insoluble organic rhizodeposits within 10 days of incubation (in terms of quantity, but could be also in terms of bioaccessibility of the compounds) than the roots of *J. effusus*. This finding agrees with the results in section 3.3.3.

Better understanding of the differences in the photosynthetic activity between the different helophyte species and their subsequent below-ground carbon allocation might help understanding the differences in the amounts of rhizodeposition between the plant species. On the other hand, differences in root structures of the different macrophytes could serve as an additional explanatory factor for the differences in insoluble rhizodeposits, if one can assume that the fine roots have shorter life spans than the bigger roots, and thus die faster and end up sooner as rhizodeposits. This area of research is however still young and not much is known about the root structures of the different macrophytes. ZhangHe et al. (2004) have reviewed the research on macrophyte roots in CWs. They distinguished two types of morphologies of wetland plants roots: rhizomatic-root plants and fibrous-root plants. Rhizomatic-root plants have rhizomes or thicker root systems, whilst fibrous-root plants have thinner roots (Root diameter $\leq$ 3 mm). They postulated that there are significant differences between these two types in root morphology, structure, growth and biomass as well as radial oxygen loss and nutrient removal. In general, the *P. australi*s species can be categorized as rhizomatic-root plants, whilst the *J. effusus* species can be classified as fibrous-root plants.

3.4.5 Conclusion

- Dead roots of *P. australis* provided in 10 days' incubation higher bioavailable organic rhizodeposits than dead roots of *J. effusus*; in similar trend to what was previously found for the overall rhizodeposits of the two helophytes (section 3.3.3);

- It is important to further investigate the differences between these two helophytes for the remainder portions of rhizodeposits as well as to investigate other frequently applied helophytes to provide better understanding of their rhizodeposition capacities.

3.5 Comparative study of the influence of three helophyte species on treatment performance and sulfur cycling in highly-loaded subsurface flow constructed wetlands

3.5.1 Introduction

In this investigation, the influence of applying different helophytes on the treatment performance for COD, TN and with emphasis on sulfur cycling was tested in HSSF CWs with high organic (as COD: 11.52 g $O_2\ m^{-2}\ d^{-1}$) and TN loading (1.82 g N $m^{-2}\ d^{-1}$).

3.5.2 Material and methods

Experimental setup

Mesocosm HSSF CWs planted with monocultures of *Phragmites australis*, *Typha angustifolia* and *Phalaris arundinacea*, each in triplicate; with two unplanted controls were placed outdoors at ambient conditions of the city of Nantes (47° 13' 02" N, 01° 33' 14" W and 17 m above MSL). The city has temperate oceanic climate with monthly-averaged 53 ± 39 mm of precipitation and 229 ± 57 hours of sunshine during the sampling period of June to October 2014. The monthly-averaged maximum-minimum air temperatures were: 24.1-12.8, 26.3-14.6, 22.7-12.7, 25.3-13.1, and 20.5-10.4°C for June to October, respectively (meteo-bretagne.fr). The CWs were made of Plexiglas containers (1.5 cm thick) with dimensions length × width × depth of: 56.5 × 19.5 × 74.5 cm, respectively. To each CW, 83 kg of crushed glass (1-4 mm) was added as substrate, achieving a filling depth of about 60 cm. On top of that, 7 kg of coarse sand (1-2.5 mm) was added to create an additional layer of 5 cm depth, to minimize light intrusion from the top of the wetland. The water level was adjusted to 60 cm. The inflow distributing pipe was cut open at uniform intervals and the near-outlet zone was filled with gravel (10-20 mm) to promote uniform distribution of the wastewater and to create clear outflow zones. The planting was performed in September 2013. Black curtains of thin plastic sheets along with boards of thermal insulating material were used to cover the systems from the 4 sides for protection against light intrusion and extreme heat. The mean pore-water temperature throughout the experimental period was: 24.3 ± 2.3, 24.9 ± 4.1, 24.5 ± 2.8, 24.6 ± 3.1 in the *P. arundinacea*, *P. australis*, *T. angustifolia* and the unplanted systems, respectively. The range of pore-water temperature was from 20.4 (min.) to 32.4 (max.). Precipitation was measured with a special rain gauge.

The batch-operated systems were fed intermittently once a day during weekdays and resting during the weekend with inflow volume (V_{in}) of 5 L of synthetic waster according to the modified OECD guideline 303. This corresponds to a feeding rate of 25 L/week. It contained (in mg/L tap water): peptone, 160; meat extract, 110; urea, 30; anhydrous dipotassium hydrogen phosphate (K_2HPO_4), 28; sodium chloride (NaCl), 7; calcium chloride dehydrate ($CaCl_2.2H_2O$), 4; and from the tap water 19.0 ± 1.8 $SO_4^{2-} - S$. This synthetic sewage gives a mean concentration in the influent (mg/L) of: [COD, 360; $PO_4^{3-} - P$, 2.9; $NH_4^+ - N$, 3.6; $NO_3^- - N$, 2.9; TN, 56; total sulfur (TS), 20.5]. The pore-volume of the CWs was estimated assuming a theoretical porosity of sand of 35% to be 23 - 24 L. This pore-volume corresponds to a nominal hydraulic retention time 'nHRT' of the added wastewater in the CWs of 6.5 days (considering the filling volume of 25 L/week). Decreased porosity caused by plants' growth has resulted in varying the 'nHRT' among the planted systems. This effect was not considered. The synthetic wastewater was freshly prepared on a daily basis and applied directly to the systems through the inflow pipe. This wastewater corresponds to a hydraulic loading rate 'HLR' of 3.24 cm/d (32.4 L m^{-2} d^{-1}); and loading of 11.52 g COD m^{-2} d^{-1}, 1.82 g N m^{-2} d^{-1} and 0.65 g S m^{-2} d^{-1}.

Sampling and analytic procedures

Sampling was conducted in the summer-autumn of 2014 (from June to October) on a biweekly basis. For the estimation of the physical-chemical parameters, pore-water samples were collected from the 11 systems at mid-length and mid-width, at depths 5, 30 and 60 cm from the water surface. Syringes were used to withdraw water from the respective depths through especially designed sampling tubes, which were inserted in each CW at the start of the experiment. The inflow sample was defined as the feed synthetic wastewater directly after preparation. The outflow samples were collected either from the outflow tube of each CW at the time of sampling or from the outflow tanks. There was no significant difference ($p > 0.05$; tested 3 parameters, selected at random) between the outflow samples from the two cases.

Most physical-chemical parameters were preserved and analyzed as described previously except for the following differences. Redox potential was measured using an Orion platinum Redox electrodes Model 96-78-00 filled with 4 mol/L KCl saturated with Ag/AgCl reference solution (Orion Research Inc., Beverly, MA, USA) read by Consort C561 portable meter (Consort, Turnhou, Belgium). Temperature was measured with a P 300W temperature sensor (Hand-held Measuring Instruments. pH was measured by a Consort pH electrode with a Consort C561 portable meter (Consort, Turnhou, Belgium). Concentrations of ammonium (2 - 75 mg/L) were measured either using the respective Test Kit (Merck, Germany), or Accumet Ammonia Combination Ion-Selective Electrode (Fisher Scientific, Pittsburgh, PA, USA), nitrate was determined using the Accumet Nitrate Combination Ion-Selective Electrode (Fisher Scientific, Pittsburgh, PA, USA), and TN was measured with Total Nitrogen Reagent Set, LR, TNT (HACH LANGE, France). COD was analyzed from settled samples using either the test kit COD-Low range (0 - 150 mg/L O_2) (VWR, France) or the test Kit LCK 514 (100 – 2000 mg/L O_2) (HACH LANGE, Germany). All sulfur species were determined as described in section 3.1.2. Above-ground plant biomass was harvested at the end of the experiment and dried as described previously.

Calculations

For the estimation of the water loss due to evapotranspiration from the systems (or due to evaporation from the unplanted CWs), outflow water was collected after every feeding in closed tanks and the outflow volume (V_{out}) was recorded. The water loss during a defined period was calculated from the water balance equation:

$$\mathbf{ET} = \frac{(\mathbf{V_{in}} + \mathbf{P_{rain}} * \mathbf{A} - \mathbf{V_{out}})}{(\mathbf{V_{in}} + \mathbf{P_{rain}} * \mathbf{A})} * \mathbf{100} \qquad 3.5\text{-}1$$

Where:

ET: specific water loss by evapotranspiration in %

V_{in} & V_{out}: influent and effluent water volumes during the stated period in L/d

P_{rain}: area specific precipitation within the defined period in L m^{-2} d^{-1}

A: is the surface area of the wetland in m^2

The pollutant load (g m^{-2} d^{-1}) was calculated at the inflow and the outflows from a concentration $C_{in/out}$ (g/L) following equation 3.1.2.

For the *P. australis* systems, ET mounted to 100% in some occasions. It was not possible in these cases to calculate the outflow loads and it was as well not valid to assume a 100% pollutant removal, therefore these data were not considered.

Statistical procedures

Data are visualized as described in section 3.2.2. The noted variability in the data is attributed to climatic variations and to differences in the physiological status of plants during the growth season. Data from the 3-2 replicates of each treatment variant were not significantly different. Thus, for simplification, data from replicates of each variant were gathered together as one population.

All the statistical analyses were conducted using the R statistical computing environment (R-Core-Team 2013). A statistically significant difference was defined at 95% confidence (p value < 0.05). One-way ANOVA tests were carried out for individual parameters when testing outflow values and two-way ANOVA when testing values from different depths assuming normality of distribution and homogeneity of variances. When ANOVA reflected significant difference, the Tukey's honestly significant difference (HSD) test was applied to determine the significance of differences between the combinations of treatment variants via multiple comparisons of means. Correlation between $NH_4^+ - N$ and S^{2-} was tested using the Pearson's method.

3.5.3 Results

Plant biomass development

This investigation was conducted during the first growth season, which was characterized by good and healthy plant development and coverage. The above-ground dry plant biomass was analyzed at the end of the experiment and was found highest for *P. australis* (3.8 ± 0.8 kg/m^2) followed by *P. arundinacea* (2.8 ± 0.4 kg/m^2) and *T. angustifolia* (2.4 ± 0.5 kg/m^2; in means and standard deviations from three replicate HSSF CWs for each species). The below-ground dry biomass was not analyzed. However, the depth of the roots was observable through the transparent walls of each CW and was found to be higher for *P. australis* (roots penetrated the whole depth of the CWs by the end of the experiment) followed by *T. angustifolia* while the shallowest root system was from *P. arundinacea* which did not exceed the top few centimeters till the end of the period of observation (Figure 3.5-1).

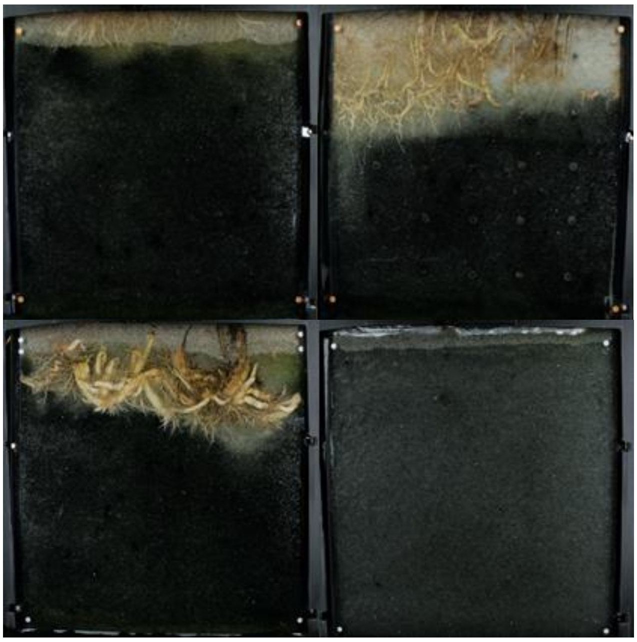


Figure 3.5-1 Side profile of one replicate of each treatment system (plots from top-left to bottom-right): CWs planted with *P. arundinacea*; with *P. australis*; with *T. angustifolia*; unplanted CWs. The images reflect the status of root development at the end of July 2014.

ET from the different treatments

Water loss due to evapotranspiration was significantly higher in all planted systems compared to unplanted control ($p < 0.05$), as shown in Figure 3.5-2. ET was as well significantly different amongst species with *P. australis* depicting the highest ET followed by *P. arundinacea* and *T. angustifolia*. Negative ET values refer to rainy events wherein outflow volumes exceeded inflow.

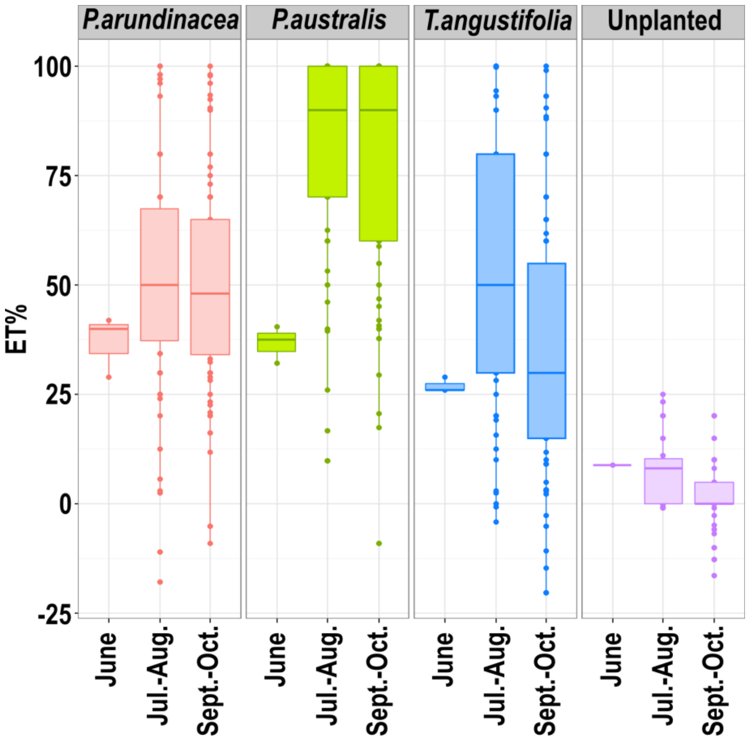


Figure 3.5-2 Water loss (ET %) from the different planted and unplanted CWs (2-3 replicates each) during the sampling period.

Pore-water conditions

The environmental conditions of the pore-water in terms of redox potential and pH levels varied among the different planted and unplanted treatment variants. The redox potential (Figure 3.5-3) was significantly higher (p value < 0.05) at depth 5 cm than the other two depths, whilst depth 30 cm and 60 cm were not significantly different. In addition, redox potential showed significant differences between all combinations of systems with the exception that *P. arundinacea* were not significantly different from unplanted systems.

The redox potential correlated positively with the presence of plant roots at a sampled location (the presence or absence of roots was based on regular visual inspection of the sampling depths). The rooted zones thus had a significantly higher redox potential of -46 ± 176 mV than the unrooted zones of -277 ± 96 mV (in means and standard deviations of all rooted and all unrooted sampled locations, $p < 0.05$).

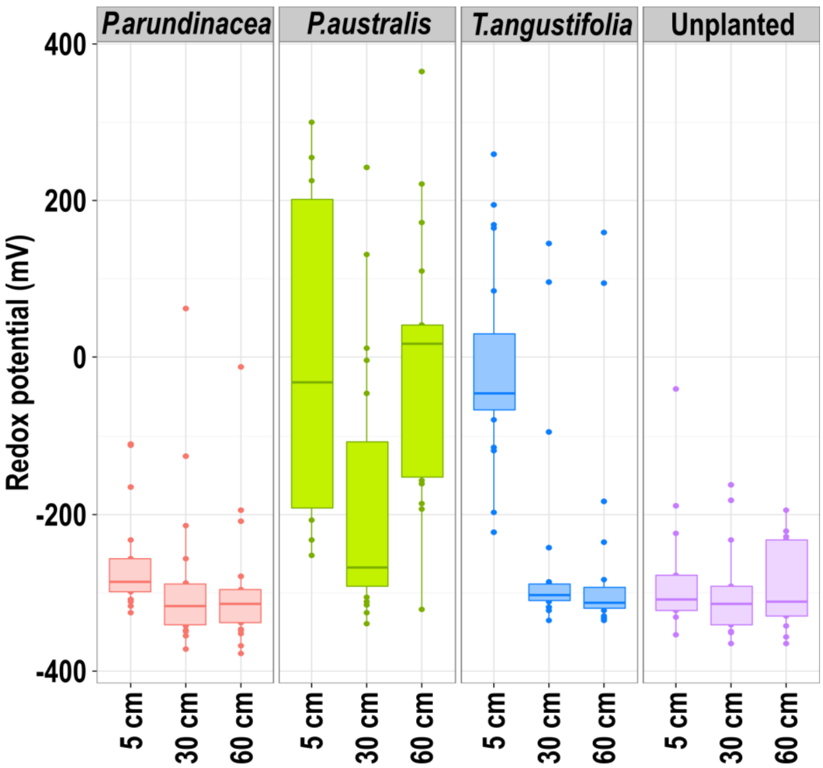


Figure 3.5-3 Redox potential (E_h) at the different depths of the planted and unplanted CWs (n ≤ 21; from 7 sampling events and 2-3 replicates).

The pore-water pH remained in the neutral range within all CWs and no significant differences were noticed between the systems. A pH increase was measured inside the CWs and at the outflows compared to the pH at influent (pH of 5.7). The unplanted systems showed the highest increase of about 2 units to pH 7.9 ± 0.5 at outflow whereas the planted systems reflected pH increase values of above 1 unit to pH 7.1 ± 0.1; 6.9 ± 0.2; and 7.1 ± 0.3 in outflows of *P. arundinacea*, *P. australis* and *T. angustifolia* CWs, respectively.

COD and TN removal

Regarding the wastewater treatment performance, the planted systems performed generally better than the unplanted controls in COD and TN removal (Figure 3.5-4). COD and TN removal were highest in *P. australis* followed to similar extents by *T. angustifolia* and *P. arundinacea* and least in unplanted systems. Whilst only *P. australis* COD removal was significantly higher than unplanted (p < 0.5); all planted systems reflected statistically significant higher removal of TN than unplanted systems. Amongst species, *P. australis* had significantly higher COD and TN removal than the other two helophytes systems, whereas *T. angustifolia* were not significantly different than *P. arundinacea* systems.

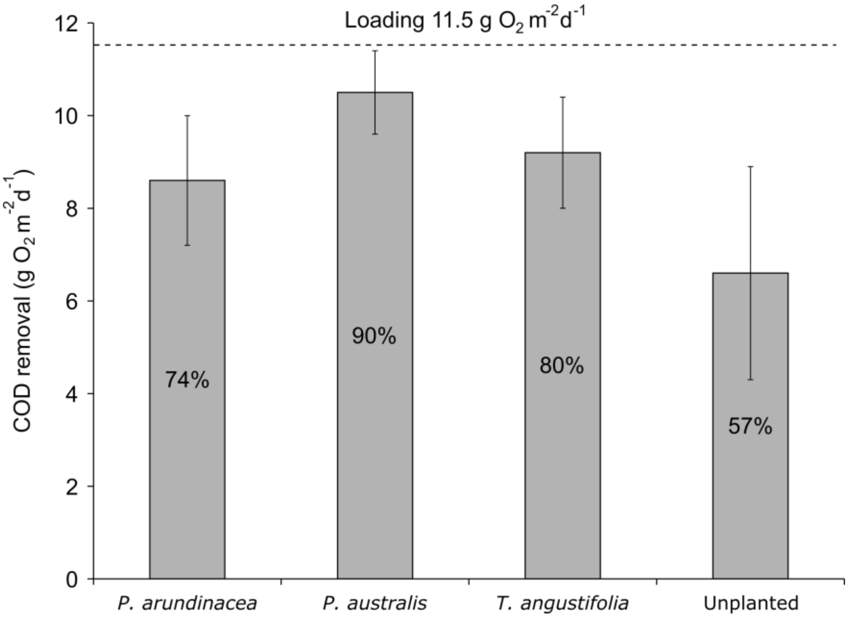


Figure 3.5-4 COD and TN removal from the different CWs (columns show means and error bars represent standard deviations).

The $NH_4^+ - N$ concentration in the influent was 3.6 mg/L. This value increased by a factor of 10 in the CWs planted with *T. angustifolia* and *P. arundinacea* and the unplanted systems (Figure 3.5-5). The main processes affecting the $NH_4^+ - N$ concentration are ammonification of organically-bound nitrogen in the influent, nitrification and evapotranspiration.

Internal nitrogen cycle processes were not monitored, however coupled nitrification/denitrification and plant uptake were assumed to be the main processes contributing to the noted TN removal, although $NO_3^- - N$ and $NO_2^- - N$ remained mostly below detection during the experiment timeframe. Ammonia volatilization was considered to be insignificant since the pH of the pore-water remained below 8 (Reddy et al. 1984).

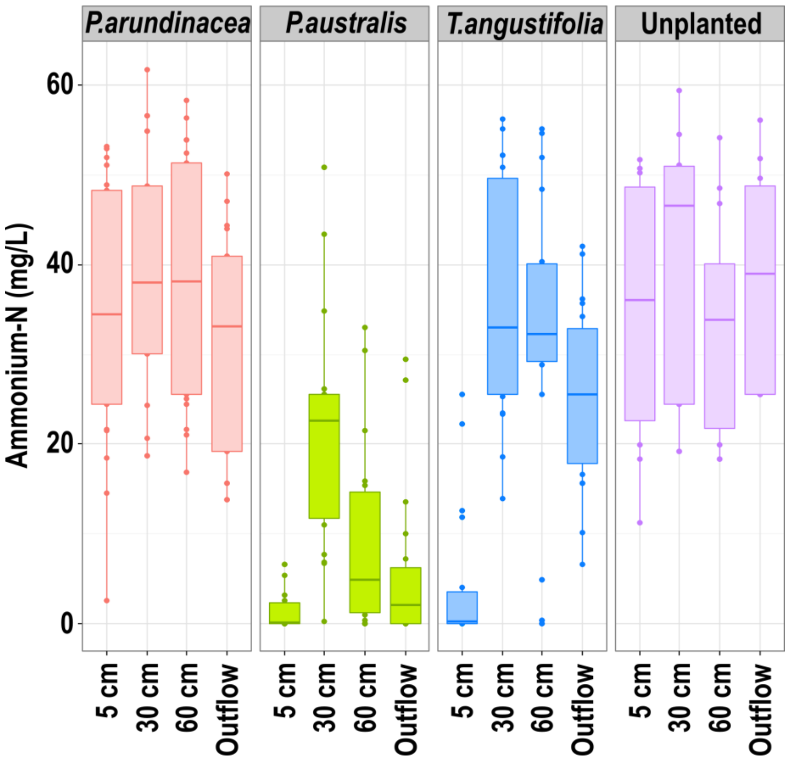


Figure 3.5-5 $NH_4^+ - N$ concentrations inside and at outflow of the different CWs (n ≤ 21; from 7 sampling events and 2-3 replicates).

Sulfur cycling

Significant differences in the internal sulfur cycling and overall sulfur removal was noted among the treatment variants. The synthetic wastewater contained sulfur mainly in the form of sulfate and to limited extent as organically-bound sulfur. The organically-bound sulfur was not analyzed inside the systems and was assumed to be transformed into inorganic sulfur. Effective DSR took place in all systems, as its end product sulfide was detected in concentrations up to 94% of the influent sulfate-S concentrations. Sulfide concentrations were significantly lower in the top layer (the sampled depth 5 cm) of *P. australis* and *T. angustifolia* systems than in the rest of the sampled depths of all systems (Figure 3.5-6). This is in correlation with the higher redox levels at the top layer of the *P. australis* and the *T. angustifolia* CWs. On the other hand, the analyzed intermediately oxidized sulfur species (elemental sulfur, sulfite and thiosulfate) were detected at moderate concentrations inside all the systems. Since the influent did not contain any of the latter species, and as they can only be the product of abiotic or biotic oxidation of sulfide, it was concluded that DSR and sulfide oxidation processes occurred simultaneously.

The mass removal of sulfate was highest in the *P. australis* systems followed by the *P. arundinacea* systems the *T. angustifolia* and the unplanted systems (Table 3.5-1). The differences among the plant species were not found statistically significant, possibly due to high variability in the data. However, significant differences in sulfate removal capacity were found between the *P. australis* and the unplanted systems (*p* value < 0.05). In addition, unlike any other treatment variant where sulfur mainly changed forms from sulfate to less oxidized compounds, all the analyzed sulfur compounds were removed from *P. australis* CWs leading to TS removal of about 85%. Hence, sulfur transformations were more intensified in *P. australis* systems, since both sulfate and sulfide concentrations were the lowest ones measured in the effluent, reflecting concurrently more intensive DSR and sulfide oxidation processes. Contrastingly, the unplanted CWs demonstrated not only the lowest sulfate removal but also no TS removal. In other words, the sulfur underwent speciation changes but was not removed from the unplanted controls.

Table 3.5-1 Sulfur mass balance of the different planted and unplanted CWs

	Influent $\mathbf{g\ S\ m^{-2}\ d^{-1}}$	**Effluent** $\mathbf{g\ S\ m^{-2}\ d^{-1}}$					**Mean removal**
	TS	$\mathbf{S^0}$	$\mathbf{S^{2-}}$	$\mathbf{SO_3^{2-}-S}$	$\mathbf{S_2O_3^{2-}-S}$	$\mathbf{SO_4^{2-}-S}$	***TS**
P. arundinacea	0.65 ±0.04	0.015 ±0.023	0.119 ±0.062	0.020 ±0.013	0.111 ±0.046	0.146 ±0.078	37%
P. australis	0.65 ±0.04	0.018 ±0.025	0.000 ±0.001	0.001 ±0.001	0.030 ±0.053	0.059 ±0.075	85%
T. angustifolia	0.65 ±0.04	0.012 ±0.020	0.095 ±0.044	0.017 ±0.014	0.134 ±0.060	0.202 ±0.100	29%
Unplanted	0.65 ±0.04	0.015 ±0.021	0.212 ±0.112	0.029 ±0.023	0.163 ±0.072	0.288 ±0.109	0%

Means ± standard deviations. *TS is calculated as the sum of the 5 analyzed inorganic sulfur species, since the organic sulfur and the other inorganic sulfur compounds are assumed to exist at negligible concentrations.

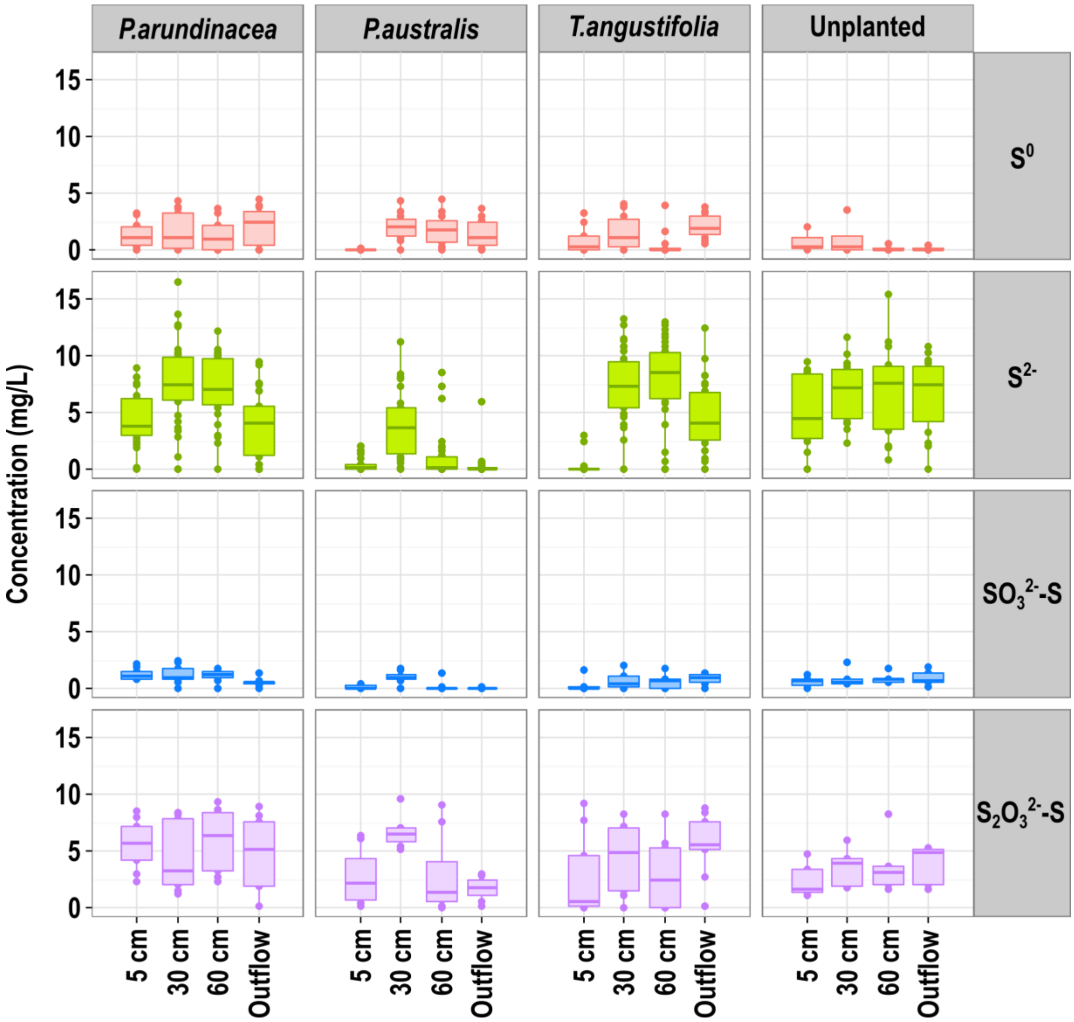


Figure 3.5-6 Concentrations of measured reduced sulfur compounds: $\mathbf{S^{2-}}$ (n ≤ 33); $\mathbf{S^0}$, $\mathbf{SO_3^{2-} - S}$ and $\mathbf{S_2O_3^{2-} - S}$ (n ≤ 21; from 7 sampling events and 2-3 replicates).

3.5.4 Discussion

The performance of unplanted controls was regarded as a background against which the planted systems were evaluated.

The redox potential encapsulates the balance of all the reducible-oxidizable components found in the pore-water; the existence of helophytes alters this balance in three main aspects. Firstly, they release oxygen to their rhizosphere (Wiessner et al. 2006, Wiessner et al. 2002a); secondly, they deposit organic compounds (Cheng and Gershenson 2007); and thirdly, they take up some of the pollutants (Brix 1997). Differences exist between helophyte species in terms of patterns and magnitudes of all three aspects (Bertin et al. 2003, Brix 1997, Lai et al. 2012). In the present investigation, the net effect of the presence of plants on the redox potential was found position dependent. Lower redox potential was detected in the unrooted zones. In part due to the high organic loading of the systems, the oxygen released from the roots was apparently not sufficient to diffuse to the non-rooted portions of the CWs. This is mostly evident in *P. arundinacea* systems, wherein the root structure was shallow and the majority of its wetlands' depth remained anoxic, with redox potentials similar to unplanted systems.

The ranking of the redox potential conditions inside the planted systems (*P. australis* followed by *T. angustifolia* followed by *P. arundinacea*) agreed with the ranking of the observed depths of the root systems and did not correlate with the above-ground biomass size (*P. australis* followed by *P. arundinacea* and least for *T. angustifolia*). It appears that the below-ground biomass (given that the only indicator of it considered here is the root depth and not the root dry biomass) had higher influence than the above-ground biomass on the redox potential levels. Hence, how deep a helophyte species' roots can grow can be decisive of the depth of the oxygenated portions of the CWs planted with it. However, if the oxygen release factor is considered alone, the present results contradict the findings of Wiessner et al. (2002b), who observed that the amount of root-released oxygen was rather affected by the size of the above-ground biomass than by the size of the root systems for two tested helophytes.

The redox potential is also affected by the microbial transformations (Reddy and D'angelo 1994). The microbial community structures are expected to vary among the different treatment variants. Gagnon et al. (2007) found that microbial density and activity in CWs increase in the presence of helophytes and can be significantly different among different helophyte species. They investigated the same helophyte species as here, but in smaller systems (microcosms of 1.8 L) and found *P. arundinacea* to be the superior plant species in terms of the microbial density and activity supported in the microcosms planted with it. In their experiment, *P. arundinacea* was as well the biggest in size in terms of above- and below-ground dry biomass and as well as in terms of root surface area. Here, *P. australis* was the superior plant in terms of above-ground dry biomass and root depth and in terms of CWs performance. It can then be postulated that the physical size of the helophytes impacts the size and activity of the supported microbial community which is the major drive force for wastewater treatment processes (Faulwetter et al. 2009, Reddy and D'angelo 1994).

The implications of helophytes size need to be investigated further for both above- and below-ground components. One of the motivations to seek this is that the size of plants is projected to be proportional to their photosynthetic activity, which is in turn estimated to be proportional to the carbon allocation to underground tissue, i.e. rhizodeposition (Helal and Sauerbeck 1989, Michaletz et al. 2014). In addition, the different helophyte species have different seasonal patterns that can influence both their biomass and their activity in term of oxygen supply, rhizodeposition and associated activity of microorganisms. Such effects need be further investigated, at best in collaboration with plant physiologists and plant biologists.

Vymazal (2005) has documented COD removal efficiencies of HSSF CWs from 131 systems worldwide to be in the range of 75%. The higher COD removal efficiencies in the investigated planted systems -although the systems' organic loading was at the upper limit for design standards- can be attributed to existence of mainly easily biodegradable substrate in the synthetic wastewater. In addition, this investigation was conducted during the growth season, whilst the documented values were mostly yearly-averaged.

Although the planted systems received an additional organic carbon load from the rhizodeposits, they showed considerable COD removal which was higher than removal rates of unplanted systems. Thus, the net effect of helophytes was to intensify the COD removal presumably by boosting microbial community growth as found by Gagnon et al. (2007) and via cometabolism.

Analogously, the nitrogen transformations and eventually TN removal from the planted systems can as well be impacted by the plants presence related to the three stated aspects of helophytes' influence. The nitrogen uptake and storage in the plant tissues differ among helophyte species (Tanner 1996) and may differ for the same species based on nitrogen concentrations in the medium, plant growth rate and stage of season, and the existing nitrogen content in the plant tissues (Langergraber 2005, Vymazal 2007). Findlay et al. (2002) documented that *P. australis* sequesters nearly twice the amount of nitrogen per unit marsh area in its above-ground biomass compared to *T. angustifolia*. Here, the plants nitrogen uptake was not quantified, but was estimated to be one of the main routes of the observed TN removal in the planted systems. In addition, the processes of nitrification and denitrification, also assumed as a main pathway of TN removal, can be stimulated by plants presence. Nitrification and denitrification could not be proven nor the rates for it calculated, as the nitrate and nitrite were detected at few occasions and at very low concentrations that were not different from influent nitrate levels (2.9 mg/L). However, it is assumed that some nitrification took place at the oxygenated root surfaces and that the produced nitrite and/or nitrate were denitrified in the anoxic zones of the CWs consuming organic carbon and reduced inorganic sulfur compounds as electron donors. This is a plausible assumption since plant uptake alone is very unlikely to explain the noted TN removal (analogous to findings of section 3.3.3 related to calculated N uptake of *P. australis*) and since ammonia volatilization was not expected to be very high.

The intermediate inorganic sulfur cycling was monitored in more detail. One of the major implications of sulfur cycling on the performance of HSSF CWs is the production of sulfide. Sulfide is known to be toxic to helophytes at concentration ranges of 10 - 50 mg/L (Armstrong et al. 1996, Wiessner et al. 2008a). On the other hand, the nitrifying microbial community is found to be very sensitive to sulfide and suffers inhibitory effects at sulfide concentrations as low as 0.5 mg/L (Æsøy et al. 1998). In this study, the sulfide concentrations were found to correlate positively with the ammonium concentrations (Pearson's r of 0.72) and hence negatively with the TN removal. A similar correlation was documented by Wiessner et al. (2008b).

Higher sulfur turnover in planted systems reflected their higher microbial DSR activity. The boosting effect of *P. australis* on sulfur cycle processes was visible in TS removal rates about two times higher than for *P. arundinacea* and about three times higher than for *T. angustifolia* systems, whilst the unplanted systems showed no TS removal. This can be due to provision of rhizodeposits by *P. australis* at higher quantity or better quality (bioavailability); or due to higher release of oxygen; or a combination of both. Brix et al. (1996) have reported the outstanding capacity of *P. australis* to ventilate its underground tissues, Saad et al. (2016) documented its considerable rhizodeposition capacity and this study has revealed its superior role in boosting the treatment processes in HSSF CWs as compared to the other two tested helophytes.

DSR process could have been more efficient in the unplanted systems had the microbial activity not been the limiting factor (sulfate and biodegradable organic carbon were both in excess, pH was not inhibitive and redox potential ranges were favorable). Yet, the observed DSR in unplanted systems utilized less than 50% of influent sulfate.

One of the limitations of this investigation is that it was conducted only in the plants' growth season and has considered neither the cold season (when plants are dormant and ET is minimal) nor the early spring season (when regrowth may be associated with higher nutrient uptake). Another limitation is that the root systems were not analyzed -apart from the depths observed from the side of the CWs- which is insufficient to give conclusive information about the linkage between roots' size and the capacities of helophytes. In order to comprehend the differences amidst the helophyte species, it is crucial to identify the differences in their root growth. For instance, *P. australis* roots grew vigorously and developed an extensive root system by the end of the growth season, whilst root growth of *P. arundinacea* was very limited in terms of depth.

3.5.5 Conclusion

- The incorporation of helophytes in the investigated HSSF CWs has led to higher performance regarding COD and TN removal compared to unplanted controls. Helophyte presence also increased the rates of both the reductive and the oxidative sulfur cycle processes and increased TS removal. This advocates that the existence of plants supports the microorganisms responsible for sulfur transformations;

- In addition, significant differences were noted amongst the planted systems regarding the COD and TN removal as well as the rates of sulfur processes, with *P. australis* CWs standing out as most efficient systems. Generally, *P. australis* showed higher capacities to prevent the development (accumulation) of sulfide in their rhizosphere, as reflected by the lower sulfide concentrations and the higher redox levels in the rooted zones of its CWs. *P. arundinacea* showed modest capacity to prevent sulfide accumulation; which may be attributed to the fact that this helophyte had the shallowest root system. It still needs to be investigated whether the plants' root depth, size of above- or below-ground components are determinant factors for the magnitude of helophytes' influences;

- Investigating the sulfur cycling in detail was a useful approach to assess the internal conditions of the systems and to illuminate the helophyte interspecies differences. In the investigation a small buildup of reduced sulfur species in the CWs correlated with high COD and TN removal rates;

- The noted significant differences in treatment performance and process intensities of HSSF CWs planted with different helophytes foster the deliberate selection of helophytes. In this respect it is assumed that further understanding of microbial agents responsible for main pollutant cycles and of how the different helophytes support these microbial agents is of high interest.

3.6 Fate of the sulfur removed from pore-water of a horizontal subsurface flow constructed wetland receiving contaminated groundwater

3.6.1 Introduction

An overall understanding of sulfur cycling in HSSF CWs cannot be achieved without knowing the quantities and speciation of the different sulfur pools that are incorporated within the soil matrix. Several studies in which sulfur removal was documented have concluded that a considerable part of the sulfur removed from the water phase was immobilized in the beds (Wiessner et al. 2010, Wu et al. 2011).

In this investigation, a closer look into the sulfur content within the soil of HSSF CW receiving contaminated ground water (GW) with high sulfate and low organic carbon loads was assessed to test the hypothesis that most of the sulfur removed from the water phase is trapped inside the beds. Alongside the soil analysis, pore-water concentrations of sulfur compounds were as well assessed.

3.6.2 Material and methods

Description of the pilot-scale CWs

This pilot-scale experimental plant was built south-east of the city of Bitterfeld (51° 37' 0" N, 12° 19' 0" E and 79 m above MSL) in Saxony-Anhalt, Germany. It was established in December 2002 under the UFZ research project SAFIRA (SAnierungs Forschung In Regional kontaminierten Aquiferen) in order to develop and implement *in-situ* techniques for the remediation of contaminated GW.

The base of the wetlands was a stainless steel container of the dimension 6 m (length) × 2 m (width) × 0.5 m (depth) (Figure 3.6-1). It was equally divided into two segments, one was operated as a HSSF CW and the other one represented a FHRM CW (was not investigated within the framework of this chapter), both planted with *P. australis*. The wetland parts had dimension of 5 m (length) × 1 m (width) × 0.5 m (depth) which was followed by a free water body with the dimension of 1 m (length) × 1 m (width) × 0.4 m (depth) to the outflow. The last meter of each part was left as an open water (FW) compartment (Vogt et al. 2002). However, during the soil sampling there was some soil in the section intended as FW section, from which core-samples were taken as well.

The HSSF CW was fed continuously with GW pumped up from an approximately 22 m-depth well of the SAFIRA research site with the average inflow rate of around 4.7 L/h. The average HRT was about 6 days. The effluent was regulated by a filling-level meter, shaped as a floater on the surface of the open water compartment. The filter media used for the HSSF CW consisted of 36% gravel, 58% sand and 6% clay. Due to residues of the local lignite seam, TOC content in the media varied between 1.5 and 2.0 % by weight (Braeckevelt et al. 2008, Vogt et al. 2002). Influent ground water composition in the period April-June 2012 is summarized in Table 3.6-1. A detailed composition of the GW analyzed from the neighboring monitoring-wells is indicated in Table A-1 in appendix 0. The composition of the monitoring wells show however slight differences to the influent water to the pilot-scale facility.

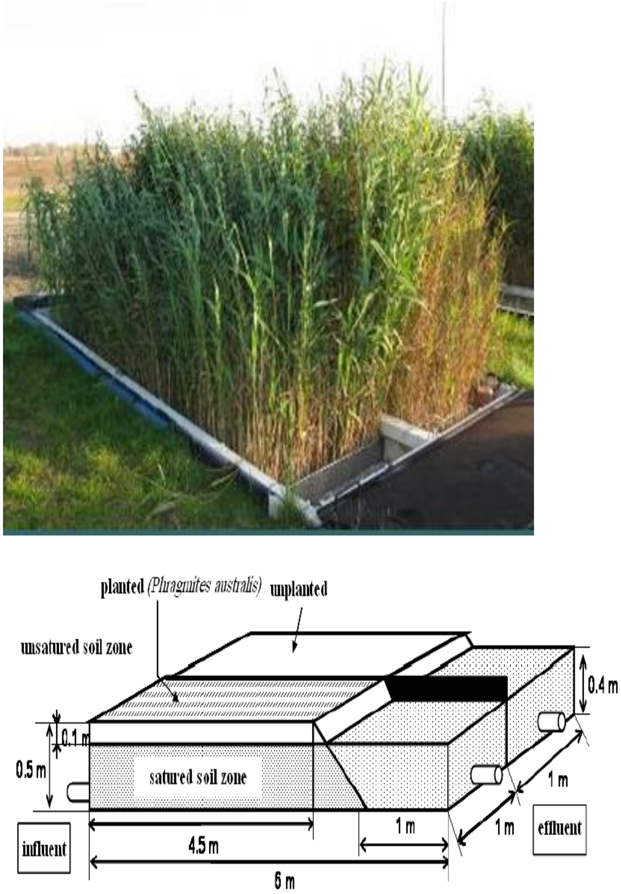


Figure 3.6-1 The pilot scale CW systems in Bitterfeld: HSSF CW (left) and FRM CW (right), planted with *Phragmites australis.* Photos: Dr. Peter Kuschk (left); Braeckevelt et al. (2008) (right).

Table 3.6-1 Mean influent ground water characteristics April-June 2012

	COD mg/L	$NO_3^- - N$ mg/L	$NH_4^+ - N$ mg/L	E_h mV	pH	S^{2-} mg/L	$SO_4^{2-} - S$ mg/L	S^0 mg/L
Avarage	67.4	3.6	11.1	10.1	6.8	0.2	372.6	0.0
Standard Deviation	47.7	4.4	4.7	33.1	0.4	0.1	72.3	0.0

Sampling procedure

Pore-water sampling was conducted throughout the period April – June (total of 7 sampling events). Samples were taken at 25 cm depth and at inflow and at distances 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 m from inflow with identical procedure to that described in section 3.1.23.2.2. Redox potential, pH and temperature along with COD and some N and S compounds were monitored.

Soil sampling was conducting by taking core samples using a stainless steel cylinder driven with a drilling machine (Figure 3.6-2 left). Triplicate samples (width-wise; to investigate the horizontal profile) were taken from the HSSF bed at distances: 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 m from inflow (total number of soil cores: 18, Figure 3.6-2, right). Each soil core had diameter of 10 cm and depth of 40 cm. The core samples were sectioned *in-situ* depth-wise. As each core came out with different height (it was not always physically possible to get a full-depth (40 cm) core), the sections were not always 10 cm in height as planned, and it was not always 4 sections (therefore: 65 sections instead of 18*4=72, and not all equal in size). Upon sectioning, samples were directly placed in leak-proof containers (sealable plastic bags with air extruded). The polymer bags were of a thickness and composition to minimize subsequent diffusion of oxygen into the samples. The samples were kept cold (0 – 4°C) in the field (by storing in containers filled with dry ice and frozen cooling cells) (Ahern et al. 2004). Water-proof and oven-proof labels remained with samples at all times. Samples were stored at -20° C until further analysis.

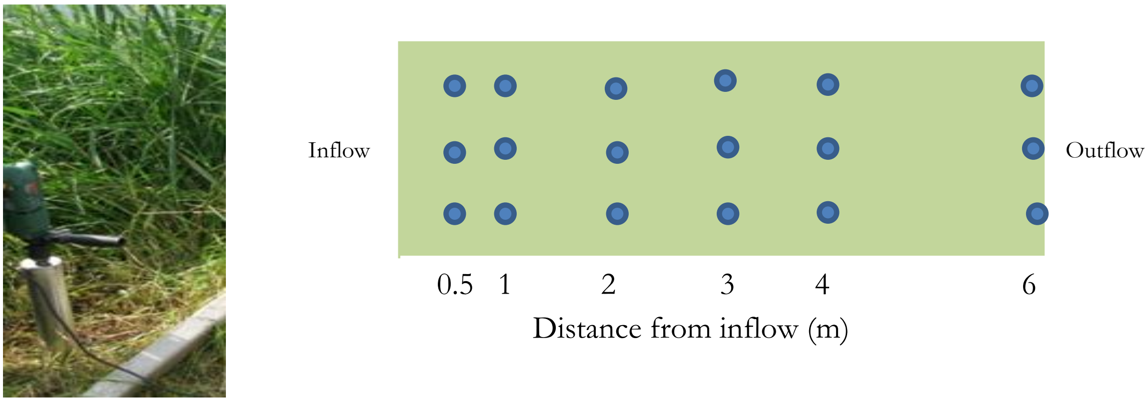


Figure 3.6-2 The cylinder sampler (left) and the sample taking locations from the bed (right).

Analytic procedure

Analysis of pore-water physical-chemical parameters is done as described previously. COD was analyzed from settled samples using the test kit COD-Low range LCK 314 (15 - 150 mg/L O_2) or the test Kit LCK 514 (100 – 2000 mg/L O_2) (HACH LANGE, Germany).

Soil samples were dried, after separating the plant root material, to constant weight as stated in section 3.3.2. The separated plant material was as well dried with the same procedure to obtain the dry mass of the plant mater of each sample. After drying and recording the dry weight, the soil samples were sieved and size-separated at (4 mm, 2 mm, 500 µm, 200 µm) and the weight of each segment was recorded. The fraction < 200 µm was used for analysis. The element content of total sulfur, total iron, and other relevant elements of each sample was measured using X-ray fluorescence spectrometry (method described by Wagner and Mages (2010)) and inductively coupled plasma atomic emission spectroscopy (ICP AES) as a validation method. Complete sulfur speciation was not possible as an oven was used for the drying of the samples, which lead to oxidation of all portions of acid volatile sulfide (AVS). However, by applying a distillation procedure as described in section 3.2.2 it was possible to determine the chromium reducible sulfur (CRS) portion. It was assumed that the rest of the total sulfur was already converted to sulfate. CRS values were used only to indicate the distribution within the wetland, but since the AVS was not possible to estimate and the estimated sulfate was form both originally existing sulfate and oxidized AVS, no conclusions are based on this speciation.

3.6.3 Results

Pore-water parameters

The results from the sampling are summarized in Figure 3.6-3 and 3.6-4. Redox potential inside the CW was not different from that of the influent GW. In addition, pH remained at neutral levels comparable to that of the influent wastewater and was predominantly between 6.5 and 7.0.

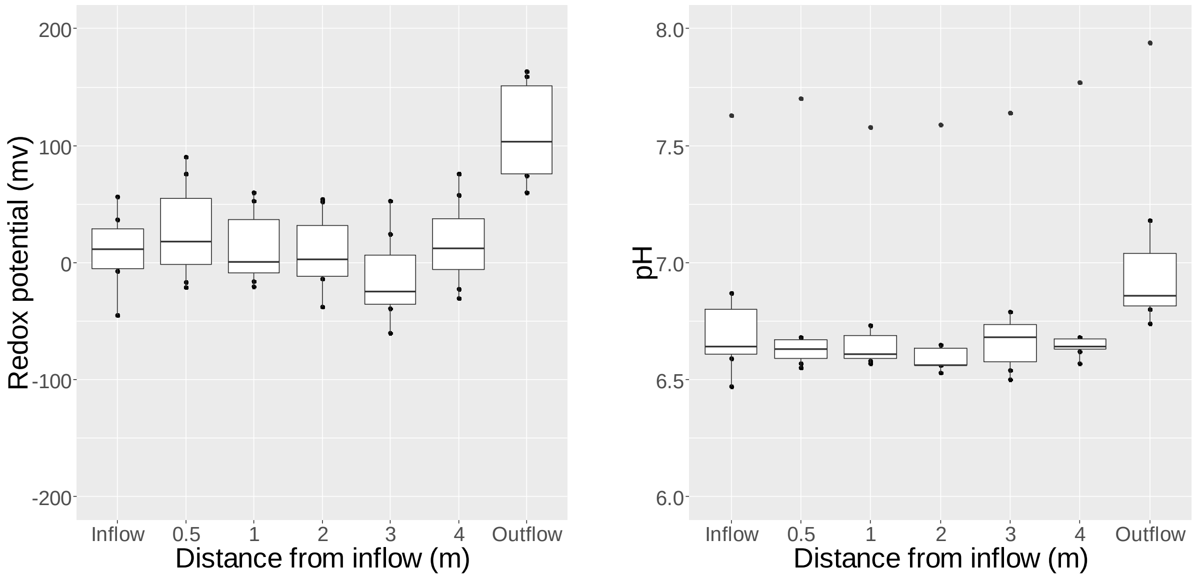


Figure 3.6-3 Redox potential (left) and pH (right) in the pore water at depth 25 cm of the HSSF CW (n=7).

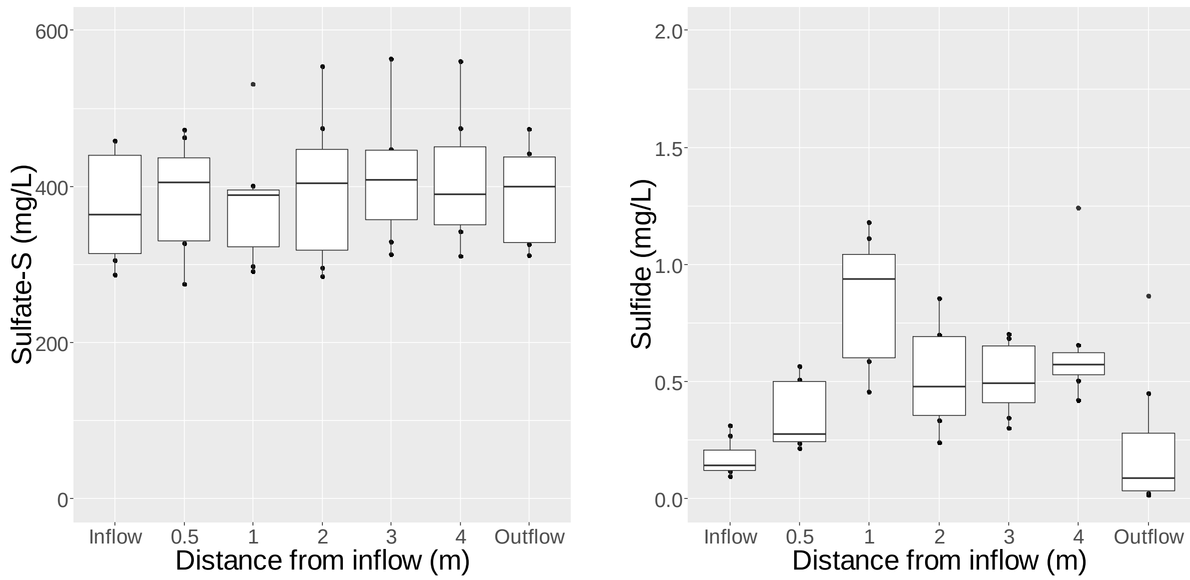


Figure 3.6-4 $SO_4^{2-} - S$ (left) and sulfide (right) concentrations in the pore-water at depth 25 cm of the HSSF CW (n=7).

The concentrations of $SO_4^{2-} - S$ remained similar to inflow levels. In addition, limited sulfide concentrations were measured and S^0 remained below detection limit of 1 mg/L at the HSSF CW throughout the period of observation (April - June 2012). Since the water balance data were not available, DSR activity and load removal of sulfate are viewed and discussed based on findings from Wu et al. (2011) who sampled the same CW at depths 30 and 40 cm. These authors reported higher concentrations of reduced sulfur compounds in these depths than what was found here at depth 25cm. The conditions at the outflow point of the HSSF CW (left) are different from the system, and therefore the 4 m point can be considered as the outflow point, instead of the 6 m point analogous to Wu et al. (2011).

Soil analyses of sulfur and iron

Sufficient material of the soil filter media used to fill the HSSF CW upon construction was protected from any sources of humidity. This material was used as control to define the content of sulfur and iron of the soil prior to the operation of the CW. The assumed accumulation of sulfur in the soil was not proved, as rather a depletion of the original sulfur content than accumulation took place over the span of about 10 years of operation. The measurements of X-ray fluorescence and ICP AES methods were slightly different, with values of the first method always higher than the estimation of the second method (appendix B). Here, only the results of the mid-width samples from X-ray fluorescence spectrometry are presented and discussed.

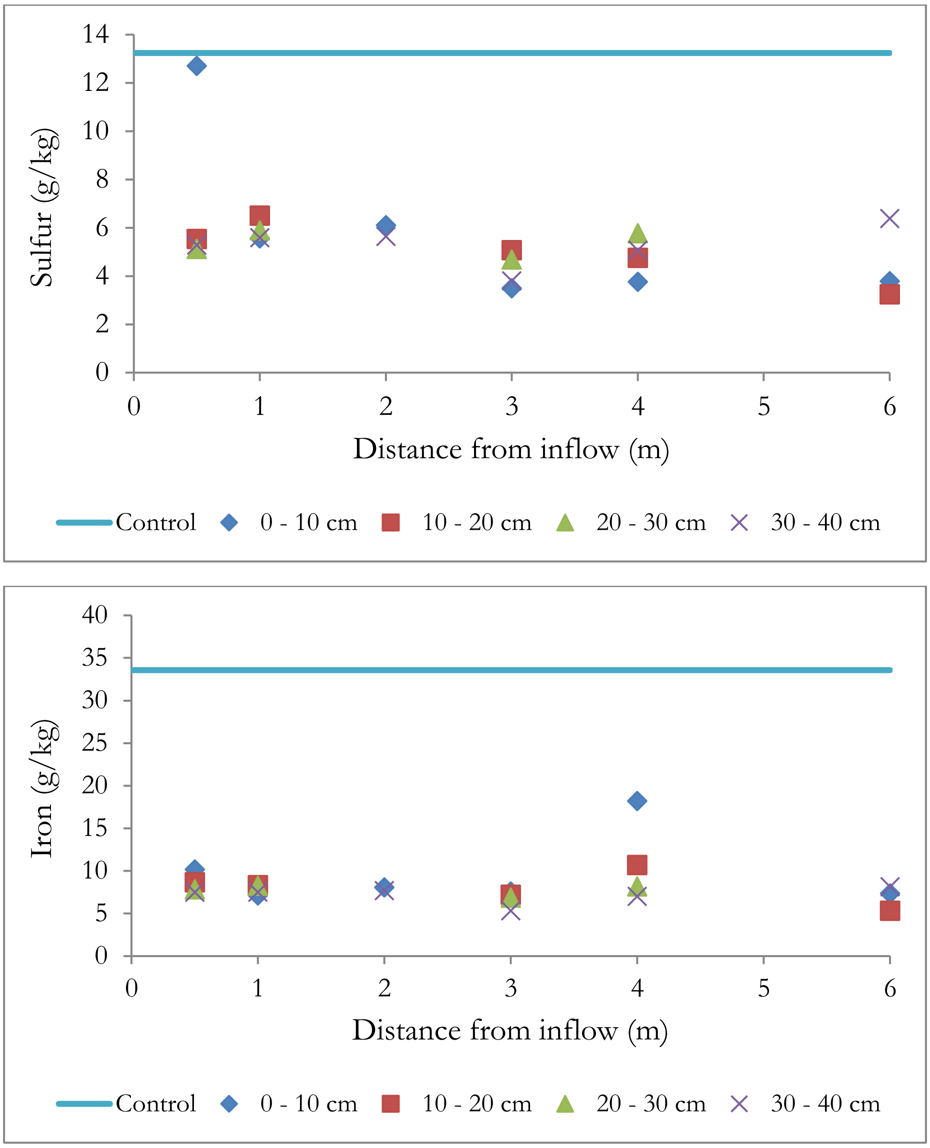


Figure 3.6-5 Sulfur (top) and iron (bottom) distribution in mid-width samples (fraction <200 µm) at different depth fractions. The control values are obtained by analyzing sulfur and iron content in the pristine soil.

As stated previously, it was not possible to capture any AVS content due to the applied drying method, but CRS was preserved and it showed depth-wise distribution with the lower depth reflecting higher CRS content than the top depth layer.

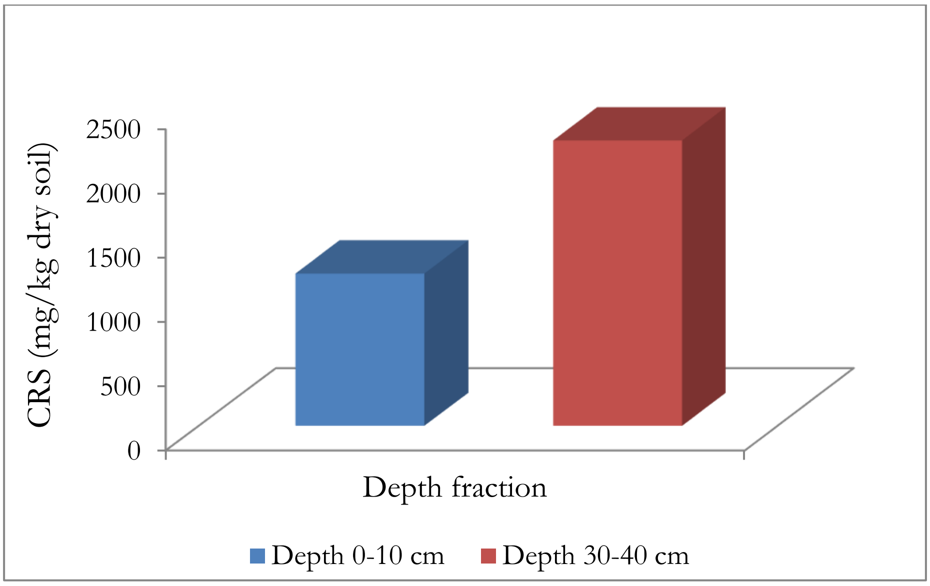


Figure 3.6-6 Depth-wise distribution of CRS in mid-width samples.

On the contrary, the plant matter distribution reflected higher plant matter content in the top depth layer and lower plant matter presence in the deeper layers.

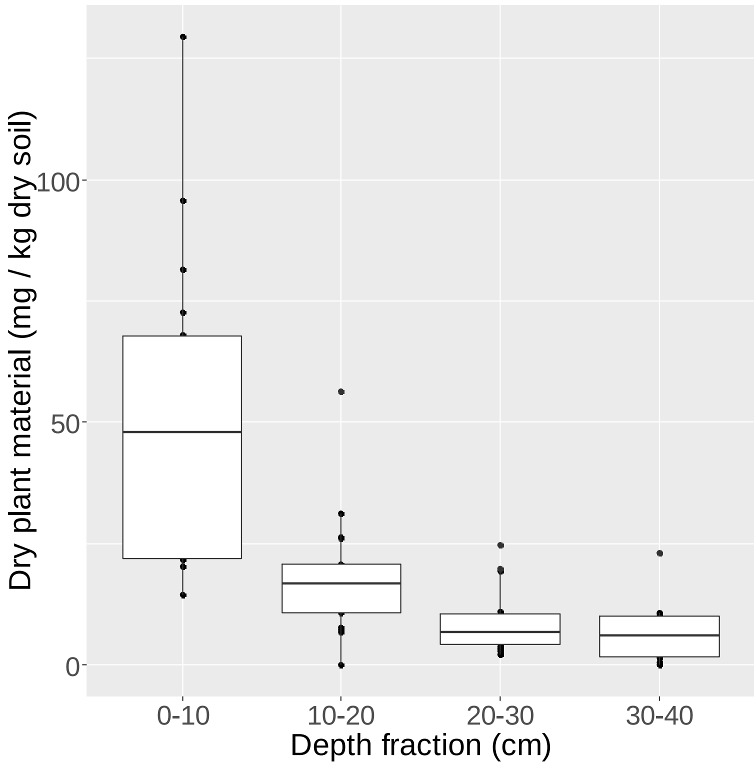


Figure 3.6-7 Plant matter distribution in all 65 samples.

3.6.4 Discussion

The sulfate concentrations showed no significant decrease over the length of the bed, similar to findings of Wu et al. (2011). These authors had included the water balance analyses and calculated a sulfate removal of 21% of the influent GW sulfate (corresponding to 1.75 g S m^{-2} d^{-1}). Their results were from a previous year, but since the system was operating at a steady-state after several years of operation with no major operational changes, their findings are estimated to be representative of the succeeding years. The main organic contaminant was reported to be monochlorobenzene (MCB) (values in Table A-1 from monitoring wells in the region are smaller than values at the pilot-scale site reported by Chen et al. (2012)) and some other chlorinated hydrocarbons. In addition, Chen (2012) has indicated preference of aerobic pathways for dechlorinating processes. The noted removal of sulfate was thus expected to have been boosted by the rhizodeposits from *P. australis* since these compounds were at low concentrations in addition to being at lower bioavailability.

Wu et al. (2011) have as well calculated about 30% of the removed sulfate in other measured reduced sulfur compounds. They then estimated the remaining 70% of the removed sulfate to be immobilized in the CW soil matrix. Firstly, this 70% is an overestimation since other important sulfur compounds such as thiosulfate were not included in the calculated sulfur balance (sections 3.2.3 and 3.5.3 reflected that thiosulfate exist in the pore-water of HSSF-CWs at considerable concentrations). Secondly, Figure 3.6-5 shows that the sulfur was not accumulating in the soil. In addition, soil sulfur content reflected no depth-wise or length-wise spatially distributed and values were in a narrow range between 3 – 6.5 g/kg dry soil. It appears that the sulfur reached steady-state concentrations in the soil and that the remainder of sulfur was rather in other compartments than the soil compartment.

In general, CW systems can be divided into 4 main compartments:

- The pore-water compartment representing the contaminated water intended for treatment;
- The helophyte (from section 3.3.3 the sulfur uptake of *P. australis* was estimated as 0.094 ± 0.019 g S m^{-2} d^{-1}, about 5% of the reported sulfate removal by Wu et al. (2011); in addition, *P. australis* plants here may have even lower sulfur uptake since they are subjected to some phytotoxins in the contaminated GW that are absent in the synthetic wastewater in section 3.3.3);
- The soil compartment;
- The air compartment (including both air volume inside the CW if available and the atmosphere nearby and around the CW).

Thereby, the sulfur release to the atmosphere compartment was underestimated. Additional studies need to be dedicated to measuring H_2S volatilization and phytovolatilization from HSSF CWs.

The opposite trends of CRS and plant material reflect the effect of plants in boosting aerobic conditions and the prevalence of anaerobic processes in the less- or unrooted zones. AVS is expected to be as well higher in the deeper layers of the CW where plant activity is absent. AVS includes metal sulfide precipitates such as iron mono sulfides (e.g. FeS) (Ahern et al. 2004, Morse and Rickard 2004) which is highly reactive and readily oxidizes at exposure to air; whereas CRS includes the more stable forms of sulfur such as pyrite (FeS_2). Further speciation of soil sulfur was not conducted, and the molar ratios of sulfur and iron give incomplete information about their existence.

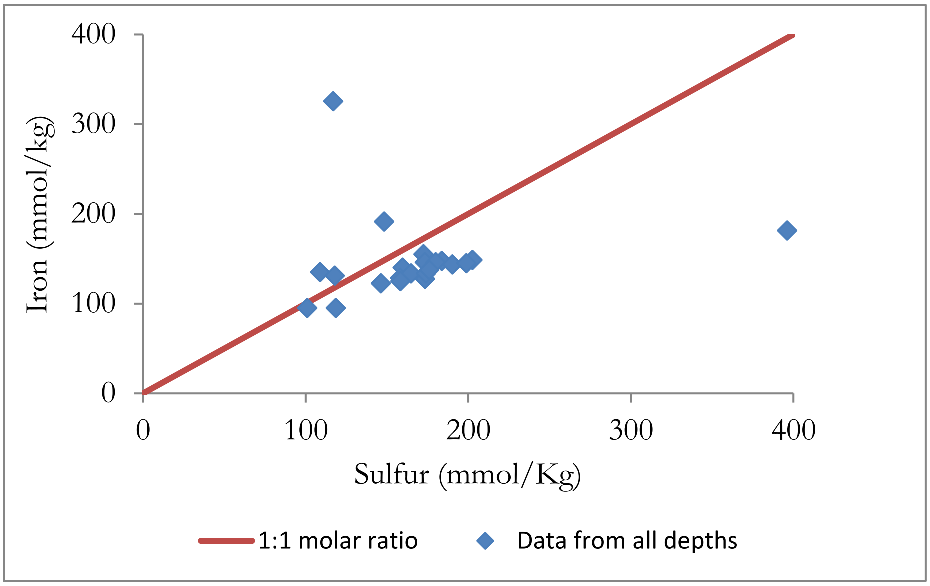


Figure 3.6-8 Coexistence of sulfur and iron in the mid-width soil samples.

In addition, sulfate salt precipitates may represent an important form of oxidized sulfur in the soil. For instance, the reaction of calcium (Ca) with sulfate in acid sulfate soils was reported by Ahern et al. (2004) to produce gypsum ($CaSO_4$) which has low solubility in water (2.6 g/L). From X-ray fluorescence data, Ca was found in the mid-width samples at concentrations of 159 ± 77 mmol/kg dry soil (mean ± standard deviation) and was not depleted in comparison to the pristine soil (control sample had 158 mmol/kg dry soil).

3.6.5 Conclusion

- There was no accumulation of sulfur in the soil matrix. Therefore, a bigger portion of the sulfur load that was removed from the pore-water than previously estimated must have escaped to the atmosphere;
- CRS was considerably higher in deeper layers than in top layers of soil. Oxidized sulfur forms such as sulfate salts are then expected to have had higher concentrations at top layers of the CW, where the plant presence and activity were higher;
- The quantification of H_2S emissions from HSSF CWs and the resulting implications on the environment need to be further investigated.

4. General Discussion and Outlook

Outlook

Alongside VF CWs which are commonly applied in Europe, the two most applied systems worldwide are HSSF CWs and FWS CWs (Kadlec 2009). Based on the findings from this research work, a main focus of future research on sulfur cycling in CWs should be set on HSSF systems, since these systems were found to have dynamic sulfur transformations; and on FHRM systems, to provide better understanding of the sulfur processes in these novel systems. Saturated VF CWs and FWS CWs may be scanned to identify the relevance of sulfur transformations in them, as these systems were not considered in this work. Unsaturated vertical flow systems need not to be allocated much effort related to the sulfur research unless they are applied to treat certain wastewater types that contain sulfur as a major role player (e.g. AMD or influent wastewaters with very high sulfide content).

In addition to limitations discussed in chapter 3 and including among others the short time span of some experiments and the limited number of replicates, another limitation of this research is lack of information about the organic portion of the sulfur cycle. Based on an assumption that the organic sulfur cycling is of smaller proportion to the total sulfur cycling than inorganic sulfur, the former was not investigated. However, the links and feedbacks between the organic and inorganic sulfur cycling need to be further elaborated to identify their importance. Another major limitation of this research is the lack of information on microbial communities at work. The inclusion of microbial consortia identification techniques need be augmented in future research.

Nevertheless, black box investigations are as well beneficial and needed to define design criteria of future CWs. There is room for future optimization and innovation in CWs configurations beyond those summarized by Wu et al. (2014) based on better understanding of the treatment processes in CWs. In general, CWs for wastewater treatment can be engineered in such a way that different types of redox conditions prevail, in order to enhance the removal of several pollutants at the same time. Alternatively, they can be designed in such a way that only selected redox conditions occur and thus only the targeted contaminants will be removed.

The future of wastewater treatment worldwide

The current situation worldwide, as illustrated in appendices D and E, is that sanitation coverage is not coping with the increasing rates of urbanization. The lack of access to proper sanitation starts at not having the very basic sanitation facilities and expands to the unavailability of any infrastructure for wastewater collection and treatment. Due to several advantages of applying CWs for wastewater treatment, they represent an attractive cost-effective alternative technology. In addition to need for wastewater treatment to protect the receiving environments, the wastewater itself is viewed as an indispensable resource in water scarce regions and the reuse of wastewater can be made possible after treatment with CWs as conducted by Masi and Martinuzzi

(2007). It is thus the task of researchers to introduce and make CWs systems more attractive to stakeholders in rural and urban settlements that require sanitation infrastructure.

Potentials for CWs application for wastewater treatment

CWs in tropical and subtropical climates

Considerable portion of the application, research and development of CW technologies takes place in regions with temperate climates (such as Europe and North America). However, a great potential for application of CWs in regions with tropical and subtropical climates exit. Firstly, most of the world's developing countries exist in tropical and subtropical climatic regions; secondly, in these countries the need for improvement in sanitation and wastewater treatment to decrease pollution is greater (www.un.org); and thirdly, the CW technology can witness more efficient application in tropical and subtropical climates since most of the treatment process rates in CWs can be enhanced at higher temperatures and prolonged growth seasons of plants. Kivaisi (2001) reported a strong potential for application of CWs in developing countries, particularly as decentralized option for small rural communities. She attributed the delay of widespread application of these systems to lack of awareness and lack of local expertise to develop the technology on a local basis.

In general, semi-natural wastewater treatment systems such as WSPs and CWs have proven to be better options for developing countries, if land area is not limiting, as their main investment cost lies in their land requirement whilst they require very low running costs and no highly-skilled employees. Additionally, when compared to WSPs, CWs may provide better pathogen removal. Moreover, CWs include types where water losses are minimised and exposure of water surface to environment is prevented (e.g. subsurface flow systems). Such subsurface flow feature which is unique for CWs wastewater treatment technologies implies two big benefits: firstly, as temperatures are relatively high all over the year, lowering the water losses from the treatment systems reduces the risk of soil salinization and is beneficial if water reuse is targeted; and secondly, having plants as a buffer (wastewater surface is not exposed) implies that an important vector is eliminated (e.g. reduce the risk of outbreaks of water-related vector diseases).

Research requirements for CWs application in tropical and subtropical climates

One of the main findings of current research is that the helophytes play several roles in CWs treatment performance (Brix 1997) and that different capacities of different helophytes suggest the conscious selection of helophytes (Brisson and Chazarenc 2009). Hence, it is very beneficial to conduct local research with the locally existing helophytes in a specific region, in order to better evaluate the CWs performance. This will lead to provision of region-specific design standards, since the current design criteria of CWs are rules of thumb obtained from experience and research conducted in countries such as Germany or USA.

In addition, the porous media such as sand or gravel which is incorporated in soil-based CW technologies is one of the major cost items in the construction phase of CWs. Significant savings in construction investments can be provided by applying local material, so if sand and/or gravel are unavailable in a region, economic alternatives could be researched. For instance, crushed glass was used as porous media and did not show higher tendencies to clogging than sand or gravel (Gagnon et. al, in press). This was as well incorporated with the recycling industry, which can add economic value to CWs application. In addition, soil free systems such as FHRMs can be promoted (Chen et al. 2016).

Wastewater treatment in an uncertain world

As mentioned, CWs can be characterized as more flexible systems than the current state of the art high-tech wastewater treatment plants (WWTPs, such as activated sludge systems). For instance, uncertainties in storm events predictions is making urban sewerage and WWTP infrastructure more expensive in combined sewer applications, especially under uncertainties of climate change scenarios. Given land is available at reasonable cost, applying CWs coupled to or instead of conventional systems can help decrease such uncertainty since CWs are more robust to flow fluctuations than conventional WWTPs. Planning of future smart cities (Nam and Pardo 2011) in which control over most of the urban infrastructure will be automated is currently being discussed, but the focus on urban sanitation infrastructure is missing.

In addition, future energy production at sufficient levels to supply a growing population is uncertain. Hence applying CWs is advantageous over systems with high energy demand for operation.

CWs for wastewater treatment in crisis situations

Currently, several conflicts and wars occurring worldwide lead millions of people to flee their homes and gather as refugees, in some cases overstraining the receiving regions. In such cases, if affordable, provision of additional sanitation facilities and wastewater management infrastructure may be implemented. Here, CWs can be as well an attractive alternative.

Coupling CW technologies to other wastewater treatment concepts

A major disadvantage of CWs is the requirement for collection and transport of the wastewater (unless applied at household level). Concepts such as settled sewerage (Mara 1996) can be applied to decrease the costs of wastewater transport.

In addition, the Ecosan concepts and the source separation of contaminated streams in general can be applied in consortium with CWs technologies for wastewater management and resource retrieval and recycling (Masi 2009).

Moreover, the wildlife incorporation (Kadlec and Wallace 2008) and the aesthetic aspects of CWs make them fit in many urban landscape designs, leading to improving both the appearance and the environments of villages and cities. Incorporation and utilization of the aesthetic value of CWs was advocated by several authors such as Brix et al. (2011), Thompson and Sorvig (2007) and Bishop et al. (2012).

Appendices

A. Ground water composition in monitoring-wells in Bitterfeld

Table A-1

Parameter	Unit	SafBit 26 / 98	SafBit 33 / 98
Ethene	µg/L	< 1,0	< 1,0
Vinyl chloride	µg/L	< 5,0	< 5,0
1.1- Dichloroethene	µg/L	< 0,8	< 0,8
Trans-1.2-Dichloroethene	µg/L	< 0,95	< 0,95
cis-1.2-Dichloroethene	µg/L	2.04	3.21
Benzene	µg/L	< 1,30	< 1,30
Trichloroethene	µg/L	< 2,70	< 2,70
Tetrachloroethene	µg/L	< 1,65	< 1,65
Chlorobenzene	µg/L	5.73	6.07
2-Chlorotoluene	µg/L	< 1,40	< 1,40
1,4-Dichlorobenzene	µg/L	< 1,80	< 1,80
1,2-Dichlorobenzene	µg/L	< 1,90	< 1,90
Methane (1. vial)	µg/L	1533	178
Methane (2. vial)	µg/L	1330	192
Chloride	mg/L	116	114
Nitrite	mg/L	<0.01	<0.01
Nitrate	mg/L	<0.15	0.61
Sulfate	mg/L	700	777
Ammonium	mg/L	5.19	3.76
Phosphate	mg/L	0.58	0.43
Fe^{2+}	mg/L	2.2	1.1
Mn	mg/L	0.36	0.373
pH		7.3	7.1
Electric conductivity	µS/cm	1897	2190
O_2	mg/L	0.02	0.07
Redox potential	mV	-80	-3.2

*SafBit refers to GW monitoring wells in the Bitterfeld region. Date of sampling: 30.07.2012. Data directly at the pilot-scale facility are reported by Chen et al. (2014).

B. Differences in soil analyses between X-ray fluorescence and ICP AES methods

From the comparison between the X-ray fluorescence and the ICP AES results, the trend from analyses is similar, and the values from the latter are slightly (15% ± 5.5%) lower than the values from the former method.

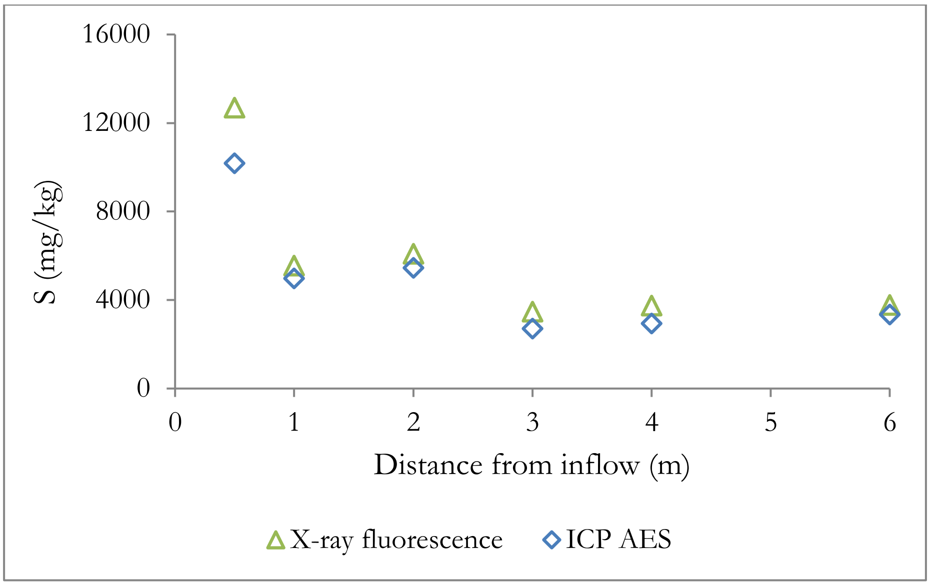


Figure B-1 Sulfur distribution in mid-width samples from 0-10 cm depth fraction

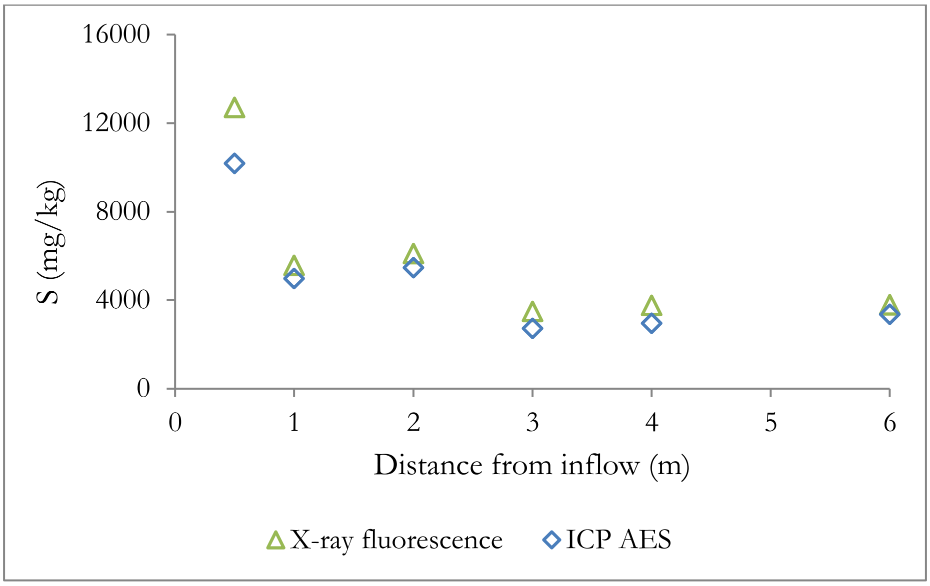


Figure B-2 Sulfur distribution in mid-width samples from 30-40 cm depth fraction

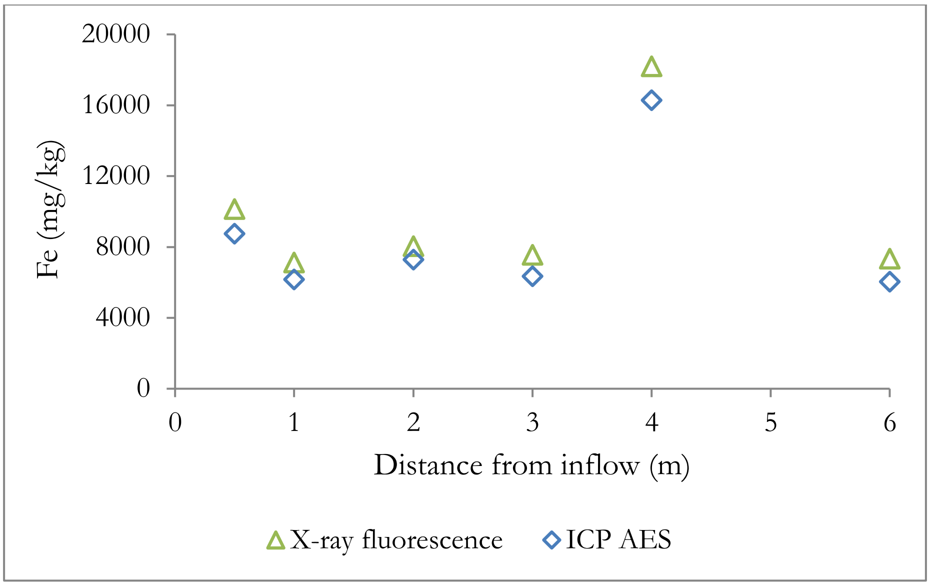


Figure B-3 Iron distribution in mid-width samples from 0-10 cm depth fraction

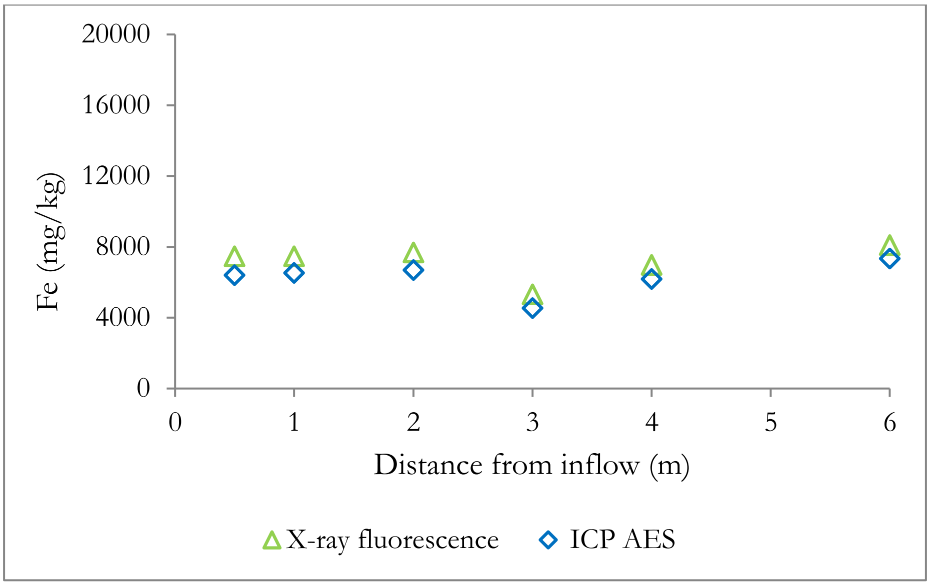


Figure B-4 Iron distribution in mid-width samples from 30-40 cm depth fraction

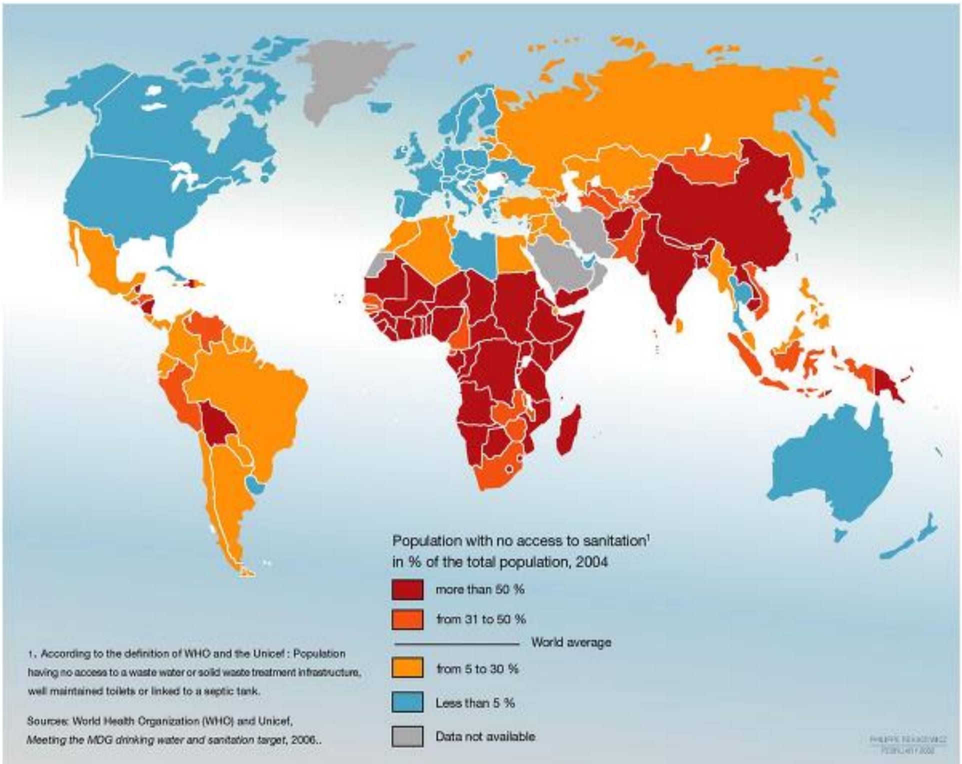
C. Note on water loss and pollutant loads calculations

The equations used for water loss and pollutant loads calculations in the FHRMs in section 3.3.2 were different from the equations used for calculating pollutant load in sections 3.1.2 and 3.2.2 and as well from calculations in section 3.5.2 for the following considerations:

- The FHRMs were indoors and hence rain water consideration was excluded;
- For the systems in Langenreichenbach, the flow data received from collaborators were influent and effluent flow volumes; rain water was already comprised in the effluent water volume. Sulfur input from rainwater was thus not considered in influent load calculation but this is assumed negligible in proportion to the sulfate in the influent wastewater. In addition measurements of sulfate in rainwater was not conducted although Marquardt et al. (2001) have documented the occurrence of sulfate in the rainwater in East Germany resulting from SO_2 emissions from previous decades and succeeding air movement. Therefore, it is recommended in future research to investigate the sulfur content in rainwater.
- The rainwater was separately collected in the pilot-scale systems in the city of Nantes and hence rainwater information was included in the water balance calculations. In addition, samples of rainwater were tested for sulfate content and barely detectable values of sulfate were identified which were estimated below the precision of utilized spectrometer and were thus not considered in influent load calculations.

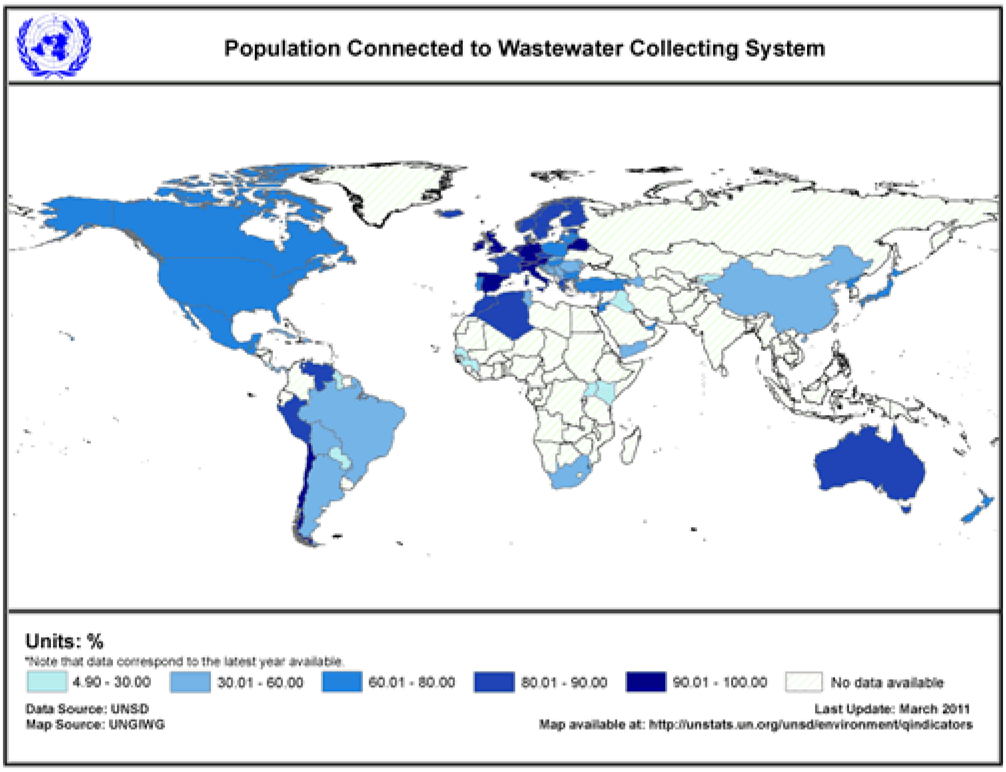
D. Urban population and sanitation coverage worldwide

Source http://www.scidev.net

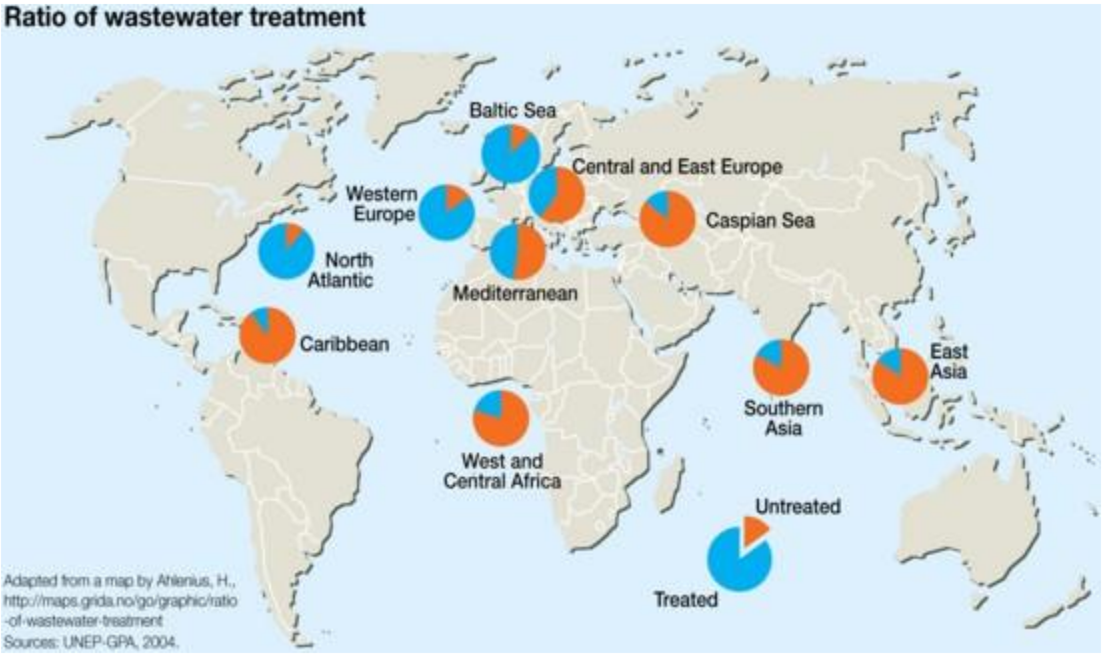


Source: http://www.who.int

E. Wastewater collection and wastewater treatment coverage worldwide



Source: http://unstats.un.org/unsd/environment/wastewater.htm



Year: 2010
From the collection: Sick Water - The Central Role of Wastewater Management in Sustainable Development.
Author: UNEP/GRID-Arendal, Hugo Ahlenius

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Curriculum vitae

Conference contributions

Published proceedings

Pérez Sierra, J., Saad, R.A.B., Arias Escobar, M.A. and Mirzabaev, A. Towards a nexus approach to land, water and waste treatment: economic and methodological aspects. Proceedings of the International Kick-off Workshop: Advancing a Nexus Approach to the Sustainable Management of Water, Soil and Waste. Session Summaries and Case Studies. UNU-FLORES. Dresden from November 11th to 12th, 2013.

Oral presentations

Saad, R.A.B., Kuschk, P., Nivala, J. and Köser, H. Evaluation of sulfur turnover in different types of pilot-scale constructed wetlands treating domestic wastewater. The 5th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL), Nantes from October 13th to 17th, 2013.

Saad, R.A.B., Gagnon, V., Chazarenc, F., Kuschk, P. and Köser, H. Sulfur transformations in horizontal subsurface flow constructed wetlands planted with different macrophyte species. The 6th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL), York from September 13th to 18th, 2015.

Poster presentations

Saad, R.A.B., Kuschk, P., Nivala, J. and Köser, H. Evaluation of technology variation impact on sulphur cycling in pilot-scale constructed wetlands receiving pre-treated domestic wastewater. The 9th International Phytotechnology Society (IPS) Conference. Hasselt University, from September 11th to 14th, 2012.

Saad, R.A.B., Kuschk, P. and Köser, H. Sulfur amount and distribution in the soil-matrix of a pilot-scale horizontal subsurface flow constructed wetland. The 5th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL), Nantes from October 13th to 17th, 2013.

Saad, R.A.B., Kuschk, P., Wiessner, A., Müller, J.A., Kappelmeyer, U. and Köser, H. Plant species influences on constructed wetlands performance. HIGRADE Spring Conference. Leipzig KUBUS, April 29th, 2015.

Awards and Certificates

The 9th International Phytotechnology Society (IPS) Conference: Poster Award, 2nd place.

HIGRADE Spring Conference 2015: Poster Award, 3rd place.

IPSWaT Certificate: PhD scholarship holder from January 1st 2012 to December 31st 2014.

HIGRADE Certificate: awarded 25 CPs on academic and soft skill courses.

Published manuscripts

Wu, S., Kuschk, P., Wiessner, A., Mueller, J., Saad, R.A.B. and Dong, R. (2013) Sulphur transformations in constructed wetlands for wastewater treatment: A review. Ecological engineering 52, 278-289.

Saad, R.A.B., Kuschk, P., Wiessner, A., Kappelmeyer, U., Müller, J.A. and Köser, H. (2016) Role of plants in nitrogen and sulfur transformations in floating hydroponic root mats: a comparison of two helophytes. Journal of Environmental Management.

Submitted manuscripts

Saad, R.A.B., Gagnon, V., Chazarenc, F., Kuschk, P. and Köser, H. Comparative study of the influence of three helophyte species on treatment performance and sulfur cycling in highly-loaded subsurface flow constructed wetlands. To the Journal of Ecological Engineering.

Saad, R.A.B., Wiessner, A., Müller, J.A., Kuschk, P., Hamad, Z.M.A. and Köser, H. Constructed wetlands: an alternative wastewater treatment technology. To the Sudanese Engineering Society Journal.

Manuscripts in preparation

Saad, R.A.B., Nivala, J., Köser, H., Müller, J.A., Pascke, H. and Kuschk, P. Inorganic sulfur turnover in dependence on system type in pilot-scale constructed wetlands receiving domestic wastewater with high organic load.

Saad, R.A.B., Wu, S., Knöller, K., Nivala, J., Köser, H., Wiessner, A. and Kuschk, P. Dynamics and stable isotope abundance patterns of inorganic sulfur pools in pilot-scale horizontal subsurface flow constructed wetlands.

Saad, R.A.B., Kuschk, P., Knöller, K., Wu, S., Mothes, S. and Köser, H. Fate of the sulfur removed from pore-water of a horizontal subsurface flow constructed wetland receiving contaminated groundwater.

Research stays

June – October 2014: guest researcher at the École des Mines de Nantes, Département Systèmes Energétiques et Environnement.

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Postgraduate education

January 2012 – May 2016	PhD student at the Helmholtz Centre for Environmental Research - UFZ, Leipzig and Martin-Luther-Universität Halle-Wittenberg, Halle-Saale, Germany
June – November 2011	Pre-doctoral internship at the UFZ
October 2008 – June 2010	MSc. in Municipal Water and Infrastructure, specialization Sanitary Engineering, UNESCO-IHE Institute for Water Education, Delft, the Netherlands
February – October 2008 and October – December 2010	Postgraduate Diploma in Business Administration (DBA), School of Management Studies, University of Khartoum, Khartoum, Sudan

Graduate studies

December 1998 – May 2003	BSc. (Honours) in Civil Engineering, Faculty of Engineering and Architecture, University of Khartoum, Khartoum, Sudan

Employment information

February – August 2004	Office Engineer at SAS Engineering, Khartoum, Sudan
November 2004 – October 2008 and July 2010 – May 2011	Site Engineer (18 months); then Planning and Supervision Engineer at the Public Corporation for Irrigation Works and Earthmoving (PCIWE), Khartoum, Sudan

Languages

Arabic	Mother tongue
English	*Level C1-C2: proficient user International Diploma in Professional English (for Everyday and Business Use), International Cambridge College, September 2008
French	*Level B2-C1 : proficient user Diplôme D’études en Langue Française (DELF B2), May 2011
German	*Level C1: proficient user
Spanish	*Level B1: independent user

*Self-assessment; levels of the Common European Framework of Reference for Languages.