Treatment of arsenic containing artificial wastewater in different laboratory-scale constructed wetlands

Von der Fakultät Bau- und Umweltingeieurwissenschaften der Universität Stuttgart zur Erlangung der Würde eines Doktors der Ingenieurwissenschaften (Dr.-Ing.) genehmigte Abhandlung

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CONTENTS

List of Abbreviation

AAS	atomic absorption spectrometer/spectrometry
AES	atomic emission spectrometry
As	arsenic
As(III)	arsenite
As(V)	arsenate
AVS	acid volatile sulphide
BOD	biochemical oxygen demand
CW's	constructed wetlands
COD	chemical oxygen demand
DO	dissolved oxygen
DOC	dissolved organic carbon
Eh	redox potential
et al.	and others, (Latin: et alteri)
Fig.	figure
FSW	free surface wetland
F_o	fluorescence of photosystem II
Fm	fluorescence following a pulse of saturation light
Fv/Fm	photochemical efficiency of photosystem II
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
IC	ion chromatography
ICP	inductively coupled plasma
ICP-MS	inductively coupled plasma-mass spectrometry
J. effusus	*Juncus effusus* sp.
MMA	monomethyl arsine
MMAA	monomethylarsonic acid
PAM 2000	pulse amplitude modulated fluorometer
PFBR	planted fixed bed reactor
ppm	part per million
sp.	species, (Latin: species)
SRB	sulphate reducing bacteria
SSW	subsurface wetland
TC	total carbon
TOC	total organic carbon
TMA	trimethyl arsine
TMAO	trimethylarsine oxide
UASB	up flow anaerobic sludge blanket

UFZ	Helmholtz Centre for Environmental Research
WHO	World Health Organisation
WW	wastewater
WWT	wastewater treatment
W1	horizontal flow laboratory-scale constructed wetland one
W2	horizontal flow laboratory-scale constructed wetland two
W3	horizontal flow laboratory-scale constructed wetland three

Summary

Knowledge regarding dynamics of As-species and their interactions under gradient redox conditions in the rhizosphere of treatment wetlands is still insufficient. Therefore, it is necessary to understand the fundamental processes and mechanisms operative in treatment wetlands to realise long-term stability and high As-removal efficiencies, both in the presence and absence of wetland plants. In the past, little attention has been paid to the biotransformation processes and metabolism of As in constructed wetlands. Hence, the aim of this investigation was to gain more information on the biotransformation of As and the dynamics of As- species in predominantly multi-gradient (both micro- and macro-) horizontal subsurface-flow constructed wetlands. Experiments were carried out in laboratory-scale wetland systems, two planted with *Juncus effusus* and one unplanted, using an artificial wastewater containing As (0.2 mg l^{-1}) under defined conditions of organic C- and SO_4^{2-}- loading.

Immobilization of As was found in all systems (both planted and unplanted) under conditions of limited C, obviously due to adsorption and/or co-precipitation. Removal efficiencies were substantially higher in the planted systems (60-70%) as compared to the unplanted system (37% on average). Immobilization under such conditions appeared to decrease over time in all systems.

At the beginning, the dosage of organic carbon (COD ~ 340 mg l^{-1}; SO_4^{2-}-S ~ 50 mg l^{-1}) immediately caused intensive microbial dissimilatory sulphate reduction in all systems (in the range of 85-95%) and highly efficient removal of total arsenic (81-96% on average), most likely as As_2S_3 precipitation. A significant amount of reduced As [As(III)] was found in the effluent of the planted systems (>75% of total As) during this period of efficient microbial sulphate reduction, compared to the unplanted system (>25% of total As). Later on in this operation period, the intensity of sulphate reduction and simultaneous removal of As decreased, particularly in the planted wetlands (ranging from 30-46%). One reason could be the re-oxidation of reduced compounds due to oxygenation of the rhizosphere by the emergent water plants (helophytes). Only traces (2-3 µg As l^{-1}) of DMA were found in the temporal dynamics of the planted wetlands under these conditions.

The immobilization of arsenic was found to be more stable in the planted beds than in the unplanted bed in a more oxidised environment with and restricted microbial sulphate reduction (stopping of inflow C-dosage; SO_4^{2-}-S ~ 0.2 mg l^{-1}).

In principle, both systems (planted and unplanted) were suitable to treat wastewater containing As, particularly under sulphate reducing conditions. The unplanted system seemed to be more efficient regarding the immobilization of As, but planted systems showed a better stability of immobilized As.

Total As-mass retention in planted wetlands was substantially higher (nearly 60% of the inflow) than in the unplanted bed (only 43%). In general, a higher level of As was found in the roots than in the shoots in the planted beds. Only 1% of the inflow As-mass was retained in the shoots while more than 55% was sequestered in and/or on the roots and sediments. However, the retention of As varied widely between each segment along the flow path of the planted wetlands. It was shown that there was a substantial increase in the As-accumulation level in the inflow regions (first half of the bed) and the declination of the As-concentration level was more pronounced along the flow path and in the outflow regions.

In the frame of investigating As-species dynamics within the horizontal subsurface-flow planted soil beds of treatment wetlands, redox dynamics of As-species particularly in the root-near environment of the rhizosphere were also investigated. Therefore, long-term experiments were carried out using a specially designed macro-gradient-free rooted gravel bed reactor, planted with *Juncus effusus* to treat an artificial wastewater containing As (0.2 mg l^{-1}). The exceptional quality of the biofilm processes at the root-surface of helophytes in treatment wetlands were of special importance in this investigation.

The experimental results showed that under carbon surplus and strict microbial dissimilatory sulphate reducing conditions (COD ~ 340 mg l^{-1}; SO_4^{2-}-S ~ 25 mg l^{-1}), a higher As-removal efficiency (up to 82%) was attained, probably due to idealized flow conditions within the rhizosphere (macro-gradient-free rooted gravel bed reactor) compared to the whole multi-gradient prevailed horizontal subsurface-flow planted wetlands (44 - 47%). Under such carbon surplus conditions (COD ~ 340 mg l^{-1}), it was also observed in this specially designed rooted gravel bed reactor that there was a better As deposition and accumulation under a high SO_4^{2-}-S (25 mg l^{-1}) load compared to a low SO_4^{2-}-S (0.2 mg l^{-1}) inflow concentration.

Therefore, it can be concluded from this investigation that SO_4^{2-} loading under carbon surplus conditions is a promising strategy to improve the retention of As in the rhizosphere of constructed wetlands. However, the plants exhibited apparent toxic symptoms, probably due to toxic sulphide formation along with reduced As-species, arsenite [As(III)].

As mentioned earlier, a potentially higher stability of immobilized As was observed in the horizontal subsurface-flow planted wetlands under more oxidized environment with restricted microbial sulphate reduction (stopping of inflow C-dosage; SO_4^{2-}-S ~ 0.2 mg l^{-1}). However, in the root-near environment of the rhizosphere (macro-gradient-free rooted gravel bed reactor), immobilized As showed greater instability, releasing up to 85% total As effluent predominantly as As(V) under such conditions. The findings of this study highlighted the importance of the plants in multi-redox gradient prevailed horizontal subsurface-flow planted soil beds in treatment wetlands.

Based on As mass-balance analysis, the rooted gravel bed reactor with high SO_4^{2-} loading along with wetland vegetation (*Juncus effusus*) was found to retain more than 76% of the total As input, while the 9% unaccounted values were postulated to be due to some other microbial reactions, adsorption and even volatilizations. Similar to the multi-gradient prevailed horizontal subsurface-flow planted wetlands, both plant biomass (specifically roots) and sediments comprise the ultimate sink for As in the rhizosphere (macro-gradient-free rooted gravel bed reactor). A substantially higher mass of arsenic is retained in the roots and sediment (>60% altogether) as compared to shoots (<1%) in the rooted gravel bed reactor system.

In conclusion, the obtained results demonstrated that horizontal subsurface-flow constructed wetlands seem to be viable alternatives for effective elimination of elevated As concentration from secondary domestic wastewater effluents prior to disposal to the receiving water bodies (rivers, lakes etc.) or application for agricultural field irrigation purposes. Long-term accumulation of As in the wetland vegetation (mostly in the below-ground biomass) and soil sediments may reduce widespread distribution of As in the environment.

Zusammenfassung

Motivation – Das weltweite Arsenproblem

Die umwelt- und gesundheitsschädlichen Auswirkungen von Arsen sind in den letzten Jahren immer deutlicher geworden. Das toxische Element hat sich zu einem Anliegen für das Gesundheitswesen entwickelt und wird auch weiterhin ein Gesundheitsproblem darstellen, wenn nicht effektive und innovative Möglichkeiten gefunden werden, um diese giftige Verbindung aus Böden, Sedimenten und Grundwasserquellen zu entfernen bzw. zu reduzieren. Die Situation erweist sich in den Regionen West Bengal und Bangladesh als besonders problematisch, da dort mehrere Millionen Menschen arsenverschmutztem Trink-, Grund- und Oberflächenwasser ausgesetzt sind (Ahmad et al. 1997, Chowdhury et al. 1999, Nickson et al. 1998).

Das Problem hat in den letzten Jahren durch den vermehrten Gebrauch von Handpumpen zur Förderung von Grundwasser zur Versorgung mit Trinkwasser und zur Bewässerung stark zugenommen. Das mit den Handpumpen geförderte Wasser weist in vielen Fällen Arsengehalte auf, die über dem empfohlenen Niveau liegen. Hinzu kommt, dass täglich große Mengen arsenverschmutzten Abwassers generiert werden. In jedem Haushalt wird das verschmutzte Grundwasser für häusliche Zwecke genutzt und anschließend direkt in nahe Gewässer (Flüsse, Seen usw.) entsorgt oder unbehandelt zur Bewässerung von Feldern eingesetzt.

Entwicklungsländer sehen sich enormen Herausforderungen gegenüber gestellt. Eine Verbesserung der Lebensqualität ist normalerweise nur unter schwierigen Bedingungen möglich, da finanzielle Ressourcen nur schwer verfügbar sind. Wasser- und Umweltschutz und der Schutz natürlicher Ressourcen haben nur wenig Aufmerksamkeit erfahren und es wurde weniger investiert, als es für die Verbesserung der Lebensbedingungen notwendig wäre. Für diese Länder ist die Option auf kostengünstiges Wasser und Abwassersysteme, die die Wasserqualität verbessern und Umwelt und natürliche Ressourcen schützen, zwingend notwendig und von großer Wichtigkeit.

Einführung

Arsenverunreinigungen in Böden, Sedimenten und Gewässern entstehen durch verschiedene geogene und anthropogene Ursachen (Chowdhury et al. 1999). Die Adsorption und Copräzipitation von As an Hydroxiden von Fe und Mn, Al-Oxiden und Fe-Sulfiden stellen eine wichtige Senke zur Immobilisierung von As dar (Jacks et al., 2003). Die Redoxtransformation von As ist oft mit der von Sulfat und Eisen gekoppelt (O'Day et al., 2004; deLemos et al., 2006). Die dissimilatorische Reduktion durch Eisen- und Sulfat-reduzierende Bakterien wird weitgehend als der primäre Mechanismus für die in anaeroben Umgebungen stattfindende rapide Reduktion und Freisetzung von As angesehen (Kirk et al., 2004; Islam et al., 2004).

Die Abwasserbehandlung in Pflanzenkläranlagen ist eine relativ neue Technologie mit einigen Vorteilen: es wird keine Energie für die Belüftung benötigt, da die Wurzeln spezieller Pflanzen (Helophyten) den Transport des Sauerstoffs in den durchwurzelten Boden bewerkstelligen (Armstrong 1990a). Doch die Kenntnisse zur Dynamik von As-Spezies und deren Wechselwirkungen unter schwankenden Redoxbedingungen in der Rhizosphäre von Pflanzenkläranlagen sind unzureichend. Deshalb ist es notwendig, die grundlegenden Prozesse und Mechanismen, die in Pflanzenkläranlagen zur Wirkung kommen, zu verstehen, um eine langfristige Stabilisierung und hohe Effizienzen bei der Entfernung von As aus dem Abwasser zu erreichen. In der Vergangenheit blieben die Biotransformationsprozesse und der Metabolismus von As in Pflanzenkläranlagen eher unbeachtet. Das Ziel dieser Untersuchung ist es deswegen, weitere Informationen zur Biotransformation von As und zur Dynamik von As-Spezies in vorwiegend multigradienten (sowohl mikro- als auch makrogradient) horizontal durchflossenen Pflanzenkläranlagen zu gewinnen.

Im Rahmen der Untersuchung der Dynamik der As-Spezies in horizontal durchflossenen bepflanzten Bodenkörpern von Pflanzenkläranlagen wurde auch die Redoxdynamik von As-Spezies, insbesondere in der wurzelnahen Umgebung der Rhizosphäre, untersucht. Dafür wurden längerfristige Experimente mit einem speziell angefertigten makrogradientenfreien, durchwurzelten Kiesbettreaktor, bepflanzt mit *Juncus effusus,* durchgeführt, in dem ein künstliches As-haltiges Abwasser (0,2 mg l^{-1}) behandelt wurde. Die außerordentliche Qualität der Biofilmprozesse an der Wurzeloberfläche von Helophyten in Pflanzenkläranlagen war bei dieser Untersuchung von besonderer Bedeutung.

Ziele

Die spezifischen Ziele dieser Arbeit lagen deshalb in der Untersuchung und der Bewertung folgender Aspekte:

- Charakterisierung der As-Biotransformationsprozesse in der wurzelnahen Umgebung der Rhizosphäre von Modellen von Pflanzenklärsystemen.

- Demonstration der optimalen Bedingungen zur As-Fixierung in Modellanlagen, sowohl mit als auch ohne Bepflanzung.

- Bewertung der Wichtigkeit eines Gradienten (Mikro- und Makro-) bezüglich der Effektivität der Arsenentfernung in Pflanzenkläranlagen mit Mikrogradienten und auch Mehrfachgradienten.

- Ein besseres Verständnis der Bioakkumulation und der Speziation von Arsen in Sumpfpflanzen und die Demonstration der As-Bindung in verschiedenen Teilen von Pflanzenkläranlagen, die zur Arsen-Massenbilanz beitragen.

Material und Methoden

Im Rahmen dieser Arbeit wurden zwei verschiedene Pflanzenkläranlagenmodelle im Labormaßstab untersucht. Dies waren:

1. Laminar horizontal durchströmtes Subsurface-Flow-System:

Aus dem Namen lässt sich ableiten, dass der Fließpfad in diesem System horizontal entlang des Pflanzenklärbeetes verlief. Dieses Pflanzenklärsystem stellte ein realistischeres und praxisnaheres Design dar. Das verschmutzte Abwasser durchläuft bei der Passage durch das System ein Netzwerk von aeroben, anoxischen und anaeroben Zonen (Makro- und Mikro-Redoxgradienten) im Kies, in dem eine Vielzahl von suspendierten und im Biofilm fixierten Mikroorganismen und Pflanzenwurzeln gewachsen sind.

Es wurden Versuche in Pflanzenklärsystemen im Labormaßstab durchgeführt. Dabei wurden zwei mit *Juncus effusus* bepflanzte Systeme (W1, W3) und ein unbepflanztes System (W2), sowie ein künstliches Abwasser mit As ($0{,}2$ mg l^{-1}) und einer festgelegten organischen C- und SO_4^{2-}-Belastung verwendet. Die Experimente wurden über einen Zeitraum von 463 Tagen durchgeführt und die Anlagen W1, W2 und W3 wurden in fünf verschiedenen Experimentphasen (Phasen A, B, C, D und E) betrieben (siehe Tabelle 1). Der Phasenbetrieb wurde durch festgelegte Konzentrationen an organischem C und SO_4^{2-} im künstlichen

Abwasser realisiert, bei einer hydraulischen Verweilzeit (HRT) von 5 Tage während aller Phasen des Experiments.

Anlage	Zulauf Konz. (mg l^{-1})	Experimentphasen				
		A	B	C	D	E
Bepflanzt W1	As(V)	0.2	0.2	-	0.2	-
	SO$_4^{2-}$-S	50	50	0.2	0.2	-
	CSB	-	340	-	340	340
	NH$_4^+$-N	5	30	30	30	30
Unbepflanzt W2	As(V)	0.2	0.2	-	0.2	-
	SO$_4^{2-}$-S	50	50	0.2	0.2	-
	CSB	-	340	-	340	340
	NH$_4^+$-N	5	30	30	30	30
Bepflanzt W3	As(V)	0.2	0.2	-	0.2	-
	SO$_4^{2-}$-S	50	50	0.2	0.2	-
	CSB	-	680	-	340	340
	NH$_4^+$-N	5	30	30	30	30

Tabelle 1: Betriebsbedingungen der horizontal durchflossenen Subsurface-Flow-Systeme während verschiedener Phasen des Experiments (Phasen A, B, C, D und E) im zulaufenden künstlichen Abwasser.

Der Hintergrund für die verschiedenen Phasen war, zu untersuchen, wie die As-Dynamik bei C-Mangel und oxidierten Bedingungen (Phase A), bei C-Überschuss und anaeroben Bedingungen mit einer festgelegten SO$_4^{2-}$-Konzentration (Phase B und Phase D), ohne Zufuhr von organischem C und As(V) (Phase C; SO$_4^{2-}$ nur in Spuren) und ohne Zufuhr von As(V) und SO$_4^{2-}$ (Phase E; nur Zufuhr von organischem C) im Zulauf beeinflusst wurde, sowie, die Stabilität des immobilisierten As in den Anlagenbeeten zu beobachten. Jede Phase hatte eine ausreichende Dauer, um sicherzugehen, dass eine repräsentative Anzahl von Proben von jeder Anlage genommen werden konnte. Porenwasserproben wurden wöchentlich in den Zulauf- und Ablaufzonen jeder Anlage (W1, W2 und W3) in einer Tiefe von 15 cm unter der Oberfläche genommen.

2. Planted Fixed Bed Reactor (PFBR):

Die verschiedenen Prozesse des komplexen Redoxsystems in der Rhizosphäre sind schwer bewertbar, z.B. aufgrund der spezifischen Betriebsbedingungen von Pflanzenkläranlagen, die durch gewöhnlich langsame Fließraten, Makrogradienten der Konzentrationen, die

Möglichkeit von Kurzschlussströmungen und instabile Umweltbedingungen (jährliche und tägliche Zyklen, Wetterereignisse/Fluktuationen) gekennzeichnet sind. Um die temporären Aspekte der variablen Redoxbedingungen in der Rhizosphäre erfolgreich zu untersuchen, wurde ein durchwurzeltes Kiesbettreaktorsystem mit idealisierten Fließbedingungen verwendet (Kappelmeyer et al., 2002). Um den Fließinhomogenitäten (Kurzschlussströme) entgegenzuwirken, verwirklichte dieses System eine permanente Durchmischung bzw. kontinuierliche Rezirkulation des Flüssigkeitsstroms, wodurch Makrogradienten im Wurzelbeet verhindert wurden und daher auch Mikrogradientenprozesse bewertet werden konnten (Kappelmeyer et al., 2002).

Drei Reaktoren im Labormaßstab (PFBR1, PFBR3 und PFBR4) wurden unter der Bedingung der vollständigen Durchmischung des Filterbetts durch kontinuierliche Zirkulation des Porenwassers aufgebaut. Da die internen Fließbedingungen mit den Bedingungen in einem kontinuierlich gerührten Reaktor oder einem ideal durchmischten Gefäß vergleichbar waren, wurden die Makrogradienten bei den Konzentrationen, Eh, pH usw. ausgeglichen und die Effekte der Gradientenänderungen konnten in diesem System leicht bestimmt werden. Das Design und die Prinzipien für den Betrieb des Reaktors sind zuvor schon im Detail beschrieben wurden (Kappelmeyer et al., 2002; Wiessner et al., 2005).

Die Planted Fixed Bed-Reaktoren wurden in sechs verschiedenen Experimentphasen (Phasen A, B, C, D, E und F) betrieben, die durch variierende Konzentrationen von organischem

Reaktor	Zulaufkonzentration (mg l^{-1})	Experimentphasen					
		A	B	C	D	E	F
PFBR1	CSB	-	340	340	340	340	340
	SO_4^{2-}-S	0.2	0.2	0.2	0.2	0.2	0.2
	As(V)	0.2	0.2	0.2	0.2	0.2	0.2
PFBR3	CSB	-	340	340	-	340	340
	SO_4^{2-}-S	5	0.2	5	0.2	5	-
	As(V)	0.2	0.2	0.2	-	0.2	0.2
PFBR4	CSB	-	340	340	-	340	340
	SO_4^{2-}-S	25	0.2	25	0.2	10	-
	As(V)	0.2	0.2	0.2	-	0.2	0.2

Tabelle 2: Betriebsbedingungen der Reaktoren während verschiedener Phasen des Experiments (Phasen A, B, C, D und E) im zulaufenden künstlichen Abwasser.

Kohlenstoff (CSB) und SO_4^{2-}-S mit As(V) im künstlichen Abwasser im Zulauf realisiert wurden (siehe Tabelle 2).

Jede Phase hatte eine ausreichende Dauer, um sicherzugehen, dass eine repräsentative Anzahl von Proben von jedem Reaktor genommen werden konnte. Die Reaktoren wurden in einem Gewächshaus unter festgelegten Umweltbedingungen bei einer Temperatur von 16-22°C betrieben, wodurch ein durchschnittlicher Sommertag in einem gemäßigtem Klima simuliert wurde (Wiessner et al., 2005a). Die Wasserproben wurden wöchentlich in den Zulauf- und Ablaufzonen jedes Reaktors genommen.

Ergebnisse und Diskussion

<u>As-Dynamik in horizontal durchflossenen Subsurface-Flow-Systemen im Labormaßstab:</u>

In allen Systemen (bepflanzt und unbepflanzt) wurde eine Immobilisierung von As unter C-Mangel festgestellt, die offensichtlich auf Adsorption bzw. Copräzipitation zurückzuführen ist. Die Entfernungseffizienz lag bei den bepflanzten Systemen wesentlich höher (60-70%) als bei dem unbepflanzten System (durchschnittlich 37%). Unter diesen Bedingungen schien die Immobilisierung in allen Systemen im Laufe der Zeit abzunehmen (siehe Phase A, Abb. 1).

Zu Beginn verursachte die Dosierung von organischem Kohlenstoff (CSB~340 mg l^{-1}; SO_4^{2-}-S~50 mg l^{-1}) sofort eine intensive mikrobielle dissimilatorische Sulfat-Reduktion in allen Systemen (im Bereich von 85-95%) und eine sehr effiziente Entfernung des Gesamtarsens (durchschnittlich 81-96%), höchstwahrscheinlich durch Fällung als As_2S_3 (Phase B). Während dieser Periode der effizienten mikrobiellen Sulfat-Reduktion wurde im Ablauf der bepflanzten Systeme eine signifikante Menge reduzierten Arsens [As(III)] gefunden, mit >75% des Gesamtarsens im Vergleich zu dem unbepflanzten System mit >25% des Gesamtarsens.

Im späteren Verlauf des Versuchs nahm die Intensität der Sulfat-Reduktion und der gleichzeitigen As-Entfernung ab, insbesondere in den bepflanzten Versuchsanlagen (im Bereich von 30-46%). Ein Grund dafür könnte die Re-oxidation der reduzierten Verbindungen durch Sauerstoffanreicherung in der Rhizosphäre durch die Helophyten sein. Unter diesen Bedingungen konnten nur Spuren (2-3 µg As l^{-1}) von DMA in der zeitlichen Dynamik der bepflanzten Systeme gefunden werden.
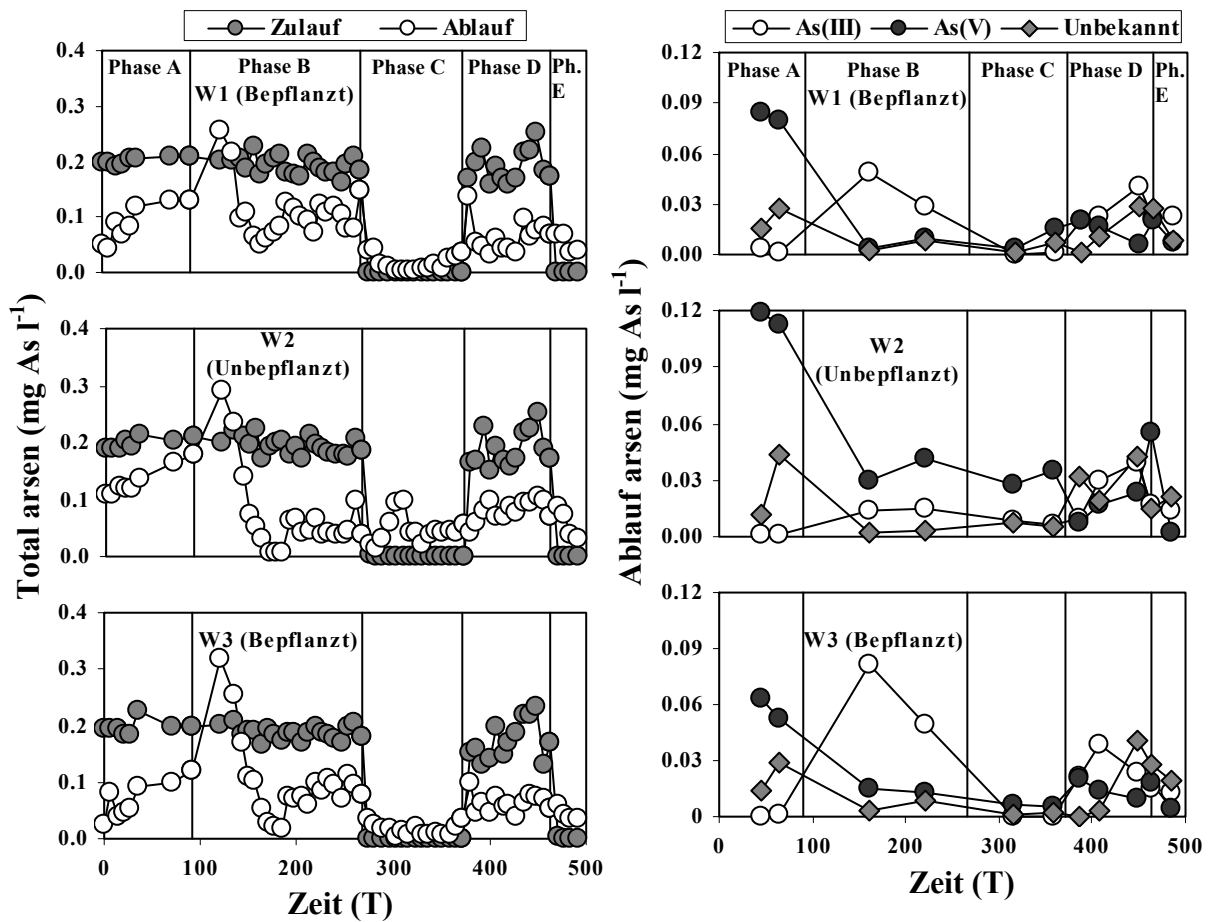
Abb 1: Dynamik des Gesamt-As und der As-Spezies in den entsprechenden Modellanlagen

Die Immobilisierung von Arsen unter stärker oxidierten Bedingungen und bei einer begrenzten mikrobiellen Sulfat-Reduktion (ohne C-Dosierung; SO_4^{2-}-S ~ 0,2 mg l^{-1}) erwies sich in den bepflanzten Systemen als stabiler als in der unbepflanzten Variante (Phase C). Prinzipiell eigneten sich beide Systeme (bepflanzt und unbepflanzt) zur Behandlung von As-haltigem Abwasser, insbesondere unter sulfatreduzierenden Bedingungen. Das unbepflanzte System schien effizienter, was die Immobilisierung von As anbelangt, die bepflanzten Systeme zeigten jedoch eine bessere Stabilität des immobilisierten As.

Die Retention der Gesamt-As-Masse war in den bepflanzten Systemen wesentlich höher (fast 60% des Zulaufs) als in dem unbepflanzten System (nur 43%). Im Allgemeinen wiesen die Wurzeln in den bepflanzten Beeten eine größere Menge As auf als die Triebe. Nur 1% der zulaufenden As-Masse wurde in den Trieben zurückgehalten, während über 55% in bzw. an den Wurzeln und dem Sediment abgelagert wurden. Die Retention von As variierte jedoch stark zwischen den Segmenten entlang des Fließpfades in den bepflanzten Systemen. Es

konnte nachgewiesen werden, dass es einen substantiellen Anstieg in der As-Anreicherung in der Zulaufregion (erste Hälfte des Beetes) und ein sinkendes As-Konzentrationsniveau entlang des Fließpfades und in der Ablaufregion gab.

As-Dynamik im Planted Fixed Bed Reactor:

Bei C-Mangel und unter oxidierten Bedingungen zeigten die Ergebnisse des Experiments eine bessere Leistung bei der Entfernung von As aus dem Abwasser, mit einer Effizienz von über 85%, wobei die Konzentration von SO_4^{2-}-S im Modellabwasser keine Rolle spielte (siehe Phase A, Abb. 2). Trotz der ungünstigen sulfatreduzierenden Umgebung ist die Entfernung von As aus dem Abwasser unter diesen Bedingungen offensichtlich mit der Adsorption von Arsen an Bakterien, Pflanzenwurzeln, organischem Bodensubstanzen und/oder der Adsorption an Oxidmineralien und gleichzeitiger Copräzipitation speziell mit Fe(III)-Oxyhydroxiden zu erklären.
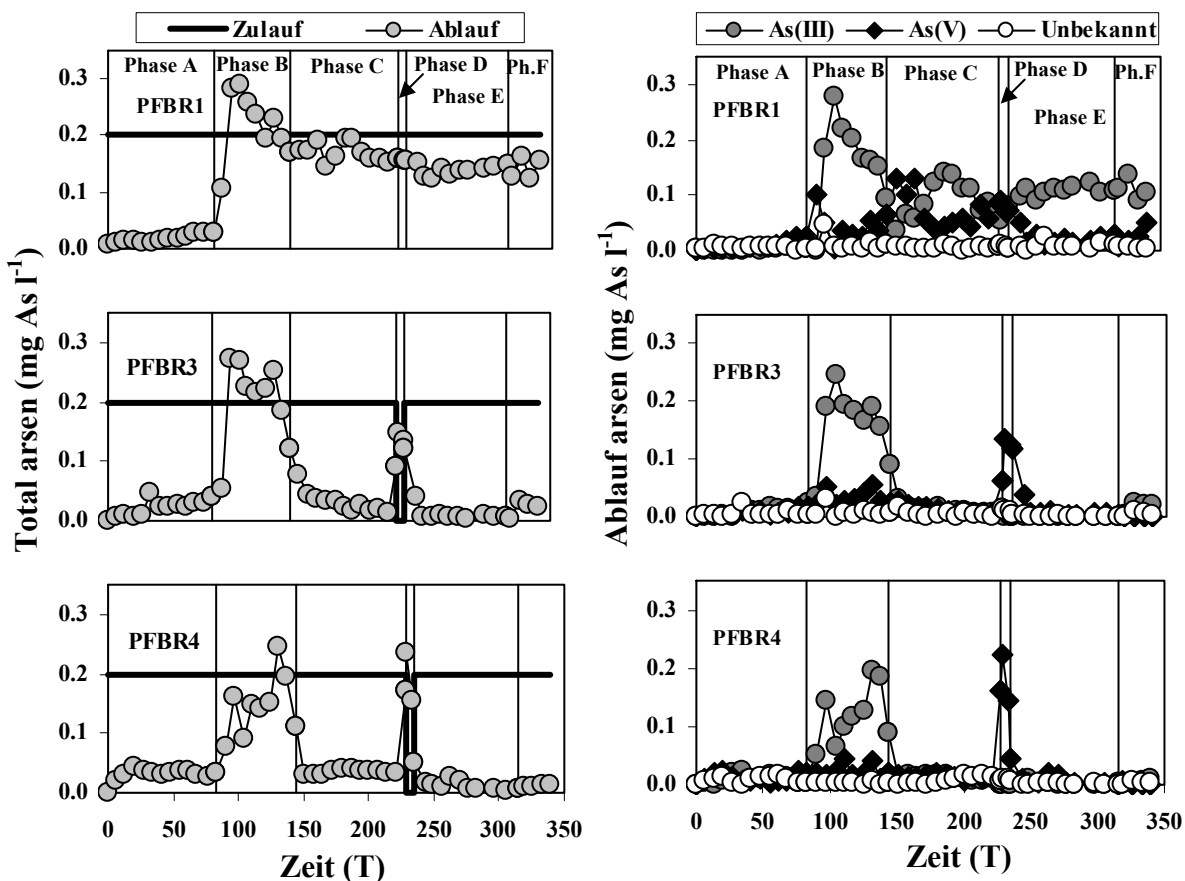
Abb 2: Dynamik des Gesamt-As und der As-Spezies in den entsprechenden PFB-Reaktoren

Bei Kohlenstoffüberfluss und unter den Bedingungen mikrobieller dissimilatorischer Sulfat-

Reduktion (CSB ~ 340 mg l^{-1}; SO$_4^{2-}$-S ~ 25 mg l^{-1}) wurde eine höhere Entfernungseffizienz für As erreicht (bis zu 82%). Dies ist höchstwahrscheinlich den idealen Fließbedingungen in der Rhizosphäre des makrogradientenfreien durchwurzelten Kiesbettreaktors zuzuschreiben (siehe Phase C, in PFBR4).

Unter diesen Bedingungen des Kohlenstoffüberflusses (CSB ~ 340 mg l^{-1}) konnte in dem speziell angefertigtem durchwurzelten Kiesbettreaktor auch beobachtet werden, dass As bei hoher SO$_4^{2-}$-Belastung (25 mg l^{-1}) besser abgelagert und angereichert wurde als bei einer geringen SO$_4^{2-}$-Konzentration im Zulauf (0,2 mg l^{-1}). Es kann deswegen geschlussfolgert werden, dass Bedingungen der SO$_4^{2-}$-Belastung und des Kohlenstoffüberflusses eine vielversprechende Strategie für die Verbesserung der Retention von As in der Rhizosphäre von Pflanzenkläranlagen bilden. Die Pflanzen wiesen jedoch Toxizitätssymptome auf, die wahrscheinlich auf toxische Sulfidbildung und reduzierte As-Spezies, Arsenit [As(III)], zurückzuführen sind.

Wie bereits erwähnt, wurde in den bepflanzten Systemen eine stabilere Immobilisierung von Arsen unter stärker oxidierten Bedingungen und bei einer begrenzten mikrobiellen Sulfat-Reduktion (ohne C-Dosierung; SO$_4^{2-}$-S ~ 0,2 mg l^{-1}) beobachtet. In der wurzelnahen Umgebung der Rhizosphäre (makrogradientenfreier, durchwurzelter Kiesbettreaktor) zeigte das immobilisierte As jedoch eine stärkere Instabilität unter solchen Bedingungen und es wurden bis zu 85% des Gesamtarsens im Ablauf, vorwiegend als As(V), freigesetzt (siehe Phase D, in PFBR3 und PFBR4). Die Ergebnisse dieser Studie heben die Bedeutung der Pflanzen in horizontal durchflossenen bepflanzten Bodenkörpern von Pflanzenkläranlagen mit schwankenden Redoxbedingungen hervor.

Auf Grundlage der Massenbilanz für As wurde festgestellt, dass in dem durchwurzelten Kiesbettreaktor mit hoher SO$_4^{2-}$-Belastung und Vegetation (*Juncus effusus*) über 76% des gesamt zulaufenden As zurückgehalten wurden, während 9% nicht belegte Werte anderen mikrobiellen Reaktionen, Adsorption und sogar Volatilisation zuzuschreiben sind. Ähnlich wie in den multigradienten horizontal durchflossenen, bepflanzten Systemen bildeten die Pflanzenbiomasse (besonders die Wurzeln) und das Sediment die hauptsächliche Senke für As in der Rhizosphäre (des makrogradientenfreien durchwurzelten Kiesbettreaktors). Eine wesentlich höhere Masse Arsen wurde in den Wurzeln und dem Sediment (insgesamt >60%) als in den Trieben (<1%) des durchwurzelten Kiesbettreaktorsystems zurückgehalten.

Schlußfolgerungen und Ausblick:

Zusammengefasst zeigen die Ergebnisse, dass horizontal durchflossene Pflanzenkläranlagen eine realisierbare Alternative für die effektive Eliminierung erhöhter As-Konzentrationen aus häuslichen Abwassern darstellen, bevor diese in Gewässer eingeleitet werden (Flüsse, Seen usw.) oder zur landwirtschaftlichen Bewässerung eingesetzt werden. Die langfristige Anreicherung von As in der Vegetation (vor allem in der unterirdischen Biomasse) und in Bodensedimenten kann die Ausbreitung von As in die Umwelt reduzieren.

Bei der weiteren Forschung sollte Folgendes untersucht werden: (i) großflächige Systeme und deren Kapazitäten bei der Entfernung von Arsen aus dem Abwasser, der Deposition und Remobilisierung von Arsen, Reduktion von As(V) zu As(III) und anderen methylierten Verbindungen, wie MMA, DMA, TMAO usw., (ii) potenzielle Interaktionen und Einflüsse von Schwefelspezies, wie elementarem Schwefel, Sulfit und Thiosulphaten, bei der Entfernung und Transformation von Arsen bei variierenden organischen C- und S-Frachten, (iii) die potenzielle Hemmung von Nitrifikation-Denitrifikation, Sulphidogenese, Methanogenese durch Arsen, (iv) die Muster in der mikrobiellen Ökologie und die Arsentoxizität bei Pflanzen und mikrobieller Biomasse unter dynamischen Redoxbedingungen, (v) toxische Effekte inorganischer Arsenspezies und –sulfide an Pflanzen und Mikroorganismen sowohl unter makrogradientenfreien als auch Mehrfachgradienten-Bedingungen, sowie (vi) intensivere und umfassendere analytische Methoden für methylierte polare und flüchtige Arsenverbindungen.

1 Introduction

Arsenic contaminations of soils, sediments and waters occur from diverse geogenic and anthropogenic sources. The situation becomes particularly problematic in the regions of West Bengal and Bangladesh, where several millions of humans are exposed to arsenic contaminated drinking, ground and surface water (Ahmad et al. 1997, Chowdhury et al. 1999, Nickson et al. 1998). The adsorption and co-precipitation of As on hydrous oxides of Fe, Mn, Al oxides and Fe sulfides are an important sink for As immobilisation (Chao and Theobald, 1976; Forstner, 1985; Jacks et al., 2003). The most important organic complexing agents of As are humic substances, which derive from vegetation (Forstner, 1985).

The redox transformation of As is often coupled with that of sulfate and iron (O'Day et al., 2004; deLemos et al., 2006). Dissimilatory reduction caused by iron- and sulfate-reducing bacteria is widely considered the primary mechanism responsible for the rapid As reduction and release observed in anaerobic environments (Ahmann et al., 1994; Laverman et al., 1995; Oremland and Stolz, 2003; Kirk et al., 2004; Islam et al., 2004).

Due to very limited financial budgets simple methods/systems for wastewater treatment like ponds or wetlands are often preferred (Kadlec and Knight, 1996a; Al-Malack et al., 1998; Mbuligwe, 2004; Davidson et al., 2005; McCardell et al., 2005). The wastewater treatment in constructed wetlands is a relatively new emerging technology with some advantages: no energy for aeration is needed – roots of special plants (helophytes) allow the transport of oxygen to the rooted soil (Armstrong 1990a). Numerous studies have shown the effectiveness of constructed wetlands in terms of sulphur, carbon and nitrogen removal (Kadlec et al., 2000b; Vymazal, 2002; Stottmeister et al., 2003; Sousa et al., 2003; Mashauri et al., 2003; García et al., 2004; Kaseva, 2004, Wiessner et al. 2005a). Sulphate in wetlands initiates Eh and pH changes, C-transformation and, indirectly the mobilization of nutrients (Feng and Hsieh, 1998; Lamers et al., 1998) – all these processes incorporated with arsenic and influences on S, C, N-removal under dynamic gradient redox conditions in constructed wetlands are not yet well understood.

Moreover, it is necessary to understand the fundamental processes and mechanisms operative in treatment wetlands to realize long-term stability of arsenic within wetland beds and highly effective removal efficiencies, both in presence and absence of wetland plants. Because of the very different site-specific wastewater qualities and the very great

variability according to sizing, design and fundamental flow characteristics of the constructed wetlands used, it is quite a challenge to compare the efficiencies, specific removal rates and optimize arsenic removal efficiencies by varying different physico-chemical parameters both in planted and unplanted wetlands. Till now no fundamental data for evaluating the particular importance of the plants, gravel materials and microorganisms are available.

In the case of a domestic and even an industrial wastewater loaded with arsenic and moderate organic carbon and sulphate, constructed wetlands can act as an important sink for arsenic removal. However, knowledge regarding the dynamics of arsenic species and their interactions under gradient redox conditions in planted soil filters is highly limited.

In the past, little attention has been paid to the arsenic bio-transformation processes and metabolism in constructed wetlands. Buddhawong (2004) showed that the reduction of total arsenic concentrations and transformation processes occurred in constructed wetlands, but in fact, virtually no information is available that directly addresses the fate of arsenic and the influences of dynamic redox reactions on root-microbe induced transformation processes.

The role of organic C and S-loads for the post-treatment of anaerobically treated domestic wastewater should be investigated in terms of arsenic removal effectiveness. Both chemical and microbial transformation processes of arsenic within the rhizosphere under dynamic redox conditions and their role in the complex network of As-fixation by varying organic C- and S-load are necessary for better understanding of the "black box" rhizosphere and for optimum design and operation of constructed wetland systems. Sulphide may be highly toxic to microorganisms and macrophytes, and is a competitor for the consumption of oxygen (Armstrong et al., 1996b; Chambers et al., 1998; Lee, 1999; Koch et al., 2001; Pedersen et al., 2004); along with more toxic and mobile As(III) within the root vicinity, the exhibited toxicity upon wetland plants and microorganisms should also be investigated.

This study evaluates the ability of macro-gradient free planted fixed bed reactors (PFBR) and multi-redox gradient (both micro- and macro) prevailed horizontal subsurface-flow model constructed wetlands to remove As from a synthetic wastewater which resembled an arsenic-containing secondary domestic effluent. Plant growth tolerance, efficiencies and behavior in dynamic redox conditions, biotransformation and probable detoxification of arsenic under constructed wetland conditions and a quantitative mass-balance in planted

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fixed bed reactors and also in both planted and unplanted wetlands were prime focus of this investigation. Using a system of planted fixed bed reactor- PFBR and laboratory-scale horizontal subsurface-flow constructed wetlands, both with the presence and absence of plants, As-removal efficiencies and constitution of plants were investigated and evaluated.

Moreover, transformation of As(V) to As(III) and other methylated species and possible formation of As_2S_3 after dissimilatory sulphate reduction under reducing environmental conditions within the rhizosphere which resulted from different carbon and sulphate loading rates, plant root activity etc. were closely investigated in this study.

Therefore, this study was undertaken to gain a greater understanding of the fate of As in the rhizosphere of *Juncus effusus* under constructed wetland conditions and to elucidate the effects of differing redox conditions on arsenic resulting from different organic C- and SO_4^{2-} concentrations. To our knowledge the influences of different org. C- and SO_4^{2-} loading on the arsenic removal had not been explored in horizontal subsurface-flow wetlands prior to this study.

1.1 Objectives

The specific objectives of this work were to focus and assess following aspects:

- To characterize As-biotransformation processes in the root-near environment of the rhizosphere of model wetland systems

- To provide a demonstration of optimum conditions for As-fixation within model wetlands, both in presence and absence of wetland plants

- To evaluate the significance of gradient (both micro- and macro) on the effectiveness of arsenic removal under both micro-scale gradient and multi-gradient prevailed constructed wetland conditions

- To achieve better understanding on the bioaccumulation and speciation of arsenic in wetland plants and to demonstrate As-sequestration in different parts of constructed wetland which contribute towards a quantitative mass-balance of arsenic.

1.2 Motivation – arsenic problem worldwide

The detrimental health effects of environmental exposure to arsenic have become increasingly clear in the last few years. This toxic element is a public health concern and will remain a health concern if an effective and innovative way to remove or reduce this toxic compound in soils, sediments, and groundwater sources is not found. High concentrations detected in groundwater from a number of aquifers across the world, including in South and East Asia, have been found responsible for health problems ranging from skin disorders to cardiovascular disease and cancer. Countries affected include Bangladesh (the worst affected), West Bengal-India, Myanmar, Nepal, Cambodia, China (including Taiwan), Vietnam and parts of Argentina, Chile, Hungary, Mexico, Western USA etc. Smedley and Kinniburgh (2002) and Mandal and Suzuki (2002) provided reviews of typical arsenic concentrations in natural waters, as well as in different countries world-wide.

The problem has increased greatly in recent years with the growing use of tubewells (hand pumps) to extract groundwater for water supply and irrigation. The water delivered by these tubewells has been found in many cases to be contaminated with higher than recommended levels of arsenic. Moreover, huge amount of arsenic contaminated wastewater are being generated on daily basis in every household after domestic purposes of using contaminated groundwater and dispose directly to the nearby water bodies (rivers, lakes etc.) or uses in agricultural fields untreated. Developing countries face enormous challenges of promoting quality of life usually under very adverse condition of financial resources availability. Water and environment protection, natural resources conservation are among those items that have deserved less attention and received less investment that needed for the improvement of living conditions. For these countries, the option for low cost water and wastewater treatment systems that provide water quality improvement associated to environmental protection and natural resources conservation is mandatory and of high importance.

Effluents from anaerobic bioreactors cannot be discharged into receiving water bodies without further post-treatment. In this way, the search for post-treatment alternative arose to permit the application of anaerobic reactors even under very restrictive situations. The search for alternative solutions related to wastewater treatment systems has taken advantages of some favorable environmental condition that amplify the range of applications of non-conventional systems. In order to remove contaminants (e.g. arsenic),

passive biological treatments, for instance, are preferred technologies rather than physico-chemical methods, which are expensive and may generate toxic residues. The anaerobic digestion step can be interlinked with further treatment in a constructed wetland but due to lack of sufficient knowledge and experiences some questions arises such as possible toxicity effects on plants, the transformation processes of the arsenic and sulphur compounds, the different redox processes within the rhizosphere of wetland plants and other influencing factors are not yet well understood and special attention must be paid to achieve highly efficient and sustainable arsenic removal from the wetland systems which may contribute and promises to a far better environment.

2 Literature Review

2.1 Arsenic and arsenic species

Arsenic is a ubiquitous trace metalloid and is found in virtually all environmental media. For an introduction to the element arsenic (As), first discovered in 1250 by the German scholastic Albertus Magnus, several detailed reviews exist, e.g., Cullen and Reimer (1989); Fowler (1983); Korte and Fernando (1991); Mandal and Suzuki (2002); Matschullat (2000); Merkel and Sperling (1998); Nriagu (1994a); Nriagu (1994b); Rüde (1996); and Smedley and Kinniburgh (2002). Drinking of As-contaminated groundwater is perhaps the most common exposure pathway of humans to arsenic toxicity. The biggest known As calamity occurred in the Bengal Delta (Bangladesh/West Bengal) where millions of people depend on As-rich drinking water (Chakraborti et al., 2001).

Many As-species appear in the environment. Different pathways of As transformation and speciation are illustrated in Figure 2.1. The pathways are either promoted by microorganism or are abiotic chemical reactions. The two major chemical pathways of As or heavy metal transformation are oxidative and reductive pathways depending on the redox state of the environment. Adriano (1986) postulated different pathways of As-species production from As compounds. It was found that 14% to 15% of the As applied in soil could be lost through volatilization of arsines each year.

2.1.1 Geogenic occurrences

The semi-metal or metalloid As ranks 44th in abundance in the earth's crust with average concentrations of 2.0 ppm in the upper crust and 1.3 ppm in the lower crust (Wedepohl 1995). It is mainly associated with magmatic or sedimentary rocks, especially iron ores (detailed review in Smedley and Kinniburgh 2002). Average concentrations in soils vary significantly, depending on the parent rock and inhomogenities. Geochemical sources of As-contaminated soils include this arsenic-rich parent material as 'As' easily substitutes for Si, Al or Fe in silicate minerals (Bhumbla and Keefer, 1994). Arsenic is also commonly associated with sulfides, e.g. in sulfidic ore deposits. Other natural sources of As include volcanic activities, windborn soil particles, sea salt sprays and microbial volatilisation of arsenic (Nriagu, 1990; Frankenberger and Arshad, 2002).

Arsenic is mobilized in the environment through a combination of natural processes. These include weathering reactions, biological activity, and volcanic emissions. Arsenic can be

found in more than 245 minerals. About 60% of these are arsenates, 20% sulphides and sulfosalts and the remaining 20% include arsenides, arsenites, oxides and elemental arsenic (Onishi, 1996). Major As containing minerals are the sulfidic ores of which arsenopyrite is the most common, realgar, and orpiment, as well as arsenides of copper, lead, silver, or gold (World Health Organisation, 1981). Primary source of terrestrial arsenic is mineral ores, including Arsenopyrite ($FeAsS$), Realgar (AsS), Orpiment (As_2S_3), and Arsenolite-Claudetite (As_2O_3) (Mandal and Suzuki, 2002).

Arsenic occurs in soils in some regions naturally from weathering of arsenic-rich mineral deposits. Inorganic As exists primarily as thermodynamically stable pentavalent arsenate and more soluble trivalent arsenite. These oxyacids interconvert and equilibrate depending on the prevailing redox potential and pH.

2.1.2 Sources of and anthropogenic uses

Presence of As at elevated concentrations in soils is due to both anthropogenic and natural inputs. Number of human activities i.e. anthropogenic sources include mining and smelting processes, application of As-based insecticides, herbicides, fungicides, algaecides, crop desiccants, sheep dips, wood preservatives, dyestuffs, the combustion of fossil fuels, feed additives and compounds for the eradication of tapeworm in sheep and cattle (Adriano, 2001). Arsenic was also used as a prominent medicine to treat rheumatism, malaria, sleeping sickness, and syphilis until the introduction of Penicillin in 1909 (Gorby 1994). More recent applications range from metal processing to chemical industries.

However, As is still released to the environment from numerous sources, e.g., high-temperature combustion (oil, coal, waste, cement), compost and dung (growth stimulant e.g., for poultry), glass ware production (decoloring agent), electronic industries (GaAs or InAs as semiconductor material), ore production and processing, metal treatment (lead and copper alloys, admixture in bronze production), galvanizing industry, tanning industry (depilation agent), ammunition factories, chemical industry (dyes and colors, wood preservatives, pesticides, pyrotechniques, drying agent for cotton, oil, and solvent recycling), and pharmaceutical and cosmetic industry (Ishiguro 1992; Léonard 1991).

The use of arsenical pesticides and herbicides has decreased significantly in the last few decades, but their use for wood preservation and feed additives is still common. The environmental impact of using arsenical compounds can be major and long lasting, although the effects of most are relatively localized. Most environmental As problems

recognized today are the result of mobilization under natural conditions.

2.1.3   Geochemistry of arsenic

Arsenic belongs to group V of the periodic table, has the atomic number 33 and an atomic mass of 74.92. It is considered a semi-metallic compound or metalloid and is widely distributed in the earth's crust and presents at an average concentration of 2 mg kg^{-1}. Arsenic has five valence states: -3, 0, +1, +3, and +5 (Welch et al., 1988). Under aerobic environment, the predominant form of arsenic is As (V). Arsenate (AsO_4^{3-}) and its various protonation states, H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, AsO_4^{3-} precipitate when metal cations are present (Evanko and Dzombak, 1997). Metal arsenate complexes are stable under specific conditions. As(V) possesses the ability to co-precipitate with or adsorb onto iron (III)oxyhydroxides under acidic conditions. Most of inorganic arsenic compounds that are widely present in soil and groundwater are As(III) and As(V) depending on the environmental pH and its redox conditions (Wang, 2006).

Under reducing conditions, thermodynamically stable form of arsenic is As(III) when it exists as arsenite (AsO_3^{3-}) and in its protonated forms, H_3AsO_3, $H_2AsO_3^-$, and $HAsO_3^{2-}$. As(III) can precipitate with sulphides and co-precipitate with metal sulphides and has a high attraction to other sulphur compounds (Evanko and Dzombak, 1997, Inskeep et al., 2002). Under extreme reducing conditions, elemental arsenic and arsine AsH_3 may be present. Elemental arsenic occurs rarely, and arsines have been identified from fungal cultures and other strongly reducing environments (Bentley and Chasteen, 2002).

At near-neutral pH the solubility of most trace-metals is severely limited by precipitation or co-precipitation (e.g. oxide, hydroxide, carbonate or phosphate mineral, strong adsorption to hydrous metal oxides, clay or organic matter). Arsenate, in contrast, tends to become less strongly sorbed as the pH increases (Dzombak and Morel, 1990). The binding of As to different solid phase metal oxides via the formation of thermodynamically stable inner-sphere complexes is well documented in literature (Fendorf et al., 1997; Manning and Goldberg, 1997; Manning et al., 1998; Raven et al., 1998).

Among the As-species, As(V) binds more strongly with the metal oxides of Fe and Mn, as compared to the As(III) species. However, the binding mechanisms are dependent on the pH and redox potential of the environment. Adsorption affinity for As(V) is higher at low pH and for As(III), at higher pH values (Masscheleyn et al., 1991; Yang et al., 2002). An increase in pH can result in desorption of arsenic due to the lower stability of otherwise

stable metal oxide-arsenic complexes (Pierce and Moore, 1982; Masscheleyn et al., 1991; Raven et al., 1998). Arsenate is generally the stable oxidation state in oxygenated environment. In a reducing environment, reductive dissolution of As containing iron oxides and hydroxides can also result in increased concentrations of dissolved As (Pierce and Moore, 1982; Nickson et al., 2000; Meng et al., 2001). Arsenic and its species occur in crystalline, powder, amorphous or vitreous forms. In the presence of extremely high concentrations of reduced sulphur, dissolved arsenic-sulphide species can be significant. The precipitation of orpiment (As_2S_3), realgar (AsS) or other sulphide minerals may be favored by acidic, reducing conditions. However, the theoretical behavior is not necessarily followed because of the affects of biological processes on individual arsenic species.

All these facts suggest that As is capable of transforming to an immobilized precipitate form. However, arsenic mobility increases as pH increases (Evanko and Dzombak, 1997). This provides a limitation to the bioremediation of As because once the compound is immobilized; variations in pH can reverse arsenic's immobility to mobility.

2.1.4   Speciation chemistry of arsenic

Arsenic may undergo several complex transformations such as oxidation-reduction and biochemical methylation (Anawar *et al.*, 2004). It is a redox-sensitive trace element and its fate in the environment strongly depends on its oxidation state and speciation. Inorganic As-species usually prevail in soils, unless they were contaminated with organic arsenical pesticides. Under waterlogged conditions, the microbial decomposition of organic matter leads to the depletion of O_2 and to the reductive dissolution of Fe(III)oxyhydroxides in soils (Ponnamperuma, 1972). Adsorbed As(III) and As(V) are concomitantly released into soil solution and As(V) gets reduced to As(III) (Masscheleyn et al., 1991; McGeehan and Naylor, 1994; Onken and Hossner, 1996).

Arsenic speciation is controlled by pH and Eh. $H_2AsO_4^-$ is the dominant species under oxidizing conditions at low pH. At higher pH (> ~ 9.9) $HAsO_4^{2-}$ becomes dominant. H_3AsO_4 and AsO_4^{3-} may be present in extremely acidic and alkaline conditions, respectively. At pH less than ~ 9.2, H_3AsO_3 will predominate (see Fig 2.2). Generally, Eh-pH conditions determine the As(V)/As(III) ratio but microbial action can also promote oxidation/reduction and methylation (Ebdon et al. 2001)

However it was not until 1973 that As speciation became an issue, when Braman and Foreback (1973) introduced the first hydride generation technique, capable of separating

different inorganic and methyl As compounds even in small concentrations. Even though mobility, degradability, and toxicity vary significantly between the different species, there is still a lack of standard procedures for As speciation, especially for the methylated or volatile As-species.
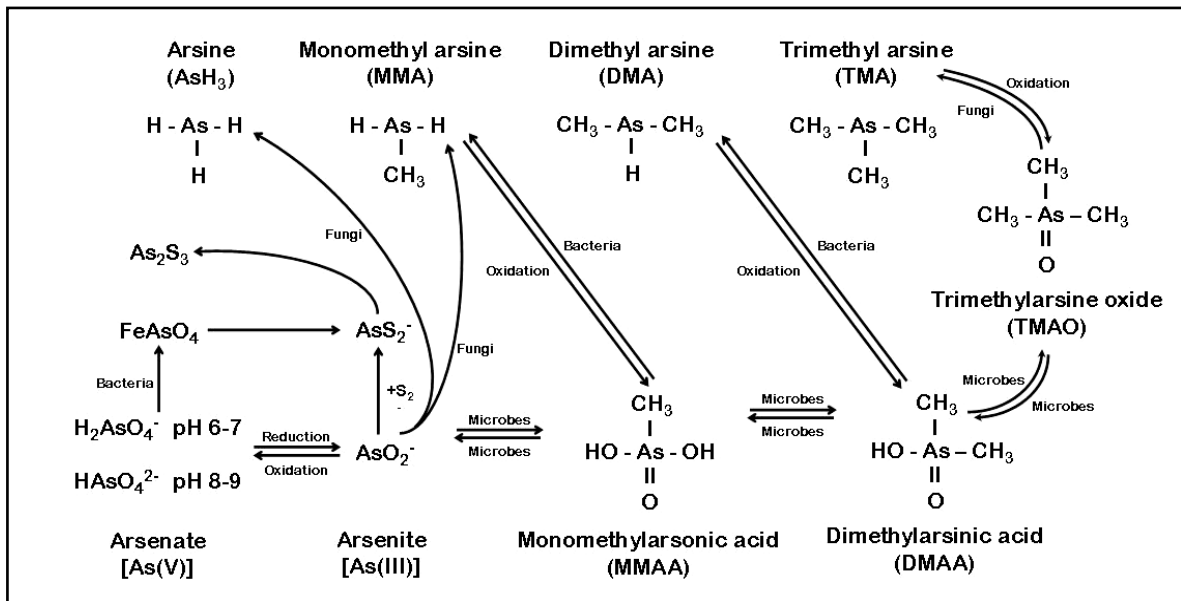
Figure 2.1 Chemical forms of arsenic and their transformations (Source: Modified from Bhumbla and Keefer., In: Nriagu ed., 1994)

Bio-transformation through methlylation of arsenic can result in methylated compounds of arsenic, such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsenic oxide (TMAO) (Evanko and Dzombak, 1997). As (V) can be mobilized under reducing conditions and reducing conditions consisting of alkaline, promote the formation of As (III) with the presence of organic compounds that form compound units with arsenic (Evanko and Dzombak, 1997).

2.1.5   General toxicity of arsenic

Toxicity and chemical behavior of As compounds are largely influenced by the form and speciation of As. As(III) is more mobile and considered to have a higher acute toxicity than As(V) (Penrose, 1974, Adriano, 1986). Compared to As(V), As(III) was found to be about 18-50 times more toxic by Petrick et al. (2000) and 25-60 times more toxic by Morrison et al. (1989). Moreover, inorganic As species are more toxic and less mobile than organic As species (Wauchope, 1975; Holm et al., 1980; Chiu and Hering, 2000; Mandal and Suzuki, 2002). Gaseous arsines are the most toxic (see Fig 2.1) whereas arsenobetaine and

arsenocholine (mainly found in marine organisms) are the least toxic or non-toxic.

$H_3AsO_4^0$

$H_2AsO_4^-$

$HAsO_4^{2-}$

AsO_4^{3-}

$H_3AsO_3^0$

$H_2AsO_3^-$ AsO_3^{3-}

$HAsO_3^{2-}$

Eh (mV)

pe

pH
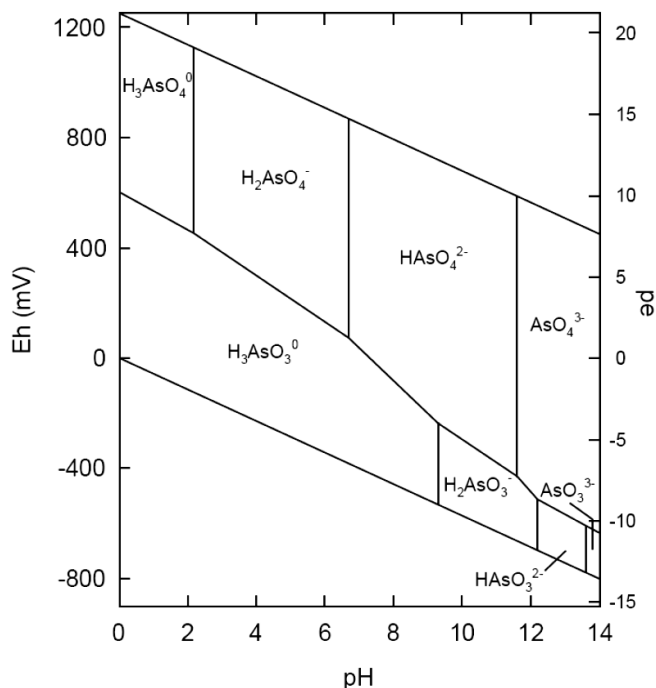
Figure 2.2 Eh-pH diagram of aqueous arsenic species (World Health Organisation, 2001)

The toxic effects of As on living organisms are well documented (Niragu, 1994), with each valence state having distinct toxic properties. Much of the toxicity of As(III) is associated with the ability of this trivalent oxyanion to form bonds with functional groups of proteins. Chronical effects include bronchitis, myocardial infarction, arterial thickening, peripheral neuropathy, hyperkeratosis, hyper-pigmentation, the so-called "black foot" disease (necrosis, mainly on palms and soles, first identified in Taiwan), skin (Col et al. 1999; Tsuruta et al. 1998), lung, bladder (Hopenhayn-Rich et al. 1996a), liver and kidney cancer, as well as teratogenic effects (inorganic As can cross the placenta), mutagenic changes, and genotoxicity (Carson et al. 1986; Florea et al. 2004; Mandal and Suzuki 2002).

Metabolism of inorganic As to methylated forms by living organisms adds to many soil environments a range of organic arsenic compounds of generally lower toxicity (Cullen and Reimer, 1989). A detailed recent review about arsenic toxicology is provided in Chou et al. (2000). There are many species and strain differences in the toxicity of arsenic compounds. The purity, physical form and solubility of the compounds also influence toxicity (Stoeppler, 2004).

The most toxic form of As are gaseous As compounds, followed by dissolved organic trivalent, inorganic trivalent, inorganic pentavalent, and organic pentavalent arsenic

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compounds, and last but not least elemental arsenic and arsenosugars. Therefore, the toxicity of As compounds can be described in the following decreasing order (Stoeppler, 2004, Hindmarsh and McCurdy, 1986): $AsH_3 \gg As_2O_3 >$ easily soluble As(III) (e.g. K and Na arsenite) > less soluble arsenite (e.g. Cu arsenite) > As(V) > As-sulfide > metallic arsenic > organic pentavalent arsenic > elemental arsenic > arsenosugars. The fatal human dose for ingested arsenic trioxide or an alkaline arsenite for an adult is assumed to range from 60 to 300 mg As_2O_3 (Baselt and Gravey, 1995).

2.1.6 Bio-transformation of arsenic species

2.1.6.1 Methylation of arsenic

Both inorganic trivalent and pentavalent As compounds can be methylated. The process is not purely chemical but requires the involvement of a living organism and, presumably, the intervention of As within the metabolic pathways of the cells. Cullen and Reimer (1989) as well as Bentley and Chasteen (2002b) provide excellent reviews about the As bio-methylation pathway. Methylation of inorganic As species by aerobic and anaerobic microorganisms produce monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAO) (Cullen and Reimer, 1989; Sadiq, 1997; Bentley and Chasteen, 2002). Tetramethylarsonium ion (TETRA), arsenobetaine (AsB), arsenocholine (AsC), and arsenosugars, are thought to be originated from biosynthesis, e.g., by algae or microorganisms (Pongratz, 1998; Geiszinger et al., 2002). Abiotic As methylation is possible, e.g., in the presence of methyliodide, but is rarely observed. Bacteria, algae, fungi, vascular plants, and animals can methylate arsenic like other metalloids/metals such as mercury (Cullen et al., 1979; Frankenberger and Arshad, 2001). There are two series of methylated arsenic compounds. The methylated As(V) compounds include:

- Monomethylarsonic acid (MMA), $CH_3AsO(OH)_2$ and its salts

- Dimethylarsinic acid (DMA), $(CH_3)_2AsOOH$ and its salts

- Trimethylarsenic oxide (TMAO), $(CH_3)_3AsO$

These As(V) compounds are produced by algae, cyanobacteria, arthropods, fish, mammals, and other organisms. They are much less toxic than As(V); each added methyl group showed about 10-fold decreases of the toxicity. The monomethyl forms are produced by many aerobic organisms as a way of detoxifying arsenic.

Hasegawa et al. (2001) reported that methylarsenic (III) species could be produced by phytoplankton in freshwater. MMA (III) and DMA(III) were released as metabolites from the biosynthetic pathway for methyl-arsenicals by *Closterium aciculare*. The methylated species are believed to be detoxification products (Kneebone and Hering, 2000). In most streams, less than 1% of the arsenic is methylated. In lakes, particularly eutrophic ones, over 50% of the arsenic may be methylated. There is definitely a seasonal variation in the amount of methylation that seems to be related to variations in the water's microbial ecology as temperatures change.

Sohrin et al. (1997) studied the seasonal variations of arsenic species in lake water in the mesotrophic northern and eutrophic southern basins of Lake Biwa in Japan. It was found that within the eutrophic zone, As(III) increased in spring and fall, and dimethylarsinic acid (DMA) became the dominant form in summer. Monomethylarsonic acid (MMA) and trivalent methylarsenic species such as [monomethylarsonous acid, MMA(III), and dimethylarsinous acid, DMA(III)] also appeared, although they were always minor fractions.

Temperature seems to be one decisive factor in the methylation process. Howard et al. (1982, 1984) described a temperature threshold, stating that no methylation occurs below 9-12 °C. The methylation reaction pathway as indicated already in Figure 2.1 is a sequence of alternating reduction steps (including the replacement of OH-groups by CH_3-groups) and oxidizing steps. The principle was already proposed by Challenger (1945), however, detailed information about the compounds and enzymes involved was only gathered in more recent research studies on laboratory animals or humans.

Like all organometallics, organic As compounds are thermodynamically unstable. However, their decomposition kinetics is so slow that they may have a transient existence under a variety of environmental conditions. For microbial de-methylation from DMAA to As(V) by an estuarine microbial culture, Sanders (1979) reports a rate of 1 ng l^{-1} d^{-1}. Chemical de-methylation is very slow. However, oxidation, reduction, hydrolysis, and reactions with sulphur compounds can significantly alter distribution, volatility, and mobility of biologically produced compounds.

2.1.6.2 Volatilization of arsenic

Volatile arsenicals, so-called As hydrides, are As compounds with a boiling point below 150°C. They can be formed either as intermediates or end products of the As

biotransformation pathway as indicated in Figure 2.1 or in a purely chemical reaction. This chemical hydride generation is commonly used in analytical techniques. Volatile methyl and hydride derivatives of metal(loid)s are found in gases released from natural environments, such as sediments, wetlands, and hydropogenic springs, as well as from anthropogenic environments such as wastewater treatment plants and waste deposits (Michalke et al., 2000). The first indication about the existence of volatile As and it's garlic smell is cited in Gmelin (1839).

In aquatic environments, algae and cyanobacteria (blue-green algae) methylate arsenate to monomethyl and some dimethyl As(V), some of which is excreted and some of which is retained. The plankton (e.g. shrimp) consumes the algae and produces a higher percentage of dimethyl (As). The animals higher on the food chain consume the plankton and methylate the arsenic further, producing some trimethylarsine oxide. According to Cheng and Focht (1979), *Pseudomonas* and *Alcaligenes* (*Achromobacter*) produce arsine (AsH_3) both from arsenite and arsenate under anaerobic conditions. Michalke et al. (2000) incubated sewage sludge anaerobically. They detected 0.76 ng l^{-1} AsH_3 and according to this study, sulfate-reducing bacteria are not able to form AsH_3. It is unstable under atmospheric conditions and is readily oxidized by oxygen. There is also a series of methylated As(III) compounds:

- Monomethylarsine, CH_3AsH_2
- Dimethylarsine, $(CH_3)_2AsH$
- Trimethylarsine, $(CH_3)_3As$

All these compounds are extremely toxic. AsH_3 is the most toxic inorganic form of As, causing immediate hemolysis. Fewer than 250 cases of arsine gas poisoning have been reported in the past 65 years, half of which were fatal (Gorby 1994). Immediate death occurs at 150 $\mu g\ m^{-3}$. Extensive hemolysis ending in death follows 30 minutes of exposure to 25 to 50 $\mu g\ m^{-3}$ and less than 30 minutes after 100 $\mu g\ m^{-3}$ (Ellenhorn 1997). Like AsH_3, Monomethylarsine [$(CH_3)AsH_2)$], Dimethylarsine [$(CH_3)_2AsH$] can be produced by methanogenic bacteria *Methanobacterium formicicum* (Michalke et al. 2000) and other anaerobes. Trimethylarsine [$(CH_3)_3As$] is the most stable volatile As compound. It is also the most toxic form, except AsH_3 because arsines gain toxicity with each added methyl group. Like the other volatile As compounds it can be produced by *Methanobacterium formicicum* (Michalke et al. 2000). According to Wickenheiser et al. (1998), it was the

only volatile As species formed by sulphate-reducing bacteria and the peptolytic bacteria *Clostridium collagenovorans, Desulfovibrio gigas* and *Desulfovibrio vulgaris*. But small amounts of AsH_3 were also detected in cultures of *Desulfovibrio gigas* (Michalke et al., 2000).

Methanogenic and sulphate-reducing bacteria, for instance, are able to transform inorganic As to volatile species (Michalke et al. 2000; Wickenheiser et al. 1998) and thereby it was suggested that this volatilization can be an important process removing arsenic from wetlands. Arsines may travel in air for long distances or they are oxidized rapidly depending on environmental conditions (Pongratz, 1998). Oxidation returns arsenic back to inorganic species and the cycle of arsenic is completed because atmospheric inorganic arsenic is deposited back to soil by rain or by dry deposition (Pongratz, 1998)

2.1.7   Role of micro-organisms in arsenic transformation and mobility

The importance of arsenic in microbial ecology and its biogeochemical cycle have also been realized only recently (Oremland and Stolz, 2003, Oremland et al. 2004, Oremland et al. 2005, Rhine et al. 2005). The activities of arsenic-metabolizing microbes can affect the speciation and mobility of arsenic. Arsenate-respiring bacteria can liberate arsenic [As(III)] from sediments (Ahmann et al. 1997, Jones et al. 2000), from adsorptive sites of aluminum oxides or ferrihydrite (Zobrist et al. 2000), or from arsenate-containing minerals (i.e., scorodite) (Newman et al. 1997). Recent studies in Bangladesh have implicated microbial processes as a key contributor to arsenic contamination in near- and sub-surface aquifers (Harvey et al. 2002, Islam et al. 2004, Nickson et al. 2000, Oremland and Stolz, 2003, 2005). Aerobic and anaerobic respiratory processes form one central class of processes that strongly influence arsenic biogeochemistry, both directly and indirectly. As stated earlier, As(III) is more toxic and soluble than As(V) (Lovely, 2001). Recent studies suggest that the As(V) reducing microorganism, *Sulfurospirillum barnessi*, has the capability to reduce and mobilize arsenate As(V), which was co-precipitated on ferrihydrite.

Within the past seven years, microorganisms have been discovered in a great diversity of anoxic environments that are able to generate energy by coupling the oxidation of H_2 or organic carbon to the reduction of inorganic As(V), forming inorganic As(III) (Ahmann et al. 1994, Cummings et al. 1999, Dowdle et al. 1996, Laverman et al. 1995, Macy et al. 1996, Newman et al. 1997, Newman et al. 1998). Certain bacteria and fungi appear to detoxify arsenicals by reducing them to arsine, As(-III), in both inorganic and methylated

forms (Cheng and Focht. 1979, Cullen and Reimer. 1989). In addition, some algae have been shown to reduce arsenate to arsenite, presumably for detoxification purposes, but this purpose has not been confirmed (Sanders and Windom. 1980). Finally, certain bacteria and algae, as well as many higher organisms, may incorporate arsenic into organic compounds such as arsenocholine, arsenobetaine, and other arsenosugars (Andreae and Klumpp 1979, Cullen and Reimer 1989).

Therefore, microbes play an important role in many reactions that have an influence on the speciation of arsenic. The inorganic forms of arsenic, As(III) and As(V), can be oxidized or reduced due to microbial activity (Woolson, 1977). Inorganic arsenic species can also be biomethylated to monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and trimethylarsine oxide (TMAO) (Woolson, 1977; Cullen and Reimer, 1989; Gadd, 1993; Turpeinen et al., 1999), while the other microbes can demethylate organic forms back to inorganic species (Sohrin et al., 1997). Water-soluble arsenic species can also be volatilized by microbes to gaseous arsines (Bachofen et al., 1995; Gao and Burau, 1997, which are extremely toxic compounds to mammals (Buchet and Lauwerys, 1981). As(V) and As(III) can be volatilized to arsine [AsH_3], MMAA to monomethylarsine [MMA, $(CH_3) AsH_2$], DMAA to dimethylarsine[DMA, $(CH_3)_2 AsH$] and TMAO to trimethylarsine [TMA, $(CH_3)_3As$] (Cullen and Reimer, 1989).

It has been concluded that microorganisms play the defining role in catalysing the redox transformations that ultimately control the mobility of metalloid (Oremland and Stolz, 2003)

2.1.7.1 Arsenate reducing bacteria

Research has discovered anaerobic bacteria species that can achieve respiratory reduction of As (V) to As (III) (Zobrist, 2000). Respiratory reduction of As (V) to As (III) is called dissimilatory reduction and involves bacteria strains, *Sulfurospirillum arsenophilum* strain MIT-13 (Zobrist 2000; Ahmann et al., 1994) and *Sulfurospirillum barnesii* strain SES-3 (Zobrist 2000; Laverman et al., 1995; Oremland, 1994), *Desulfotomaculum auripigmentum* strain OREX-4 (Newman et al., 1997b) and *Chrysiogenes arsenatis* strain BAL-1T (Macy et al., 1996).

Sulfurospirillum barnesii strain SES-3 was found in selenate-respiring enrichment from the Massie Slough marsh in the Stillwater Wildlife Management Area of western Nevada. It belongs to the epsilon subdivision of the Proteobacteria. SES-3 also grows on nitrate but

not on sulphate (Laverman et al., 1995; Oremland, 1994).

Sulfurospirillum arsenophilus strain MIT-13 was isolated from arsenic contaminated sediments near the Industry-Plex Site, a superfund site in Woburn, MA. It is in the epsilon subdivision of the Proteobacteria and MIT-13 grows on nitrate, but not on sulphate (Ahmann et al., 1994).

Desulfotomaculum auripigmentum strain OREX-4 is a newly discovered bacterium and was isolated from surface sediments of the Upper Mystic Lake in Winchester, MA. It is a gram-positive bacterium and has a hexagonal S-layer on its cell wall. It grows on scorodite mineral. Moreover, it grows on lactate with arsenate or sulphate as an electron acceptor but does not respire nitrate (Newman et al., 1997b). This bacterium can precipitate arsenic trisulphide (As_2S_3), as a result from the reduction of As(V) to As(III), both intra- and extracellularly. It is suggested that As_2S_3 formation might be important in the biogeochemical cycle of arsenic.

Chrysiogenes arsenatis strain BAL-1T was isolated from a reed bed at the Ballarat Goldfields in Australia. It is gram-negative and appears to be the first representative of a new deeply branching lineage of the bacteria (Macy et al., 1996).

2.1.7.2 Arsenite oxidizing bacteria

Arsenite [As(III)] is more toxic than arsenate, because it inhibits dehydrogenises and some other enzymes due to its ability to react with the functional −SH groups of cystein residues in proteins (Ehrich, 2001; Santini et al., 2001). There are a number of bacteria which are able to oxidize arsenite into the less toxic pentavalent form, arsenate [As(V)].

Bacterial oxidation of arsenite to arsenate was first described in 1918 (Green, 1918). *Bacillus arsenoxydans*, was isolated from an arsenical cattle dip in South Africa by including organic matter in the form of dung extract in the medium. Turner (1949, 1954) assigned the isolates of 15 arsenite-oxidizing bacterial strains which were isolated by including organic matter in the medium and were therefore heterotrophic arsenite oxidizers. *Pseudomonas arsenoxydans-quinque* was presumably the most rapid oxidizer. This is considered synonymous with *Alcaligenes faecalis* (Ehrlich, 1996).

Pseudomonas arsenitoxidans was found as being able to grow using energy gained from arsenite oxidation. It was isolated from a gold-arsenic deposit and found to grow chemo-litho-autotrophically with oxygen as the terminal electron acceptor, arsenite as the electron

donor, and carbon dioxide as the sole carbon source (Ilyaletdinov and Abdrashitova, 1981).

Another chemo-litho-autotrophic arsenite oxidizer, designated NT-26, is the fastest arsenite oxidizer reported to date with a doubling time of 7.6 hr when grown chemo-litho-autotrophically. This organism was isolated from the Granites gold mine in the Northern Territory, Australia (Santini et al., 2000).

2.1.7.3   Mobilisation of arsenic in the environment

Sorption onto iron and manganese oxide solids and precipitation in sulphide solids in anoxic environments, appear to be the two primary mechanisms governing As mobility in aqueous, soil, and sedimentary environments (Bodek et al. 1998, Sadiq 1997). Oxidation of As and environmental conditions affects its mobility.

Arsenic is mobilized in the environment through a combination of natural processes. These include weathering reactions, biological activity, and volcanic emissions.

The oxidation of arsenic-rich pyrite in aquifer sediments has been proposed as one of possible mechanisms of arsenic mobilization (Dus et al. 1996, Chowdhury et al. 1999). Other studies have suggested that the reductive dissolution of arsenic-rich Fe(III) oxyhydroxides deeper in the aquifer may lead to the release of arsenic into the groundwater (Smedly and Kinniburgh 2002, Nickson et al. 1998, 2000, Harvey et al. 2002). Addition of acetate as a potential electron donor for metal reductin and a proxy for organic matter resulted in a marked stimulation in the rate of Fe(III) reduction followed by arsenic release (Lovely and Chapelle 2003, Islam et al. 2004)

Biological processes aid with the facilitation and mobilisation of As. The case study '*Mobilisation of arsenite by dissimilatory reduction of absorbed arsenate'* discusses microbes that can mobilize arsenite. This experiment discovered that Fe(III)-respiring bacterium mobilizes As. The bacterial strains *Sulfurospirillum arsenophilum* and *Sulfurospirillum barnessii* both can release As into the environment. This suggests that microorganisms can be responsible for spreading of As in soil and groundwater sources. It also confirms that microorganisms hold the ability to transform As(V) to As(III).

2.1.8   Kinetics of arsenic precipitation during bacterial sulphate reduction

Sulphate-reducing bacteria fulfill their energy needs by reducing sulphate. Sulphate reduction goes along anaerobic oxidation of organic compounds, with alkalinity production

and arsenic precipitation as sulphides (e.g. orpiment As_2S_3) like:

$$3SO_4^{2-} + 6CH_2O \rightarrow 3H_2S + 6HCO_3^{-} \quad (1)$$

$$2As^{3+} + 3H_2S \rightarrow As_2S_3 + 6H^+ \quad (2)$$
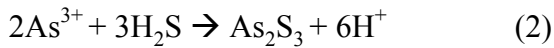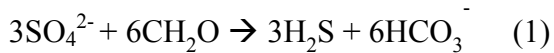
where CH_2O is a simple organic molecule.

pH values between 6 and 9, permanent reducing conditions, prone to react organic substrates favor the reaction (1). Bacterial catalysis is essential, since geological times are required to reduce any significant masses of sulphate at low temperatures, even as high as 100°C (Powell and Macqueen, 1984; Krouse et al., 1988).

2.2   Technologies for arsenic removal from the environment

2.2.1   Background

Of the various sources of arsenic in the environment, drinking water probably poses the greatest threat to human health. Drinking water is derived from a variety of sources depending on local availability: surface water (rivers, lakes, reservoirs and ponds), groundwater (aquifers) and rain water. These sources are very variable in terms of arsenic risk. Alongside obvious point sources of arsenic contamination, high concentrations are mainly found in groundwaters. These are where the greatest number of, as yet unidentified, sources are likely to be found (Smedley and Kinniburgh, 2002).

The groundwater pollution caused by arsenic in a number of Asian countries has led to a major environmental crisis. Some recent estimates indicate that more than 35 million people in West Bengal (India), Nepal and Bangladesh are potentially at risk from drinking arsenic-contaminated water (Smith et al., 2000).

In some arsenic affected areas, substitution of drinking water source by a safe and easily available one may not be possible during part or all of the year, or may be very expensive. Arsenic removal may be a more appropriate water supply option in these situations. In areas where the drinking water supply contains unsafe levels of arsenic, the immediate concern is finding a safe source of drinking water. There are two main options: finding a new safe source, and removing arsenic from the contaminated source. In either case, the drinking water supplied must be free from harmful levels of arsenic, but also from bacteriological contamination, and other chemical contaminants (Johnston and Heijnen, 2001).

In all cases, technologies should meet several basic technical criteria as followings:

- First of all, the systems must be economically feasible

- Should able to produce water of the required quality, both chemical and bacteriological

- Must able to supply water in adequate quantity, throughout different seasons

- Technologies should be robust and must be convenient

- It is important that operational safety be ensured

- Technologies should not have an undue adverse effect on the environment

- Finally, technologies must be socially acceptable to community members in order to be successful as a long-term safe water supply option.

The biggest challenges ahead lie however in applying the technologies described in poor, rural settings, and in enabling those communities to choose safe sources of water for drinking and cooking.

2.2.2 Technologies available

There are several methods available for removal of arsenic from water in large conventional treatment plants. The most commonly used technologies include oxidation, co-precipitation and adsorption onto coagulated flocs, lime treatment, adsorption onto sorptive media, ion exchange resin and membrane techniques (Cheng et al., 1994; Hering et al., 1996, 1997; Kartinen and Martin, 1995; Shen, 1973; Joshi and Chaudhuri, 1996). A detailed review of arsenic removal technologies is presented by Sorg and Logsdon (1978). Jackel (1994) has documented several advances in arsenic removal technologies. In view of the lowering the drinking water standards by USEPA, a review of arsenic removal technologies was made to consider the economic factors involved in implementing lower drinking water standards for arsenic (Chen et al., 1999). There are nine general categories of remediation processes for arsenic removal:

- Precipitation/Co-precipitation;

- Sedimentation;

- Coagulation;

- Filtration;

- Adsorption;

- Oxidation;

- Ion exchange;

- Membrane Processes;

- Biological processes.

Most of the established technologies for arsenic removal make use of several of these processes, either at the same time or in sequence.

2.2.2.1 Precipitation / Co-precipitation

Chemical precipitation is the process by which dissolved ions in solution form an insoluble solid via a chemical reaction. Naturally occurring dissolved iron in groundwater (in Bangladesh, for example) when exposed to oxygen, forms a precipitate. Simple sedimentation for at least 12 hours will allow the arsenic to combine with any iron present and settle to the bottom. This may reduce the concentration of arsenic in the water by as much as 50%. Precipitation causes dissolved arsenic to form a low-solubility solid mineral, such as calcium arsenate. This solid can then be removed through sedimentation and filtration. When coagulants are added and form flocs, other dissolved compounds such as arsenic can become insoluble and form solids, this is known as co-precipitation. The solids formed may remain suspended, and require removal through solid/liquid separation processes, typically coagulation and filtration.

2.2.2.2 Sedimentation

Sedimentation is the gravity separation of solids from liquid by settling. It is generally used in conjunction with precipitation or coagulation.

2.2.2.3 Coagulation

Historically, the most common technologies for arsenic removal have been coagulation with metal salts, lime softening, and iron/manganese removal. The most commonly used metal salts are aluminum salts such as alum, and ferric salts such as ferric chloride or ferric sulfate. Ferrous sulfate has also been used, but is less effective (Jekel, 1994; Hering et al., 1996; Hering et al., 1997). Excellent arsenic removal is possible with either ferric or aluminum salts, with laboratories reporting over 99% removal under optimal conditions,

and residual arsenic concentrations of less than 1 μg/L (Cheng et al., 1994). Full-scale plants typically report a somewhat lower efficiency, from 50% to over 90% removal. Coagulation encompasses all reactions, mechanisms and results in the overall process of particle growth (floc formation) and particle aggregation within a water being treated, including in situ coagulant formation, chemical particle destabilization and physical interparticle contacts (Johnston and Heijnen, 2001). Coagulation converts soluble As into insoluble reaction products, allowing separation by sedimentation and/or filtration. The general approach typically involves the addition of ferric or alum salts as the coagulant and may include pre- and post-oxidation treatment and/or pH adjustment.

During coagulation, arsenic is removed through three main mechanisms (Edwards, 1994):

- precipitation: the formation of the insoluble compounds $Al(AsO_4)$ or $Fe(AsO_4)$

- co-precipitation: the incorporation of soluble arsenic species into a growing metal hydroxide phase

- adsorption: the electrostatic binding of soluble arsenic to the external surfaces of the insoluble metal hydroxide.

All three of these mechanisms can independently contribute towards contaminant removal. In the case of arsenic removal, direct precipitation has not been shown to play an important role. However, co-precipitation and adsorption are both active arsenic removal mechanisms.

Factors affecting arsenic removal by coagulation include i) coagulant type and dose, ii) mixing time and speed, iii) pH, iv) arsenic oxidation state and concentration, v) presence of inorganic solutes etc. The optimal conditions vary for removal of different constituents, and coagulation to remove arsenic may not be optimal for removal of other compounds, notably phosphate and fluoride. Disposal of the arsenic-contaminated coagulation sludge may be a concern.

2.2.2.4 Filtration

Numerous studies have shown that filtration is an important step to ensure efficient arsenic removal. Conventional filtration is the separation of solid particulates from a liquid by passing the mixture through a medium, e.g. sand, anthracite coal, activated carbon, cloth, paper, which retains the solid on its surface and allows the liquid to pass through. After coagulation and simple sedimentation, HAO and HFO – along with their sorbed arsenic

load – can remain suspended in colloidal form. Hering and others showed that coagulation and sedimentation without filtration achieved arsenate removal efficiencies of 30%; after filtration through a 1.0 micron filter, efficiency was improved to over 96%. Only marginal improvements were made by reducing the filter size to 0.1 micron (Hering et al., 1996). In field applications, some plants improve arsenic removal with two-stage filtration (Sancha, 1999b).

2.2.2.5 Adsorption

Various solid materials, including iron and aluminum hydroxide flocs, have a strong affinity for dissolved arsenic. Arsenic is strongly attracted to sorption sites on the surfaces of these solids, and is effectively removed from solution. Adsorption is a mass transfer process where a substance is transferred from the liquid phase to the surface of a solid and becomes bound by chemical or physical forces. In water treatment in the developed countries, the adsorbent (solid) is typically activated carbon, either granular (GAC) or powdered (PAC) and it is used for taste and odor removal. Activated alumina is a granulated form of aluminum oxide (Al_2O_3) with very high internal surface area, in the range of 200-300 m^2/g. This high surface area gives the material a very large number of sites where sorption can occur, and activated alumina has been widely used. In the early 1970s Bellack accidentally discovered that activated alumina could remove arsenic from water (Bellack, 1971; Sorg and Logsdon, 1978). Arsenic is adsorbed onto the surface of granular materials, clays and processed cellulosic materials including:

- activated carbon; metal-treated activated carbon;

- oxides (e.g. hydrated ferric oxide, titanium oxide, silicium oxide);

- Granular ferric hydroxide (Driehaus et. al. (1998);

- clay minerals (e.g. kaolinite, bentonite, Bijoypur clay);

- bauxite, hematite, feldspar;

- synthetic anion exchange resins;

- chitin and chitosan;

- iron oxide-coated or MnO_2-coated sand;

- cellulose materials (sawdust, newspaper pulp).

Each media has different associated performances and costs. Some are now available in small packet or tablet form for arsenic removal from drinking water (Ahmed, M.F., 1999). The efficiency of each media depends on the use of oxidizing agent(s) as aids to the sorption of arsenic. However, adsorption is well-suited for other applications either to assist in the coagulation process and/or as an innovative, small-scale alternative to conventional larger-scale ractices.

2.2.2.6 Oxidation

Oxidation is simply the addition of oxygen to a compound, or more generally, any reaction involving the loss of electrons from an atom. Aeration, the supplying of air, oxidizes arsenic and the iron which co-occurs. This is precipitated as $FeAsO_4$. Most arsenic removal technologies are most effective at removing the pentavalent form of arsenic [As(V)], since the trivalent form arsenite [As(III)] is predominantly non-charged below pH 9.2. Therefore, many treatment systems include an oxidation step to convert arsenite to arsenate. Oxidation alone does not remove arsenic from solution, and must be coupled with a removal process such as coagulation, adsorption or ion exchange.

Arsenite can be directly oxidized by a number of other chemicals, including gaseous chlorine, hypochlorite, ozone, permanganate, hydrogen peroxide, and Fenton's reagent (H_2O_2/Fe^{2+}). Some solids such as manganese oxides can also oxidize arsenic. Ultraviolet radiation can catalyze the oxidization of arsenite in the presence of other oxidants, such as oxygen. Direct UV oxidation of arsenite is slow, but may be catalyzed by the presence of sulfite (Ghurye and Clifford, 2000), ferric iron (Emett and Khoe, 2001) or citrate (EAWAG, 1999).

2.2.2.7 Ion exchange

Coagulation processes are sometimes unable to efficiently remove arsenic to these low levels. As a result, various alternate technologies have been developed or adapted that are capable of removing arsenic to trace levels. These advanced treatment options include ion exchange, membrane methods etc. Ion exchange can be considered as a special form of adsorption, though it is often considered separately. The technology involves the reversible displacement of an ion adsorbed onto a solid surface by a dissolved ion. Other forms of adsorption involve stronger bonds, and are less easily reversed.

Various strong-base anion exchange resins are commercially available which can

effectively remove arsenate from solution, producing effluent with less than 1 μg/L arsenic. Arsenite, being uncharged, is not removed. Analysts have taken advantage of this specificity to develop procedures for analytical differentiation of arsenite and arsenate (e.g. Ficklin, 1983; Edwards et al., 1998). Therefore, unless arsenic is present exclusively as arsenate, an oxidation step will be a necessary precursor to arsenic removal (Johnston and Heijnen, 2001).

Removal of the arsenic is relatively independent of the pH and initial concentration, and is almost complete (85% to 100%). The advantages of ion exchange resins are easy regeneration using sodium chloride, wide pH range and the improvement in water quality through removal of chromate, selenate, nitrate and nitrite. This method is relatively costly and regeneration of the resin produces salt solutions that are rich in arsenic (EPA 1997, Johnston et al. 2001, Vance 2002).

2.2.2.8 Membrane processes

Some synthetic membranes can act as molecular filters to remove arsenic and other dissolved particulate compounds, as they may be permeable to certain dissolved compounds but exclude others. Two classes of membrane filtration can be considered: low-pressure membranes, such as microfiltration and ultrafiltration; and high-pressure membranes such as nanofiltration and reverse osmosis. Low-pressure membranes have larger nominal pore sizes, and are operated at pressures of 10-30 psi. The tighter high-pressure membranes are typically operated at pressures from 75 to 250 psi, or even higher (Letterman, 1999).

Reverse osmosis and nanofiltration provide removal efficiencies of the order of 95% when the operating pressure is at 1 psi from the ideal (75 to 250 psi). Removal of arsenic is independent of the pH and the presence of other solutes. The use of membranes requires that the water should not contain excessive quantities of colloidal material, especially organic material. With nanofiltration, arsenic removal efficiency reaches 90% (EPA 1997, Johnston et al. 2001). These systems remove between 91 and 98% of As(V) in high pressure reactors and between 77% and 87% in low pressure reactors. This separation is attributed to the high molecular weight of As(V) and As(III), rather than rejection by load. Therefore, these membranes are ideal for groundwater where As(III) predominates since no prior oxidation is needed.

Membrane filtration requires a relatively high-quality influent water. Membranes can be

fouled by colloidal matter in the raw water, particularly organic matter. Iron and manganese can also lead to scaling and membrane fouling. To prevent fouling, reverse osmosis filters are almost always preceded by a filtration step. Membrane filtration has the advantage of lowering the concentrations of many other components in addition to arsenic. Membrane process treatment performance is dependent on the quality of the feed water and the desired quality of the product water. Generally the more contaminated the feed water and the higher the desired product water quality, the greater the likelihood of membrane fouling caused by particulate matter, scaling and biofouling.

2.2.2.9 Biological processes

Bacterial activity may play an important role as a catalyst in several of the processes used to remove arsenic, but little is known of the viability of biological processes in eliminating arsenic from water (Johnston et al. 2001).

It has lately been advanced as an alternative process for removing arsenic. Small-scale studies show that optimum conditions of pH, temperature and oxygen enable biological filtration and the simultaneous elimination of As(III) and iron. The critical parameter is the initial concentration of iron. At higher concentrations, the efficiency of arsenic removal reaches >90% and at lower concentrations efficiency is approximately 40%. For water systems with low iron concentrations, the addition of ferrous sulfate is recommended to complete removal of arsenic. Fixation of As(III) in the iron oxides produced by bacterial activity is the main mechanism. Biological filtration in the treatment of arsenic can be applied to any groundwater system for the bacteriological oxidation of iron (Lehimas et al. 1992). Methods of filtration using spores to remove arsenic are also being successfully developed (Bencheikh-Latmani & Rizlan 2004, Katsoyiannis et al. 2004).

While these technologies have all been shown to be effective in lab or pilot studies, there is still relatively little experience with full-scale treatment. In addition, a number of novel removal technologies are under development, some of which show great promise.

2.2.3 Emergent technologies

In recent years, a tremendous amount of research has been conducted to identify novel technologies for arsenic removal, particularly low-cost, low-tech systems that can be applied in rural areas. Most of these technologies rely on oxidation of arsenite, followed by filtration through some sort of porous material, where arsenic is removed through

adsorption and co-precipitation. Many of these systems make use of iron compounds, which have a very strong affinity for arsenic (Johnston and Heijnen, 2001). Some of the most documented technologies is given below:

2.2.3.1 Fe-Mn oxidation

Conventional iron and manganese removal can result in significant arsenic removal, through coprecipitation and sorption onto ferric or manganic hydroxides. The mechanisms involved are the same as in coagulation and filtration. Most low-cost technologies for arsenic and manganese removal rely on aeration and filtration through porous media such as sand and gravel. Any technology that effectively removes iron and manganese could be evaluated to see if arsenic is also removed effectively (Johnston and Heijnen, 2001).

In Bangladesh and West Bengal, elevated arsenic concentrations are often associated with high iron and manganese levels. One survey in Bangladesh found that over 80% of arsenic-affected tubewells (>50 µg/L) also contained iron levels of 2 mg/L or more. However, iron alone is not a good indicator of arsenic: 30% of the wells with safe levels of arsenic also had 2 mg/L iron or more (DPHE/BGS/MML, 1999). Ahmed (1999) reports that tubewell water in 65% of the areas in Bangladesh show dissolved iron concentrations greater than 2 mg/l and, in some areas, the dissolved iron concentration is higher than 15 mg/l. Because of the link between arsenic and iron levels, and the affinity of arsenic for iron hydroxides, there have been calls for a simple solution to arsenic contamination: simple storage of pumped water to allow iron to settle out, scavenging arsenic in the process. Ahmed (1999) mentions that iron removal plants in areas of high iron concentrations in Bangladesh are constructed on the principle of aeration, sedimentation, filtration.

While this is an appealing idea, successful application of this type of 'passive Fe-Mn oxidation' is not simple, for several reasons:

- iron removal is not always easily accomplished. Some waters contain iron in a form that is slow to oxidize, or may be complexed with organic material that impedes oxidation and filtration. Precipitation may not occur if alkalinity is low;

- without a filtration step, much of the iron can remain suspended as colloidal matter, even after oxidation;

- arsenite is not as strongly bound to iron as arsenate, if the waters contain mostly arsenite arsenic removal will be less efficient; and

- when water is stored in household containers, there is a high risk of bacterial contamination.

When considering passive Fe-Mn oxidation, particularly at the household level, careful pilot studies should be made using the local waters and local storage conditions, in order to assess the effectiveness of this technique, and the possibility of pathogenic contamination (Johnston and Heijnen, 2001).

2.2.3.2 Sorption onto other metal oxides

Besides activated alumina, other metal oxides have strong affinities for arsenic, and can serve as effective sorbents, and in some cases as oxidants. Quartz is very poor at removing arsenic under most environmental conditions, because the mineral surface is negatively charged above a pH of 2. However, quartz sand, or indeed any other granular media, can be made highly sorptive by coating the grains with metal oxides. In recent years many researchers have used this principle to develop low-cost arsenic removal methods using locally available materials.Vaishya showed that sand from the Ganges river, which presumably is rich in iron coatings, could remove arsenite from solution, with a reported capacity of 0.024 mg/g. Removal was found to be pH-dependent, and best from pH 7-9 (Vaishya and Agarwal, 1993). Joshi and Chaudhuri showed that iron oxide coated sand (IOCS) is able to remove both arsenite and arsenate. A simple fixed bed unit was able to treat about 160-190 bed volumes of water containing 1000 μg/L arsenite and 150-165 bed volumes of water with 1000 μg/L arsenate. Flushing with 0.2 N sodium hydroxide regenerates the media. The authors propose that this media would be very useful for domestic arsenic removal units (Joshi and Chaudhuri, 1996).

A similar coated sand material can be prepared using manganese dioxide instead of iron. Since MnO_2 is a good oxidant, this material can remove arsenite as well as arsenate. In fact, the treated sand was able to remove 80% of a 1 mg/L solution of arsenite within two hours, but slightly less than 70% of an equivalent solution of arsenate. A prototype household unit was developed, which could treat about 150 bed volumes of 1 mg/L arsenic (half arsenite and half arsenate) before breakthrough (Bajpai and Chaudhuri, 1999).

Several proprietary iron-based adsorption materials have been developed recently. Granular ferric hydroxides are being used in full scale systems in Germany (Driehaus et al., 1998), and similar materials have been developed in Canada and the United States. These materials generally have high removal efficiency and capacity.

2.2.3.3 Sorption onto reduced metals

Most of the above processes rely on arsenate adsorption onto surfaces of metal oxides. However, arsenic also has a strong affinity to reduced metal surfaces, such as sulfides. A few researchers have taken advantage of this property to remove arsenic through reduction and sorption.

Lackovic and others have demonstrated that zero-valent iron filings can be used either in situ or ex situ to reduce arsenate, and produce ferrous iron. The ferrous ions precipitate out with sulfide, which is also added to the system. Arsenite is removed either through co-precipitation or adsorption onto pyrite. This system is promising for use in rural areas, because of the low cost of materials, and the simple operation. However, treated water is very high in ferrous iron, and must undergo iron removal treatment before distribution or consumption (Lackovic et al., 2000).

A similar system using zero-valent iron to treat water stored in individual homes was tested in Bangladesh and West Bengal (the so-called: three kolshi filter). Arsenic removal was approximately 95% for highly contaminated waters, containing 2000 µg/L arsenic in the presence of sulfate at pH 7. Removal is rapid, but if batches are left for too long, dissolved iron concentrations become unacceptably high (Ramaswami et al., 2000).

2.2.3.4 In-situ arsenic removal

When arsenic is mobilized in groundwater under reducing conditions, it is possible to immobilize the arsenic by creating oxidized conditions in the subsurface. In Germany, in order to remediate an aquifer containing high arsenite, high ferrous iron, low-pH groundwater, Matthess injected 29 tons of potassium permanganate directly into 17 contaminated wells, oxidizing arsenite, which coprecipitated out with ferric oxides. Mean arsenic concentrations were reduced by over 99%, from 13,600 to 60 µg/L (Matthess, 1981). More recently, atmospheric oxygen was used to reduce arsenic concentrations in-situ from approximately 20 to 5 µg/L, while iron and manganese levels were also lowered (Rott and Friedle, 1999). Under reducing conditions, and in the presence of sulfur, arsenic can precipitate out of solution and form relatively insoluble arsenic sulfides.

In-situ removal has been successful in decreasing arsenic concentrations from ground water containing high concentrations of both arsenic and iron. Pumping water from one well into a second well after adding atmospheric oxygen can result in arsenic removal from

the ground water (Rott and Friedle 1999). The recharged well can then be used for water supply. One advantage of this approach is that 10 or more gallons of low-arsenic water can be obtained for each gallon recharged. Additionally, removal efficiency increases with successive cycles of recharge and withdrawal.

Removal of arsenic from ground water within an aquifer, or in-situ remediation, can result in significant cost benefits compared with aboveground treatment. Lower costs may be realized because of lower capital and operating costs, a simpler and less-expensive operation, and avoidance of sludge and wastewater disposal (Rott and Friedle 1999).

This in-situ treatment (subterrestrial groundwater treatment) is a multi-functional technology which is effective for the removal of such water constituents as iron, manganese, arsenic and ammonia. The treatment technology can be applied efficiently for decentralised water supply. Within the framework of a recent research project, biofilms (which play an important role in the in-situ treatment) has been localised, isolated and examined using model investigations. Microorganisms were identified and characterised in order to make the in situ treatment controllable regarding both planning and design of facilities, and to increase the efficiency of the technology (Rott and Meyer, 2002).

HFO (hydrous ferric oxide) appears to be the most important phase responsible for removing the arsenic from the ground water (Appelo and de Vet, 2003). The arsenic removal process associated with iron removal may be described as a series of reactions involving dissolved oxygen, aqueous and exchangeable Fe and other cations, and arsenic. Injection of water containing dissolved oxygen can lead to rapid exchange of Fe^{2+} for cations in the injected water with subsequent Fe^{2+} oxidation to form HFO. Upon reversing the flow direction, the injected water has a lower iron concentration. Continued pumping can produce water with a lower iron concentration because Fe^{2+} is removed by exchange. Arsenic can co-precipitate with the HFO during injection and adsorption onto the HFO during withdrawal.

A second approach that can lead to arsenic removal is lowering of pH where alkaline ground water contains high arsenic concentrations (Welch, Stollenwerk et al. 2000; Welch, Stollenwerk et al. 2003). In the system studied, ground water had a high pH (about 9.2) and contained > 100 ppb arsenic. The HFO content of the aquifer was increased through the injection of $FeCl_3$ followed by injection of oxic ground water with a lower pH.

In-situ immobilization has the great advantage of not producing any wastes that must be

disposed of. However, experience is limited, and the technique should be considered with caution. Oxidants are by definition reactive compounds, and may have unforeseen effects on subsurface ecological systems, as well as on the water chemistry. Care must also be taken to avoid contaminating the subsurface by introducing microbes from the surface. Also, at some point pore spaces can become clogged with precipitates, particularly if dissolved iron and manganese levels are high in the untreated water (Johnston and Heijnen, 2001).

2.2.3.5 Wetlands for arsenic removal from wastewaters

Several treatment technologies have been applied for the removal of arsenic from contaminated waters, such as precipitation/co-precipitations, coagulation/filtration, ion exchange, lime softening, adsorption on iron oxides or activated alumina, and reverse osmosis (Jekel, 1994; Zouboulis and Katsoyiannis, 2002). Most of these technologies are not efficient enough for the removal of As(III); hence, they are mainly applied for the removal of As(V). Therefore, a pre-oxidation step is usually required to transform the trivalent form to the pentavalent one. The oxidation procedure is mainly performed by the addition of chemical reagents, such as potassium permanganate, chlorine, ozone, hydrogen peroxide, or manganese oxide (Jekel, 1994; Kim and Nriangu, 2000). Although these reagents are effective for the oxidation of trivalent arsenic, they may also cause several secondary problems, arising mainly by the presence of residuals or from byproducts formation, inducing simultaneously a significant increase to the operational costs of respective methods.

Precipitation/co-precipitation has been the most frequently used method to treat arsenic-contaminated water, including groundwater, surface water, leachate, mine drainage, drinking water and wastewater in numerous pilot- and full-scale applications. Thereby chemicals are used such as ferric salts, ammonium sulphate, alum, limestone, manganese sulphate etc. But the presence of the more soluble trivalent state of arsenic and pH level in water may reduce the overall removal efficiency. Adsorption on activated alumina (AA), activated carbon (AC), granular ferric hydroxides etc. is less frequently used than precipitation/co-precipitation. Several factors (As-oxidation state, wastewater pH etc.) are affecting arsenic removal efficiency (U.S. EPA 2000, 2001, 2002). As mentioned earlier, other treatment systems like ion exchange, reverse osmosis, membrane filtration etc. are very expensive and not suitable for low-income developing countries.

Two broad categories of wastewater treatment systems are active and passive. Active systems of wastewater treatment require continuously supportive operation and maintenance during the treatment. For instance, pH modification, ion exchange, and electrochemical treatment are the supportive maintenance. Unlike active systems, passive systems are intended to be self-sustaining after an initial startup period (Brown *et al*., 2002). Among the passive and biological treatment systems, constructed wetlands are promised to be very simple, cost effective and environment friendly and hence constructed wetlands are of special interest for arsenic removal in this study.

2.2.4   Outlined remarks

Some of the key technologies for arsenic removal from the environment have recently gained plenty of experiences from field level application. But still, research needs to be done to identify certain emerging problems and their probable solutions. Arsenic removal efficiency varies according to many site-specific chemical, geographic, and economic conditions, so actual applications may vary from one technology to another. Because of the many factors that can affect arsenic removal efficiency (e.g. arsenic concentration, speciation, pH and co-occurring solutes), any of the above mentioned technology should be tested depending on the actual water to be treated (e.g. groundwater, surface water and/or wastewater), before implementation of arsenic removal systems at the field scale.

2.3   Constructed wetlands for wastewater treatment

Constructed wetlands are an exciting new application of technology that is very effective at improving water quality. While they don't solve all water quality problems, they hold much promise as a new type of water treatment system that combines low cost and high efficiency. Those attributes alone make them attractive systems, especially to small and medium-sized cities and many industries.

Constructed wetlands (CWs) are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and the associated microbial assemblages to assist in treating wastewaters (Kadlec and Knight, 1996). These systems can be used for secondary or tertiary treatment of wastewater from households and/or municipalities, a function they have in common with natural wetlands. Unlike natural wetlands, treatment in constructed wetlands is performed under more controlled environments, which allow for greater treatment efficiency and constancy of

wetland functions across the entire bed (Vymazal et al., 1998). They are designed to take advantage of many of the same processes that occur in natural wetlands, but do so within a more controlled environment.

Constructed wetlands have been used for decades mostly for the treatment of domestic or municipal sewage. However, recently CWs have been used for many other types of wastewater including industrial and agricultural wastewaters, landfill leachate or storm water runoff. The performance of constructed wetlands for wastewater treatment depends on many factors, including the type of pre-treatment, influent concentration, flow, wetland type, wetland size, and soil (Brown, 1994).

The pollutants are removed from the inflowing water by a combination of processes (chemical, physical and biological) within the wetland, such as sedimentation, precipitation, adsorption to soil particles, assimilation by plant tissue and microbial transformations. Heavy metals in a wetland system may be sorbed to wetland soil or sediment, or may be chelated or complexed with organic matter. Metals can precipitate out as sulphides and carbonates, or uptake by plants. Macrophytes can enhance pollutant removal within the system by either assimilating them directly or by providing an environment for surface microbial attachment to transform and uptake pollutants. The rhizosphere of aquatic plants is also a primary site for pollutant uptake and transformation as it is a zone of oxygen transfer between the plant and sediment which is a requisite for sediment microbial activity and pollutant oxidation (Brix 1994a).

High productivity results from having high availability of light, nutrients, and water, and from the plant's morphological and physiological ability to take advantage of this environment. High levels of activity also occur at the microbial level resulting in the decomposition of organic matter and other substances. For these reasons, aquatic ecosystems (wetlands, in particular) have been considered as alternatives and/or supplements to a variety of water treatment and recycling processes (Bavor et al. 1995; Kadlec 1995; Wood 1995; Brix 1994b; Cullen 1989).

Under anaerobic conditions, sulphate-reducing bacteria (SRB) oxidise simple organic compounds by utilising sulphate as an electron acceptor and generate sulphide (S^{2-}) and alkalinity. This biogenically produced sulphide can react with dissolved metals to form metal sulphide precipitates since the solubility of most toxic metal sulphides are generally very low (Kim et al. 1999).

Some advantages of constructed wetlands are:

- their low cost of construction and maintenance when compared to the costs of treatment plants,

- their low requirements for energy,

- their flexibility

- the fact that they are low-technology based systems,

- their high process stability (buffering effect) and

- their optimal aesthetic appearance.

The disadvantages include:

- the requirement for large amounts of land, depending on their use,

- seasonal variability in their effectiveness,

- temperature and fluctuations in flow affect their function and display inconsistent contaminant removal rate,

- their aging problem may contribute to a decrease in effectiveness.

2.3.1   Wetland definition, classification, design and sizing

Constructed wetlands for wastewater treatment may be classified according to the life form of the dominating and emergent macrophytes into systems with free-floating, rooted emergent and submerged macrophytes (Kadlec, 1987; Wissing, 1995; Brix and Schierup, 1989), but the design of the systems in terms of media as well as the flow regime varies. The most common systems are designed with horizontal subsurface-flow (HF CWs) but vertical flow (VF CWs) systems are getting more popular in recent times. Constructed wetlands with free water surface (FWS CWs) are not used as much as the HF or VF systems despite being one of the oldest designs in Europe (Brix, 1994; Vymazal et al., 1998; Vymazal, 2001a). The schemes of some systems are shown in Figure 2.3.

The dominant form of macrophyte within the wetland classifies the wetland treatment system. There are free-floating macrophyte systems, rooted emergent macrophyte systems, submerged macrophyte systems and multi-stage systems, which are a combination of the preceding systems, and other kinds of low technology systems (oxidation ponds, sand filters, etc.).
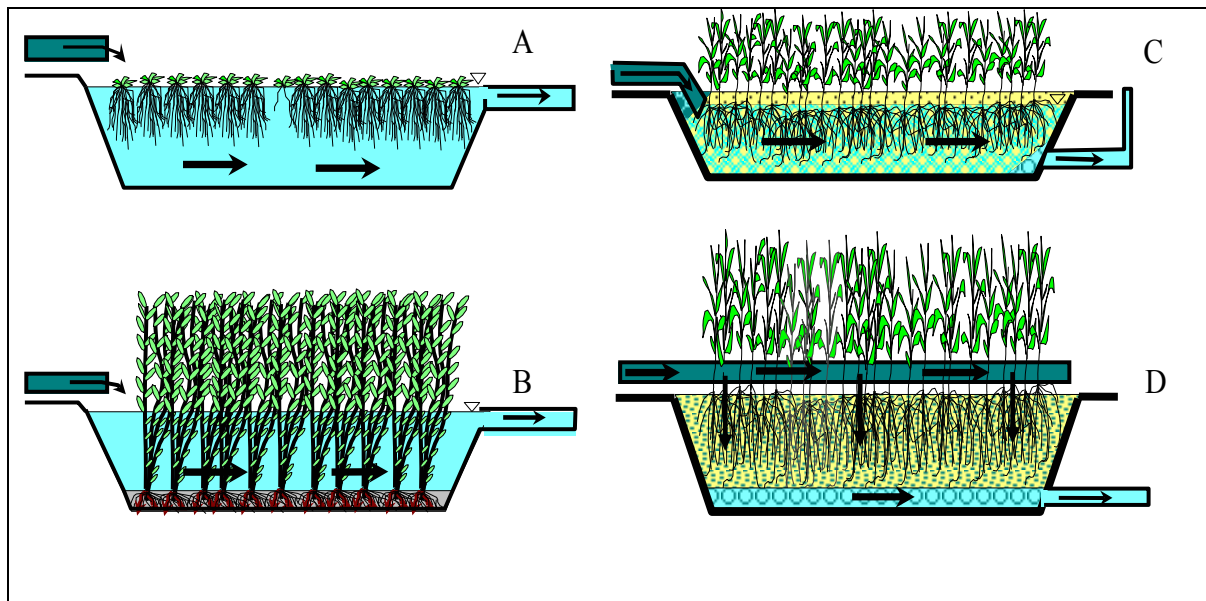
Figure 2.3 Wetland systems for wastewater treatment (A, bed with free-floating plants; B, horizontal surface flow wetland or bed with emergent plants; C, horizontal subsurface-flow wetland; D, vertical flow wetland).

A free-floating macrophyte system typically consists of basins or channels, with a natural or constructed subsurface barrier of clay or impervious geotechnical material to prevent seepage, soil or another suitable medium to support the emergent vegetation, and water at a relatively shallow depth flowing over the soil surface. The shallow water depth, low flow velocity, and presence of the plant stalks and litter regulate water flow and, especially in long, narrow channels, ensure plug-flow conditions (Reed et al. 1987).

The free-floating macrophyte systems are generally limited to systems based on water hyacinth (*Eichhornia crassipes*) or duckweed (*Lemna*, *Spirodella* or *Wolffia* spp.) in which the plants are allowed to grow in shallow ponds or cells within treatment ponds. Much has been documented on the productivity and effectiveness of these systems, particularly the water hyacinth (Karpiscak *et al.* 1994, Aoyama and Nishizaki 1993, Reddy and Sutton 1984). Hyacinth is more effective in warmer climates making it an ideal system for tropical environments but, as it is a declared noxious weed in Australia, it cannot be used. *Lemna* spp. and other duckweeds are more manageable; however, they are a minute plant, and therefore require barrier systems or compartments to minimise movement on the water by wind to ensure water surface coverage. These systems represent the oldest type of artificial wetland design. The bottoms of the channels are generally sealed to prevent wastewater leakage.

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Treatment wetlands have some properties in common with facultative lagoons and also have some important structural and functional differences (see Fig 2.3 A). Water column processes in deeper zones within treatment wetlands are nearly identical to ponds with surface autotrophic zones dominated by planktonic or filamentous algae, or floating or submerged aquatic macrophytes.

The submerged macrophyte system uses plants which have their photosynthetic tissue entirely submerged. The diversity of plants available for use is great and includes low-productivity oligotrophic water species (*Lobelia dortmanna*), commonly occurring species in freshwater systems (*Potamogeton* spp., *Ceratophyllum* spp., and *Myriophyllum* spp.) and high productivity species that thrive in eutrophic waters (*Elodea canadensis*; *Hydrilla verticillata*). Emergent plants partly submerged under water and partly above the wetland surface in case of bed with emergent plants. These plants can assimilate nutrients directly from the water but only grow well in oxygenated waters. Therefore, these systems are not suitable for receiving wastewater with a high loading of organic matter. Their primary use could be to polish treated water, whether derived from a secondary treatment system or a low pollutant effluent source.

Horizontal subsurface-flow wetlands are the most widely used concept of constructed wetlands in Europe (Fig. 2.3C). This design was pioneered in Germany by Seidel in the 1950s and developed further in the 1970s (Brix 1994b). The design typically consists of a shallow rectangular bed with gravel or other medium to support the roots of vegetation, planted with the macrophytes, lined with an impermeable membrane and water control structure that maintains a shallow depth of water. Mechanically pre-treated wastewater is fed in at the inlet and passes slowly through the filtration medium under the surface of the bed in a more or less horizontal path until it reached the outlet zone where it is collected before discharge via level control arrangement at the outlet. Water level always remains below the surface of the wetland bed and during the passage of wastewater through the reed bed the wastewater makes contact with a network of aerobic, anoxic and anaerobic zones (Vymazal, 1999).

During the passage of the wastewater through the rhizosphere, the wastewater is cleaned by microbiological degradation and by physical and chemical processes (Brix 1987, Cooper et al. 1996).

Two important functions occur through this system as a result of :

- oxygen is supplied to the heterotrophic organisms in the rhizosphere, and

- hydraulic flow through the medium is increased and stabilised.

Organic matter and suspended solids are removed effectively via these systems but the removal of N and P varies greatly depending on the loading rate of the wastewater, type of substrate, and the type and composition of the wastewater. The flow rate is an important factor as high input resulting in surface flow has to be avoided as this prevents the wastewater coming into contact with the sediment and the rhizosphere.

Vertical subsurface-flow systems (see Fig 2.3 D) allow for improved hydraulic conditions and water/rhizosphere contact. These systems composed of a flat bed of gravel topped with sand, with macrophytes growing at the same sort of densities as like horizontal subsurface-flow system. This design provides percolation flow with intermittent loading, flooding the surface which improves soil oxygenation when compared to horizontal subsurface-flow systems. The liquid then gradually drains vertically down through the bed and is collected by drainage network at the base. Therefore, during the loading period, air is forced out of the soil and during the percolation phase the surface soil dries out drawing air back into the soil pore spaces. This process therefore provides alternating oxidising/reducing conditions in the soil promoting alternating nitrification and de-nitrification reactions and P adsorption. Vertical flow, and more significantly, vertical up-flow systems are currently being developed for Freshwater Ecology and preliminary findings appear to indicate that these systems are promising as single-use, low load systems such as household treatment systems, particularly for P removal (Breen and Chick 1995; Chick and Mitchell 1995; Mitchell *et al.* 1995; Heritage *et al.* 1995).

The water/sediment interaction and associated microbial activity is the driving force behind water purification processes and therefore a sink for nutrients in both constructed and natural wetlands. Another significant factor which determines the effectiveness of a wetland as a water treatment system is the amount of time that the water stays in contact with the wetland, and this is related to the size of the wetland and the amount of water it receives.

In order to overcome the overland flow, wetland systems were designed with a low aspect ratio (length to width ratio). It resulted in a very wide beds and short passage length (Brix,

1998). However, the design with a very long inlet trenches caused problems with water distribution and, therefore, the inlet trench was subdivided into two or more separate units that could be loaded separately in order to get better control on the distribution of water (Brix, 1998).

The following equation, first proposed by Kickuth (1977), has been widely used for sizing of horizontal subsurface-flow systems for domestic sewage treatment:

$$A_h = Q_d \left(\ln C_{in} - \ln C_{out} \right) / K_{BOD}$$

where A_h is the surface flow of bed (m^2), Q_d the average flow ($m^3\ d^{-1}$), C_{in} the influent BOD_5 ($mg\ l^{-1}$), C_{out} the effluent BOD_5 ($mg\ l^{-1}$) and K_{BOD} is the rate constant ($m\ d^{-1}$).

2.3.2   Technological aspects/ removal mechanisms

Roots and rhizomes of reeds and all other wetland plants are hollow and contain air-filled channels that are connected to the atmosphere for the purpose of transporting oxygen to the root system. The majority of this oxygen is used by the roots and rhizomes themselves for respiration, but as the roots are not completely gas-tight, some oxygen is lost to the rhizosphere (Brix, 1994, 1997). According to the working principle of horizontal subsurface-flow constructed wetlands (HF CWs), the amount of oxygen released from roots and rhizomes should be sufficient to meet the demand for aerobic degradation of oxygen consuming substances in the wastewater as well as for nitrification of the ammonia. However, many studies have shown that the oxygen release from roots of different macrophytes is far less than the amount needed for aerobic degradation of the oxygen consuming substances delivered with sewage and that anoxic and anaerobic decomposition play an important role in HF CWs (Brix, 1990; Brix and Schierup, 1990). As a results organic compounds are degraded aerobically as well as anaerobically by bacteria attached to plant underground organs (i.e. roots and rhizomes) and media surface and the removal of organics is generally very high in HF CWs.

Anaerobic degradation is a multi-step process. In the first step the primary end products of fermentation are fatty acids, such as acetic, butyric and lactic acids, alcohols and the gases CO_2 and H_2 (Mitsch and Gosselink, 2000; Vymazal, 1995; Vymazal et al., 1998b).

Acetic acid is the primary acid formed in most flooded soils and sediments. Strictly anaerobic sulfate reducing bacteria and methane-forming bacteria then utilize the end-products of fermentation and, in fact, depend on the complex community of fermentative

bacteria to supply substrate for their metabolic activities. Both groups play an important role in organic matter decomposition (Valiela, 1984; Grant and Long, 1981; Vymazal, 1995).

The acid-forming bacteria are fairly adaptable but the methane-formers are more sensitive and will only operate in the pH range 6.5–7.5. Over-production of acid by the acid-formers can rapidly result in a low pH value. This stops the action of the methane-forming bacteria and will result in production of odorous compounds from the constructed wetland. Anaerobic degradation of organic compounds is much slower than aerobic degradation. However, when oxygen is limiting at high organic loadings, anaerobic degradation will predominate (Cooper et al., 1996).

Total suspended solids and carbon removal:

Solids that are not removed in pre-treatment system are effectively removed by filtration and settlement (Cooper et al., 1996; Vymazal et al., 1998b). Most of the suspended solids are filtered out and settled within the first few meters beyond the inlet zone. The accumulation of trapped solids is a major threat for good performance of HF CWs systems as the solids may clogg the bed. Therefore, the effective pretreatment is necessary for HF CWs systems (Vymazal et al., 1998b).

Organic compounds can be broken down for consumption by microorganisms in a wetland system. This biodegradation removes the organic compounds from water as they provide energy for the organisms. Organics can also be degraded when taken up by plants. They can also sorbs to surfaces in the wetland, usually to plant debris.

The primary removal mechanisms for BOD and TSS are flocculation, settling, and filtration. As the wastewater slowly flows horizontally through the wetland bed, it acts as a horizontal gravel filter, there by providing opportunities for TSS separation by sedimentation, physical straining and capture, and adsorption on biomass attached to the gravel and root system (US EPA, 2000).

Sulphur removal:

Wetlands can function as sulphur sink through their internal production and release of hydrogen sulphide as a gas, release of elemental sulphur or methyl sulphide gas, precipitation of elemental sulphur, and precipitation and burial of insoluble metallic sulphides. Physical transport processes and biogeochemical reactions, many of them driven by aquatic plants, may result in the extensive sulphur cycling between oxidizing and

reducing conditions. Oxidation of sediment sulphide produces oxidized sulphur species (i.e. SO_4^{2-}, S^o) and may release associate metals or metalloids to the water column (Simpson et al., 1998).

In constructed wetlands, especially subsurface horizontal flow systems, very little attention has been paid to the sulphur metabolism. In the case of an industrial wastewater loaded with SO_4^{2-} and $S_2O_3^{2-}$ (area-specific load of 1.1 g S $m^{-2}d^{-1}$), Winter (1985) showed that constructed wetlands can act as a sink for sulphur. Two percent of the load was retained in the soil, 31 % as S^o, 25 % as organic S (mainly in humic matter), 15 % as sulphate, 11% as sulphide and only a small fraction was released by volatilization to the atmosphere or taken up by plants (1%). Both microbial and abiotic processes are responsible for these transformation processes.

Nitrogen removal:

Nitrogen transformation in constructed wetlands has already been the subject of several papers. The main removal mechanism is microbial nitrification-denitrification; in contrast, incorporation into the plant biomass is only of minor importance (Cooper and Maeseneer, 1996; Laber et al., 1999; Urbanc-Bercic and Bulc, 1994; Bayley et al., 2003).

Constructed wetlands for the treatment of domestic sewage usually cause the removal of ammonia due to nitrification and also the removal of nitrate and nitrite owing to denitrification (Brix, 1994; Börner, 1992).

The major removal mechanism of nitrogen in HF CWs is nitrification/denitrification (Vymazal, 1999). Field measurements have shown that the oxygenation of the rhizosphere of HF CWs is insufficient and, therefore, incomplete nitrification (i.e. oxidation of ammonia to nitrate) is the major cause of limited nitrogen removal. Zhu and Sikora (1994) pointed out that no obvious nitrification could be observed when dissolved oxygen concentration is lower than 0.5 mg l^{-1}.

In general, nitrification which is performed by strictly aerobic bacteria is mostly restricted to areas adjacent to roots and rhizomes where oxygen leaks to the filtration media. On the other hand, prevailing anoxic and anaerobic conditions offer suitable conditions for denitrification but the supply of nitrate is limited as the major portion of nitrogen in sewage is in the form of ammonia. In addition, mineralization of organic nitrogen (ammonification) which proceeds both under aerobic and anaerobic conditions actually adds ammonia to the system.

Volatilisation, adsorption and plant uptake play much less important role in nitrogen removal in HF CWs (Cooper et al., 1996; Vymazal, 1999; Vymazal et al., 1998a). Volatilization is limited by the fact that HF CWs do not have free water surface. Hence, algal activity is negligible in these systems and, therefore, pH values do not increase. The adsorption capacity of the commonly used media (gravel, crushed rock) is very limited.

Greenhouse gas production

The aerenchyma tissue also plays a role in the methane emission through helophyte plants in wetlands which were estimated at 940 mg CH_4 $m^{-2}d^{-1}$ for a cattail wetland (Yavitt and Knapp, 1995). Thomas et al., (1996) summarized and cited other papers in which helophytes are responsible for 50-90 % of the total methane flux from wetlands. Tanner et al., (1997) estimated methane emission from constructed wetlands used to treat agriculture wastewater to account for around 2-4 % of wastewater carbon loads in vegetated wetlands and 7-8 % of loads in un-vegetated systems.

2.3.3 Role of plant biomass in treatment processes

The choice of plants is an important issue in constructed wetlands, as they must survive the potential toxic effects of the wastewater and its variability. The most widely used constructed wetlands design in Europe is the horizontal subsurface-flow system vegetated with the common reed (*Phragmites australis*) (Vymazal, 2005), although other plant species, such as cattails (*Typha spp.*) bulrushes (*Scipus spp.*) and reed canarygrass (*Phalaris arundinacea*) have been used for both domestic and industrial wastewater treatment (Mbuligwe, 2005; Vymazal, 2005; Vymazal and Kropfelova´, 2005; Shepherd et al., 2001).

The choice of different plant species should take into account some factors such as the rooting depth, plant productivity and tolerance to high loads of wastewater (Brix, 1994). The main emergent macrophyte species used in CWs in the Mediterranean countries are *Canna spp., Iris spp., Cyperus spp., Typha spp., Phragmites spp., Juncus spp., Poaceae spp. and Paspalum spp.* (Korkusuz, 2005).

Questions remain concerning what role plants play in the treating of wastewater. The major role of plants is probably to act as "ecological engineers" (Tanner, 2001), through the release of oxygen and other compounds from the plant roots. All plant species are adapted

to growing in water-logged anoxic sediments as they have large internal air spaces for the transportation of oxygen to the roots and rhizomes. Oxygen is transported to the roots and surrounding rhizomes by either direct diffusion and/or by convective air flow. Air diffusion by the roots and rhizomes to the rhizosphere creates an envelope of aerated soil in the otherwise anoxic soil which stimulates organic matter decomposition and the growth of nitrifying bacteria (Brix 1994a). However, even under waterlogged conditions, the diffusion of O_2 through plant roots into the surrounding soil matrix may keep the rhizosphere soil oxidized (Kirk, 2004).

Also, plants provide carbon compounds to the substrate through plant litter and root exudates. The amount of these carbon inputs from plants to the substrate is related to plant growth (Lu et al., 2002; Jones et al., 2004). Depending on plant species and growth stage, on average 10–25% of C assimilated by photosynthesis is translocated to the roots and exuded to the adjacent substrate (Kuzyakov et al., 2001). Root exudates are a primary driver of microbial growth and elevated microbial activities, and can also affect nutrient acquisition by both microbes and plants (Jones et al., 2004).

Arsenic at the root surface was found to be predominantly As(V) associated with the Fe-plaque, and Fe-plaque was suggested to cause an attenuation of the As mobility (Hansel et al., 2002; Blute et al., 2004).

The macrophytes growing in constructed treatment wetlands have several properties in relation to the treatment processes that make them an essential component to the design. The most important effects of the macrophytes in relation to the wastewater treatment processes are the physical effects that the plant tissues give rise to erosion control, filtration effect and provision of surface area for attached microorganisms. The macrophytes have other site-specific valuable functions, such as providing a suitable habitat for wildlife and giving systems an aesthetic appearance. The major roles of macrophytes in constructed treatment wetlands are summarized in Table 1.

Table 1 Summary of the major roles of macrophytes in constructed treatment wetlands (Brix 1997).

Macrophyte property	Role in treatment process
Aerial plant tissue	• Light attenuation→ reduced growth of phytoplankton • Influence on microclimate→ insulation during winter • Reduced wind velocity→ reduced risk of resuspension • Aesthetically pleasing appearance of system • Storage of nutrients, translocated metals or metalloids
Plant tissue in water	• Filtering effect → filter out large debris • Reduce current velocity → increase rate of sedimentation, reduces risk of resuspension • Provide surface area for attached biofilm • Excretion of photosynthetic oxygen → increases aerobic degradation • Uptake of nutrients
Root and rhizomes in the sediment	• Provide surface for attached microorganisms • Prevents the medium from clogging in vertical filter systems • Release of O_2 increase degradation and nitrification • Accumulation and uptake of metals or metalloids and nutrients • Release of carbon compounds

The general requirements of plants suitable for use in constructed wetland wastewater treatment systems include (Tanner, 1996):

- Ecological acceptability; i.e., no significant weed or disease risks or danger to the ecological or genetic integrity of surrounding natural ecosystems; tolerance of local climatic conditions, pests and diseases; ready propagation, and rapid establishment, spread and growth; and

- High pollutant removal capacity, either through direct assimilation and storage, or indirectly by enhancement of microbial transformations such as nitrification (via root-zone oxygen release) and denitrification (via production of carbon substrate).

The hydraulic retention times, including the length of time the water is in contact with the plant root, affects the extent to which the plant plays a significant role in the removal or breakdown of pollutants. Whereas plants significantly affect the removal of pollutants in horizontal subsurface systems with long hydraulic retention times used to clean municipal wastewater, their role is minor in pollutant removal in periodically loaded vertical filters, which usually have a short hydraulic retention time (Wissing, 1995).

Emergent and floating leaved species have been preferentially used in pilot studies of constructed wetlands. Potentially useful emergent species include many members like common reed (*Phragmites australis*), cattail (*Typha latifolia*), reed (*Cyperus sp.*), rush (*Juncus effusus*), sedge (*Carex rostrata*) and grass families. They have potentially high uptake and production rates. Plants are widespread, able to tolerate a wide range of environmental conditions, and can alter their environment in ways suitable for wastewater treatment. Tanner (1996) indicated that *Juncus effusus* showed the highest mean shoot density (4534 m^{-2}) of the eight tested species. Above-ground tissue nutrient concentrations were high but there was a low level of biomass production, and it was capable of growth in ammonium-rich organic wastewater, producing a compact stand without major seasonal die back. *Juncus effusus* is an evergreen plant which grows very well in advance of the frost-free period, especially spring-bloomers.

2.3.4   Role of microorganisms in treatment process

Microbial processes are vitally important for the proper functioning of constructed wetlands. Because of the presence of ample water, wetlands are typically home to a variety of microbial and plant species. The diversity of physical and chemical niches present in wetlands results in a continuum of life forms. This biological diversity creates inter-specific interactions, resulting in greater diversity, more complete utilization of energy inflows, and ultimately to the emergent properties of the wetland ecosystem.

In constructed wetlands, the main role in the transformation of nutrients and mineralization of organic pollutants is played not by plants but by microorganisms. It has been shown that in the rhizosphere, the zone near the root cells, the density of microorganisms is higher than in the zone far from the roots.

Depending on the oxygen input by helophytes and availability of other electron acceptors, the contaminants in the wastewater are metabolized in various ways. In subsurface flow systems, aerobic processes only predominate near roots and on the rhizoplane (the surface of the root). In the zones that are largely free of oxygen, anaerobic processes such as denitrification, sulphate reduction and/or methanogenesis take place.

Under aerobic conditions, ammonium is oxidized by microorganisms to nitrate, with nitrite as an intermediate product. Two different groups of bacteria play a role in the nitrification step: ammonium oxidizers and nitrite oxidizers. Recently, a new pathway was discovered by Mulder et al. (1995) that anamox bacteria can use nitrite as an electron acceptor and anaerobically convert ammonium and nitrite to nitrogen gas. In contrast to the traditional nitrification-denitrification route, Anamox is an autotrophic process. The microorganisms use bicarbonate as a carbon source.

Jackson and Myers (2002) reported that sulphate reducing bacteria were present throughout the free-water surface pilot wetland soil and water. The water chemistry suggested that conditions were well suited for these organisms to thrive in all parts of the wetlands. The high concentration of sulphate in the produced water ensured that there was a ready supply of substrate for sulphate reducing bacteria.

Less is known about the microorganisms involved in the S-transformation reactions in constructed wetlands.

2.3.5 Removal of arsenic and heavy metals

The benefits of using wetlands to remove a wide range of water-borne contaminants have been long recognized, especially for heavy metals (Sobolewski, 1999; Zhu et al., 1999; Kadlec and Knight, 1996). The anoxic environment and organic matter production in wetlands promote chemical and biological processes enhancing metal removal from the impounded waters. A few cases using field-scale wetlands for Se removal from wastewaters have been reported (e.g., Hansen et al., 1998). Additional studies have been done on the biogeochemistry of Se, such as immobilization by reduction into elemental Se,

the association of Se with organic phase, volatilization, and plant uptake (e.g., Zhang and Moore, 1996; Zawislanski and Zavarin, 1996; Banuelos et al., 1997; Frankenberger and Engberg, 1998).

Physical, chemical and biological processes are involved in the removal of arsenic and heavy metals in constructed wetlands. The major mechanisms are:

- Adsorption and binding to soil and gravel matrices, sediments, particulates, algae, bacteria and oxide minerals.

- Precipitation as insoluble sulphides, carbonates and co-precipitations with Fe oxyhydroxides.

- Uptake and accumulation by plants and microbial biomass.

- Volatilization as volatile species as a result of microbial action or by plant, phyto-volatilisation.

Phyto-volatilisation occurs as plants take up contaminated wastewater. Plants take up heavy metals, metalloids and other components through their roots and shoots and the heavy metals or metalloids are released as volatile species to the atmosphere. The relative importance of and removal by these mechanisms will vary from wetland to wetland, based upon: media selection, influent water composition, and biological activity in the wetland.

2.3.6   Physico-chemical factors effecting performances of constructed wetlands

2.3.6.1   Adsorption

Removal of arsenic under oxic condition is perhaps best explained by the adsorption of arsenic with algae, bacteria, plant roots, organic substrates, and/or adsorption onto oxide minerals and concomitant co-precipitation specifically with Fe(III)oxyhydroxides. Reasons for the immobilization of arsenic under oxic condition were similarly explained by other authors (Wilkie and Hering, 1996; Bednar et al., 2005, Hering et al., 1996; Driehaus et al., 1998).

2.3.6.2   Precipitation and co-precipitation

Precipitation is an important process for metal removal. Removal of metals such as copper or zinc can also take place through sorption or co-precipitation on the surface of iron and

manganese oxides (Sobolewski, 1996).

Aerobic processes in the wetland system cause the precipitation of some metals and concomitant co-precipitations of several metals or metalloids, for example, iron and arsenic. The oxidation of ferrous iron to ferric iron and the subsequent precipitation of iron oxyhydroxides is a dominant process:

$$4Fe^{2+} + O_2 + 6H_2O \rightarrow 4FeOOH + 8H^+$$
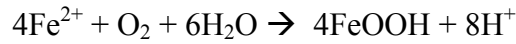
In wetlands, formation of sulphide may provide long-term metal removal, and many metals found in mine drainage form highly insoluble precipitates in the presence of dissolved sulphide (Stumm and Morgan, 1996). Sulphide precipitation accomplish on production of S^{2-} in the sulfate reduction zone of the wetland soil profile. This requires low redox potentials associated with anaerobic conditions.

Anaerobic conditions enhance microbial sulphate reduction where sulphate-reducing bacteria fulfill their energy needs by using sulphate as electron acceptor coupling with anaerobic oxidation of organic matter during their respiration. This process promote alkalinity production and potentially generate sufficient sulphide (S^{2-}) which might lead to the arsenic precipitation as sulphides (likely orpiment As_2S_3) with low solubility (Moore et al. 1988, Kim et al. 1999). The arsenate-respiring, anaerobic organisms described by Macy et. al. (2000) and Newman et. al. (1997) can utilize both arsenate and sulphate as electron acceptors, and may subsequently precipitate As_2S_3 in the surrounding media. The As-sulphide phases have bee suggested as important sinks for As(III) in reduced environment (McCreadie et al. 2000, Rittle et. al. 1995). These processes of arsenic immobilization can also be accomplished and tested in constructed wetland systems. In one specific example, Langner et. al. (1999) performed a greenhouse study using controlled wetland chambers in the presence and absence of wetland plants and observed rapid reduction of As(V) and SO_4^{2-} to As(III) and S^{2-} species, respectively, followed by a slower but significant decrease in arsenic concentration in the pore water.

It should be noted that the beneficial decrease in As(III) concentrations observed as a result of As_2S_3 formation is extremely sensitive to re-oxidation and solubilisation of As_2S_3 solid phases. Nevertheless, the formation of As(III)-sulphide phases under wetland environments may represent one possible immobilization strategy for minimizing As transport to surface water or groundwater (McCreadie et al. 2000, Langner et. al. 1999).

The As-sulfide precipitation also requires a sufficient source of SO_4^{2-} to match the

requirements. It is a fact that these metal or metalloid sulphides will remain permanently in wetland sediments as long as they are not re-oxidized or as long as the sediments remain anaerobic (Sobolewski, 1996). Consequently, it is important to prevail anaerobic conditions in wetlands for a higher capacity of arsenic and heavy metal removal.

2.3.6.3 Redox potential and pH

Redox conditions and pH have been shown to be crucial factors influencing the release and transformation of arsenic from contaminated sediments (Mok and Wai 1989; Masscheleyn et al. 1991). These are important factors controlling As and heavy metal speciation and their distribution. The redox state of the environment is the result of an energy demand from both aerobic and anaerobic microbes that can mediate arsenic transformation towards thermodynamic equilibrium. Therefore, evaluation of the redox state may serve as a quantitative measure for As mobility under various redox conditions. The redox condition (Eh) of wetland soil and sediment vary widely from approximately +500 mV (surface soil) to approximately -320 mV (strongly reducing soil).

Sediment redox levels can greatly affect toxic metals uptake by plants (Guo et al., 1997). Plant arsenic tissue concentrations and uptake were highest under reduced soil conditions (Marin et al., 1993). Redox conditions can affect the degradation and solubility of organic material and then influence the release of arsenic and heavy metals. Heavy metals or metalloids can also exist as sulphides under anaerobic conditions, which are susceptible to Eh and pH changes (Gambrell et al., 1980).

Numerous studies shows that As(V) and As(III) removals as a function of pH. As(V) removal strongly depends on the pH. Highly efficient As(V) observes at pH 5.5, while no removal occurred at pH 9.6. As(III) removal, in contrast, is extremely low at pH 5.5 and changes very little in the pH range of 7.5 – 9.6. This is because As(III) exists predominantly as the neutral species $H_3AsO_3^0$ when the pH of water is below 9.2 and is a poor ligand when compared with As(V). Under oxidizing conditions, $H_2AsO_4^-$ is dominant at low pH (less than about pH 6.9), whilst at higher pH, $HAsO_4^{2-}$ becomes dominant ($H_3AsO_4^0$ and AsO_4^{3-} may be present in extremely acidic and alkaline conditions, respectively). Under reducing conditions at pH less than about pH 9.2, the uncharged arsenite species $H_3AsO_3^0$ will predominate (Smedley and Kinniburgh, 2002).

2.3.6.4   Evapo-transpiration

Evapo-transpiration is the net water loss caused by the evaporation of moisture from the wetland surface and also by the transpiration of wetland plants due to their physiological activity. In case of high water loss from the wetland systems, concentrations of the contaminants substantially increases and thus sometimes prevents to achieve required effluent concentrations and hence area specific mass loading rates are calculated by taking water loss into considerations. It is assumed that, for a wetland system, although the presence of vegetation retards evaporation, by increasing shade and humidity and reducing wind near the surface, transpiration by the vegetation compensates for the difference. It is also influenced by vegetation on the disposal field.

Evapo-transpiration can remove high volumes of effluent in the late spring, summer, and early fall, especially if large silhouette and good transpiring bushes are used (EPA, 1998).

2.3.7   Biotic factors effecting arsenic removal in constructed wetlands

2.3.7.1   Plant biomass

Most plant species have a restricted translocation of metals and arsenic to the shoots (Stoltz and Greger, 2002) but were found to be root accumulators. Moreover, the plant rhizome and root provides surface for bacterial growth as well as for filtration of solids. More importantly, plants are known to translocate oxygen from the shoots to the roots (Gersberg et al., 1986). The root zone will offer an oxidized micro-environment in an otherwise anaerobic substrate, which stimulates the decomposition of organic matter and the growth of nitrifying bacteria, which can convert ammonia to nitrate.

Some terrestrial plants are able to accumulate arsenic to a substantial extent (Brooks et al. 1977, Visoottiviseth and Sridokchan 2002, Wagemann et al. 1979) but survive the stress to differing degrees of vitality. The influence of arsenic on important energy and metabolic cycles does not yet have sufficient explanation. So-called hyper-accumulators take up more than 1000 mg kg^{-1} dry weight of the pollutant (Brooks et al. 1977). Since these plants bear such high arsenic quantities, they must have a strategy for detoxification (Visoottiviseth and Sridokchan 2002)

Arsenic can be taken up by plants and subsequently introduced into a food chain. This may be cause for concern, particularly when considering that as much as 99.7% of all biomass

is found in terrestrial plants; any contamination of these organisms will have far reaching effects (Trapp and McFarlane, 1995). Arsenate, arsenite, dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) are all arsenic compounds that can be taken up by a number of plant species either from solution or arsenic amended soils (Marin et al., 1992; Carbonell-Barrachina et al., 1998; Tlustoš et al., 2002; Tu et al., 2004). It is not surprising then that arsenate is often one of the main arsenic compounds reported in terrestrial plants, with arsenite, another inorganic species, frequently detected (Meharg and Hartley-Whitaker, 2002).

Arsenite can be found in anaerobic soils, and it was determined that rice plants actively transport arsenite into the roots (Abedin et al., 2002). In addition, arsenate can be reduced to arsenite soon after transport into the plant (Pickering et al., 2000). Further evidence for the metabolism of arsenic compounds in terrestrial plants was observed in phosphate-starved tomato (*Lycopersicum esculentum*) plants and cell suspension cultures of periwinkles (*Catharanthus roseus*) which were found to methylate inorganic arsenic to some extent (Cullen et al., 1989; Nissen and Benso, 1982). A pathway involving alternate reduction and oxidative methylation produces simple methylated arsenic compounds such as DMA and MMA identified in rice, grass (e.g. tufted hair), and trees (e.g. apple, cedar) (Meharg and Hartley-Whitaker, 2002).

Aquatic macrophytes play an essential role in creating and maintaining wetland conditions favorable for contaminant removal, for example, contribution of organic detrital matter producing reducing conditions. Phyto-accumulation also contributes to Se removal in wetlands.

Terrestrial plants are able to accumulate arsenic to a substantial extent (Wagemann *et al*., 1979; Brooks *et al*., 1997; and Visoottiviseth *et al*., 2002). Up to now, the only two species of arsenic hyper-accumulators were reported: *Pityrogramma calomelanos* and *Pteris vittata* (Flores-Tavizón *et al*., 2003). Arsenic concentration in different parts of plants depends on its arsenic exposition. Higher values were found by Dushenko et al. (1995) in an aquatic system highly polluted with arsenic from a gold-mine effluent.

Most plant species have mechanisms to restrict translocation of metals and arsenic to the shoots (Stoltz and Greger, 2002). The amount of arsenic in plants depends almost solely on the amount of arsenic to which it is exposed. The concentration of arsenic in plants varies from less than 0.01 to about 5 mg kg^{-1} dry weight (Stoltz and Greger, 2002). Very little is known about the processes of arsenic within a plant or the tolerance mechanisms when

there is a high arsenic content in the soil. Furthermore, no concrete data exists concerning the effects of arsenic on the metabolism of plants.

However, from a technological point of view, the accumulation of arsenic and heavy metals by plants is usually insignificant when industrial effluent and mine drainage are being treated. This is because the amount that can be accumulated is only a fraction of the total load of heavy metal or metalloid in wastewater. Nevertheless, a number of terrestrial plants are known which can accumulate relatively high amounts of metals in their biomass.

2.3.7.2 Toxicity of arsenic on plants

Toxicity and accumulation of arsenic by plants depends on the plant species, concentration of arsenic and the presence of other ions. At low concentration, arsenic is not essential for plants and appeared not to be involved in specific metabolic reactions; however, it interferes with metabolic processes and inhibits plant growth and sometimes leads to plant death, at higher concentration (Marin, 1993).

Arsenic speciation was more important than the As level in solution in determining the phyto-toxic effect of As on turnip cultiva. Arsenic chemical form in solution influenced root and shoot dry weights (Carbonell-Barrachina, 1999). In the case of well-grown plants, arsenic exists mainly in the three-valence state. The main arsenic component in plants with poor growth or which have died was found to be arsenate (Mattusch et al., 2000). Protection mechanism of the pants from interruption by arsenic is also illustrated by Mattusch et al. (2000). This is an efficient way for plants to protect themselves from interruption of the oxidative phosphorylation by arsenate (Dixon, 1997). After the plant's death, arsenite can be rapidly oxidized again to arsenate by the loss of enzymatic activity.

Mattusch et al. (2000) reported that *Juncus effusus* accumulated moderate total arsenic concentration (around 250-270 $\mu g\ kg^{-1}$ dry mass) in their shoots, and the predominant arsenic species found in the shoots was arsenite even though the roots are mainly surrounded by arsenate adsorbed on ferric oxyhydrate.

Phyto-toxic symptoms from arsenic to *Typha latifolia* were observed already at concentrations exceeding 300 mg kg^{-1} in sediment, and 400 $\mu g\ l^{-1}$ in the water (Dushenko et al., 1995). Similar results were obtained with *Spartina patens* whereby the organic form dimethyl arsinic acid showed the highest toxicity (Carbonell et al., 1998).

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2.3.7.3 Microorganisms

Toxic compounds in soils are often modified by microbes (Van Zwieten et al., 2003), but many such toxins also may hinder growth of soil microbes and impair their ability to promote plant growth. Additionally, fungi associated with roots have the potential to either increase or ameliorate the uptake of inorganic contaminants by plants. Consequently, mycorrhizal fungi are crucial in maintaining diverse populations of indigenous vegetation and act as a barrier to the uptake of toxic heavy metals by plants (Leyval et al., 1997). Sharples et al. (2000) presented evidence that the ericoid mycorrhizal fungus *Hymenoscyphus ericae* acts as a filter to maintain low arsenic uptake rates by roots of the plant *Calluna vulgaris* when growing in arsenic contaminated soil. In a study of evolved arsenate resistance in cultivars of the grass *Holcus lanatus*, Gonzalez-Chavez et al. (2002) found that colonization by the arbuscular-mycorrhizal fungus *Glomus* suppressed high-affinity arsenate and phosphate transport into the roots. Conversely, mycorrhizal association with the fern *Pteris vittata* has been reported to stimulate arsenic accumulation by the host (Liu et al., 2005).

The mechanism of accumulation is poorly understood, but is mediated by rhizosphere microorganisms (Walter and Wenzel, 2002; Liu et al., 2005). Those microorganisms living in symbiotic association with plant roots in soils with long-term arsenic contamination.

Microorganisms play an important role for arsenic and metal removal. It has been shown that in the rhizosphere, the zone near the root cells, the density of microorganisms is higher than in the zone far from the roots. The microorganisms can transform heavy metals and arsenic. There are four mechanisms involved with the removal; i.e. adsorption to the cell surfaces, complexion, precipitation and volatilization (Bitton, 1994).

- **Adsorption to the cell surface:** microorganisms bind metals as a result of interaction between metals ions and the negatively charged microbe surfaces. Gram-positive bacteria are particularly suitable for metal binding. Fungal and algal cells also have a high affinity for arsenic and heavy metal removal.

- **Complexation:** microorganisms can produce organic acids (e.g., citric acid), which may chelate toxic metals and arsenic, resulting in the formation of metalorganic molecules. Metals may also be complexed by carboxyl groups found in microbial polysaccharides and other polymers.

- **Precipitation:** some bacteria promote arsenic and metal precipitation by producing

hydrogen sulphide, which precipitate arsenic as their sulphides (e.g. As_2S_3). Sulphate reducing bacteria (SRB) transform SO_4^{2-} to H_2S, which promotes the extra-cellular precipitation of arsenic and metals from solution.

- **Volatilization:** arsenic and some metals are transformed to volatile species as a result of microbial action. For example, microbially mediated methylation converts inorganic arsenic As(V) and As(III) to volatile and toxic species arsine (AsH_3), MMAA to monomethylarsine [MMA, $AsH_2(CH_3)$], DMAA to dimethylarsine [DMA, $AsH(CH_3)_2$] and TMAO to trimethylarsine [TMA, $As(CH_3)_3$] (Cullen and Reimer, 1989).

2.3.7.4 Toxicity of arsenic on microorganisms

The toxicity and behavior of arsenic in the environment depends on their species. Treatment of arsenic-contaminated wastewater with As(III) oxidase producing bacteria can improve certain arsenic removal methods (Brown *et al*., 2002). This enzyme catalyses the conversion of As(III) to As(V), which is easy to precipitate with ferric ion, consequently reduces arsenic concentration (Buddhawong, 2004). However, many microorganisms have such mechanisms to reduce the toxicity from arsenic and heavy metals. For examples, some enzymes that involve with oxidation and reduction of metal ions are one of the important mechanisms.

2.3.7.5 Sulphide toxicity to plants

If organic matter accumulates and decomposes under anoxic conditions, phytotoxins are released into the soil. In healthy sites, reeds are able to oxygenate the rhizosphere by convective flow through rhizomes (Armstrong et al., 1992), which may hence decrease concentration of sulphide in the rhizosphere.

Although sulphide may act as an inhibitor of N-uptake (Chambers et al., 1998; Mendelssohn and McKee, 1988), root absorption of both N and P did not seem to be hindered at die-back sites.

Sulphide may act as major phytotoxin, especially when environmental conditions such as waterlogged soil and high temperature affect gas diffusivity in roots, eventually enhancing the entrance of phytotoxins into the plant. High sulphide concentration may lead to toxic effects to aquatic plants, such as root decay (root blackening and increased flaccidity of the

roots) and mortality (Armstrong et al., 1996b; Smolder and Roelofs, 1996a), reduced growth (Koch and Mendelssohn, 1989; Koch et al., 1990; Van der Welle et al., 2006) or even mortality (Lamers et al., 1998; Smolders et al., 1995a).

Both sulphide and organic acids induce the formation of abnormal anatomical features such as callus blocking aerenchyma channels, lignifications and suberification of the surface layer of the root cells (Armstrong et al., 1996; Armstrong and Armstrong, 1999). On the other hand, callus blockage can also be induced by insect damage (Armstrong et al., 1996). It is known that sulphide is an inhibitor of aerobic respiration and nutrient uptake (Allan and Hollis, 1972; Mendelssohn and McKee, 1988). However, sulphide usually accumulates under anoxic conditions in brackish wetlands because of high sulphate concentration in the water (Armstrong et al., 1996).

Sulphide concentrations in sediment pore-water >1 mM have been found to induce stunted growth adventitious roots, lateral roots and buds, as well as callus formation in root and rhizomes, besides blockages in the vascular system (Armstrong et al., 1996; Armstrong, Armstrong and Pittaway, 1996; Armstrong, Armstrong and Van der Putten, 1996). Additionally, Fürtig et al., (1996) found that energy metabolism in *Phragmites australis* is negatively affected even at sulphide concentration in pore-water as low as 1 mM.

Goodman et al., 1995, found negative effects of sulphide on sea-grass photosynthesis and increased mortality during die-back event have also been related to sulphide exposure (Carlson et al., 1994, 2002; Holmer et al., 2001). Intrusion of sulphide is considered to be the main cause for rapid die-back event of *Thalassia testudinum* in Florida Bay (Borum et al., 2005).

Van der Welle (2007) investigated the responses of the freshwater wetland species *J. effusus* L. and *Caltha palustris* to iron supply in sulphidic environments. *J effusus* showed a double advantage under sulphide-rich condition: it does not suffer from sulphide toxicity since it can oxidize potentially harmful reduced compounds in its rhizosphere.

Sulphide toxicity, however, can be mitigated by the formation of highly insoluble metal sulphides like iron sulphides (FeS, FeS_2 or pyrite) or metal sulphide complexes (Huerta-Diaz et al., 1998; Smolders and Roelofs, 1995b; Wang and Chapman, 1999), thereby reducing both sulphide and metal toxicity. In areas where iron-rich groundwater is discharged, free S^{2-} concentration are usually low, as a result of iron sulphide precipitation.

2.3.7.6 Sulphide toxicity to microorganisms

The toxicity of sulphide in anaerobic reactors has been well studied. Koster et al., (1986) reported that a free sulphide of 250 mg S l^{-1} caused 50 % inhibitions of methanogenesis in UASB granules. In a lactate-fed serum vial test, McCartney and Oleszkiewicz (1993) observed a 50 % inhibition of the methanogenic activity at 100 mg l^{-1} free sulphide. In an acetate-fed UASB reactor, a free sulphide of 184 mg l^{-1} was also found to cause a 50 % inhibition of methanogenesis at neutral pH (Visser et al., 1996).

2.3.8 Application of the technology

There are an expanding number of application areas for constructed wetlands technology. During the early years (1985) of the development of the technology, virtually all emphasis was on the treatment of domestic and municipal wastewater. Later the emphasis was on domestic wastewater, agriculture wastewater and mine drainage water (Mandi et al., 1998; Gearheart, 1992; Knight et al., 2000). In recent years there has been a branching to include a very broad spectrum of wastewater, including industrial and storm-waters. Increasing attention is now also being paid to using constructed wetlands to treat leachate, contaminated groundwater and industrial effluents.

There are several roles for constructed wetlands in the treatment of domestic and municipal wastewaters. They can be positioned at any of several locations along the water quality improvement path. Constructed wetland technology is generally applied in two general themes for domestic and municipal wastewaters: for accomplishing secondary treatment and for accomplishing advanced treatment.

Constructed wetland treatment systems can provide secondary treatment of arsenic containing domestic wastewater after mechanical pre-treatment consisting of a combination of screen, grit and grease chambers, sedimentation, septic and Imhoff tanks.

3 Material and Methods

3.1 Treatment of arsenic containing model wastewater in the Planted Fixed Bed Reactor (PFBR)

The different processes of the complex redox system inside the rhizosphere are difficult to evaluate because of, for instance, the specific operating conditions of the constructed wetlands, which are characterized by usually slow flow rates, macro-gradients of concentrations, the possibility of short-circuit flows and unstable environmental conditions (annual and daily cycles, weather incidents/fluctuations). To successfully study the temporal aspects of redox variability inside the rhizosphere, a small planted gravel laboratory system with idealized flow conditions was used (Kappelmeyer et al., 2002). In order to counteract the flow inhomogeneties (short-circuit flow), this experimental system comprised with permanent mixing or continuous recirculation of fluid flow which prevented macro-gradients within the root bed and hence micro-gradient processes could also be evaluated (Kappelmeyer et al., 2002).

3.1.1 Synthetic wastewater

The artificial wastewater simulated a typical secondary effluent of domestic wastewater (DIN-38412-T24, 1981) with a theoretical COD value of about 340 mg l^{-1} which was derived from a combination of two different organic carbon sources (122.3 mg TOC l^{-1}), acetate and benzoate was used in this investigation (see Table 2). As(V) concentration of 0.2 mg l^{-1} was prepared from the stock solution of Titrisol [®] (Arsenic standard 1000 mg As l^{-1}, As_2O_5 in H_2O, Merck, Germany). The resulting concentration of parameters were (in mg l^{-1}): 340 COD, 30.8 ammonia-N, 5 phosphate-P, 0.2/5/25 sulphate-S and 0.2 As(V).

Table 2: Chemical composition of the synthetic wastewater

Reagents	Concentration (mg l^{-1})
CH_3COONa	204.9
C_6H_5COONa	107.1
NH_4Cl	117.8
K_2HPO_4	28
NaCl	7
$MgCl_2 \times 6H_2O$	3.4
$CaCl_2 \times 2H_2O$	4
As(V)	0.2
Na_2SO_4	0.89/22.19/110.94
Trace mineral solution (see Table)	1 ml l^{-1}

The composition of the trace mineral solution is shown in the Table 3.

Table 3: Chemical composition of the trace mineral solution

Compound	Concentration (mg l^{-1})
EDTA-Na	100
$FeSO_4 \cdot 7H_2O$	100
$MnCl_2 \cdot 2H_2O$	80
$CoCl_2 \cdot 6H_2O$	170
$CaCl_2 \cdot 2H_2O$	70
$ZnCl_2$	100
$CuCl_2 \cdot 2H_2O$	150
$NiCl_2 \cdot 6H_2O$	30
H_3BO_3	10
$Na_2MoO_4 \cdot 2H_2O$	10
$Na_2SeO_3 \cdot 5H_2O$	2
HCl	3 ml l^{-1}

These compounds were dissolved in deionised water by varying the concentration of sulphate-S and with/without organic carbon, arsenic during different experimental phases in the model reactor.

3.1.2 Experimental design: laboratory-scale reactor

Three laboratory-scale model wetlands (PFBR1, PFBR3 and PFBR4) were established under the condition of complete mixing of the filter bed by continuous circulation of the pore water. Since the internal flow conditions were comparable to conditions of a continuously stirred tank reactor or ideal mixed vessel, macro-scale gradients of concentrations, E_h, pH, etc. were equalized and the effects of the gradient changes could easily be determined in this system. The design and the principles of operation of the reactors were previously described in detail (Kappelmeyer et al., 2002; Wiessner et al., 2005). Figure 3.1 shows the process scheme of the Planted Fixed Bed Reactor -PFBR.

The reactors were consisted of a cylindrical glass vessel (diameter: 30 cm and height: 30 cm). A perforated stainless steel basket (diameter: 28 cm and height: 30 cm) was placed centrally inside the glass vessel. A suction cylinder (diameter: 4 cm and height: 30 cm) was placed at the center of the metallic basket from which the "internal" circulation flow was pumped by the peristaltic pump and was re-circulated into the distribution ring. This permanent mixing of the pore water made for hydrodynamic condition similar to an ideal mixed vessel inside the rhizosphere (Kappelmeyer et al., 2002). The basket was completely filled with 21 kg of gravel (2-6 mm in diameter, density 1.665g cm^{-3}, porosity 0.39) around

the perforated suction cylinder. The reactors were closed tightly with a Teflon lid containing five circular openings through which the plants (14 shoots per hole) were grown in the gravel bed. The height of the gravel bed was 28 cm and the water level was adjusted to 2.5 cm below the surface of the gravel bed.
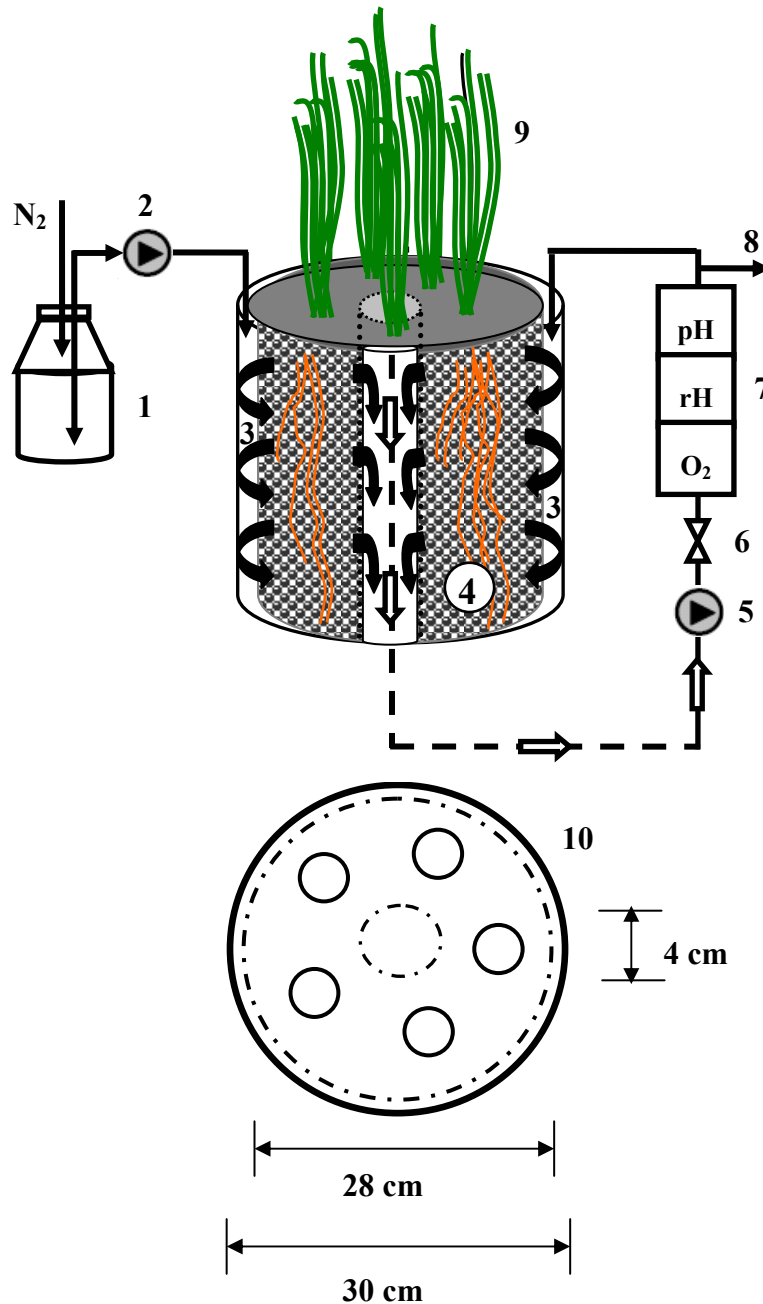

Figure 3.1 Schematic diagram of the Planted Fixed Bed Reactor- PFBR (1 Feeding storage tank, 2 Pump, 3 Distribution chamber, 4 Gravel bed, 5 Recirculation pump, 6 Magnetic valve, 7 On line measurement , 8 Outflow , 9 Plants, 10 Layout (plan view)) (adapted from Kappelmeyer et al., 2002).

The pore water volume in the planted bed amounted to 10 l and the hydraulic retention

time was adjusted to 5 days. The influent was pumped from the inflow feeding tank to the inlet of the reactors by means of a pre-calibrated piston pump at 1.4 ml min^{-1} (approximately 0.2 pore volumes per day). The water level in the reactor and the outflow was controlled by a level control system that comprised with a bottom pressure sensor, which controlled the outlet-valve in the internal circulation flow. The circulation flow was adjusted to 10 times the inflow. The recirculation system was connected to a microprocessor Standard (WTW, pH-Ionen-to Put pMX 3000/pH). The circulation flow represented the actual concentration of the pore water inside of the reactor and thus the pH, redox potential (E_h), and the oxygen concentration (DO) were controlled continuously in the circulation flow and the data were recorded online twice per hour. The reactors were completely filled with arsenic containing artificial wastewater (see Table 2) up to the defined level.

Plant transpiration represented 98 % of the total water loss (Wießner et al., 2005) and was controlled by balancing the inflow and the outflow amounts of water. The physical and operational characteristics of the PFBR are shown in Table 4.

Table 4: Physical and operational characteristics of the PFBR

Characteristics	
Soil material	Gravel
Gravel diameter (mm)	2 – 4
Uniformity coefficient of the gravel (D_{60}/D_{10})	1.1
Porosity (%)	0.39
Total reactor volume (L)	20.1
Effective reactor volume (L)	13.2
Pore water volume (L)	10
Height of the reactor (mm)	280
HRT (days)	5
Flow rate (ml/min)	1.4

3.1.3 Plant biomass

All the experimental reactors were planted with well-developed macrophytes (*Juncus effusus*) which were pre-grown in a hydroponic culture under greenhouse condition at a temperature of 23 ± 2°C. Plants used for plantation to the reactors were apparently well established in their place of origin. The macrophytes were planted with a range of 14 green shoots per circular hole of Teflon lid which resulted in an approximately 70 green shoots of *Juncus effusus* in each reactor. This particular species of macrophytes was selected on the basis of their common occurrence, rapid growth (plant biomass production), rooting

depth, tolerance to high loads of wastewater and abundance in natural wetlands. Numbers of green shoots were counted on monthly basis and also after the termination of the experiment; the total number of shoots and the total dry weight of the shoots and roots were estimated.

3.1.4 Experimental conditions

The planted fixed bed reactors were fed and run under six different experimental phases (phase A, B, C, D, E and F) realised by varying organic carbon and sulphate-sulphur concentrations in the simulated wastewater of inflow feeding tank (see Table 5). Each phase had a sufficient duration to guarantee a representative number of samples taken from each experimental reactor.

Table 5: Operating conditions of the planted fixed bed reactors during different experimental phases (Phase A, B, C, D, E and F) realised by different organic carbon, sulphate-sulphur and arsenic concentrations in the artificial wastewater

Reactor	Inflow concentration (mg l^{-1})	Experimental phases					
		A	B	C	D	E	F
PFBR1	COD	-	340	340	340	340	340
	SO_4^{2-}-S	0.2	0.2	0.2	0.2	0.2	0.2
	As(V)	0.2	0.2	0.2	0.2	0.2	0.2
PFBR3	COD	-	340	340	-	340	340
	SO_4^{2-}-S	5	0.2	5	0.2	5	-
	As(V)	0.2	0.2	0.2	-	0.2	0.2
PFBR4	COD	-	340	340	-	340	340
	SO_4^{2-}-S	25	0.2	25	0.2	10	-
	As(V)	0.2	0.2	0.2	-	0.2	0.2

(-) below detection limit (BDL)

Prior to start of the experimental phases, the reactors were newly planted after highly efficient operating for several months using similar artificial wastewater (see Table 2) without any carbon, sulphate-sulphur or arsenic (Wießner et al., 2005) and ensured the same initial conditions. The experimental period with a continuous arsenic-contaminated inflow supply started in March 2007 and continued until February 2008 for a total operation time of 340 days. Table 6 shows the starting and ending dates, time duration of

the different experimental phases in the reactors. PFBR1 was represented as a control reactor with the same arsenic, sulphate-sulphur and organic carbon loading throughout all the experimental phases, except in Phase A where no additional organic carbon sources were added.

Table 6: Operating time of the Planted Fixed Bed Reactors during different experimental phases (Phase A, B, C, D, E and F)

Phase	Starting date	Ending date	Time duration (days)
Phase A	07/03/2007	30/05/2007	0 - 83
Phase B	30/05/2007	31/07/2007	83 - 144
Phase C	31/07/2007	23/10/2007	144 - 228
Phase D	23/10/2007	30/10/2007	228 - 235
Phase E	30/10/2007	18/01/2008	235 - 315
Phase F	18/01/2008	12/02/2008	315 - 340

PFBR3 and PFBR4 were used for varying the sulphate-sulphur concentration from a substantially medium to high level and kept the same arsenic and organic carbon concentration in order to investigate how increasing in the sulphur concentrations influence the arsenic dynamics and removal efficiency in root near environment of the rhizosphere, both in carbon limited and carbon surplus conditions.

The reactors (see Fig 3.2) were placed in a greenhouse and operating under defined environmental conditions with a temperature of 16-22°C simulating an average summer day in moderate climates (Wiessner et al., 2005a). The temperature was set to 22°C from 6 am to 9 pm to simulate daytime and to 16°C at night. One lamp (Master SON-PIA 400 W, Phillips, Belgium) was switched on during daytime as an additional artificial light source whenever the natural light fell below 60 klx. Day/night length was controlled and maintained as 16/8 hours.

Figure 3.2 Experimental set up of the Planted Fixed Bed Reactor – PFBR

The different experimental phases were established in order to compare the arsenic removal efficiency in these reactors by varying organic carbon and sulphate concentrations in the influent. In experimental phase A, all the three reactors were fed without any additional organic carbon sources and associated with different sulphate-sulphur concentrations as 0.2, 5 and 25 mg l^{-1} in PFBR1, PFBR3 and PFBR4 respectively. The resulted molecular ratio of sulphate-sulphur to arsenic varied as 1:1, 25:1 and 125:1 in the three respective experimental reactors. This phase continued for the first 83 days under persistent oxic conditions (Eh~280-300 mV) that prevailed inside the reactors prior to changing to the experimental phase B. The effects of increasing sulphate-sulphur concentration on arsenic fixation in root near environment of the rhizosphere under carbon deficient conditions were demonstrated and studied in this phase A. Adsorption and complexation reactions of arsenic on oxides/hydroxides of Fe(III) were also investigated. A visibly stable condition achieved in the outflow dynamics of arsenic after 83 days of operation and then conditions were changed to the next experimental phase B.

Organic carbon sources (CH_3COONa and C_6H_5COONa) were added in all three reactors in phase B which, in total, provided a theoretical COD value of 340 mg l^{-1}, a moderate carbon constituent in domestic wastewater. Instead of 5 and 25 mg l^{-1} (in phase A operation), the

concentration of sulphate-sulphur was lowered down to 0.2 mg l^{-1} in both PFBR3 and PFBR4 in this phase which was similar to PFBR1, the control reactor. Addition of organic carbon sources enhanced the drastic changes in redox condition dynamics in all the reactors and stimulated the growth of microbial biomass especially in root near environment of the rhizosphere which is of our particular interest from the viewpoint of arsenic transformation processes and fixation mechanism. For a better understanding of rhizosphere interaction and the fate, transformation and removal of arsenic under reduced conditions were addressed and documented in this Phase B which continued for 61 days (corresponding to days 83-144) in particular. Rapid depletion of oxygen by microbial anaerobic degradation of organic matter resulted in a sharp decreasing of redox potential E_h in this phase and a steady state condition approached in the redox state and outflow dynamics of arsenic. All three reactors were possessing similar running conditions prior to changing for the next experimental phase.

Then in phase C (duration of 84 days), concentration of sulphate-sulphur was again increased in this already changed reducing condition in PFBR3 and PFBR4 as 5 and 25 mg l^{-1} respectively and maintained the same (0.2 mg l^{-1}) in PFBR1. COD value and arsenic concentration remained the same in the artificial wastewater feeding solution in order to investigate the influences of high sulphate loading on the enhancement of arsenic removal in carbon surplus conditions. Special attention was paid on studying the transformation and removal mechanism of arsenic by dissimilatory sulphate reduction and sulphide formation, plant root activities etc. in this phase C (corresponding to days 144-228).

Phase D duration was the shortest of all the phases which operated only for 7 days (between days 228-235), a short-term monitoring campaign of 7 successive days. Cancellation of electron donor and arsenic in both PFBR3 and PFBR4 concomitantly and only a trace amount of sulphate-sulphur (0.2 mg l^{-1}) were added in the synthetic wastewater for this particular phase D operation. The behavior of already immobilised arsenic in the reactor deposit was closely investigated in this phase to observe the consequences if the carbon deficient and arsenic limited condition had any significant influences on the dynamics of arsenic or not. This phase was interestingly poised to monitor the stability of arsenic inside the reactors on daily basis and also any dominant re-oxidation of reduced sulphur compounds to sulphate were studied. However, an unexpected energy failure enforced the early termination of this phase leaving behind the trail of fully oxic conditions in the reactors by atmospheric oxygen inclusion and

overflowing the reactors resulted in the unrest of experimental conditions and subsequently the dynamics of arsenic.

A steady state condition established again after getting rid of that technical difficulty and reactors were prepared again for the next phase of operation, total duration of 80 days (corresponding to days 235-315). Addition of sulphate-sulphur with a concentration of 0.2, 5 and 10 mg l^{-1} in PFBR1, PFBR3 and PFBR4 respectively in the model wastewater solution and a moderate COD (340 mg l^{-1}) with the same arsenic concentration (0.2 mg l^{-1}) were used in this experimental phase which was represented as phase E. Sulphate-sulphur concentration was decreased to 10 mg l^{-1} in PFBR4 instead of 25 mg l^{-1} (in phase A and C) due to the fact that a probable high sulphide (S^{2-}) toxicity effect on plant growth was observed when added with high sulphate-sulphur (25 mg l^{-1}) in a rapid sulphate reducing conditions. Dynamics of arsenic along with fast sulphate reduction and interactions between arsenic and reduced sulphur species were the main objectives of this phase in operation. There were no elementary differences between phase C and this particular phase E, only exception was the reduced concentration of sulphate-sulphur in PFBR4. The new resulted molecular ratio of sulphate-sulphur to arsenic was established as 50:1 in PFBR4. Duration of this phase was 80 days and ended after day 315 of total experimental time.

Cancellation of sulphate-sulphur in the wastewater inflow feeding of PFBR3 and PFBR4 and keeping the same organic carbon (COD $\sim$ 340 mg l^{-1}) and arsenic (0.2 mg l^{-1}) concentration were performed in last and final experimental phase F. Probable re-oxidation of reduced sulphur species and arsenic bound sulphur to sulphate by microbial activity, stability of fixed/immobilised arsenic in the reactor bed were investigated due to the consequences of sulphur deficiency in this particular experimental phase F which lasted for 25 days (corresponding to days 315-340), till the termination of total operation on day 340.

COD, total carbon (organic and inorganic) removal, nitrogen removal via nitrification/denitrification, plant health status (number of green shoots, transpiration), total arsenic, all inorganic and methylated organic arsenic species, sulphur species and physical parameters like pH, E_h and DO were closely monitored, analysed and documented the correlations with one another throughout all the experimental phases in the experimental reactors.

Plants were checked twice in a week if growth status were hampered due to the toxic effects from endangering arsenic, sulphide or high carbon load that might be proved fatal for the plants. Stress symptoms of *J. effusus* were noticed by a general yellowing

(chlorosis) of shoots. Frequently this yellow shoots turned to dark brown (necrosis). A "die-back" response was defined by visual loss of green color (chlorosis) and necrotic symptoms as indicator of plants death.

3.1.5 Sampling

Water samples were taken from the inlet and outlet zones of each reactor (PFBR1, PFBR3 and PFBR4) with a syringe and N_2 gas was purged in the sample headspace to minimize autoxidation of sample ingredients, especially for arsenic species analysis. Samples were taken once in a week, except for phase D when samples for total arsenic, arsenic species, sulphur species were taken and analysed on daily basis. For dissolved gas analysis, samples were collected from the outlet of the reactors in a glass bottle completely full with water samples such that no void spaces were left in the bottle. Bottles were closed tight enough to ensure no atmospheric oxygen could penetrate in and altering the composition of dissolved gases of a particular sample. Due to the reactor design, the circulation flow represents the actual concentration of the pore water inside of the reactor and thus the pH, redox potential, and the oxygen concentration were controlled continuously in the circulation flow and the data were recorded online twice an hour.

After the termination of all experimental phases, plant biomass samples (shoots and roots) and sludge sediment were collected from each reactor. Plant samples were sectioned into their components shoots and roots after collecting and roots were thoroughly washed to remove any soil or gravel aggregates. All samples were freshly weighed (wet weight) and dried for three days at 110°C, allowed to cool, re-weighed, calculated the water content and then ground to a fine powder to obtain a homogeneous sample and preserved in sealed plastic bottles for analysis. Retaining liquid in the reactors (pore water) after the termination of total investigations were also collected, measured the volume and samples were taken for the parameters to be analysed. However, all these analytical results and calculations attributed towards a probable mass balance of total As, S, C and N for each experimental reactors.

3.2 Treatment of an arsenic containing model wastewater in the Laboratory-scale Horizontal Subsurface Flow Wetland

Constructed wetland used in this study was called laminar stream subsurface horizontal flow system. From the name, the flow path through the operational systems was horizontal along the wetland bed. In contrast to the planted fixed bed reactor (PFR) with homogeneous flow and without macro-gradients which was ideal for fundamental investigations to characterise the ongoing contaminant transformations, the laminar stream horizontal flow constructed wetland systems represented a more realistic and near to practice design. Both macro- and micro gradients prevailed in this system. During the passage in the system, wastewater contaminants came in contact with a network of aerobic, anoxic and anaerobic zones (macro- and micro- redox gradients) in the gravel media where big variety of suspended and in biofilm fixed microorganisms and plant roots were grown.

3.2.1 Synthetic wastewater

Model constructed wetlands were fed with tap water, trace mineral (TSM 3, 1 ml l^{-1}, see Table 3) and nutrient salt (Hakaphos, 0.1 g l^{-1}) for 3 months (August to October 2006) prior to starting of the experiments from November 2006. No addition of any organic carbon sources and arsenic were made in the model wastewater feeding during this particular time period.

Arsenic containing (0.2 mg l^{-1}) artificial wastewater (see 3.1.1) simulating a secondary domestic effluent with a theoretical COD value of about 340 mg l^{-1} which was derived from a combination of two different organic carbon sources (122.3 mg TOC l^{-1}), acetate (204.9 mg l^{-1}) and benzoate (107.1 mg l^{-1}) was prepared both in tap water and deionised water in this investigation.

3.2.2 Experimental design

Three laboratory-scale horizontal subsurface flow model wetlands (planted W1, unplanted W2 and planted W3) were established to investigate the fate of arsenic in the rhizosphere of wetland vegetation by varying organic carbon and sulphate loading rates under constructed wetland conditions (both macro- and micro-gradients) in this study.

The schematic diagram of a laboratory-scale subsurface horizontal flow wetland is shown in Figure 3.3.
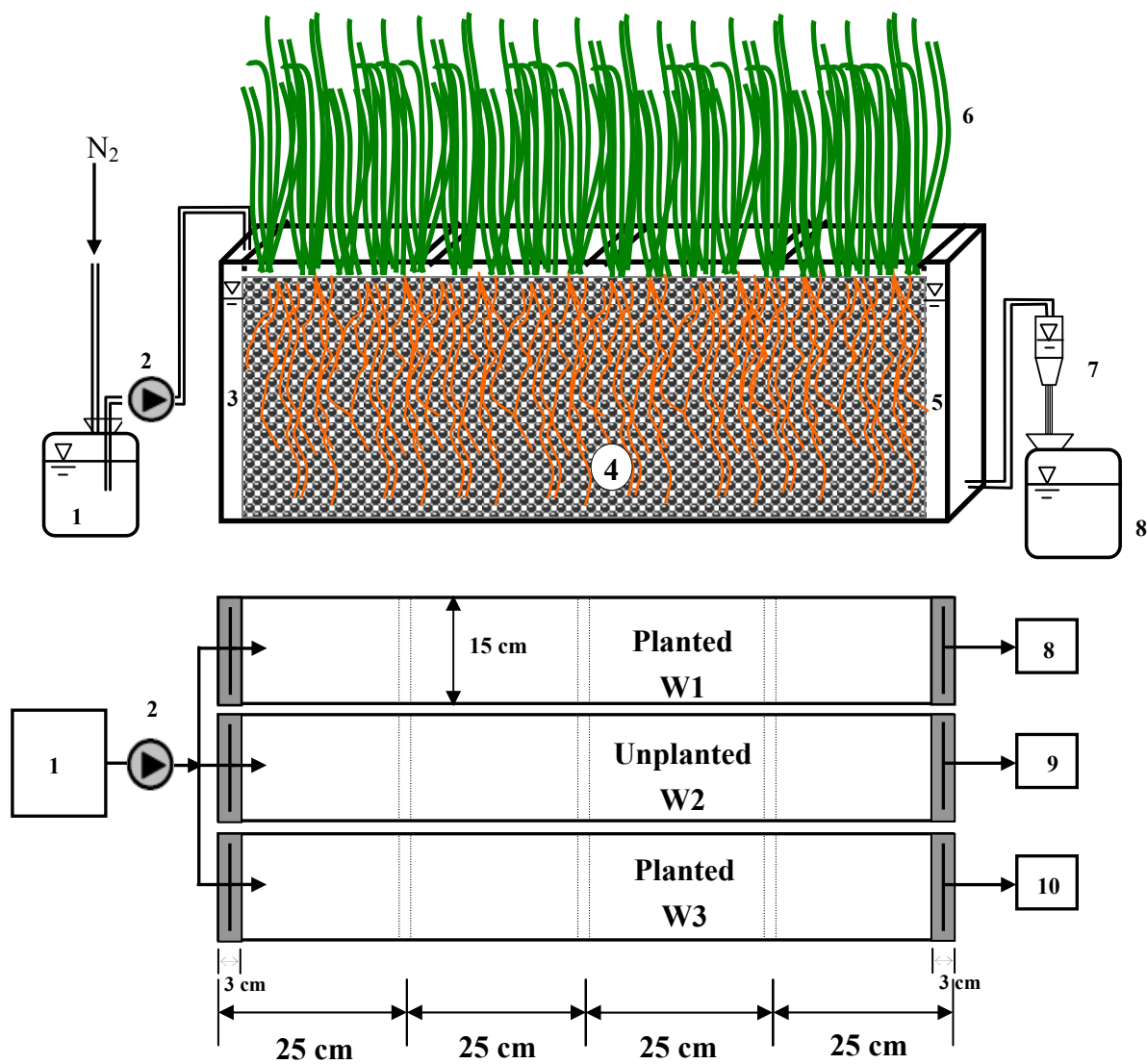
Figure 3.3 Schematic diagram and layout (plan view) of horizontal subsurface flow laboratory-scale constructed wetland. (1 Feeding storage tank, 2 Pump, 3 Inflow distribution chamber, 4 Gravel bed, 5 Outflow chamber, 6 Plant biomass, 7 Flow meter, 8-10 Outflow storage tanks)

The wetlands consisted of plastic containers of 100 cm in length, 15 cm in width and 35 cm in height. The containers were uniformly filled with 65.7 kg gravel (2-6 mm in diameter, 1.665 g/cm^3 in density, 0.39 in porosity) up to a height of 30 cm and had a free pore water volume of 25 l. The water level was adjusted to 5 cm below the surface of the gravel bed. Sieves of perforated stainless steel were placed 3 cm in front of the inflow and

outflow of the gravel bed. This free liquid volume should ensure an equal distribution of the inflow and a laminar liquid flow through the gravel bed (see Fig 3.3). Model wastewater was pumped continuously from the storage tank to the inlet of each wetland by means of a well-calibrated peristaltic pump which ensured the same flow rate for all the experimental wetlands. The inflow rate was adjusted to an average value of $5 \, l \, d^{-1}$ (~3.5 ml min^{-1}.), which provided a hydraulic retention time of 5 days.

The soil matrix used in the wetlands was washed gravel in a range between 2-8 mm in diameter. No plants were grown on Wetland 2 (W2) which was constructed in parallel representing as control wetland and the same model wastewater were fed into it. This control wetland W2 provided a baseline to compare plant performances in the treatment wetlands W1 and W3. Table 7 gives the main constructive details of the treatment units.

Table 7: Physical and operational characteristics of the laboratory-scale wetlands

Characteristics of the wetlands systems	W_1 , W_2 and W_3
Constructed height	0.35 m
Height of the gravel bed	0.30 m
Height of the water level	0.25 m
Length	1.00 m
Width	0.15 m
Hydraulic retention time	5 days
Pump inflow rate	$5 \, l \, d^{-1}$
Surface area specific hydraulic load	$3.33 \, cm \, d^{-1}$

Each laboratory-scale subsurface horizontal flow wetland was fed separately from the same storage tank (50 l capacity). The storage tank was used for storing the synthetic wastewater (see section 3.1.1) to be treated in the wetlands. The tank had to be re-filled before being empty to ensure uninterrupted flow of wastewater into the wetland beds. In order to keep an anoxic environment inside the storage tank containing synthetic wastewater, a continuous purging of nitrogen gas (N_2) through the headspace of the storage tank was maintained throughout the whole operation period.

3.2.3 Plant biomass

Wetland 1 (W1) and wetland 3 (W3) were planted uniformly with macrophytes (*Juncus effusus*) with a density of approximately 800 and 733 plant shoots m^{-2}, respectively into each of the planted wetlands in August 2005. This species of macrophytes was selected presumably on the basis of their common occurrence, rapid growth into their biomass (plant biomass production), rooting depth, tolerance to high loads of wastewater and abundance in natural wetlands. During the acclimatisation period of approximately 3 months, plants were fed only with tap water and fertilizer (NPK Wasserlösliches Nährsalz, Hakaphos, concentration of 1 g l^{-1}) as plant nutrient source.

By October 2005, the plant shoots were well established and covered the entire surface of the model wetlands. Feeding with arsenic containing synthetic wastewater (see section 3.1.1) started from November 2005. Number of green shoots were counted at least ones in every month throughout the whole operation time and also immediately after the termination of the experiment.

3.2.4 Experimental conditions

Experiments were conducted during a period of 491 days and the wetlands W1, W2 and W3 were fed and run under five different experimental phases (phases A, B, C, D and E) of operation (see Table 8) which were accomplished by varying organic carbon and sulphate-sulphur concentrations in the simulated wastewater for better evaluation of the performances of arsenic removal, both with the presence and absence of macrophyte biomass. Each phase had a sufficient duration to guarantee a representative number of samples taken from each experimental wetland.

Despite a continuous N_2 gas purging in the headspace to ensure anoxic environment in the feeding tank, artificial wastewater was freshly prepared in every 3 days to prevent microbial degradation during storage and operation. N_2 gas was vigorously purging in and bubbling out from the liquid phase of wastewater for approximately 20-25 minutes after each preparation of fresh feeding solution which ensured a complete removal the traces of dissolved oxygen from the feeding tank.

Table 8: Operating conditions of the experimental wetlands W1, W2 and W3 during different experimental phases (Phase A, B, C, D, and E) realized by different organic carbon, sulphate and arsenic concentrations of the artificial wastewater.

Wetland	Inflow concentration (mg l^{-1})	Experimental phases				
		A	B	C	D	E
Planted W1	As(V)	0.2	0.2	-	0.2	-
	SO$_4^{2-}$-S	50*	50*	0.2	0.2	-
	COD	-	340	-	340	340
	NH$_4^+$-N	5	30	30	30	30
Unplanted W2	As(V)	0.2	0.2	-	0.2	-
	SO$_4^{2-}$-S	50*	50*	0.2	0.2	-
	COD	-	340	-	340	340
	NH$_4^+$-N	5	30	30	30	30
Planted W3	As(V)	0.2	0.2	-	0.2	-
	SO$_4^{2-}$-S	50*	50*	0.2	0.2	-
	COD	-	680	-	340	340
	NH$_4^+$-N	5	30	30	30	30

(*) Tap water
(-) below detection limit (BDL)

Model wetlands were placed in a greenhouse (see Figure 3.4) with 16-h day length and operating under defined environmental conditions with a temperature of $22 \pm 2^{\circ}$C simulating an average summer day in moderate climates (Wiessner et al., 2005a). The operational periods (see Table 9) lasted from November 2006 to March 2008.

Table 9: Operating time and dates of the model wetlands W1, W2 and W3 during different experimental phases (Phase A, B, C, D, and E)

Phase	Starting date	Ending date	Time duration (days)
Phase A	06/11/2006	05/02/2007	0 - 91
Phase B	05/02/2007	31/07/2007	91- 267
Phase C	31/07/2007	13/11/2007	267 - 372
Phase D	13/11/2007	12/02/2008	372 - 463
Phase E	12/02/2008	11/03/2008	463 - 491

The key concept of how a demonstration horizontal subsurface flow wetland for arsenic fixation might work under carbon deficient and oxic environmental condition was carried

out and studied in all three model wetlands W1, W2 and W3 in the experimental phase A. Inflow concentration of arsenic in synthetic wastewater was similar (0.2 mg l^{-1}) to the wastewater that was used in PFR experiments. No addition of organic carbon sources ensured a persistent aerobic condition in the wetland beds and allowed the activity of aerobic microorganisms. Instead of deionised water, tap water was used which already provided a high sulphate-sulphur concentration of about 50 mg l^{-1} in the synthetic wastewater. The resulted molecular ratio of sulphate-sulphur to arsenic (S:As) was very high (250:1) in all three experimental wetlands. NH_4^+-N concentration of 5 mg l^{-1} was also added to investigate the nitrogen removal efficiency, rhizosphere acidification (pH). Adsorption and precipitation/co-precipitation reactions of arsenic on oxides/hydroxides of Fe(III) under aerobic condition were closely investigated both in planted wetlands W1, W3 and comparing them with unplanted wetland W2. First 91 days of the total experimental period belonged to this particular phase A.
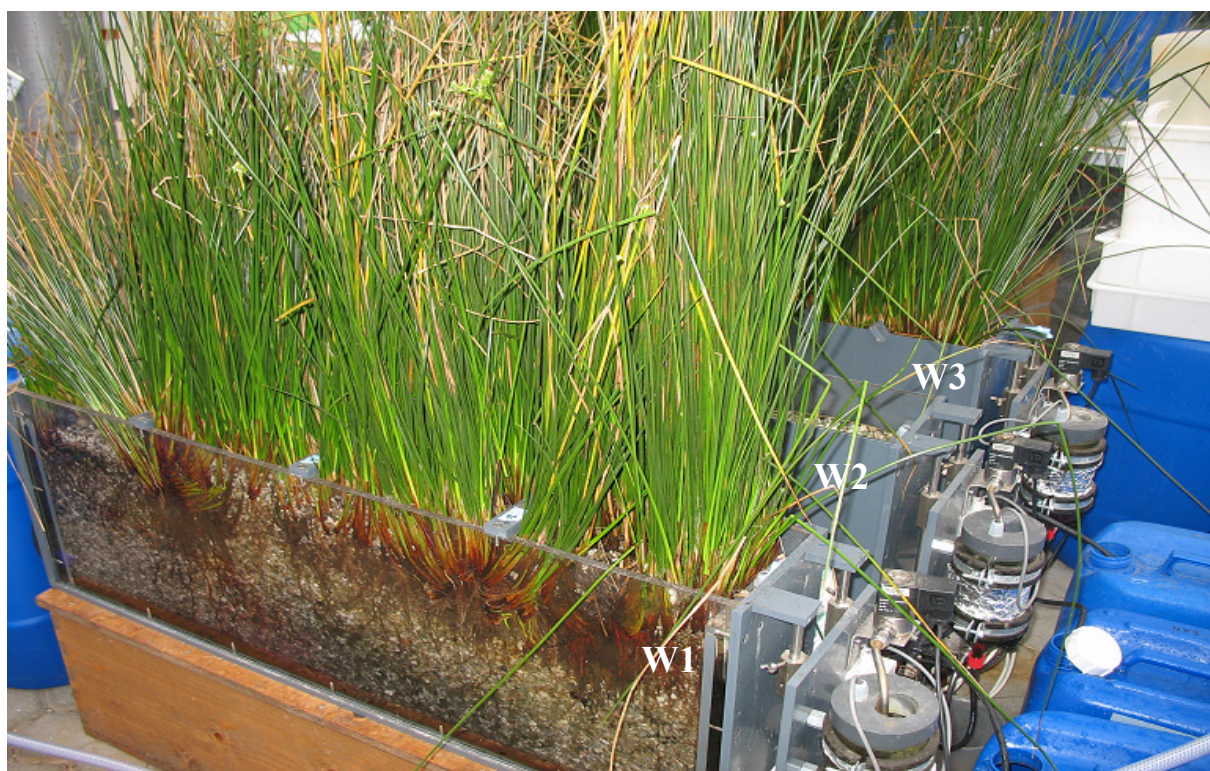
Figure 3.4: Experimental set up of the laboratory scale subsurface horizontal wetlands: W1 (planted), W2 (unplanted) and W3 (planted).

Organic carbon content in a moderate level is one of the major constituent of secondary domestic effluent. So, the next investigation objective highlighted the addition of electron donor in the simulated wastewater and studied the effects on arsenic, sulphur and nitrogen removal in parallel under C-surplus conditions in both planted and unplanted wetland.

Phase A and B differed only by the fact that organic carbon sources were included in the wetlands. Starting of phase B operation on day 91, two organic carbon sources (CH_3COONa and C_6H_5COONa with 204.9 and 107.1 mg l^{-1} respectively) were added in the model wastewater inflow of planted wetland W1 and unplanted wetland W2, which resulted a theoretical COD value of 340 mg l^{-1} in total, similar to the carbon concentration used in case of PFR experiments. Using tap water (SO_4^{2-}-S $\sim$ 50 mg l^{-1}) resulted a molecular ratio of carbon to sulphate-sulphur as 6.8:1. To further investigate the effects of high carbon loading on the dynamics of arsenic and sulphate-sulphur under stimulated microbial anaerobic degradation, carbon concentration was doubled (COD $\sim$ 680 mg l^{-1}) in planted wetland W3 (C:S $\sim$13.6:1). Continuous purging of N_2 gas in the headspace of inflow feeding tank ensured anoxic or anaerobic condition and prevented any unwanted microbial degradation inside the tank and connecting inflow tubes. Transformation behaviors of arsenic and removal efficiency were observed under dynamic redox conditions and comparison were made both in planted W1, W3 along with unplanted W2. Addition of organic carbon sources attributed to the changing of the redox dynamics rapidly in all the wetland beds and presumably stimulated the growth of anaerobic microorganisms such as sulphate reducers, Fe^{3+} reducers, methanogenic bacterias, denitrifiers and others. Methanogenesis (methane production), sulphidogenesis (sulphide formation) by dissimilatory sulphate reduction, plant activity (growth status of shoots, EVT), nitrogen removal (nitrification/denitrification), sulphur transformation processes etc. incorporated with arsenic in the systems were also closely monitored in this phase B which continued upto day 267. Strict anaerobic environment was achieved immediately after the addition of organic carbon sources in this phase and a steady state condition approached in the outflow dynamics of arsenic in all three model wetlands.

After the first experimental phase A and initial few days of phase B, plants were visibly much healthier (darker green shoots) but as phase B continued, plants were showing substantial stresses and pigmentation changed from green to yellow, followed by dark brown and sooner they died. This was probably due to the fact that a longer period of organic carbon loading and sulphate reducing anaerobic condition consequenced a substantial amount of sulphide formation which proved fatal for the wetland plants. In combination with sulphide and arsenic exposure under reduced condition in the systems, toxicity level was presumably too high for the mortality of plants. Despite the presence of sufficient plant nutrients in the model wastewater, over a period of 176 days of phase B

operation, it became apparent that wetland plants were suffering immensely and more than 25% of the plant biomass were dead and density of green shoots decreased from 9367 m^{-2} (starting of phase B) to 7007 m^{-2} (end of phase B) and EVT rate also dropped-down from 2.67 l d^{-1} to 0.87 l d^{-1} (nearly 67 %). Dead plant biomasses were removed from the wetland beds which was approximately 150-180 g dry weight.

At that point of investigation, decision was taken to change the feeding strategy from phase B to phase C where no addition of any particular contaminants that would cause persistent harm to the plant growth. Hence the cancellation of As(V), organic carbon, high sulphate-sulphur loading were made in the model wastewater composition. Only a trace amount of SO_4^{2-}-S (0.2 mg l^{-1}) and same amount of plant nutrients (like in phase B) were added in deionised water resulted a newly simulated wastewater feeding for all three model wetlands. The effects of the presence or absence of plant biomass on the outflow dynamics of arsenic and reduced sulphur species was the prime focus to investigate in this experimental phase C. This study also highlighted whether the already immobilised arsenic (in phase A and phase B) were showing stability to stay inside the wetland bed and comparison were made between planted and unplanted wetlands to conclude if plants were playing a significant and decisive role for a better immobilisation or not. Probable re-oxidation of reduced sulphur and arsenic bounded sulphur to sulphate under fully-aerobic condition (E_h ~700 mV) and plant root activity were studied in this phase C. During this 105 days of experimental phase (corresponding to days 267-372), plant biomass density was increased significantly which clearly indicated that plants were rapidly recovered from the stress generated in previous phase B, established themselves again in phase C and remained in a good condition (healthy, dark green shoots). When a stabilised condition in the outflow dynamics of arsenic and sulphur was achieved in all three wetlands, they were ready for the next experimental phase for further investigation.

In experimental phase D (starting on day 372), moderate organic carbon (COD ~340 mg l^{-1}) along with As(V) of 0.2 mg l^{-1} were added to all the model wetlands in order to investigate the outflow dynamics of newly added arsenic and already immobilised arsenic in the wetland deposit under a sulphate-reducing condition. The composition of synthetic wastewater in this phase was almost similar to the experimental phase B, only exception was the amount of sulphate-sulphur concentration which was maintained as trace amount (0.2 mg l^{-1}) in phase D, instead of 50 mg l^{-1} used in phase B. Sulphate-sulphur removal efficiency, As- and S-mass accumulation, transformation of S-species, COD and total

nitrogen outflow concentrations, plant toxicity, inhibition of nitrification/denitrification due to As and sulphide toxicity as compared to the experimental phase B were closely monitored and studied in this phase D on the basis of both concentration and loading rate. Phase D was ended up after 463 days of total operation (duration of 91 days) and leaving behind a stable arsenic outflow concentration in all three model wetlands.

In the final experimental phase E, stability of immobilised arsenic in wetland deposit was investigated under C-surplus condition (opposite to the experimental phase C). Only organic carbon sources (COD~340 mg l^{-1}) and NH_4^+-N (~30 mg l^{-1}) remained the same like the previous phase D, otherwise As(V) and sulphate-sulphate were cancelled (~BDL) from the simulated wastewater feeding solution. This phase was carried out to demonstrate how sulphur bounded arsenic and those of arsenic which mineralised with organic matter behaved along with rapid microbial anaerobic degradation of organic matter and with concomitant C-mineralisation. Re-oxidation of reduced sulphur species to sulphate by plant mediated oxygen and concomitant sulphate reduction in anaerobic environment, sulphide formation etc. were compared between both planted and unplanted wetland and afterwards with phase C. This phase duration was 28 days (corresponding to days 463-491) which continued until the end of the whole experimental period on day 491.

The scientific objective of crossing the experimental phases in all three wetlands was to confirm that the findings were not wetland specific. Plant growth (no. of green shoots), water loss (EVT) and chlorophyll *a* fluorescence were useful parameters in all experimental phases to identify probable toxic effects of inflow contaminants in the simulated secondary domestic effluent wastewater on plant biomass.

3.2.5 Maintenance

Prior to start of each experimental phase and several times during each phase operation, all the model wetland units were checked on regular basis and well-maintained to ensure the same initial and running conditions. Hence the systems were inspected on, at least, a weekly basis concerning the overall functioning. Major attention was given to the inlet and outlet flow pipes, tubes and structures, which were checked twice a week after re-filling the inflow storage tank and being made empty of the three outflow tanks, as obstruction/clogging of the pipes due to inner-surface biofilm formation from continuous organic carbon loading in the influent and effluent could occur. A general cleaning of all the inter-connected pipes and tubes was usually undertaken twice a month.

3.2.6   Sampling

During the overall study, pore water samples were collected on weekly basis at 15-cm depth from the wetland surface and five consecutive locations at 25 cm interval along the flow path at 0, 25, 50, 75 and 100 cm from inflow with a syringe and a long needle which was rinsed with deionised water and also with N_2 gas between each sampling in order to prevent autoxidation of sample ingredients. Sample preservations (unless analysed immediately) were made according to the standard preservation techniques suggested by various analytical methods for the compounds to be analysed.

Plant biomass samples (shoots and roots) and sludge sediment were collected from each wetland segments of 0-25, 25-50, 50-75 and 75-100 cm in order to investigate which segment of wetland beds had the highest rate of arsenic mass removal in the sediment deposit and amounts accumulated/fixed into their biomass. Plant samples were sectioned into their components shoots and roots after collecting from each above-mentioned segments of horizontal subsurface flow wetlands. Roots were thoroughly washed to remove any soil or gravel aggregate. All samples were freshly weighed (wet weight) to 0.1 gm and dried for three days at 110°C, allowed to cool, re-weighed, calculated the water content and then ground to a fine powder to obtain a homogeneous sample and preserved in sealed plastic bottles for analysis.

3.3   Analytical methods and calculations

All the collected samples were analysed with the methods as described in the following sections. Parameters of all physical, chemical and biological activities which were analysed during different experimental phases both in PFR and in horizontal subsurface flow model wetland operation are listed bellow along with brief analytical techniques and calculation procedure.

3.3.1   Total arsenic

The total arsenic contents of the collected samples in this study were analyzed by hydride generation atomic absorption spectrometry (HG-AAS).

A combination of an FIAS 400 for hydride generation in the flow injection mode and an atomic absorption spectrometer (ZL 4100, PerkinElmer, USA) with an electro-thermal atomizer was used. The hydrides were enriched in a graphite furnace (permanently modified with Pd). The absorbance was measured at the wavelength 193.7 nm. Arsenic

hydride was produced by a pre-reduction of As(V) with potassium iodide and ascorbic acid and by nascent hydrogen. The nascent hydrogen was formed by acidifying $NaBH_4$ with HCl. The detection limit for total arsenic was 0.3 μg l^{-1}.

3.3.2 Arsenic species (inorganic and methylated polar species)

Ion chromatography coupled with inductively coupled plasma mass spectrometry (IC-ICP-MS) is a powerful tool to investigate the distribution of arsenic species in plants and corresponding soil extracts (Mattusch *et al*., 2000). Using a simple species-preserving extraction method involving water, the proposed gradient separation of eight arsenic species is robust and provides long-term stability for the analysis of aqueous extracts of plant material.

The chromatographic system consisted of a LC 250 binary pump (Perkin Elmer), an injection valve with a 200 μl injection loop, an Ion Pac AG7 guard column (all Dionex). The anion-exchange column (250 mm x 4 mm, 10-μm particles) having an alkyl quaternary ammonium exchange site on a styrene-divinylbenzene copolymer was connected to an Elan 5000 ICPMS (Perkin Elmer) via a cross-flow nebulizer. The ICP-MS was operated at 1050 W rfpower, 1000 ms dwell time, the data acquisition in the graphic mode with argon flows of 0.85 l min^{-1} (auxiliary gas), 15 l min^{-1} (plasma gas) and 0.92 l min^{-1} (nebulizer gas). The signal at m/z 75 was monitored. The mobile phase was nitric acid solution with a concentration gradient pumped through the column at 1.0 ml min^{-1}.

The gradient consisted of two solvents (A and B). Eluents, solvent A was 0.4 mM HNO_3 and solvent B was 50 mM HNO_3 (Mattusch *et al*., 2000). The gradient was programmed as follows:

0-2 min	100% A
2-3 min	0-100% B linear gradient
3-8 min	100% B isocratic
8-10 min	100-50% B linear gradient
10-15 min	50% B isocratic
15-15.5 min	50-0% B linear gradient
15.5-20.5 min	100% A isocratic

Concentration of arsenic species is always given as the concentration of elemental arsenic.

Stock solutions of arsenic compounds with a concentration of 1000 mg l^{-1} were prepared from arsenic trioxide (Fluka), arsenate solution (Titrisol®, Merck), and dimethylarsinic acid trihydrate (Merck). Stock solution of mono-methylarsonic acid, arsenobetaine and trimethylarsine oxide were kindly provided by the Institute of Analytical Chemistry, KFUniversity, Graz, Austria. Stock solutions were stored in the dark at 4°C and final standard solutions were prepared daily (Londesborough et al., 1999). The detection limits with the optimised chromatographic separation were 0.16-0.60 µg As l^{-1} for different species.

3.3.3 Volatile arsenic species

The volatile arsenic species were analyzed by gas chromatography-mass spectrometry (GC-MS, Shimadzu-GC-17A-Shimadzu GC-MS-Qp5000 with electron ionization and quadrupole analyzer, using the method described by Pantsar-Kallio and Korpela (2000). The analyses were done isothermally at 50°C and helium was used as a carrier gas.

3.3.4 Dissolved sulphide

The concentration of free sulphide was determined with an ion-specific Ag^+/S^{2-} electrode (Silver/Sulphide-Electrode Ag 500, WTW, Germany) in a 6 ml sub-sample fixed immediately after collection with sulphide antioxidant buffer (SAOB) containing sodium hydroxide, sodium EDTA, and ascorbic acid according the WTW's instruction. The detection limit of sulphide was 0.003 mg S^{2-} l^{-1}.

3.3.5 Sulphite and thiosulphate

Inorganic highly reactive sulphur compounds sulphite (SO_3^{2-}) and thiosulphate ($S_2O_3^{2-}$) in the water samples were analyzed by high performance liquid chromatography (HPLC, modified method according to Rethmeier et al., 1997). The sulphur components were derivatised by monobromobimane to yield fluorescent derivatives. The derivatised sulphur compounds were detected by fluorescence emission at 480 nm. The HPLC (Beckman, USA) was equipped with a 250 mm*4 mm column filled with LiChrosphere® 60 RP select B (5 µm, Merck, Germany) and a fluorescence detector (Shimadzu, Japan). The eluents were 0.25 % acetic acid, pH 4 (solvent A) and 100 % methanol (solvent B). The flow rate of the eluent was 1 ml min^{-1} and the gradient was programmed as follows:

0-5 min	88 % A, 12 % B isocratic
5-13 min	12-30 % B linear gradient
13-16 min	30 % B isocratic
16-34 min	30-60 % B linear gradient
34-36 min	60-100 % B linear gradient
36-39 min	100 % B isocratic
39-39.1 min	100-12 % B linear gradient
39.1-42 min	88 % A, 12 % B isocratic

The lowest detectable concentration was 0.08 mg l^{-1} for sulphite and 0.112 mg l^{-1} for thiosulphate.

3.3.6   Elemental sulphur

Elemental sulphur (S^0) was also determined according Rethmeier et al., 1997 by extracting samples with chloroform and the subsequent detection by HPLC (Beckman, USA) using a Li Chrospher 100, RP 18 column (5 μm, Merck, Germany) and equipped with a UV-detector at 263 nm. The detection limit for elemental sulphur was about 0.064 mg l^{-1}.

3.3.7   Total carbon, total organic carbon and COD

The total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) of the inflow and outflow of the reactors were analyzed using TOC analyzers (Shimadzu, TOC 600, Duisburg, Germany). COD was determined photo-metrically by using Test No. 314 over a range of 15–150 mg l^{-1} (Dr. Bruno Lange GmbH, Düsseldorf, Germany).

3.3.8   Ion chromatography analysis (IC)

Concentration of anions like nitrite, nitrate, sulphate and phosphate and cations like ammonium, sodium, potassium, magnesium and calcium were analyzed by ion chromatography (DIONEX 100, columns AG4A-SC/AS4A-SC (for anions) and CG12A/CS12A (for cations); Idstein, Germany) using a UV detector for nitrite and nitrate at a wave length of 215 nm and a conductivity detector for the other ions. The self generating suppressor ASRS-Ultra 4mm (for anions) and CBES-I 4 mm (for cations) were used.

3.3.9 Total arsenic, sulphur, carbon and nitrogen

Total arsenic was defined as the sum of arsenate [As(V)], arsenite [As(III)], methylated species (MMA, DMA, TMAO etc.), volatile species (e.g. AsH_3). Total sulphur was calculated by summing up all analysed sulphur compounds as sulphide, sulphate-sulphur, thiosulphate-sulphur, sulphite-sulphur and elemental sulphur (S^0). Total nitrogen was defined and calculated as the sum of ammonium-nitrogen, nitrite-nitrogen, nitrate-nitrogen.

3.3.10 Extraction method for total arsenic in plant biomass and sediment

For the analysis of total arsenic concentration in plant biomass (shoots and roots) and sludge sediment, plant samples were rinsed with distilled water, dried at 105-108°C, ground into homogenised powder and digested by microwave extraction (PE Anton Paar GmbH, Graz, Austria). For digestion, 2 ml of digestion mixture (HNO_3 : HCl = 4:1) was added to 0.5 g powder in a Teflon pressure bomb and heated to 260°C for 1 h. After the digest cooled down, it was filled with deionised water to a total volume of 10 ml, mixed and filtered using a 0.45 μm syringe filter (Satorius AG, Goettingen, Germany). Total arsenic in the extracts was determined with atomic absorption technique (AAS) with a detection limit of 0.3 μg l^{-1}. Acid blanks were analysed in order to assess possible contamination. All analyses were performed in duplicate.

3.3.11 Extraction method for arsenic species in plant biomass

Arsenic species were analysed by accelerated solvent extraction method for plant biomass extraction. Fresh plant shoots and roots were collected separately and ground homogeneously in a mortar by using liquid nitrogen. 1-3 g of each ground sample was extracted for 15-20 minutes with 30 ml of deionised water. The volume of supernatant ranged from 15 to 20 ml and was filtered with a cellulose filter (pore size 0.45 μm). It was diluted to a final volume of 100 ml. Sample of 1 ml was taken into a small vial with a rubber septum, purged N_2 or Helium gas into the headspace of the vial for few minutes in order to prevent sample compounds from autoxidation. Then the vials were placed on the sampling port and the chromatograms of arsenic species were analysed by IC-ICP-MS. Concentration of inorganic and methylated organic arsenic species were obtained by calculating the area under the curve from each chromatogram using a computer software package.

3.3.12 Elemental analysis

The elemental analysis (As, S, P and Fe) of the plant shoots, roots and sediments was performed by the energy dispersive X-Ray fluorescence (EDXRF) spectrometer XLAB 2000 (SPECTRO Instruments) running with the software package X-LAB Pro 2.2 after drying the shoots, roots and sediments at 105°C for 24 h using oven MA4O (Satorius, Germany). Aliquots of the sample material (3 g) were dried, ground by means of an agate ball mill (Retsch), mixed with stearine wax (Hoechst wax for XRF-analysis) as binder in a ratio 80:20w/w and subsequently pressed at 150 MPa to pellets (i.d. 32 mm) and analysed by means of energy dispersive X-ray fluorescence analysis (EDXRF). A mixed sample was used for analysis and mean value of two replicates were calculated. The method's relative error is 2 to 3%.

The calibrations of the spectrometer based on the certified and measured data of about 50 certified reference materials including soils, river- and lake sediments and biological materials. To achieve optimum spectrometer operating conditions, the sample was excited by both polarized and monochromatic X-radiation, respectively. Depending on the sample matrix the limit of detection (LOD) of arsenic amounts to 2-3 mg kg^{-1}.

3.4   Other physico-chemical parameters

Other physico-chemical parameters that were obtained and calculated in different model experiments are listed below:

3.4.1   Redox potential (E_h) and pH

The redox potential in the Planted Fixed Bed Reactor (PFBR) was measured by the Pt4805-S7/120 combination Redox, METTLER TOLEDO, and the pH by the pH-electrode Sentix 41. Both parameter were measured on-line and recorded by a microprocessor Standard (pH-ION-Meter pMX 3000/pH, WTW) which allows the measurements on-line every 20 minutes.

In laboratory-scale horizontal subsurface flow wetland redox potential was measured in-situ by direct pumping out the pore water from the sampling point of the respective wetland with the same flow rate as like the main inflow and recorded the data every 5 minutes. A SenTix ORP electrode connected to a multiline P4 (WTW, Germany) was used in this case. To prevent air contact, the electrode was placed in a small flow-through cuvettee. The inlet of the cuvettee was connected with a long robust injection needle which

was inserted into the defined 15 cm depth of the horizontal flow model constructed wetlands. The outlet of the cuvettee was connected to a pump to suck water samples through the cuvettee and thus the pore water came in contact with the measuring electrode. Sets of readings from each wetland were recorded. Another flow through cuvettee with pH electrode was connected in series with redox measuring cuvettee in order to obtain both the pH and rH values from the same sampling position at the same time.

The proper functioning of the electrodes were tested and calibrated regularly with WTW solution for redox potential (Pt/Ag/AgCl in 1 M KCl, +220 mV/25 °C) and for the pH using standard pH buffer (pH 4.01 and pH 7.00) solutions. Redox potential (rH) values were converted to the potential relative to the normal hydrogen reference electrode (E_h) by taking the sample temperature into account.

3.4.2 Dissolved oxygen and temperature

Dissolved oxygen (DO) was measured with a portable DO meter with automatic temperature compensation. Concentrations were measured in flow through mode using an optical oxygen trace sensor system (sensor FTC-TOS7 and instrument FIBOX-3-trace, PreSens, Regensburg, Germany). A separate temperature probe and DO probe were immersed into the sampling position and pore water was pumped out from each sampling position of the model wetlands through the DO probe into a flow cell cuvettee which was connected to a laser-sensitive optode and automatically calculated dissolved oxygen and temperature online into a computer software package supplied by the manufacturer.

Pore water pumping rate was adjusted to the same rate of the main inflow rate (3.5 ml min^{-1}) so as to achieve a more real in-situ sample. The probe was calibrated against saturated water of known temperature and adjusted for atmospheric pressure according to the manufacturer's instructions and was rinsed with deionised water between each readings.

Daily average temperature of the greenhouse was also recorded with a standard laboratory alcohol-filled thermometer.

3.4.3 Dissolved gas (CH_4, CO_2) analysis

Methane, carbon dioxide in the pore water were directly analysed by membrane inlet mass spectrometry (MIMS) (Bohatka, 1997) using a QMG 422 quadrupole mass spectrometer (Pfeiffer Vacuum GmbH, Germany) fitted with a silicon covered inlet probe. The

quantification of methane concentrations was based on calibrations using synthetic gases with 4-50 % methane.

3.4.4   Chlorophyll a fluorescence

A fresh weight of 0.1 g of plant sample was dried and grinded with liquid nitrogen in a mortar. After that, 5 ml of extracting agent (250 ml of acetone with 1.25 ml of concentrated ammonia solution) was added into the ground sample and incubated in 20 min^{-1} shaker for 15 minutes. Shortly afterwards, centrifugation of this solution was made at cooling conditions (4°C, 10 minutes).

Supernatant was then measured for light absorbance with spectrophotometer at the wavelength of 470, 647, and 664 nm (Gruber, 2005). Series of equations to the calculation of pigment composition in the sample (chlorophyll a, chlorophyll b, and carotenoid) are mentioned below:

$$\text{Chlorophyll a (mg kg}^{-1}) = 12.25\, A_{664} - 2.79\, A_{647}$$

$$\text{Chlorophyll b (mg kg}^{-1}) = 21.50\, A_{647} - 5.10\, A_{664}$$

$$\text{Carotenoid (mg kg}^{-1}) = \frac{1000\, A_{470} - 1.82\, Chlorophyll\ a - 85.02\, Chlorophyll\ b}{198}$$

Where A_{470}, A_{647} and A_{664} are the absorbances of the supernatant at 470, 647, and 664 nm respectively.

3.4.5   Evapo-transpiration and water balance

Initial and final conditions (weight and volume) of inflow feeding water and outflow water (duration of 3-4 days) were measured in order to calculate the evapo-transpiration of the system. PFR reactor design ensures that evaporation can be neglected. Plant transpiration represented 98% of the total water loss (Wießner et al., 2005) in PFBR and was controlled by balancing the inflow and the outflow amounts of water. The total water loss was divided by the time and the number of plants in the reactor to calculate the theoretical specific transpiration rate per plants.

Considering water loss due to evapo-transpiration (EVT), area specific mass loading rates

were also calculated in order to achieve actual rate of inflow mass loading and thereby subsequent removal rate produced by the horizontal subsurface-flow model wetland systems.

3.4.6 Shoot density

The numbers of the shoots were obtained periodically, at approximately 30-days intervals throughout the experimental period by counting the number of total green shoots and divided by the area to calculate the density of the plants.

3.4.7 Gravel analysis

The gravel bed used in this experiment was previously washed with tap water to remove unattached small particles before processing and then heated in a drying oven set at 105°C for 2 hours. For two replicate of gravel samples, determination of the gravel size, the density, porosity and uniformity coefficient were performed.

The granulometric distribution of sizes of the gravel bed was made with the Vibratory Sieve Shaker Analysette 3 (FRITSCH, Germany) according with the procedure DIN 4188. The density of the gravel was measured based on the water replacement method proposed by Black (1986) and ASTM (1994). The porosity was calculated after dividing the volume of water that could be poured in each graduate glass by the total volume of the material. Uniformity coefficient (Cu) was defined as the ratio between materials accumulated between the 60 and 10 percent in the granulometric curve. Table 10 shows a summary of the calculated results.

Table 10: Characteristic of the size, density (ρ), porosity (P) and uniformity coefficient (Cu) of the gravel that used both in the Planted Fixed Bed Reactors-PFBR and horizontal subsurface-flow wetlands

Sample	Size (mm)	ρ (kg m^{-3})	P (%)	Cu = D_{60}/D_{10}
1	2-6	1482	42.8	1.07
2	2-6	1543	40.6	1.12
Mean	2-6	1512	41.7	1.10

3.4.8 Arsenic adsorptive capacity of gravel

Constructed wetland systems usually contain gravels or solid material as one of the major components. Since adsorption on gravel is one of the factors which could affect the

effectiveness of total removal of arsenic in wastewater, determination of adsorptive capacity of gravel used in wetland systems was necessary. The diameter of the gravel used in this study ranged from approximately 2-6 millimeters and had a mean diameter 4 millimeters.

Fuller et al. (1993) proposed the method for soil adsorptive capacity, which was used to determine the adsorptive capacity of the gravel in this study. Three different concentrations of arsenic solution (10, 50, and 100 µg l^{-1}) were prepared from arsenate stock solution. 30 g of the gravel used in model wetlands and reactors were mixed with 100 ml of each concentration of arsenate in glass bottle. After that, these bottles were shaken for 24 hours with 200 min^{-1}. Sample solution of each bottle were taken at the beginning and at the end of shaking, filtered with 0.45 µm pore filter paper prior to the analysis for total arsenic concentration by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometer) technique. The adsorptive capacity was calculated with the following equation:

$$q = \left(C_f - C_i\right)\frac{V}{M}$$

Where; q is the adsorptive capacity of arsenic on gravel (µg As kg^{-1} gravel)

C_f is the final concentration of arsenic after shaking (µg l^{-1})

C_i is the initial concentration of arsenic before shaking (µg l^{-1})

V is the volume of the solution (l)

M is the mass of the gravel (kg)

From the previous results (Buddhawong, 2005), it was found that the gravel could not absorb arsenic in substantial amounts from the solution. Therefore, this kind of gravel itself had no significant effect on the model experiments concerning arsenic removal in constructed wetland systems.

3.4.9 Removal efficiency analysis

Performance index of the constructed wetland systems for arsenic and other contaminant removal was calculated by comparing the inflow and outflow concentrations, which was termed as removal efficiency and expressed in percentage (%). The removal efficiency was

calculated using the following equation:

$$\% \; Efficiency = \frac{Inflow \; concentration - Outflow \; concentration}{Inflow \; concentration} \times 100$$

3.4.10 Specific removal rate

The specific removal rates of the wetland systems were calculated as the difference between the specific inflow and outflow loading rates ($mg \; m^{-2} d^{-1}$).

Specific (inflow/outflow) loading rate = [concentration ($mg \; l^{-1}$) x flow rate ($L \; d^{-1}$)]/area (m^2)

4 Results and discussions

4.1 Treatment of arsenic containing model wastewater in the Planted Fixed Bed Reactor (PFBR)

4.1.1 Dynamics of arsenic removal

The dynamics of total arsenic with the values of mean inflow and outflow concentrations and the removal efficiency are represented in Figure 8.1a & b. During the whole 340 days of operation, a continuous supply of synthetic wastewater provided a mean inflow concentration of 0.2 ± 0.01 mg As l^{-1} in all three reactors, the only exception was in phase D, where cancellation of arsenic loading attributed to a mean inflow concentration well below the detection limit (<0.3 µg As l^{-1}) in PFBR3 and PFBR4.

Under C-deficient aerobic condition in the experimental phase A (only varying sulphate-sulphur concentration as 0.2, 5 and 25 mg l^{-1} in PFBR1, PFBR3 and PFBR4 respectively; see Table), a highly efficient arsenic removal was observed in all three experimental reactors. The outflow concentration showed nearly 10-fold declination in total arsenic, reaching a mean value of 0.02 ± 0.01 mg l^{-1} in PFBR1 and PFBR3 and greater than 6-fold declination with a mean value of 0.03 ± 0.01 mg l^{-1} in PFBR4 (Fig 8.1a). Mean removal efficiency attained as 92%, 90% and 85% in PFBR1, PFBR3 and PFBR4 respectively. No significant trend appeared to be in the outflow dynamics of arsenic associated with varying sulphate-sulphur concentration in these reactors. High removal efficiency indicated a better performance of arsenic removal under carbon deficient and oxic conditions (see Fig 8.12) regardless to the concentration of sulphate-sulphur in model wastewater (see Fig 8.1b).

Removal of arsenic under this condition is perhaps best explained by the adsorption of arsenic with bacteria, plant roots, organic soil substances, and/or adsorption onto oxide minerals and concomitant co-precipitation specifically with Fe(III) oxyhydroxides. Reasons for the immobilisation of arsenic under oxic condition were similarly explained by other authors (Wilkie and Hering, 1996; Bednar et al., 2005). Highly oxic conditions (Eh~700 mV) are unfavorable for sulphate-reducing bacteria and hence it can be concluded that the removal of arsenic within the root near environment of the rhizosphere was accomplished mainly by mechanisms other than the precipitation as arsenic sulphide. Lack of available organic carbon and energy sources to drive the process of microbial sulphate-reduction was probably the major limiting factor in this case.

Addition of electron donor (COD~340 mg l^{-1}) in phase B caused a drastic change in the
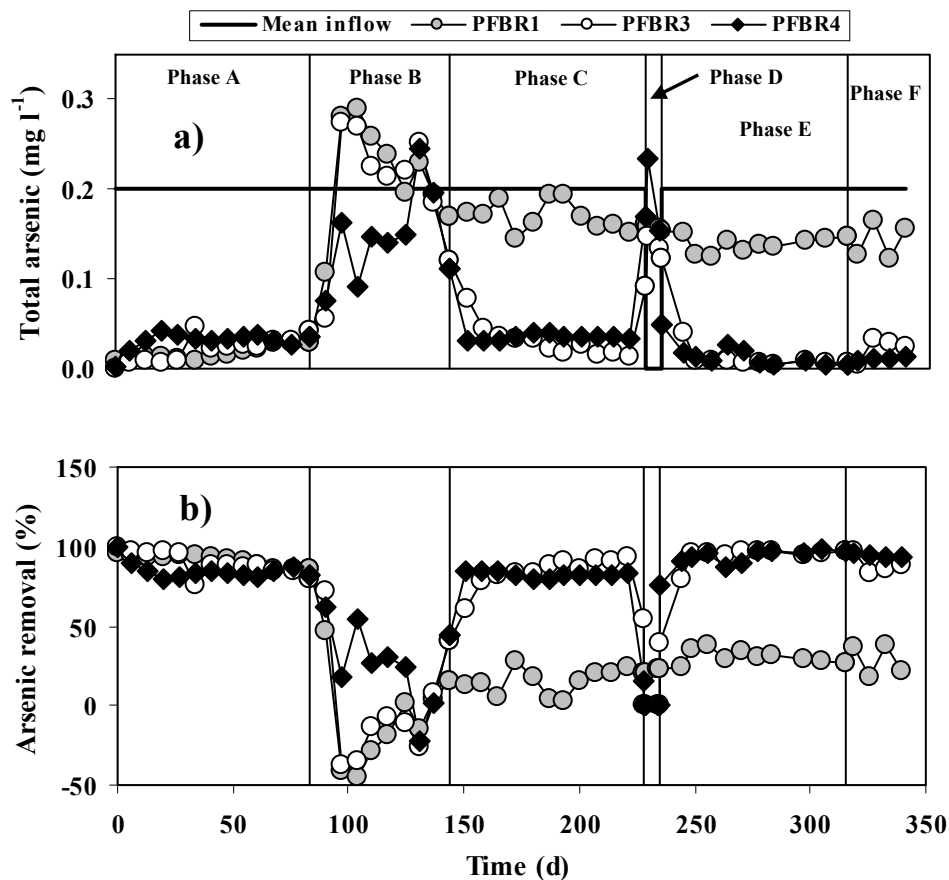
Fig. 8.1 Dynamics of total arsenic concentration with inflow and outflow a) and percent removal b) during different experimental periods in planted fixed bed reactors

redox condition dynamics in the reactors and outflow arsenic concentration exhibited surprisingly excess amount of arsenic, even higher than the mean inflow concentration of 0.2 ± 0.01 mg l^{-1}. The microbial activity for consumption and oxidation of organic matters resulted in anaerobic conditions in all three reactors (see Fig 8.1a) and under reducing conditions there was probably a shift in terminal electron acceptors from O_2 to Fe(III) oxide/hydroxides and a small fraction of the deposited arsenic was released in the aqueous phase due to microbially driven reductive dissolution of Fe(III)-oxyhydroxides. This process of arsenic release at oxic to anoxic transitions into the surrounding pore water was also explained by Smedley and Kinniburgh (2002). The maximum dissolved arsenic concentration of 0.29 and 0.274 mg l^{-1} in PFBR1 and PFBR3 respectively, was obtained in the pore water shortly afterwards of starting this particular phase B. Few days later along the flow path in case of PFBR4, concentration value also reached up to 0.245 mg l^{-1} (see Fig 8.1a). Despite the presence of organic matter in all three reactors, only a trace amount of sulphate-sulphur (0.2 mg l^{-1}) in the inflow feeding could not initiate any substantial

sulphate-reduction which might contribute to the subsequent arsenic removal. Most likely, there were several competing reactions including both dissolution or desorption in a high rate and precipitation or adsorption in a low level occurring simultaneously. Re-mobilization accounted for the elevated arsenic concentration throughout the whole phase and after 61 days of persistent unstable condition in all three reactors, feeding strategy was switched to the next experimental phase C.

Phase C (from 144-228 days) started with different sulphate-sulphur concentrations as similar to the experimental phase A (0.2, 5 and 25 mg l^{-1} in PFBR1, PFBR3 and PFBR4 respectively) but under C-surplus conditions resulted in a low Eh (see Fig 8.12). Addition of high sulphate-sulphur immediately impacted on the arsenic removal and dramatically increased (see Fig. 4.1b) in PFBR3 and PFBR4 as compared to the control PFBR1 (SO_4^{2-}-S ~ 0.2 mg l^{-1}). Fig. 4.2 illustrates that at the end of the sampling period (day-228), total As in the aqueous phase of the outflow had decreased to the range of 0.014-0.077 mg l^{-1}, with a mean value of 0.031 ± 0.018 mg l^{-1} in PFBR3, which accounted for an average concentration reduction of 84%, from an inflow concentration of 0.2 ± 0.01 mg l^{-1}. In PFBR4, outflow concentration decreased to the range of 0.031-0.041 mg l^{-1}, with a mean value of 0.035 ± 0.003 mg l^{-1} and a mean concentration reduction of 82%. On the contrary, a mean outflow concentration of 0.170 ± 0.017 mg l^{-1} resulted in a significantly lower removal of only 15% of total arsenic in control PFBR1. Arsenic levels are elevated only where sulphate content is low, as previously noted (Holm, 1995; Holm et al. 2004; Warner, 2001). This was consistent with our investigation in PFBR1.

Addition of substantially high amount of sulphate-sulphur under C-surplus and anaerobic conditions enhanced microbial sulphate-reduction where sulphate-reducing bacteria fulfill their energy needs by using sulphate as electron acceptor coupling with anaerobic oxidation of organic matter during their respiration. This process triggers alkalinity production and potentially generates sufficient sulphide (S^{2-}) which might lead to the arsenic precipitation as sulphides (likely orpiment As_2S_3) with low solubility (Moore et al. 1988, Kim et al. 1999). Although the exact processes responsible for arsenic removal were not clear, it was evident that when compared to control PFBR1 containing only traces of sulphate-sulphur, the action of bacterial sulphate-reduction in other two reactors with high sulphate-sulphur greatly enhanced arsenic removal rate. So, this experimental phase demonstrated that an elevated sulphate-sulphur concentration in the model wastewater fascilitated the arsenic retention under sulphate reducing condition. A slightly higher

concentration in the outflow of PFBR4 (SO_4^{2-}-S ~ 25 mg l^{-1}) than PFBR3 (SO_4^{2-}-S~5 mg l^{-1}) might explain by a slight increase of concentration due to water loss by evapo-transpiration (EVT) in PFBR4 (see Fig 8.11), otherwise, no clear evidence of better arsenic removal was observed due to 5-fold higher sulphate-sulphur concentration in PFBR4 than in PFBR3.
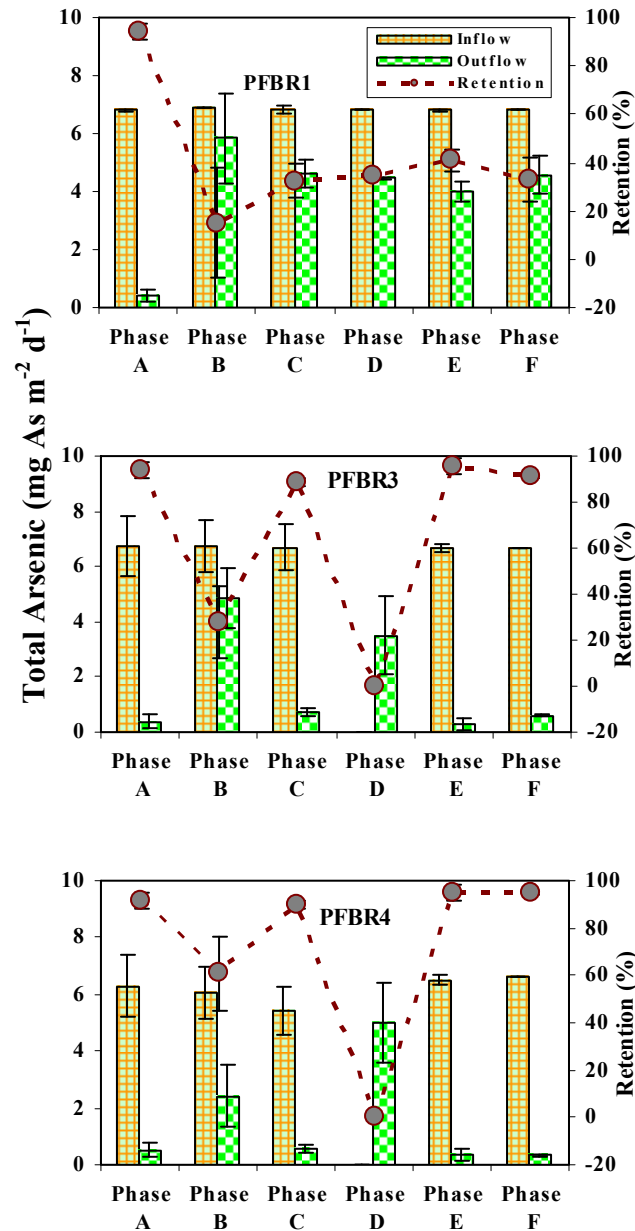

Fig. 8.2 Area specific arsenic mass loading rates and mass retention in planted fixed bed reactors PFBR1, PFBR3 and PFBR4

Results from the area specific mass loading rates (see Fig 8.2) demonstrated the mean outflow rates as 4.61 ± 0.67, 0.74 ± 0.42 and 0.55 ± 0.16 mg As m^{-2} d^{-1} in PFBR1, PFBR3

and PFBR4 respectively, as compared to the mean inflow loading of 6.74 ± 0.2 mg As m^{-2} d^{-1}, resulted in an arsenic mass retention efficiency as 32%, 89% and 90% in the respective reactors.

Comparing to the concentration basis removal efficiency, slightly improved arsenic mass retention could be observed due to a 5-fold increment of SO_4^{2-}-S loading in PFBR4 as compared to PFBR3. Since a considerable amount of water can be lost from wetland systems in terms of evapo-transpiration (Mitsch and Gosselink, 2000), which subsequently may lead to increase the concentrations of the contaminants in the outlet pore-water, the mass removal rate is a better indicator of evaluating removal efficiency than the percentage reduction in concentrations (see Fig 8.2).

A short-term experimental phase was carried out in phase D (from day 228-235) to investigate the stability of already immobilized arsenic by canceling organic carbon load and arsenic in the inflow of PFBR3 and PFBR4. Only a trace amount (0.2 mg l^{-1}) of sulphate-sulphur was included and the analytical results showed that outflow arsenic concentration drastically increased to a range of 0.092-0.148 mg l^{-1}, with a mean value of 0.141 ± 0.011 mg l^{-1} in PBR3 and a range of 0.171-0.235 mg l^{-1}, with a mean value of 0.195 ± 0.057 mg l^{-1} in PFBR4. Highly oxic conditions appeared by rising up of Eh very shortly at the transition from anoxic to oxic (see Fig 8.12) and evident of arsenic release from previous immobilized phase was obvious in these two reactors since inflow arsenic concentration was limited (below the detection limit of 1-3 µg l^{-1}). This might occured presumably due to fact that an oxidative dissolution of immobilized arsenic sulphides accelerated the arsenic re-dissolution in the aqueous phase and also the re-oxidation of reduced sulphur into other sulphur species (e.g. S^0, SO_4^{2-}) under oxic conditions. Moore et al. (1988) also demonstrated that oxidation of arsenic sulphide minerals such as arsenopyrite and orpiment favors arsenic mobilization. An energy failure at the ending part of this phase might lead to the atmospheric oxygen inclusion into the reactors and overflowing the reactors enhanced the unrest of deposited arsenic which must took into considerations to understand the dynamics of arsenic in PFBR3 and PFBR4 as compared to the running condition (like in phase C) of PFBR1.

Similar conditions like phase C were continued to maintain in phase E (corresponding days 235-315), only exception was that the sulphate-sulphur concentration lowered down to 10 mg l^{-1} instead of 25 mg l^{-1} in the inflow of PFBR4. Organic carbon (COD ~ 340 mg l^{-1}) was added again and concentration of sulphate-sulphur (SO_4^{2-}-S) kept remain the same like

in phase C as 0.2 and 5 mg l^{-1} in the inflow feeding of PFBR1 and PFBR3 respectively. Depletion of dissolved oxygen by microbial anaerobic oxidation of organic matter contributed towards a rapid transition from oxic to anoxic conditions both in PFBR3 and PFBR4 (see Fig 8.12). Outflow arsenic concentrations greatly decreased in a range of 0.005-0.122 mg l^{-1}, with a mean value of 0.011 ± 0.01 mg l^{-1} and an outstanding mean relative concentration reduction (>94%) was attained in PFBR3 as compared to the mean inflow concentration of 0.2 ± 0.01 mg l^{-1}. In PFBR4, arsenic concentration declined in a range of 0.004-0.049 mg l^{-1}, with a mean value of 0.012 ± 0.008 and a mean reduction of 94%. Contrarily in the control PFBR1 with only a trace amount of sulphate-sulphur (0.2 mg l^{-1}) showed an outflow concentration in the range of 0.125-0.154 mg l^{-1}, with a mean value of 0.138 ± 0.009 mg l^{-1} resulted in a mean concentration reduction of only 31%. By this, it can be postulated that sulphate-sulphur is playing an important role for arsenic removal under dynamic redox conditions in the root-near micro-gradient environment of the rhizosphere of constructed wetlands with dynamic fixation of arsenic precipitation as arsenic sulphides (As_2S_3).

Obtained results from area specific loading rates showed that the outflow rates decreased down to a mean value of 0.3 ± 0.29 mg As m^{-2} d^{-1} and 0.34 ± 0.21 mg As m^{-2} d^{-1} as compared to the mean inflow loading rate of 6.41 ± 0.27 mg As m^{-2} d^{-1} and 6.17 ± 0.31 mg As m^{-2} d^{-1} with a mass retention efficiency of 96% and 95% in PFBR3 and PFBR4 respectively. At least 5% more arsenic mass was retained in PFBR4 with a decreasing sulphate-sulphur concentration of 10 mg l^{-1} than in phase C where 25 mg l^{-1} was added. Relatively higher sulphate loading probably contributed to higher ratios and activities of dissimilatory sulphate-reducers which might be a limiting factor for arsenic removal under strictly anaerobic sulphate reducing environment, although other related factors should be taken into considerations for this approval.

During the last experimental phase F (days 315-340), cancellation of sulphate-sulphur from the inflow feeding of PFBR3 and PFBR4 were made and the amount of organic carbon (COD~ 340 mg l^{-1}) and arsenic (0.2 mg l^{-1}) kept remain the same like previous phase E. Stability of immobilized arsenic in absence of sulphate-sulphur was the prime concern for this investigation. Analytical results showed that a mean outflow arsenic concentration of 0.023 ± 0.013 mg l^{-1} and 0.011 ± 0.002 mg l^{-1} with a mean relative reduction of 89% and 94% as compared to the mean inflow of 0.2 ± 0.01 mg l^{-1} in PFBR3 and PFBR4 respectively (see Fig 8.1b). Area specific mass loading also showed a similar 90% and

95% mass retention in these two respective reactors. An already enriched sulphur-pool in PFBR4 might prove beneficial for a better arsenic fixation in this experimental phase in particular. Removal efficiency remained unchanged in control PFBR1 (>30%) due to lack of sufficient sulphate-sulphur throughout all the experimental phases. Despite the cancellation of sulphate-sulphur in the inflow wastewater, a highly stable arsenic in the reactor deposit reflected better immobilization due to the presence of substantial amount of sulphides and reduced sulphur species associated with the S-pool existing inside from previous phases in PFBR3 and PFBR4 under reducing conditions.

Further changing of redox condition dynamics in the rhizosphere from anoxic to oxic might lead to a release of arsenic due to oxidative dissolution of arsenic sulphide precipitates. So, dynamic redox conditions playing a significant role for arsenic fixation along with sulphate-sulphur and organic carbon loading in constructed wetlands.

4.1.2   Transformation and dynamics of arsenic species

The mobility, bioavailability, and toxicological effects of heavy metals and metalloids are largely dependent on their speciation. For instance, it is well-known that arsenite [As(III)] is a highly toxic and soluble oxyanion, whereas arsenate [As(V)] is less mobile because it tends to adsorb more strongly onto Fe(III)oxyhydroxides and methylated species are generally less toxic than inorganic species (Turpeinen et al. 1999). Both adsorption reactions and redox conditions essentially control the mobility of these chemical species (Rittle et al. 1995). Under oxidizing conditions in phase A in our investigation, arsenic solubility was low in all three reactors because As(V) was the predominant species and teds to adsorb strongly onto or forms minerals with Fe(III)-oxyhydroxide (Mok and Wai. 1994). Arsenite behaves much differently than arsenate in natural environments; in particular, its sorption onto clay minerals and metal oxides appears to be less rapid and/or less stable (Aggett and Kriegman 1988; Kuhn and Sigg 1993; Masscheleyn et al. 1991; Mok and Wai 1994; Onken and Adriano 1997; Seyler and Martin 1989).

Figure 8.3 illustrates the dynamics of arsenic species in different experimental phases in all three reactors. Mean concentration of As(III) was significantly lower than As(V) and transformation into As(III) under oxic condition in phase A was less than 12%, 35% ad 30% of total As in PFBR1, PFBR3 and PFBR4 respectively (see Fig 8.3). It is interesting to note that despite the prevailing oxic condition and C-deficient environment, As(III) can still be observed may be due to some anaerobic microbial activity inside of biofilm.

Moreover, persistent oxic condition and lack of sufficient electron donor inhibited dissimilatory sulphate-reduction despite the relatively higher sulphate-sulphur concentrations in PFBR3 (SO_4^{2-}-S: 5 mg l^{-1}) and PFBR4 (SO_4^{2-}-S: 25 mg l^{-1}) ensured that arsenic removal accomplished other than As(III) sulphide precipitation (likely as As_2S_3).
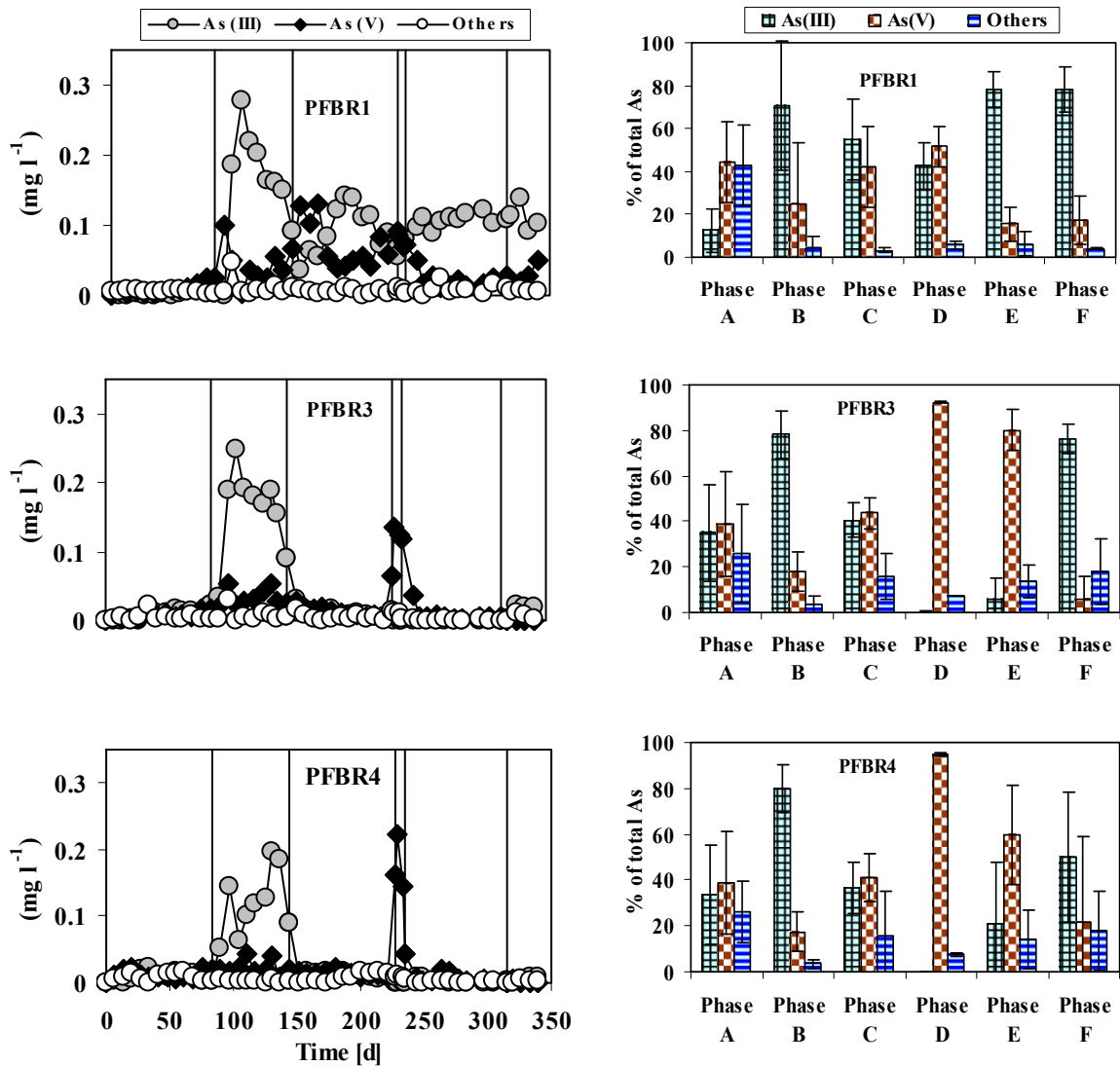

Fig 8.3 Dynamics of arsenic species in the outflow and mean conversion into different species as percentile of total arsenic in all experimental phases.

On the other hand, it is known that reducing conditions lead to the mobilization of arsenic as As(III) goes into the liquid phase (Mok and Wai 1994). Addition of electron donor in phase B as a driving force stimulated anaerobic microbial oxidation of organic matter, speeded up the reduction of As(V) to As(III) and subsequent As(III) mobilisation in the

aqueous phase after probable reductive dissolution of iron(oxy)hydroxides under reducing conditions. The biological nature of the As(V) reduction was better exhibited in between phase B and phase A. Mean As(III) concentration of 0.171 ± 0.08, 0.17 ± 0.061 and 0.124 ± 0.051 mg l^{-1} accounted for a conversion of 70%, 78% and 80% of total arsenic in PFBR1, PFBR3 and PFBR4 respectively in this highly unrest phase in terms of arsenic re-dissolution and re-mobilisation.

Apart from dynamic redox reactions which facilitate arsenic transformation, various microbial activity in the rhizosphere and/or the excretion of organic substances by the plant roots could lead to a reduction of As(V) to As(III) in the direct root vicinity. Microorganisms have been discovered in a great diversity of anoxic environments named as arsenate respiring bacteria, are able to generate energy by coupling the oxidation of H_2 or organic carbon to the reduction of inorganic As(V) to As(III) (Ahmann et al. 1994, Cummings et al. 1999, Dowdle et al. 1996, Laverman et al. 1995, Macy et al. 1996, Newman et al. 1997, Newman et al. 1998). Biogenically-produced sulphide can also reduce As(V) to As (III) (Kuhn and Sigg, 1993; Hoeft et al., 2004); Fe(III)-respiring bacteria could release As(V) by reduction of Fe(III) to Fe(II), which could subsequently be reduced to As(III) (Cummings et al., 1999). It has also been discovered that certain bacteria could use both ferric iron and As(V) as electron acceptors, and adsorbed As(V) could be reduced to As(III) without the reduction of Fe(III) (Zobrist et al., 2000). These are the most widely discovered mechanisms for arsenic reduction from As(V) to As(III) and also can react with dissolved metals or metalloids to form metal sulfide precipitates under reducing conditions.

Addition of SO_4^{2-}-S at moderately to higher concentration in PFBR3 (5 mg l^{-1}) and PFBR4 (25 mg l^{-1}) greatly decreased the concentration of As(III), whereas in PFBR1 had no noticeable effect. The utilization of sulphate-respiring bacteria to immobilize As(III) to As_2S_3 in C-surplus and reducing conditions of phase C probably lowered down As(III) concentration in both PFBR3 and PFBR4 (see Fig 8.3). Outflow concentration data indicated a mean value of 40% and 44% of total arsenic as As(III) and As(V) respectively in PFBR3 with moderate SO_4^{2-}-S (5 mg l^{-1}). About 36% and 41% of total arsenic as As(III) and As(V) respectively were found in PFBR4 with high SO_4^{2-}-S (25 mg l^{-1}). Close similarity to this phase C, As(III) was present with As(V) and constitutes a mean 21% of the total As concentrations in phase E of PFBR4 (SO_4^{2-}-S ~10 mg l^{-1}). This data suggested that more As(III) precipitates (15% less in the outflow) with reduced sulphide when SO_4^{2-}-

S concentration was 10 mg l^{-1} (in phase E) than 25 mg l^{-1} (in phase C)

In the moderate oxic environment in phase D, the shortest phase (duration only 7 days), As(V) was predominant than As(III) where cancellation of electron donor drastically changed the redox dynamics both in PFBR3 and PFBR4. More than 92% and 94% of total arsenic were found as As(V) and <1% as As(III) in PFBR3 and PFBR4 respectively. Presumable oxidative dissolution of already precipitated As$_2$S$_3$, re-oxidation of reduced sulphur compounds and sulphate and more importantly an unavoidable accident due to energy failure resulted in predominant As(V) enrichment in the systems. Withdrawal of electron donor in the transition from anoxic to oxic greatly influenced the redox condition dynamics and remobilization and/or desorption of arsenic from solid to aqueous phase. These processes inhibited microbial sulphate reduction and subsequent microbially mediated arsenic transformation.

When SO$_4$$^{2-}$-S supply was cancelled in phase F, As(III) content was increased in the outflow and a mean value of 76% and 50% of total arsenic were transformed as As(III) in PFBR3 and PFBR4 respectively. Despite sufficient electron donors (COD~340 mg l^{-1}) in both systems, lack of sulphate as electron acceptor for the respiration of bacterial sulphate-reduction were probably unable to generate enough sulphide to precipitate with arsenic as arsenic sulphide (likely As$_2$S$_3$). It is interesting to note that 25% less As(III) were detectable in the outflow of PFBR4 which might me due to the fact that in the previous phase E, a comparatively high amount of SO$_4$$^{2-}$-S (10 mg l^{-1}) was added in PFBR4 as compared to 5 mg SO$_4$$^{2-}$-S l^{-1} in PFBR3 resulted in a higher amount of sulphate-sulphur enrichment to a S-pool and thereby more sulphide formation in PFBR4 in C-surplus condition addressed in phase F. Reduced As(III) subsequently precipitate probably with sulphide and hence less amount of As(III) was observed in PFBR4 in this case. Formation of S^{2-} along with time and its correlation with As(III) dynamics might explain this even better (see Fig 8.6). In the control PFBR1, strict anaerobic condition prevailed throughout the experimental phases since after initial phase A but only traces (0.2 mg l^{-1}) of SO$_4$$^{2-}$-S in each phases exhibited poor sulphate-reduction and hence a high percentage (in a range of 55-80%) of total arsenic converted to As(III) were observed as unused and flushed out of the system in comparison to the other two reactors PFBR3 and PFBR4.

The transformation of As(V), under both aerobic and anaerobic conditions to other methylated polar compounds like monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and to even volatile arsine (AsH$_3$), trimethylarsine (TMA) was, however, less

than a mean value of 10%, 20% and 30% of total arsenic (inflow as arsenate) all throughout the experimental phases in PFBR1, PFBR3 and PFBR4 respectively.

4.1.3 Dynamics of sulphur and species formation

Dynamics of sulphur removal and various species formation incorporated to arsenic within the rhizosphere of wetland systems have a major influence for arsenic removal. Many of the physical transport processes and biogeochemical reactions driven by plants may results in the extensive sulphur cycling under dynamic redox conditions. Under C-deficient oxic
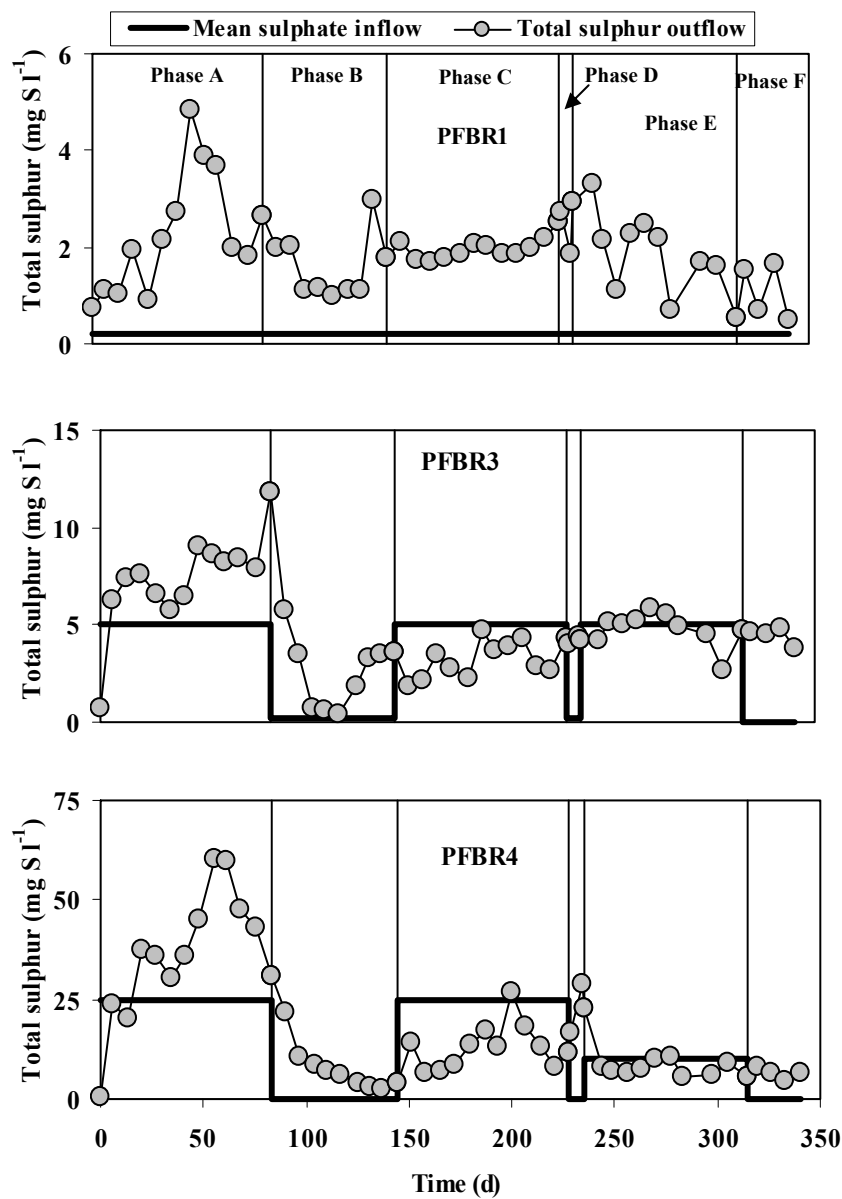

Figure 8.4 Mean total sulphur inflow and outflow concentrations in planted fixed bed reactors depending on the loading conditions (experimental phases A – F)

conditions in phase A, the extent of total sulphur outflow concentration measured between 0-83 days, increased with increasing inflow concentrations (Fig 8.4). The mean inflow concentration of total sulphur (exclusively as sulphate) was 0.2 ± 0.01, 5 ± 0.01 and 25 ± 0.01 mg S l^{-1} and subsequent mean outflow concentration was recorded as 2.2 ± 1.3, 6.9 ± 2.2 and 36.7 ± 16.7 mg S l^{-1} in PFBR1, PFBR3 and PFBR4 respectively.
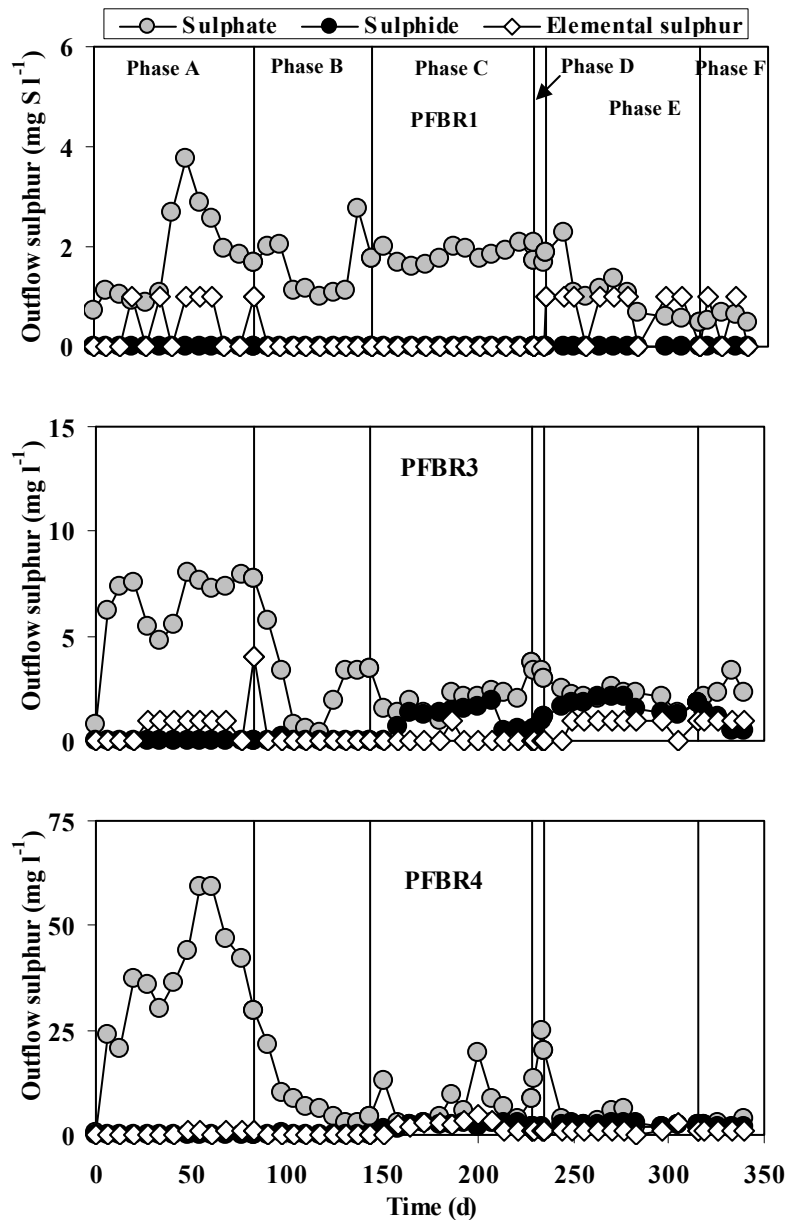

Fig. 8.5 Outflow concentration of sulphur compounds with continuous sulphate supply during the whole experimental period in planted fixed bed reactors

Under the prevailing more oxidized conditions in the wetland systems, total sulphur were not removed from the systems by sulphate-reduction and likely became concentrated by the formation of a S-pool presumably due to high EVT rate, lack of sulphate reduction in

absence of sufficient organic carbon. It revealed that high arsenic removal in this phase was not dependent on varying total sulphur concentrations or enrichment of S-pool in the root near environment. High redox potential values throughout this experimental phase did not allow substantial sulphate reduction and thereby no reduced sulphur species (with the exception of some S^0) were observed (see Fig 8.5)

After the addition of organic carbon sources (COD~340 mg l^{-1}) and a trace amount of SO_4^{2-}-S (0.2 mg l^{-1}) in all the wetland systems in experimental phase B, rapid changes in the S-dynamics along with the changes in dynamic redox conditions in all the reactors were observed. Outflow total sulphur concentrations decreased down to a mean value of 1.8 ± 0.7, 2.5 ± 1.9 and 8.0 ± 6.1 mg S l^{-1} from the previous phase A. The concentrations exhibited in the outflow were still very high in comparison to the mean inflow of only 0.2 ± 0.01 mg S l^{-1}. Already existing S-pool was playing a pivotal role for a high sulphur concentrations (mainly as sulphate) flushing out of the systems. Changes in the redox dynamics (see Fig 8.12) in the transition of oxic to anoxic resulted in a dissolution and re-mobilization of arsenic probably from the previously adsorbed Fe(III)-oxyhydroxides and reduction of As(V) to the more soluble As(III) but not initiated a substantial sulphate reduction and subsequent sulphide formation despite available electron donor for the respiration of sulphate reducers.

The changes of the inflow condition in phase C were made after adding sulphate at a concentration of 0.2 ± 0.01, 5 ± 0.01 and 25 ± 0.01 mg S l^{-1} under C-surplus (COD~340 mg l^{-1}) conditions in PFBR1, PFBR3 and PFBR4 respectively. Outflow concentrations revealed that a mean value of 3.2 ± 0.9 and 13.4 ± 5.9 mg S l^{-1} accounted for at least 36% and 46% total sulphur removal and corresponding sulphide formation in a range of 0.5-1.9 mg l^{-1} and 1.3-3.1 mg l^{-1} in PFBR3 and PFBR4 respectively. Strict and persistent reducing conditions (see Fig 8.12) fostered dissimilatory sulphate reduction by microbial activity and subsequent gradually increasing sulphide production were observed under elevated sulphate inflow conditions in PFBR3 and PFBR4 as compared to the control PFBR1. Reduced sulphur species were found in the outflow remained in excess after precipitation probably as elemental sulphur and insoluble arsenic sulphides (likely As_2S_3) in PFBR3 and PFBR4 respectively. Consistent reduction of As(V) to more soluble As(III) under this reducing conditions and probable concomitant precipitation with sulphide triggered better arsenic removal (see Fig 8.1b) in the rhizosphere of these two respective reactors with elevated sulphate inflow concentrations. Corresponding area specific mass loading rate

revealed a mean removal rate of total sulphur as 91.3 ± 20.2 and 468.3 ± 12.2 mg S m^{-2} d^{-1} which resulted in a mean sulphur mass retention nearly as 55% and 69% in PFBR3 and PFBR4 respectively (see Fig 8.4). Mean outflow sulphide mass was reported as 26.13 ± 13.5 and 39.16 ± 18.8 mg S m^{-2} d^{-1} which were 34% and 19% of total outflow sulphur mass in these two corresponding reactors respectively (see Fig 8.5).

But sulphide concentration exhibited already toxic effects on plants (decreasing water loss in terms of plant transpiration) probably due to sulphide toxicity along with available more toxic inorganic arsenic species. Outflow As(III) concentration incorporated with S^{2-} formation dynamics are shown in Figure 8.6 in all corresponding reactors.
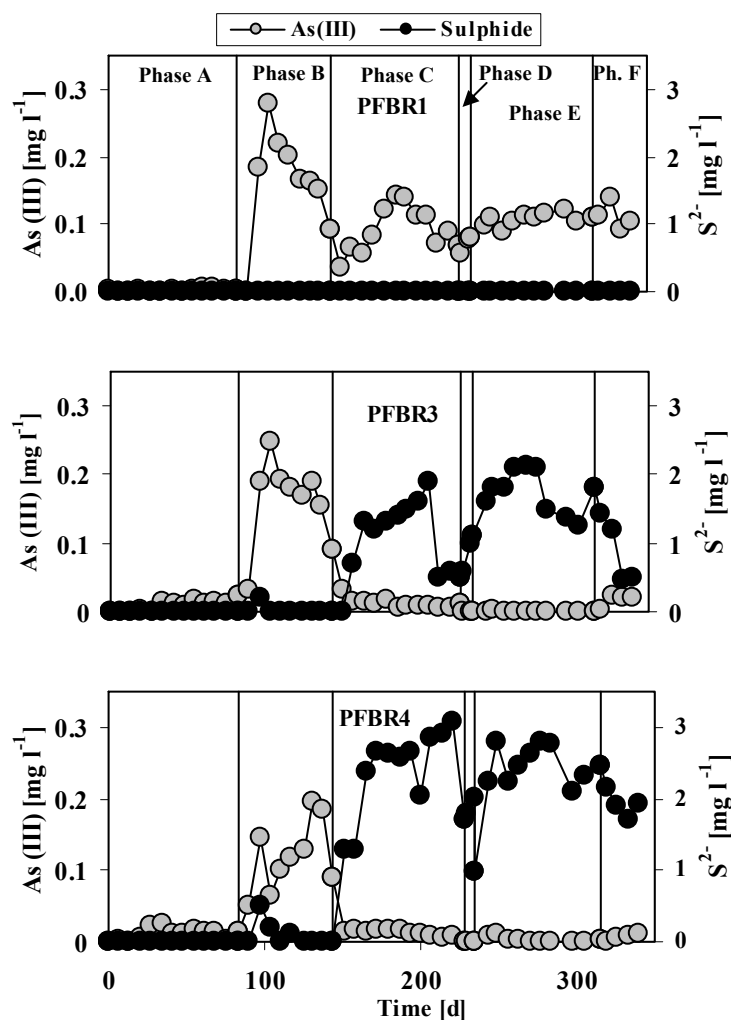

Fig 8.6 Concentration of As(III) and S^{2-} under the conditions of the different experimental phases in planted fixed bed reactors.

Higher S^{2-} production contributed to a very low As(III) in the outflow and hence better precipitation probably as As$_2$S$_3$ in PFBR3 and PFBR4 in phase C and thereafter. Inhibition

of photosynthesis or of biochemical processes linked to photosynthesis by different environmental factors may affect a plant's physiological state (Krause and Weis, 1984). Sulphide is a well known inhibitor of photosynthesis (Pezeshki et al., 1988). Plants physiological inhibition of several helophytes was shown for S^{2-} concentrations of approximately 10 to 50 mg l^{-1} (Armstrong et al., 1996; Chambers et al., 1998; Fürtig et al., 1996). But our measured S^{2-} concentrations were always <3.5 mg l^{-1} in the outflow of all experimental phases.

With a partial agreement of these above mentioned statements, it can be concluded that instantly started highly efficient sulphate reduction in this experimental phase C apparently caused sulphide and arsenic toxicity to repress plant physiological activity. A significantly low pH mean value of 4.9 ± 1.4 (see Fig 8.12) in the rhizosphere associated with a low inflow sulphate concentration (0.2 mg S l^{-1}) might not as favorable for a substantial sulphate reduction and subsequent arsenic removal in control PFBR1 as compared to other two reactors with an elevated pH and sulphate concentrations.

Withdrawal of organic carbon from the inflow feeding and a lowered down of sulphate (0.2 mg S l^{-1}) along with same arsenic (0.2 mg As l^{-1}) were the main feature of the short-termed experimental phase D (days 228-235) in PFBR3 and PFBR4 as compared to unchanged conditions in the control PFBR1. Outflow mean concentrations of total sulphur were recorded as 4.2 ± 0.3 and 22.8 ± 8.3 mg S l^{-1} in PFBR3 and PFBR4 (see Fig 8.4), associated with a declined mean sulphide concentration of 08 ± 0.3 and 1.9 ± 0.15 mg l^{-1} respectively (Fig 8.5 and 8.6). An immediate appearance of increasing Eh in these two reactors (see Fig 8.12) accelerated to a high outflow total sulphur concentrations (Fig 8.4) likely due to re-oxidation of reduced sulphur and potentially no sulphate reduction in at least favorable sulphate reducing conditions. Redox potential values rose steadily, sulphate concentration began to rise indicating that oxygen supply may have exceeded respiratory demands and was sufficient for some sulphide oxidation.

It is important to note that reduced sulphur formed during sulphate reduction (precipitated as metal sulphides) can be re-oxidized by chemoautotrophic bacteria using oxygen as the electron acceptor (Howarth et al., 1992). Oxidation of sulphides produces oxidised sulphur species (i.e. S^0, SO_4^{2-}) and may release associated metals or metalloids to the water column of pons (Simpson et al. 1998). Similarly, an oxidative dissolution of already precipitated insoluble As_2S_3 probably resulted in an increase of sulphur concentrations, subsequent mobilisation of exchangeable and oxidised As(V)-fractions in the root vicinity and

consequently enhance plant uptake.

Conditions were switched to experimental phase E which was similar to the phase C, only exception was the inflow concentration of total sulphur in PFBR4 (only 10 mg S l^{-1} instead of 25 mg S l^{-1}) (see Table 5). Reducing condition achieved shortly after addition of organic carbon (COD~340 mg l^{-1}) (see Fig 8.12) but outflow total sulphur dynamics were not showing substantial decreasing trend (Fig 8.4) as time progressed may be due to existing elemental sulphur in the enriched S-pool that previously formed. The mean outflow total sulphur concentration were measured as 4.8 ± 0.9 and 7.9 ± 1.8 mg S l^{-1} in PFBR3 and PFBR4 respectively (Fig. 4.4). Area specific mass retention showed only 28% and 32% of total sulphur mass retained in this particular phase under the dynamic redox conditions in the rhizosphere. Corresponding mean sulphide formation in the outflow were recorded as 1.7 ± 0.3 and 2.5 ± 0.3 mg l^{-1} in PFBR3 and PFBR4 respectively (see Fig 8.6).

The performance of PFBR4 in terms of arsenic removal was comparatively better when sulphate inflow concentration was 10 mg S l^{-1} (in phase E) as compared to 25 mg l^{-1} (in phase C) under the same redox conditions but total sulphur removal was other way round along with these two different concentrations (see Fig 8.4). It can be concluded from both mean concentration reduction and specific mass retention (data not shown) that high S-loading does not necessarily improve arsenic removal performances but definitely enhances high sulphate reduction in the root near environment of the rhizosphere. Transformation and subsequent removal of arsenic might be inhibited during high sulphate reductions by dissimilatory sulphate reducers under reducing constructed wetland conditions.

Sulphate was omitted from the synthetic wastewater in the last experimental phase F (days 315-340) and the concentrations of COD and arsenic remained as similar to the previous phase E in PFBR3 and PFBR4 (see Table 5). Measured mean outflow concentrations of total sulphur were 4.45 ± 0.4 and 6.5 ± 0.5 mg l^{-1} in PFBR3 and PFBR4 respectively, whereas only 1.09 ± 0.6 mg l^{-1} in control PFBR1 (see Fig 8.4). Sulphide concentrations in the outflow ranged between 0.5 to 1.8 mg l^{-1} and 1.7 to 2.5 with a mean value of 0.9 ± 0.5 and 1.93 ± 0.2 mg l^{-1} throughout the 25 days experimental period in the corresponding reactors PFBR3 and PFBR4 respectively (see Fig 8.6). Despite no total sulphur in the wastewater inflow, PFBR3 and PFBR4 exhibited substantial amount of total sulphur in the outflow which was indicating the remobilization and flushing out of sulphur from the S-deposits within the reactors. No immediate sign of arsenic dissolution or mobilization was

observed which ensured better arsenic immobilisation despite withdrawal of sulphate from the wetland systems. Already enriched S-pool and persisent sulphide availability were probably playing major roles for better immobilisation of arsenic as compared to the control PFBR1. Experimental phase E showed a less dramatic decrease in sulfide concentrations after phase C and D, and remained constant thereafter in phase F.

Exhibited sulphate removal measured in our experimental reactors added with arsenic was compared with other simultaneously running reactors without arsenic (data not shown). Extent of sulphate removal was at a lower rate in the reactors running with arsenic than to the reactors running without arsenic. This can be attributed to the fact that the rate of sulphate removal was lowered with the presence of arsenic might lead to the reduction in microbial metabolic activity as a result of arsenic toxicity on the sulphate reducers. This arsenic toxicity in the specific root-near conditions of aerobic to anaerobic micro-gradients caused by oxygen release from the roots have apparently suppressed the intensity of microbial sulphate reduction despite of a surplus of organic carbon as electron donor. It should be noted here that the dissociated sulphide has been reported to be less toxic to the sulphate reducers and also more soluble when compared with an equivalent amount of undissociated H_2S (Reis et al. 1992). Further studies need to be carried out to investigate the exact reasons and to what extent arsenic toxicity inhibits efficiency of sulphate reduction in the rhizosphere of constructed wetlands.

Nearly 100 % of outflow sulphur (see Fig 8.5) was detected as sulphate, however, during all experimental phases of PFBR3 and PFBR4, low concentrations (1-2 mg l^{-1}) of thiosulphate, sulphite and elemental sulphur were also observed. These sulphur species were presumably formed by sulphide re-oxidation and no conclusions can be made about their role as an intermediate metabolite in the various potential sulphur transformation processes and subsequent arsenic removal within the rhizosphere of wetland plants. The lack of significant amounts of thiosulphate, sulphite and elemental sulphur in pore water (see Fig 8.5) suggested that they were either not formed or other highly efficient possible transformation reactions like reduction, oxidation, disproportionation, or even other pathways finally resulted in the formation of a significant S-deposit being relatively stable under the given conditions (Weissner et al., 2005).

4.1.4 Nitrogen removal/species

The mean inflow concentration of total nitrogen (mainly as ammonium) was same as 30.8 $\pm$ 0.6 mg N l^{-1} for all experimental phases in the reactors.

In aerobic wetland environment, the inflow ammonia is likely to undergo nitrification and total nitrogen will be reduced typically through denitrification under reducing environmental conditions (Reddy and Patrick 1984). Denitrification needs organic carbon or other reduced inorganic compounds like H_2S, NH_4^+ etc. as an electron donor (Paredes et al. 2007). Under carbon deficient and aerobic conditions in the experimental phase A, exhibited mean outflow concentration of total nitrogen was 17.4 $\pm$ 8.1, 20 $\pm$ 8.9 and 19.99 $\pm$ 8.5 mg l^{-1} and accounted for a mean removal of 44%, 34% and 35% in PFBR1, PFBR3 and PFBR4 respectively (see Fig 8.7).
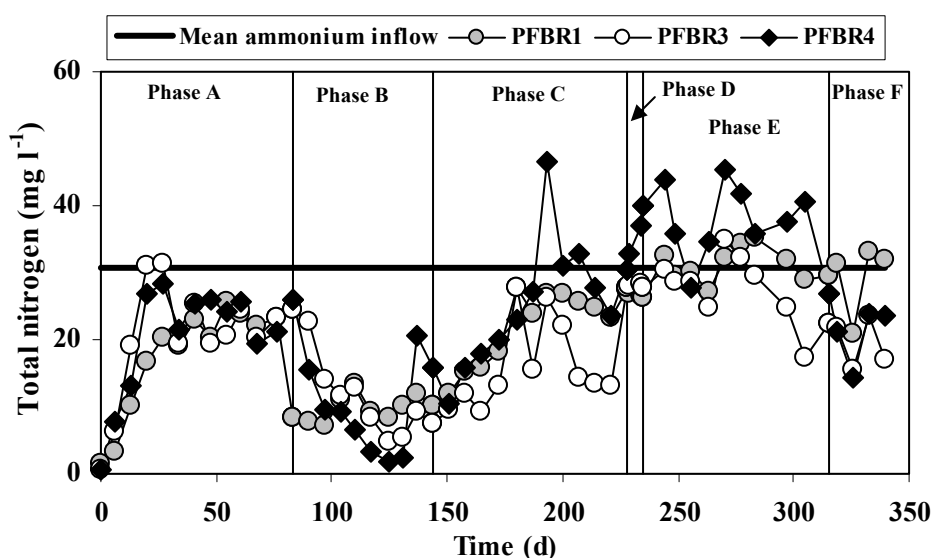

Fig 8.7 Total nitrogen mean inflow and outflow concentrations during different experimental phases in planted fixed bed reactors

However, the rate of nitrification was lower despite highly oxic conditions suggested a potential toxic effect on the nitrifiers due to arsenic in the wetland systems. Nitrifying bacteria are sensitive organisms and extremely susceptible to a wide range of inhibitors and hence it can concluded that presence of arsenic presumably inhibited nitrification under aerobic conditions (Beg et al. 2005). More importantly, nitrification might be attributed to rhizosphere acidification with a low pH value under oxic conditions in all corresponding reactors. No significant concentrations of nitrite and nitrate in the outflow indicating high efficiency of denitrification though organic carbon was limited in this
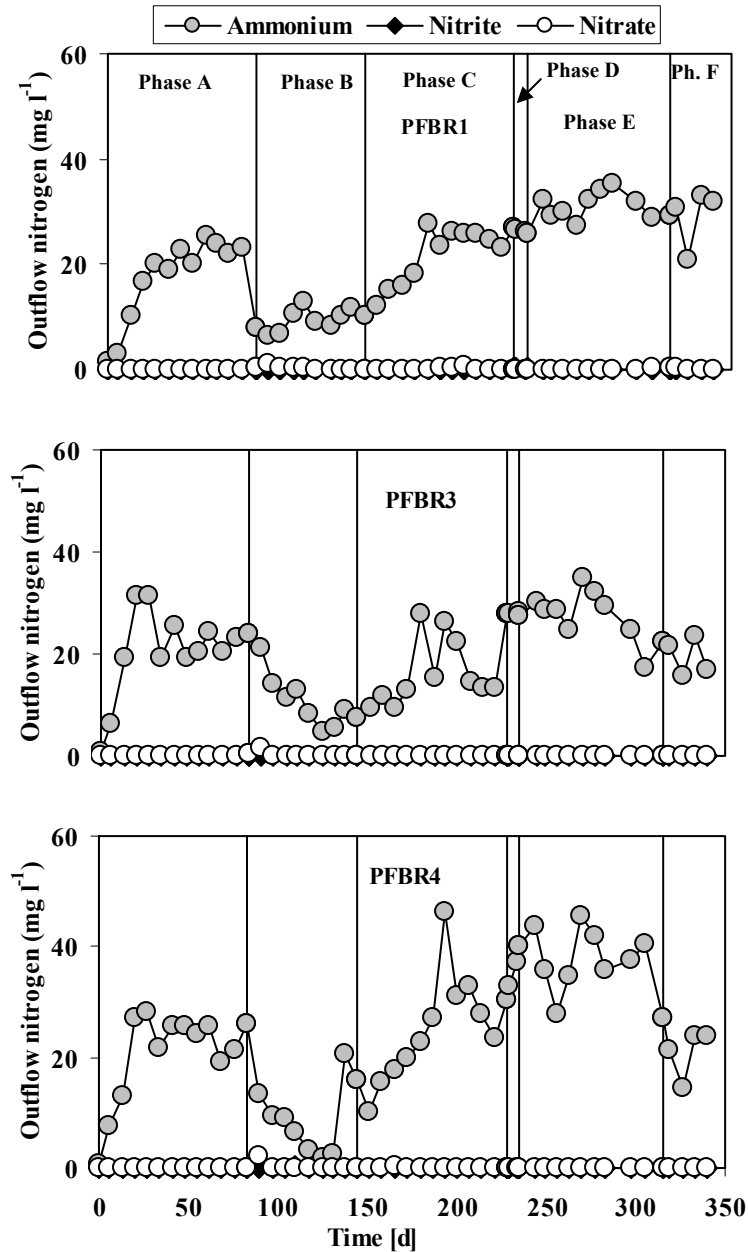
phase (see Fig 8.8).

Fig 8.8 Concentrations of outflow nitrogen compounds during different experimental phases in planted fixed bed reactors

After addition of electron donor in phase B (see Table 5), total nitrogen concentrations decreased down significantly in the outflow and a mean removal of 68%, 64% and 72% were obtained in PFBR1, PFBR3 and PFBR4 respectively. Nitrifiers were stimulated and thus resulted in a better nitrification and subsequent denitrification in all the systems. Gersberg et al. (1983) found that denitrification readily occurs in wetland systems when sufficient dissolved organic carbon was added. Nevertheless, high re-dissolution and

remobilisation of arsenic surprisingly increased better total nitrogen removal rate. In phase C with high sulphate loading in PFBR3 and PFBR4 and increased microbial sulphate-reduction, outflow total nitrogen concentrations substantially increased again and removal efficiency decreased down to 29%, 48% and 19% in respective reactors. More importantly, a very high sulphate concentration in PFBR4 resulted in a poor nitrogen removal which clearly noticed that nitrifiers were out-competed by the sulphate reducers in the system. There was sufficient carbon input into the system which could support denitrification. However, organic substrate is utilized also by other bacteria than only denitrifiers. During the whole experimental period, no significant amounts of nitrate or nitrite could be detected, indicating that all nitrite and/or nitrate formed from ammonia oxidation were consumed as an electron acceptor in anaerobic respiration processes (see Fig 8.8).

In general, the mean ammonia removal during first three experimental phases (phase A, B and C) was 45% and 65% for PFBR1 and PFBR2, respectively. The mean data of relative removal of ammonia are comparable to removal efficiencies measured in large-scale systems (Kuschk et al., 2005; Kadlec et al., 2000; Haberl et al., 1995: Börner, 1992).

However, the resulting mean removal efficiency of 45% and 65% for ammonia, used here in the idealized laboratory system, is in contrast to the much higher removal of approximately 82%, as ascertained in the same system for comparable loading condition using sulphur and arsenic-limited wastewater (Wiessner et al., 2005b).

The inhibition of ammonium removal started already at 0.5 mg l^{-1} sulphide (AEsoy et al., 1998) and this was consistent with our investigation where sulphide formation came into effect in experimental phase C (see Fig 8.6) in all reactors and continued to generate with more than 0.5 mg l^{-1} throughout the later phases. Obvious plant death due to high sulphide and arsenic toxicity reduced plant transpiration rate (see Fig 8.11) and thereby lack of sufficient oxygen in the root near environment for active ammonia oxidation. Nearly 90% of inflow ammonium was flushing out of the systems due to very poor nitrogen removal in the later experimental phases in all experimental reactors. No marked evidence of arsenic removal under reducing conditions correlated with high oscillation of outflow nitrogen can be seen in this model experiments.

4.1.5 Carbon removal

The changes over time of COD concentrations in the inflow and the outflow are shown in Fig 8.9. Efficient COD removal was observed during the experimental phases where

organic carbon was added in all reactors. The mean inflow concentration of COD was considerably stable (about 340 mg O_2 l^{-1}) throughout the experimental phases B, C, E and F. All three reactors showed outflow concentration within the range of 60 - 170 mg O_2 l^{-1} during those particular phases. Reduction of COD from a mean inflow concentration 340 mg O_2 l^{-1} to a mean outflow concentration of 64 ± 22 and 83 ± 16 mg O_2 l^{-1} accounted for a 81% and 76% removal in PFBR3 and PFBR4 respectively in phase C where high sulphate loading resulted in a better arsenic removal. Area specific COD mass removal was surprisingly similar (>86%) despite the 5-fold higher SO_4^{2-}-S in PFBR4 than PFBR3.
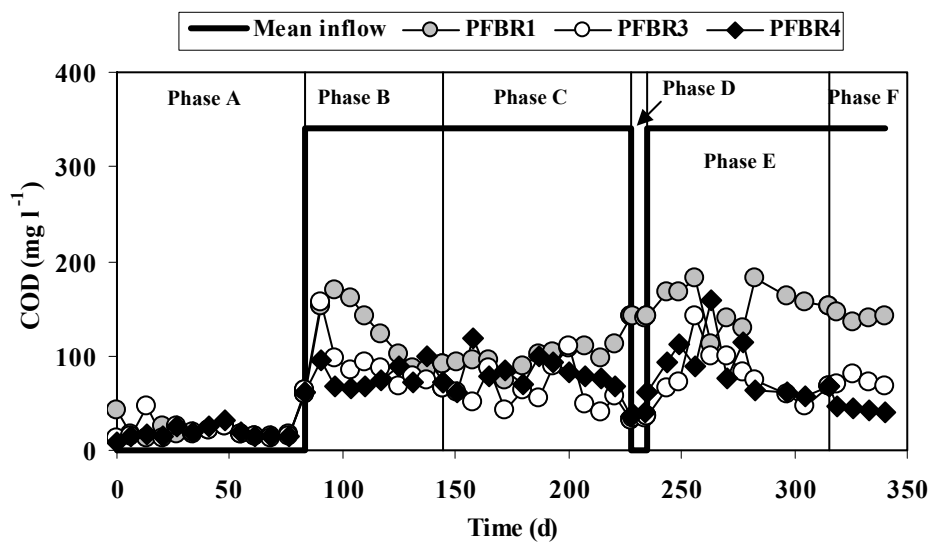

Figure 8.9 Mean COD inflow and outflow concentrations during different experimental phases in the corresponding reactors

Comparing to the control PFBR1, outflow COD concentration revealed at least 50% less in both PFBR3 and PFBR4 which denoted a greater extent of COD removal in those two high S-dosage reactors. Demand for oxygen was more due to high sulphate-reduction by dissimilatory sulphate reducing bacteria accounted for a better organic matter removal in PFBR3 and PFBR4 than the control PFBR1 with only traces (0.2 mg S l^{-1}) of SO_4^{2-}-S in the system. COD depletion slowed down in later phases (phase E and F) in PFBR1 indicating there was insufficient oxygen supply to meet all respiratory demands. Rapid plant death was also propelling oxygen deficiency within root-near environment of the rhizosphere. No clear correlation of As-dynamics with COD removal processes could be observed.

In comparison to the dynamics of the As, S and N removal, the outflow concentrations of total organic carbon (TOC) showed stable, highly effective carbon removal processes in
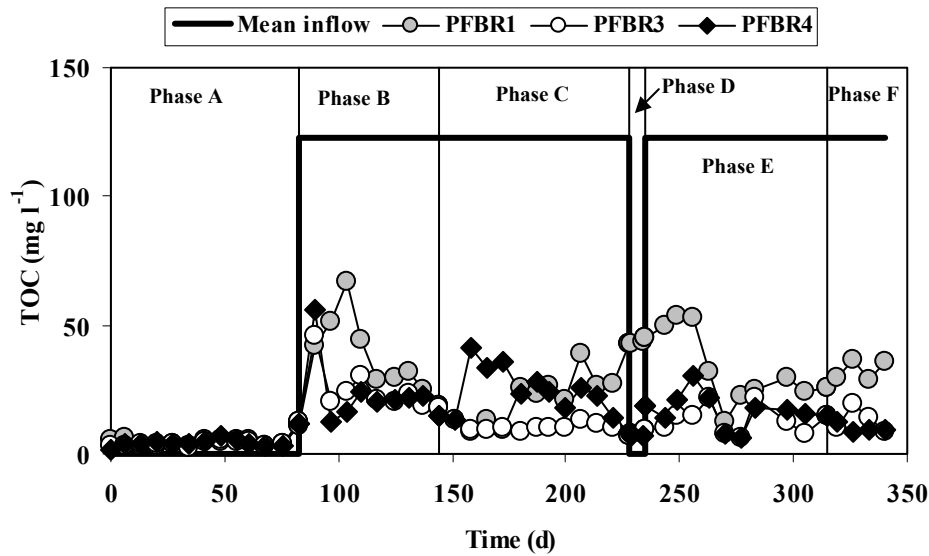
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the system (see Fig 8.10).

Fig. 8.10 Mean inflow and outflow concentrations of total organic carbon during the experimental phases in planted fixed bed reactors.

The carbon transformed (>90%) can partly be detected as enrichment of IC (>50%). Mean percent TOC removal was significantly higher in PFBR3 (87%) and PFBR4 (84%) than in the control PFBR1 (74%) indicated more organic matter degradation driven by microbial metabolism which facilitated better arsenic removal in those two reactors.

4.1.6 Further parameters (shoot density, EVT, Eh and pH)

4.1.6.1 Growth of plant biomass (shoot density) and water loss (EVT)

The growth rate of the *Juncus effusus* was monitored throughout the whole operation time. No unwanted intervention was made to hinder their normal growth. The growth status of the plant biomass in terms of total number of green and healthy shoots is shown in Fig. 8.11. By the end of the phase A and B, corresponding to 144 days of operation, the biomass production of their roots and shoots were increased steadily in all three reactors. There was a consistent 25%, 27% and 30% increase of shoot density in PFBR1, PFBR3 and PFBR4 respectively. The standing green shoot density at this point was 4926, 6021 and 5773 m^{-2} in the corresponding reactors. In general, plants were nutritionally normal and seemed undamaged by arsenic accumulation into their biomass or anaerobic root zone, both in oxic condition in phase A and anoxic condition in phase B.
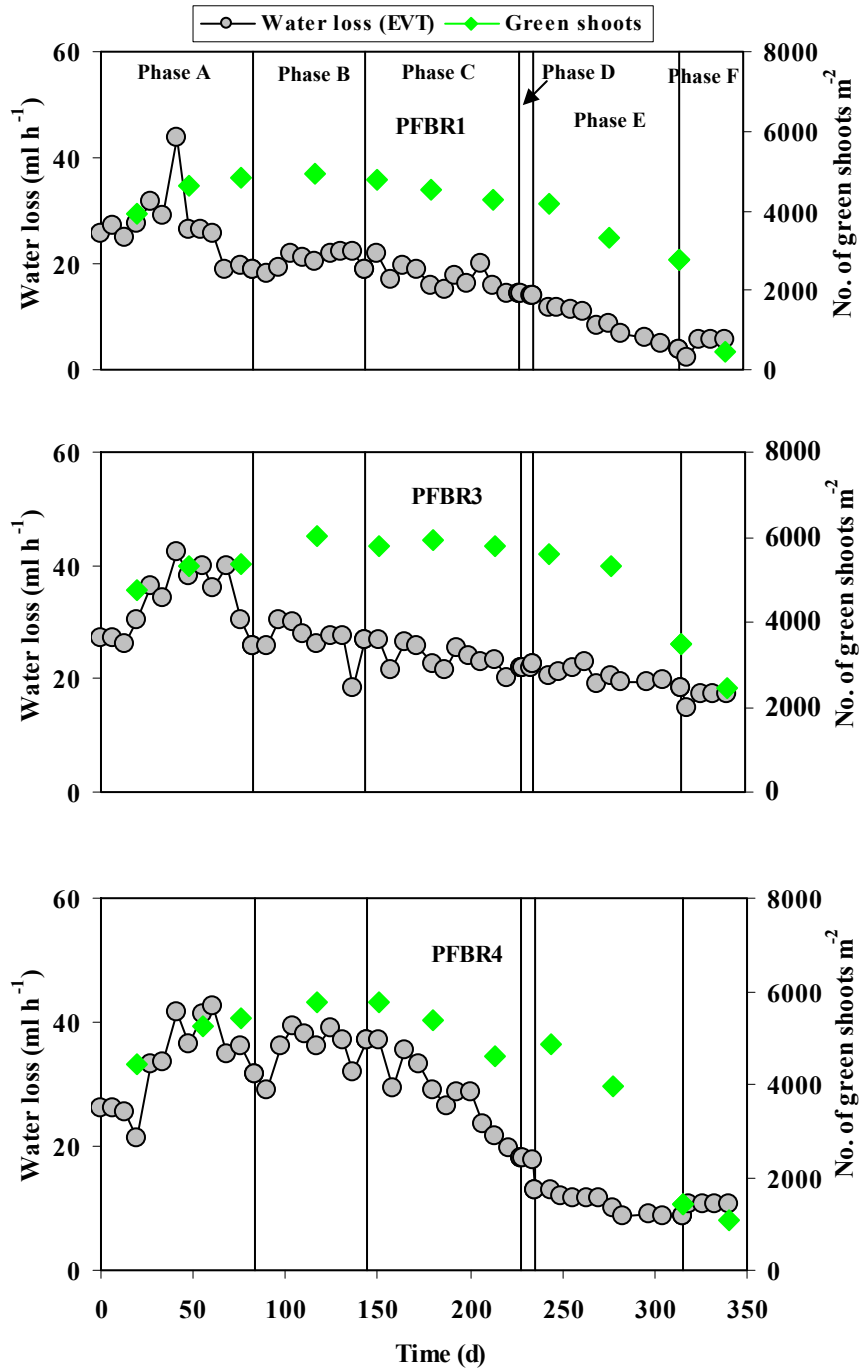
Fig. 8.11 Water loss by plant transpiration and green shoot density during whole operation period in the corresponding planted fixed bed reactors

After the starting of phase C with moderate to high sulphate loading along with arsenic and organic carbon sources in PFBR3 and PFBR4, plants seemed to be stressed and there was a pronounced declination in green shoot density 15%, 4% and 25% in PFBR1, PFBR3 and PFBR4 respectively. Formation of sulphide together with arsenic exhibited toxic effects on

wetland plants and substantially probably suppressed their growth under this strictly anaerobic condition. This decreasing tendency of shoot density continued to the next experimental phases and after phase D, a drastic 90%, 58% and 76% declination of plant shoot density leaving behind only 464, 2455 and 1078 m^{-2} prior to the termination of our investigations when plant death was obvious due to the adverse effect of high toxicity in the rhizosphere of respective reactors. Plants were dying despite low sulphate loading in control PFBR1, which was more likely indicated that accumulated arsenic to an elevated level might prove fatal for the plants than anoxic environment. Plants have toxic threshold limits at which, when exceeded, the plants normal metabolic functions were severely impaired and ultimately lead to the plant's death.

Plant biomass correlated positively with evapo-transpiration (EVT) in all three experimental reactors in this investigation (see Fig 8.11). Healthy plant constitutions (increasing water loss in terms of transpiration) were observed in phase A under oxic conditions despite the presence of arsenic contamination in model wastewater. Water loss in terms of EVT increased by as much 70, 50 and 64% in PFBR1, PFBR3 and PFBR4 respectively after initial phase A which was directly related to the growth of plant biomass and underwent a maximum decrease of about 70, 57 and 75% respectively after phase A till the end. Instantly starting highly efficient sulphate reduction in phase C of the experimental conditions immediately altered plant complexion and apparently caused sulphide toxicity along with arsenic to repress plant physiological activity. Plant transpiration declined from 40.0 and 42.7 ml h^{-1} (nearly 47 and 50 % of the inflow) after starting with organic carbon and high sulphate loading to finally 17.3 and 10.8 ml h^{-1} (nearly 20 and 13 % of the inflow) at the end of the experiment in PFBR3 and PFBR4 respectively. The corresponding specific evapotranspiration (EVT) rates (data not shown) in PFBR3 and PFBR4 exhibited higher and stable evapotranspiration rates in the range of 10 to 16 L m^{-2} d^{-1} in the first two phases (phase A and B) and 4.5 to 9 L m^{-2} d^{-1} in last experimental phases (phase D, E and F) as compared to the control PFBR 1 with 7 to 10 L m^{-2} d^{-1} in first two phases and 1 to 2 L m^{-2} d^{-1} at the end phases.

Therefore, advanced suppressions of internal gas ventilation and oxygen release by the roots of *J. effusus* can be considered as the main effect of the measured sulphide and arsenic concentrations, as also found for other helophyte-species (Armstrong et al., 1996; Chambers et al., 1998; Fürtig et al., 1996).

4.1.6.2 Redox potential E_h and pH

Redox conditions and pH have been shown to be crucial factors influencing the release of arsenic from contaminated sediments (Mok and Wai 1989; Masscheleyn et al., 1991). In our experiments, all three reactors exhibited similar behavior in terms of dynamic redox conditions within their root-zones (see Fig 8.12).
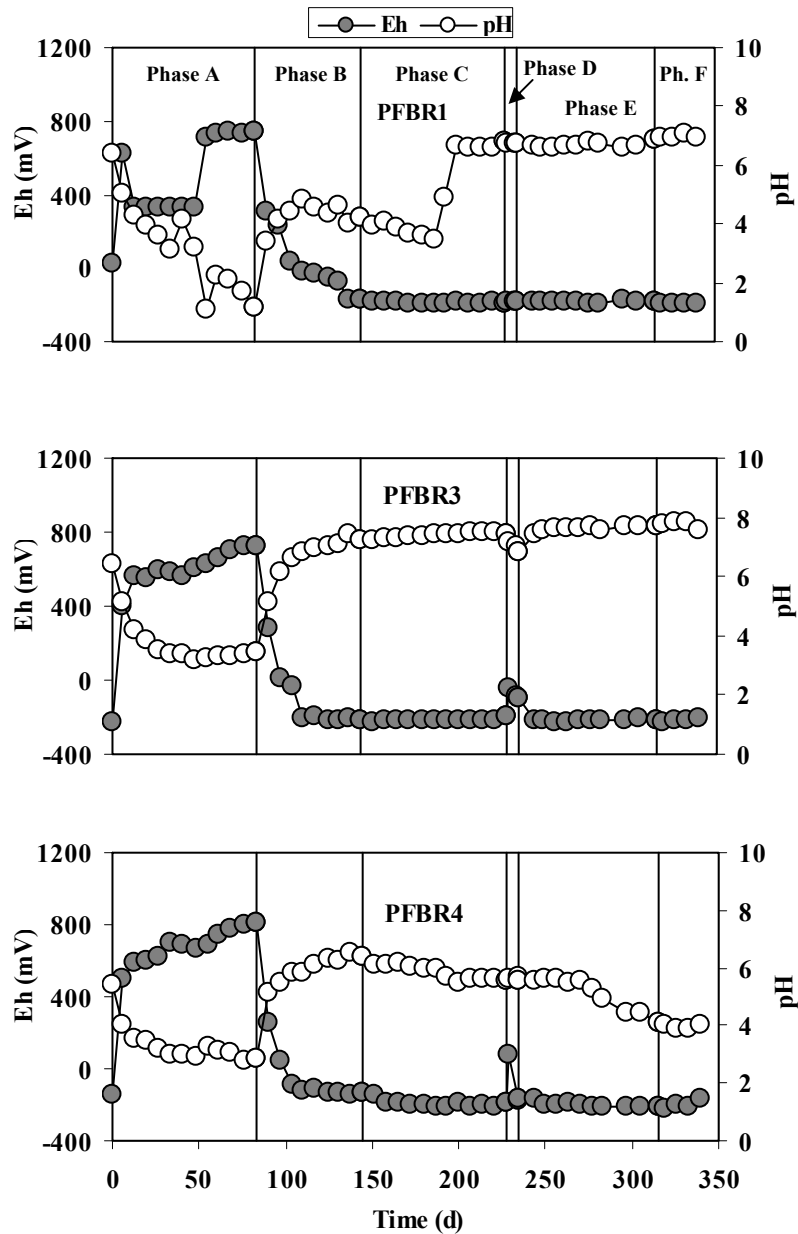

Fig. 8.12 Dynamic redox conditions measured by redox potential and rhizosphere pH during the different experimental phases in planted fixed bed reactors

During the initial experimental phase A under carbon deficient condition, very high redox potential to a maximum of 745, 725 and 814 mV were obtained in PFBR1, PFBR3 and

PFBR4 respectively, which clearly indicated an aerobic condition in the root-near environment of the rhizosphere. Adsorption and co-precipitation of arsenic specifically predominant and thermodynamically more stable As(V) with iron(oxy)hydroxide favored in this prevailing oxic condition for arsenic removal. The redox potential of the pore water was never measured below 100 mV. Therefore, redox conditions unfavorable for sulphate-reducing bacteria can be assumed and it can be concluded that the removal of arsenic was realized mainly by mechanisms other than the precipitation as sulphides.

After addition of carbon sources, depletion of dissolved oxygen by microbial oxidation of organic matter facilitated a rapid decrease in the redox potential which resulted in a minimum value of -168, -217 and -144 mV within the rhizosphere of three respective reactors in experimental phase B. Changes of redox condition dynamics in the transition of oxic to anoxic reportedly accelerated reductive dissolution of iron(oxy)hydroxides and subsequent remobilisation of arsenic from the previously immobilized phase. Redox reactions were playing a major role for this arsenic release and probable transformation of As(V) to more mobile and toxic As(III) in the aqueous phase.

A very low redox potential in the range of -190 to - 225 mV was recorded in phase C in the pore water of all three reactors which were favorable for microbial dissimilatory sulphate-reduction (Boon, 1995; Jackson and Myers, 2002; Choi et al., 2006). Better arsenic removal was observed presumably due to arsenic sulphide precipitates (As_2S_3) in this experimental phase within PFBR3 (SO_4^{2-}-S: 5 mg S l^{-1}) and PFBR4 (SO_4^{2-}-S: 25 mg S l^{-1}).

Rapid increment of redox values were obtained in phase D after cancellation of organic carbon in both PFBR3 (from -196 to -46 mV) and PFBR4 (-191 to 76 mV). Oxidative dissolution of As_2S_3 and re-oxidation of sulphur species to S^0 and SO_4^{2-} might be responsible for arsenic remobilization in this particular phase. As(V) was predominant species in this oxic condition but not a favourable condition for dissimilatory sulphate-reduction. Consistent low redox potential values were appeared and maintained for the next two phases (phase E and phase F) in all three reactors and a range of -190 to -230 mV clearly favored strict anaerobic condition for microbial sulphate-reduction and subsequent arsenic precipitation probably as As_2S_3 if sulphate-sulphur (SO_4^{2-}-S) was abundantly present within the reactors in particular.

It was assumed that under distinct conditions in the pore water within the reactor with permanent mixing of macro-gradients on the rhizoplane were continuously "disturbed" by which the conditions within the pore water very fast reflecting the status at the rhizoplane.

Especially under the conditions of low redox buffer capacity, daily variations of oxygen-input by the macrophytes were observed (data not shown). Plants directly (exploiting different volumes of gravel matrix) and indirectly (by altering rhizosphere pH and redox state) might affect the rate of arsenic transformation, mobilization and distribution in the rhizosphere.

The hydrogen ion concentration of the pore water in experimental phase A was low, which ranged from 1.7 to 6.8, 3.18 to 6.4 and 2.81 to 5.45 , with a mean value of 3.4 ± 1.5, 3.85 ± 1.0 and 3.41 ± 0.75 in PFBR1, PFBR3 and PFBR4 respectively (see Fig 8.12). This lowering of pH referred to as a rhizosphere acidification under oxic conditions which could have resulted from the effect of K^+ uptake and release of H^+ under conditions of low redox buffer capacity. As(V) removal is strongly dependent on the pH and these low pH in the rhiosphere of the experimental reactors remarkably favored arsenic removal (>90%, predominantly as arsenate) under this carbon deficient oxic condition in phase A. As(III) has a very low adsorption affinity and removal is extremely low at pH less than 5.5. This was also reported by other authors. In general, sulphate reducing microorganisms do not grow well at pH values below 5.5 and prefer higher levels of alkalinity, with 6.6 being optimal (Govind et al., 1999; Postgate, 1979). So, the rapid arsenic removal processes took place in the rhizosphere with other processes than dissimilatory sulphate reduction. But a persistently low pH would definitely affect the plant roots and hinders plants natural physiological activities.

After addition of organic carbon sources in phase B, the pH of the wetland systems were well buffered with higher organic loading and contributed to an increment of rhizosphere pH. Mean pH in this phase was 0.9 - 2.8 pH units higher than the previous phase A, resulted an average value of 4.3 ± 0.4, 6.6 ± 0.7 and 5.95 ± 0.5 in PFBR1, PFBR3 and PFBR4 respectively. Comparatively low and constant pH appeared in the control PFBR1 (SO_4^{2-}-S: 0.2 mg S l^{-1}) probably because of plant activity (K^+ exchange for H^+). Due to rapid sulphate-reduction by dissimilatory sulphate-reducers utilising already enriched S-pool from previous phase in PFBR3 and PFBR4 produced alkalinity and triggered the increment of rhizosphere pH in these two respective reactors. Alkalinity produced during highly efficient denitrification might also be resulted in a pH increment. Re-mobilisation of arsenic in the pore water and reduction of As(V) to more soluble As(III) and other methylated polar species were experienced due to the rapid increase in pH in this phase B in particular. Solubility of As(III) increases with the increasing pH and at high pH values,

As(III) can be sorbed to a greater extent than As(V) (Manning and Goldberg, 1996). Better precipitation probably as As_2S_3 in the later experimental phases were observed in a highly consistent pH with an average pH value of 7.0 ± 0.5 and 5.7 ± 0.5 in PFBR3 and PFBR4 respectively, having high sulphate loading under C-surplus conditions. So, an increase of rhizosphere pH favored mobilisation of liable and exchangeable arsenic fractions in the root vicinity and consequently enhanced plant uptake.

The pH values close to neutral in porewaters which were mainly caused by reduction of SO_4^{2-} and NO_3^- with the consumption of protons (Tiedje et al., 1984; Küsel and Alewell, 2004), might be favorable for arsenic biomethylation (Bissen and Frimmel, 2003). The reduction of SO_4^{2-} in porewaters with the activity of sulfate-reducing bacteria (Loy et al., 2004), might be able to mediate arsenic methylation (Michalke et al., 2000). Further investigations are needed to understand actual processes involved for arsenic reduction and subsequent biotransformation, identification of active microbial consortia under C-surplus, anaerobic and nearly neutral pH conditions in the root-near environment of rhizosphere in constructed wetlands.

4.1.6.3 Production of CO_2 and CH_4 gas

Microbiological process produces CH_4 and CO_2 gas as a result of decomposition of organic matter under conditions of the absence of other electron acceptors like O_2, NO_3^-, SO_4^{2-} etc. Concentrations in pore water ranged from 50 to 100 mg l^{-1} as CO_2 and 0 to 10 mg l^{-1} in terms of CH_4 in all experimental phases of our investigation (see Fig 8.13).

Maximum CH_4 concentrations of 9.8 mg l^{-1} (42 % saturation), 2.08 mg l^{-1} (9% saturation) and 8.05 mg l^{-1} (34 % saturation) were measured in the pore water of PFBR1, PFBR3 and PFBR4 respectively. Only at the final stages of the experiment, significant enrichment of CH_4 could be observed in all three reactors. At the operation time around day 190 in phase C, when plants damages became obvious by substantial declination to the transpiration rate (see Fig 8.11), substantial CH_4 started to be generated. Leading to further plant death, the concentration of methane was found to be highly enriched towards the end of the experiment simultaneously with a moderate sulphate reduction.
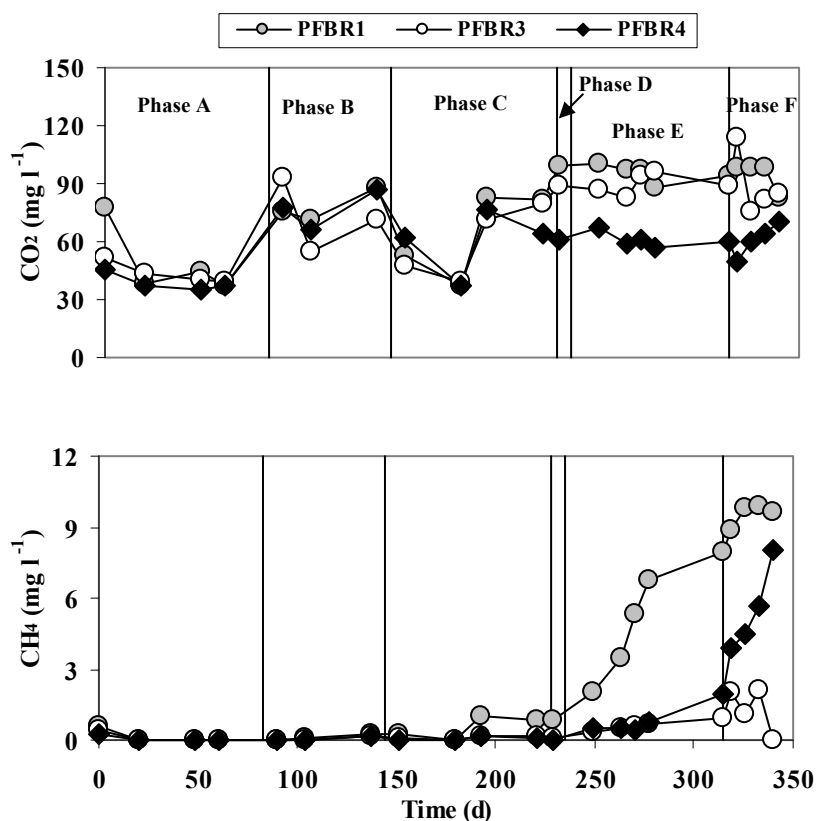
Fig 8.13 Concentrations of CO_2 and CH_4 during different experimental phases in the corresponding reactors.

Coexistence of methanogenesis and sulphidogenesis is well known (Liesack et al., 2000). Nevertheless, for natural wetlands it was shown that sulphate can substantially reduce the methane formation (Gauci et al., 2004). In our study, CH_4 production started with plant death in experimental phase C and suggests repression of methanogenesis in root near environment by oxygen released by the plants (Weissner et al. 2005). The findings underline differences of the oxygen tolerance of sulphate reducing and methane producing bacteria (Wind and Conrad, 1997; Wind et al., 1999).

Only 5-8% of total C outflow concentrations were in the form of CH_4. This low level was probably due to a relatively low loading rates and oxygenation of the substrate by active gas transport through the roots of wetland plants. Relatively much more methanogenic activity was displayed in control PFBR1 than the other PFBR3 and PFBR4 which might be due to the fact that low sulphate content in this reactor preventing high sulphate reduction and thereby stimulated more CH_4 formation. Fermentation of organic matter forms various low molecular weight acids, alcohols and subsequent CO_2. Maximum CO_2 concentrations were measured in PFBR1 as compared to other two PFBR3 and PFBR4 indicated more

decomposition of organic matter in control PFBR1.

In comparison to other reactors running simultaneously without arsenic in the wastewater inflow (data not shown), it was observed that with the addition of arsenic in our systems did not significantly affect the extent of organic matter degradation or the proportion of CH_4 and CO_2 formed. Other literature suggests that exposure to relatively high concentration of soluble arsenic inhibits methanogenic activity and decreases CO_2 and CH_4 production. With a low inflow concentration of total arsenic predominantly as arsenate [As(V)] in our investigation, apparently no sensitivity was displayed by methanogenesis.

4.1.7 Bioaccumulation of arsenic in plant biomass

Terrestrial plants are able to accumulate arsenic to a substantial extent (Brooks et al. 1977, Visoottiviseth and Sridokchan 2002, Wagemann et al. 1979) but survive the stress to differing degrees of vitality. The influence of arsenic on important energy and metabolic cycles does not yet have sufficient explanation. So-called hyper-accumulators take up more than 1000 mg kg^{-1} dry weight of the pollutant (Brooks et al. 1977). Since these plants bear such high arsenic quantities, they must have a strategy for detoxification (Visoottiviseth and Sridokchan 2002)

An important goal of this study was to investigate bioaccumulation of arsenic in the plant biomass (into their shots, roots etc.) which may contribute towards a quantitative mass balance calculation of arsenic in the systems running under constructed wetland conditions and will definitely be decisive for a meaningful post-treatment hazardous biosolid handling and management strategy. Plant tolerances to the pollutants depend on the loading conditions and there was no exception to that in our investigations with *Juncus effusus*. During the whole experimental period (341 days), arsenic was accumulated in different compartment of the plant biomass mainly into plant shoots (green and dead) and roots (see Fig 8.14).

Mean concentration of total arsenic in healthy green shoots measured as 0.91 ± 0.04 and 0.71 ± 0.04 mg kg^{-1} in PFBR3 and PFBR4 respectively, comparing to a slightly higher 2.06 ± 0.11 mg kg^{-1} in control PFBR1.
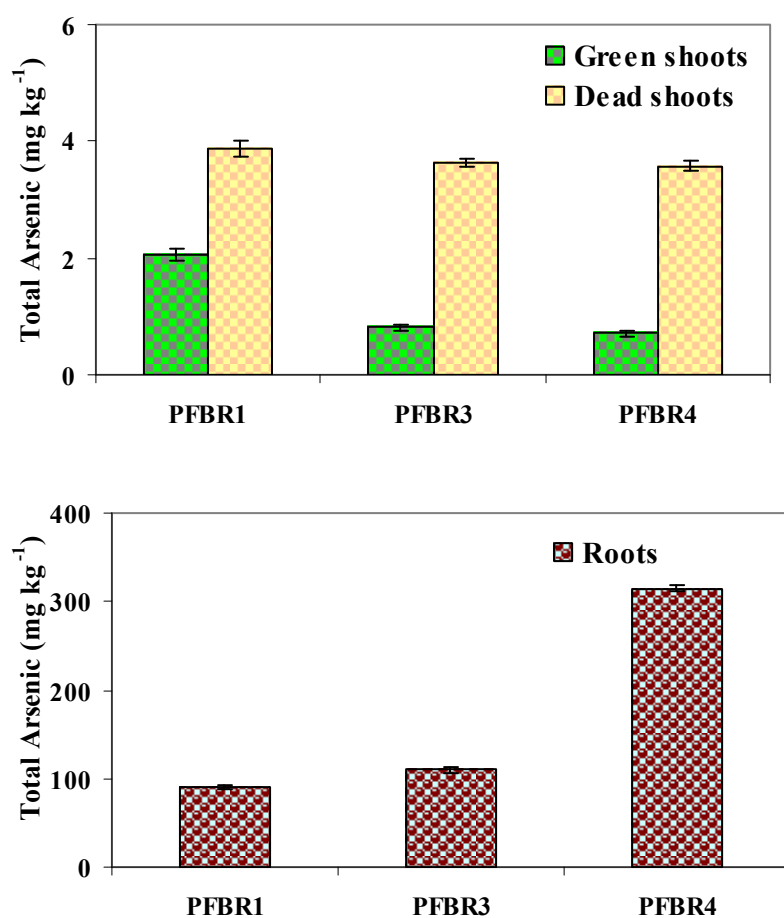
Fig 8.14 Variation of arsenic concentration in plant biomass specifically in green shoots, dead shoots and roots after the termination of the experiments in planted fixed reactors

In dead shoots, mean values of 3.88 ± 0.13, 3.63 ± 0.07 and 3.57 ± 0.08 mg kg^{-1} were obtained in PFBR1, PFBR3 and PFBR4 respectively. No significant differences can be found in terms of arsenic translocation into plant shoots within the corresponding reactors. Generally, in all reactors, higher amount of total arsenic concentrations exhibited in the roots than to their shoots (see Fig 8.14). Van den Broeck et al. (1998), Buddhawong. (2005) and other authors also reported the similar facts when dealing with arsenic and heavy metals in constructed wetlands. At the end of our investigations, high variations of mean concentration as 90 ± 2.8, 110.5 ± 3.5 and 315 ± 4.3 mg kg^{-1} were obtained in the plant roots of PFBR1, PFBR3 and PFBR4 respectively. Roots continuously remain under direct exposure to arsenic in both oxic and anoxic environment and translocation rate of arsenic into shoots presumably depends on other factors and varies within different plant species.

The elevated concentrations of total arsenic in the plants roots were extremely higher than

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in the shoots (both green and dead) in all three reactors. Carbonell et al. (1998) studied the arsenic content in *Spartina alterniflora* and found arsenic in the range of 0.80 – 1.77 mg kg^{-1} in shoots and 6.87 – 86.60 mg kg^{-1} in the roots. Our results with *Juncus effusus* showed not exactly the similar amounts, in fact, approximately 3-fold higher values but it was definitely not surprising depending on various loading conditions and exposure time.

Nearly 3-fold higher concentrations in PFBR4 than PFBR3 and 3.5-fold greater than the control PFBR1 clearly indicated a high amount of arsenic retention, accumulation, adsorbed, metabolised to other forms and/or translocated into the root vicinity of the rhizosphere of *Juncus effusus* in PFBR4 where organic carbon and sulphate concentration incorporation to arsenic were abundant all through the operation period. The much higher accumulation of arsenic in the plant roots compared to the above-ground plant biomass (shoots) corresponded to the studies for *Typha latifolia*, *Equisetum fluviatile*, *Triglochin palustre*, and *Sparganium sp*. (Dushenko et al. 1995).

4.1.8   Arsenic species accumulation in the plant biomass

Very little is known about the transformation of arsenic and tolerance mechanisms within the plant biomass. Furthermore, no concrete data exists concerning the effect of arsenic on the metabolism of plants.

Since arsenic is subjected to various metabolisation reactions in biological systems, the total arsenic content alone does not offer sufficient information on the degree of toxicity. Therefore, the differentiation between different arsenic species forms the basis for an exact evaluation of the toxicological potential of environmental samples (plant shoots, roots etc.). Different arsenic species vary in their solubility, mobility, bioavailability and phyto-toxicity. Hence, studies concerned with the uptake of different arsenic species, both inorganic and organic, by plants and their effects on plant growth and nutrition, are of essential importance for the understanding of the behavior of arsenic in the wetland systems (Carbonell-Barrachina et al. 1999). In this study the uptake and processing of As(III) and As(V) by *Juncus effusus* was investigated (see Fig 8.15).

Analytical results revealed that wetland plant (*Juncus effusus*) has a competence to transform As(V) into other species. More mobile and toxic As(III) was translocated into shoots (both green and dead).
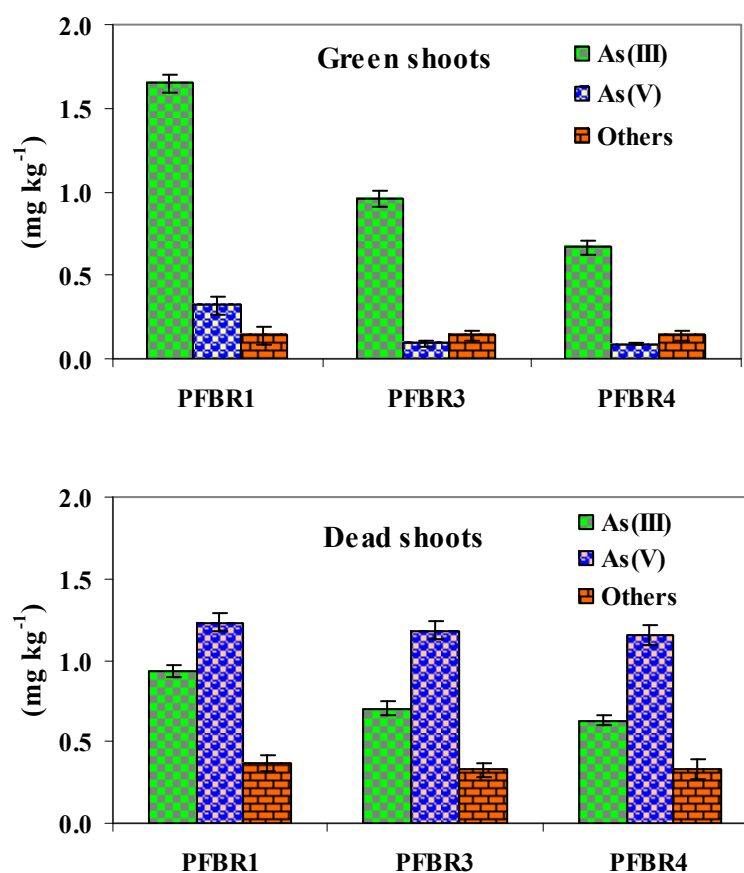
Fig 8.15 Transformation and distribution of arsenic species in green and dead shoots of *Juncus effusus* used in the experiments of planted fixed bed reactors.

Traces of methylated species (e.g. TMAO) and other unknown species were found in the shoots and roots of all experimental reactors. The results of this study indicated that plants accumulated more As(III) than As(V) into their green shoots. The As(III):As(V) ratio increased in the range 5-10:1 in green shoots of all three corresponding reactors.

The analysis of the arsenic species in dead shoots affirmed the hypothesis set up on the basis of previous experiments that in dead shoots more thermodynamically stable pentavalent form of inorganic As(V) dominated (Schmidt 1999). Calculated As(III):As(V) concentration ratio in dead shoots was very small (nearly zero). More As(III) in the green shoots would definitely reduce the quantity of this highly toxic As(III) in a wetland treating with arsenic contaminated wastewater. But prolonged highly toxic effect exhibited by As(III) might have been the reasons for the green shoots to show the signs of changing their complexion shortly afterwards, hinders plant physiological activities and leading towards obvious plant death at the end. However, there was also more As(III) than As(V) in green shoots with the observation of Mattusch et al. (2000) and Van den Broeck et al.

(1998) which agrees with our investigations. But nothing is known yet about the mechanisms of the reduction of arsenate in plants.

4.1.9   Arsenic in gravel and sludge sediment

As already mentioned (see section 3.4.8), it was found that the gravel could not adsorb arsenic in substantial amounts from the solution. Only a traces ($<$3-4 µg kg^{-1}) of total arsenic was detected which was adsorbed on the surface of gravel matrix. So, this kind of gravel itself had no significant impact on the model experiments in terms of arsenic removal in constructed wetland systems.
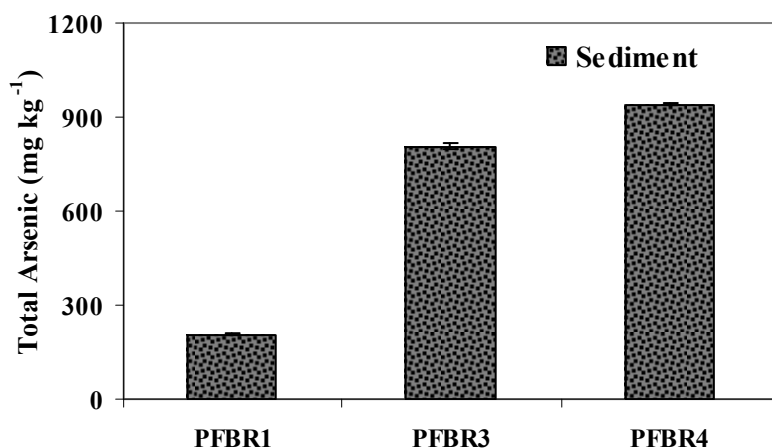

Fig 8.16 Concentration of total arsenic accumulated in the sludge sediment after the termination of the experiments in planted fixed bed reactors.

But big variety of suspended and biofilm fixed microorganisms and plant roots along with high organic carbon produced sludge sediments within the root bed which were collected from the reactors after the termination of all investigations.

Analytical results showed a wide variation of mean arsenic concentration as 206.5 ± 4.95, 806.5 ± 9.2 and 941 ± 4.24 mg kg^{-1} in PFBR1, PFBR3 and PFBR4 respectively (see Fig 8.16). Clear evidence of high arsenic adsorption and precipitation in the sludge sediment indicated more microbial activities under redox conditions in the rhizosphere of PFBR4 as compared to other two reactors.

4.1.10 As-mass balance

Analysis of arsenic mass balance was carried out for all the experimental reactors at the end of our investigations. There might be four possible fates for the contaminants removal from the model wetlands: (1) sequester into the sediments, (2) accumulate into plant biomass (shoots, roots etc.) (3) retain in the standing water at the termination of the experiment, and (4) volatilize into the atmosphere. The remainder of the contaminants passes through the reactors in the outflow where it is collected. Since the reactors were closed tightly enough and well-controlled systems in our investigations, we were able to obtain a quantitative mass balances of total arsenic by expressing its distribution as percentages of the mass loaded into the reactors (consider inflow as 100%) during the study period (see Fig 8.17).

These mass balances accounted for over 90% of the total arsenic loaded into the reactors. Fig. 8.17 illustrates a total of 140, 135 and 123 mg inflow As-mass loading and a substantially high 67.3, 107.3 and 104.3 mg As-mass was retained which resulted a 48%, 80% and 85% retention in PFBR1, PFBR3 and PFBR4 respectively.

Based on the concentration of arsenic in the plant shoots, roots and sludge sediments of three reactors at the end of the experimental period, we calculated that 13%, 17% and 43% of total inflow arsenic loading into the reactors accumulated in the roots and 4%, 23% and 17% were sequestered or deposited within the sediments of PFBR1, PFBR3 and PFBR4 respectively (see Fig 8.17). In all three reactors, total arsenic accumulation in plant shoots had a small contribution to the mass balance.

Apart from sediments, plant roots were also therefore considered to be the primary sink for the sequestration of arsenic, which partially agrees with the many other studies of treatment wetland systems (Sundaravadivel and Vigneswaran, 2001). This accumulation or deposition of arsenic in the sediments occurs due to the formation of insoluble precipitates such as As_2S_3 via abiotic processes or, more probably, driven by the high level of microbial activity and biotic processes in the sludge sediments incorporated to the organic matter content, which might be a critical consideration for improving the efficiency of arsenic removal from wetland systems.
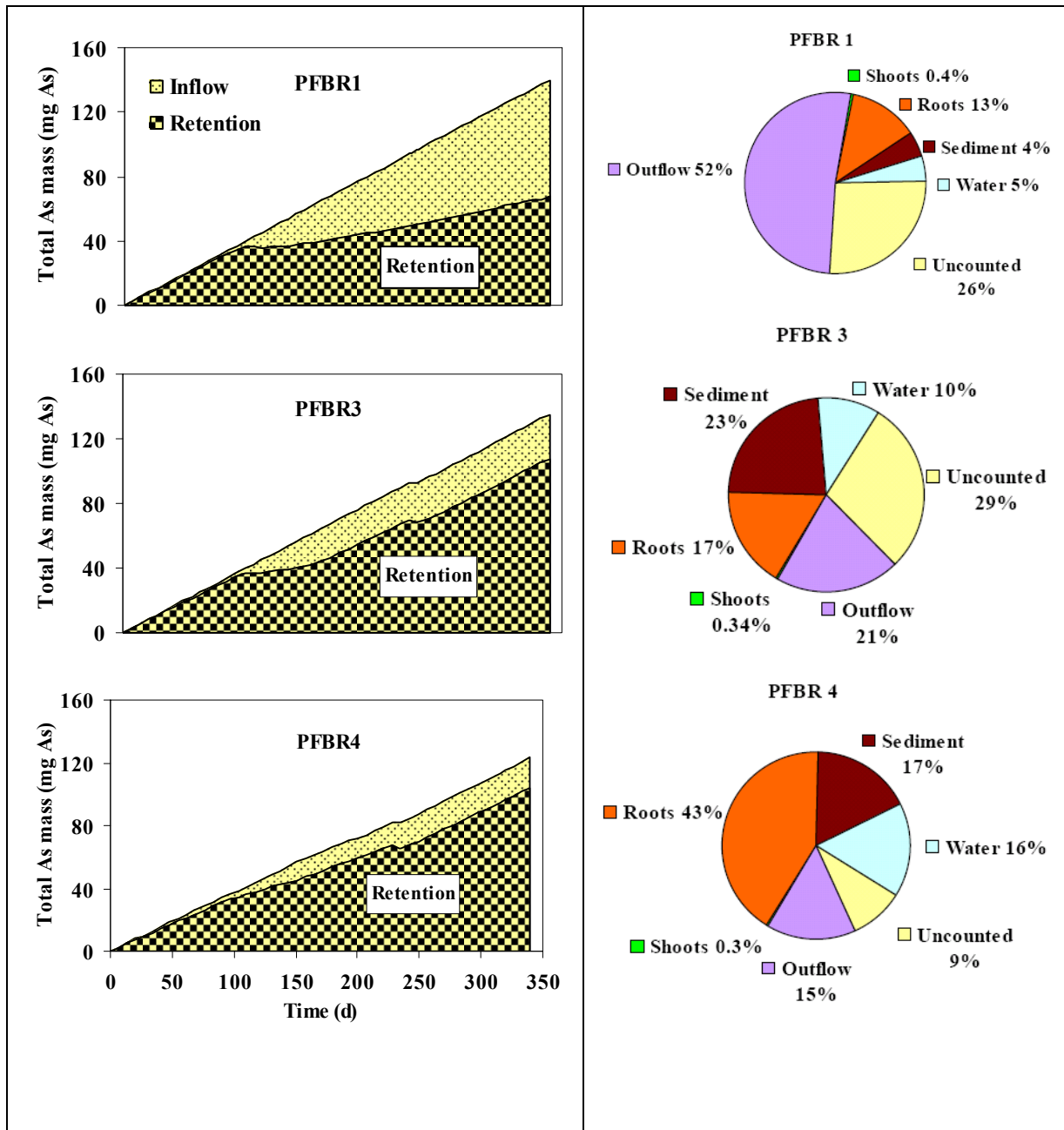
Fig 8.17 Cumulative total As-mass inflow and retention during the whole experimental period and As-mass balance estimated as percentage of inflow loading in all three reactors

What is a crucial concern are the long-term toxic effects of the build-up of As-contaminants in the sediments. Only <1% of total arsenic loaded into the reactors were accumulated into the plant shoots (see Fig 8.17). This highlights the minor role by the plant shoots in terms of arsenic uptake and very low translocation efficiency from roots-to-shoots (ranges only 4 - 6 %) in all three reactors, which is a common finding in studies of treatment wetlands (Batty and Younger, 2002). Standing water accounted for nearly 5%, 10% and 16% of total arsenic inflow loading in all three respective reactors. Only traces (2-3 µg l^{-1}) of inorganic volatile arsine (AsH$_3$) were measured in this study which resulted

in a small amount of arsenic left out of the systems in terms of volatilisation. Nearly 26%, 29% and 9% of total arsenic in these reactors were accumulated into uncounted sink which could be due to other microbial reactions, adsorption and even volatlization. A total of 52%, 21%, and 15% of arsenic were passed through the reactors of PFBR1, PFBR3, and PFBR4 respectively and were collected in the outflow. Therefore, PFBR3 and PFBR4 performed as better sink for arsenic deposition and accumulation than control PFBR1. Arsenic might be bio-transformed by plant root activity and associated microbes to other unidentified organic compounds, which prevented the calculation of a complete mass balance for arsenic.

The high total arsenic plant uptake rate (mainly by roots) as found in this study was probably due to the highly dense root biomass growth inside the reactors and high organic C and S-loading characteristics of the influent wastewater which enhanced effective total arsenic uptake by the wetland plants in all the experimental units. For long-term operation, more attention should be given to arsenic accumulation in the constructed wetland beds to avoid potential toxic effects the accumulated arsenic could pose to the wetland plants and nearby environment.

4.1.11 Outcomes and general remarks

Based on the results of this study the important observations and interpretations can be summarised as follows:

- The data suggests that constructed wetlands could be effective in removing arsenic from the secondary domestic wastewater effluents prior to their disposal into receiving water bodies or use for irrigation purposes. Under carbon limited and oxic conditions, a high mean removal efficiency (>90%) of arsenic was observed in the experiments with planted fixed bed reactors. These results encourage the construction of a pilot-scale wetland to investigate the effectiveness of constructed wetlands for treating wastewater from secondary domestic effluents. But the dynamics of the arsenic cycle in the rhizosphere under more realistic and practicable macro-gradient flow conditions should be understood in more detail.

- Addition of electron donor dramatically changes redox condition dynamics within the root-near environment of the rhizosphere and reductive dissolution of Fe(III) oxyhydroxides facilitates remobilization of arsenic, reduction of As(V) to more mobile and toxic As(III) in the aqueous phase. Most likely there are several

competing reactions including both dissolution or desorption and precipitation or adsorption occurring simultaneously. Redox conditions greatly influence arsenic transformation and bioavailability under constructed wetland conditions.

- Accumulated evidence strongly suggests that sufficient organic carbon and sulphate loading immediately impact on arsenic removal rate and the root-near micro gradient redox processes contribute to an appreciable degree of arsenic removal in ideal flow model constructed wetlands. Under reducing conditions, the mobility of arsenic is driven by the microbial sulphate reduction processes and subsequently precipitates probably as As_2S_3 with low solubility. It can therefore be concluded that sulphate loading under carbon surplus conditions is a promising strategy to accelerate the removal efficiency of arsenic in the rhizosphere of constructed wetlands. But plants exhibit apparent toxic symptoms probably due to toxic sulphide incorporation with reduced arsenic species arsenite [As(III)].

- High carbon load attributed to a stable and efficient sulphate reduction, rising of rhizosphere pH, increasing enrichment of S^{2-} and S^0 in pore water, high arsenic retention, and finally plant death by sulphide and probable arsenic toxicity. Re-oxidation of reduced sulphur to S^0 and SO_4^{2-} driven by microbial activity propels oxidative dissolution and subsequent remobilization of arsenic from already precipitated insoluble arsenic species under oxic conditions.

- The dominant redox dynamics proved decisive for the arsenic removal efficiency of treatment wetlands. The results highlight the importance of plant biomass and their physiological activities for the removal process of the contaminants despite the adverse effect of arsenic and sulphide toxicity on plant biomass.

- No clear evidence can be noted whether arsenic was inhibiting three ecologically important anaerobic processes: denitrification, sulphate reduction and methanogenesis under these special experimental constructed wetland conditions.

- Based on arsenic mass balance analysis, planted fixed bed reactors along with wetland vegetation (*Juncus effusus*) were found to retain more than 76% of the total arsenic input, while the 9% unaccounted values were postulated to be due to some other microbial reactions, adsorption and even volatilizations.

- Unlike in conventional treatment wetlands, not only sediments but both plant biomass (specifically roots) and sediments comprise the ultimate sink for arsenic in

PFBR. A substantially higher mass of arsenic is retained in the roots and sediment (>60%) as compared to shoots (<1%). High accumulation of arsenic in or on the roots (>43%) than shoots (<1%) suggests greater biological activity at root surfaces than elsewhere.

- Long-term accumulation of arsenic in wetland vegetation and sediments may reduce widespread distribution in the environment but the concentrated deposits may contribute to detrimental effects on bioaccumulation and bio-transport. For wetland systems to prove useful as an efficient arsenic removing technology, a suitable and sustainable disposal mechanism for the arsenic contaminated biomass must be developed.

- Stability and re-oxidation of immobilised S and correlation of As-dynamics and biotransformation processes along with methanogenesis in root-near environments are focusing objects of further investigation.

- Further research, therefore, should focus on the investigation (i) of large-scale systems due to capacities of arsenic removal, the deposition and remobilisation of arsenic, reduction from As(V) to As(III) and other methylated polar organic compounds like MMA, DMA, TMAO etc., (ii) of potential interactions and influences of sulphur species like elemental sulphur, sulphite and thiosulphates on arsenic removal and transformation by varying organic C and S loadings, (iii) of potential inhibition of arsenic on nitrification-denitrification, sulphidogenesis, methanogenesis, and (iv) of toxic effects of inorganic arsenic species and sulphide upon plants and microorganisms.

4.2 Treatment of artificial arsenic containing wastewater in subsurface horizontal flow laboratory-scale constructed wetlands

4.2.1 Dynamics of As-removal

The dynamics of total arsenic concentrations in the inflow, middle and outflow sections of the model wetlands are represented in Figure 9.1. During the whole 491 days of experimental period, a continuous supply of synthetic wastewater provided a mean inflow concentration of 0.2 ± 0.01 mg As l^{-1} in all three model wetlands (planted W1, W3 and unplanted W2), the only exception was in phase C and E, where cancellation of arsenic loading attributed to a mean inflow concentration below the detection limit (<0.3 µg As l^{-1}).
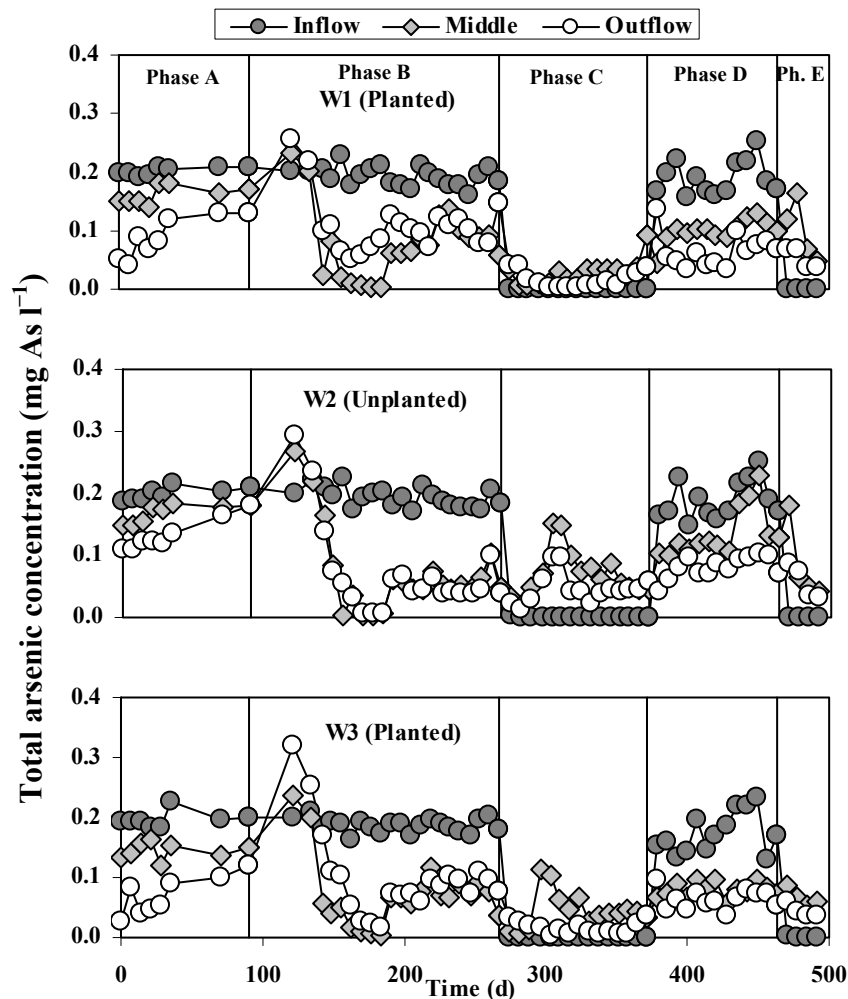

Figure 9.1 Total arsenic concentrations of the inflow, middle and outflow in planted (W1 and W3) and in unplanted control (W2) horizontal subsurface-flow constructed wetlands.

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Under the running conditions of phase A where no addition of organic carbon sources and tap water provided a sulphate-sulphur concentration of 50 mg S l^{-1}, arsenic removal in this persistent oxic condition was the chief objective in all three experimental wetlands. After an operation time of 91 days, an efficient arsenic removal accounted to concentration declination with a mean value of 0.08 ± 0.03 and 0.06 ± 0.03 mg As l^{-1}, resulted to a mean relative concentration reduction as 59% and 68% in both planted beds W1 and W3 respectively (see Fig 9.1). Only a low level of 20% and 27% removal was attained in the middle section (flow path of 50 cm from the inflow). Contrarily, in the control unplanted bed, mean concentration apparently lowered down to 0.13 ± 0.02 mg As l^{-1} from the mean 0.02 ± 0.01 mg As l^{-1}, resulted a mean removal of only 36% and showing a definite lack of effectiveness regarding arsenic removal.

These results clearly indicated that under C-deficient oxic conditions, planted wetlands with high arsenic removal efficiency performed better than unplanted wetland. This may be due to the fact that plants provide redox gradient (both micro- and macro) conditions in the root-near environment of the rhizosphere where grows big variety of biofilm fixed microorganisms and plant roots. These are all added towards an efficient arsenic removal in terms of adsorption with microorganisms, plant roots, organic substrates and/or adsorption onto oxide minerals and probable concomitant co-precipitation with Fe(III)-oxyhydroxides. Reasons for the immobilization of arsenic under oxic condition were similarly explained by other authors (Wilkie and Hering, 1996; Bednar et al., 2005). Highly oxic condition (Eh ~500 mV) (see Fig 9.8) and lack of organic carbon was the major limiting factors for sulphate reductions and hence it can be concluded that arsenic removal was realized by mechanisms other than precipitated with sulphide.

The corresponding area specific mean removal rates for both planted beds (W1 and W3) showed a value of 3.9 ± 0.7 and 4.37 ± 0.5 mg As m^{-2} d^{-1} contributed to a mean arsenic retention of 65% and 74% whereas in unplanted bed with a removal rate of 2.3 ± 0.4 mg As m^{-2} d^{-1} accounted for a mean arsenic retention of 38% (see Fig 9.2).

Addition of organic carbon sources (COD ~340 mg l^{-1}) in phase B (corresponding to days 91 – 267) caused a drastic changes in redox condition dynamics and outflow concentration showed excess amounts of arsenic, even higher than the mean inflow concentration of 0.02 ± 0.01 mg As l^{-1}. Microbially mediated reductive dissolution of Fe(III)-oxyhydroxides attributed to a release of arsenic in the aqueous phase under reducing conditions in all the respective wetlands. This release of arsenic under reducing condition was in agreement
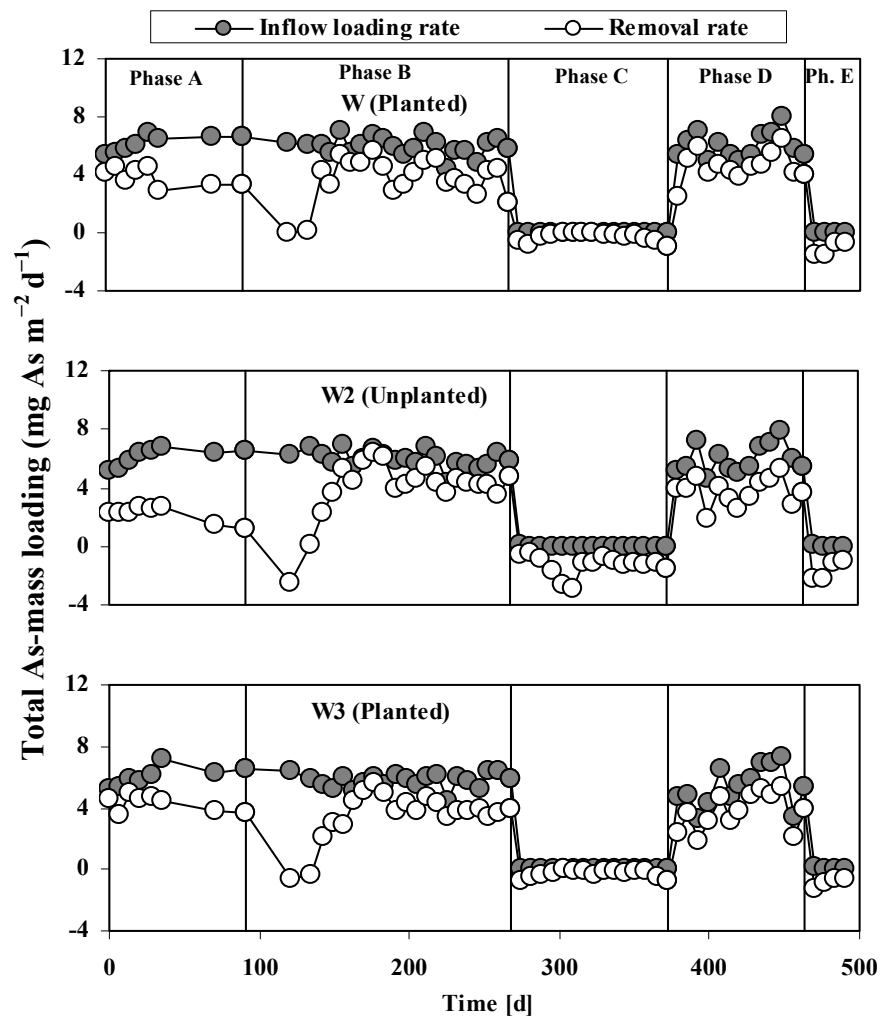
Figure 9.2 Specific loading rate and removal rate of arsenic in planted (W1 and W3) and in unplanted (W2) subsurface horizontal laboratory-scale constructed wetlands.

with Smedley and Kinniburgh (2002). The maximum dissolved arsenic concentration of 0.26, 0.29 and 0.33 mg As l^{-1} in W1, W2 and W3 respectively was obtained in pore water after starting of experimental phase B. Similar trend was shown in the middle section of all three wetland beds. Re-mobilization accounted to an elevated arsenic concentration at the beginning and soon afterwards a steady state appeared in the dynamics of outflow arsenic concentrations in all three wetlands. At the end of this phase, a mean value of 0.107 ± 0.05, 0.07 ± 0.06 and 0.1 ± 0.07 mg As l^{-1} resulted in a 44%, 64% and 47% of arsenic removal was attained in W1, W2 and W3 respectively (see Fig 9.1). Substantially high relative concentration reduction as 59%, 64% and 62% in the middle section of the respective wetlands indicated a spatial variation and better arsenic removal performances within the half of the flow path than the outflow (end section). Persistent sulphate-sulphur inflow loading via tap water (SO_4^{2-}-S ~ 50 mg S l^{-1}) under C-surplus conditions initiated

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substantial sulphate reduction by the dissimilatory sulphate-reducing bacteria and attributed towards a significant amount of arsenic reduction more likely in the form of As_2S_3 precipitation within the half of the flow path. Presumably there were several competing reactions including both dissolution or dissorption and precipitation or adsorption in a high rate occurring simultaneously.

More than 60% of arsenic deposition or precipitation occurred in the first half of the wetland beds and this result suggested that an unnecessary long wetland bed could not create any huge impact when it comes to arsenic removal under constructed wetland conditions. Shortening of wetland beds in an engineered fashion may lead towards better contaminant removal performances and cost effectiveness of the whole treatment plant.

Doubling the carbon source (COD ~ 680 mg l^{-1}) in planted bed W3 resulted no substantial changes in the arsenic removal as compared to other planted W1 and unplanted bed W2. Surprisingly, a more stable condition was appeared in the unplanted control bed W2 and better performance was shown as compared to the other planted beds (see Fig 9.1). Corresponding area specific removal rate with a mean value of 3.76 ± 1.5, 3.9 ± 2.1 and 3.52 ± 1.6 mg As m^{-2} d^{-1} resulted a mean As-mass retention of 63%, 67% and 62% for W1, W2 and W3, respectively (see Fig 9.2). More importantly, within the first half (middle section) of all the three wetland beds, moderately high arsenic retention (>65%) indicated better removal within the first half of wetland bed and suggesting that wetland beds should not necessarily construct in a huge length in order to achieve better contaminant removal performances.

Cancellation of organic carbon, arsenic and high sulphate-sulphur concentration after the prolonged toxic effects exhibited by the plants, immediately impacted on achieving oxic conditions to all of the three wetlands in experimental phase C (duration of 105 days) (see Fig 9.8). Only traces of sulphate-sulphur (0.2 mg S l^{-1}) were introduced along with sufficient plant nutrients in all three wetland beds. Moore et al. (1988) demonstrated that oxidation of arsenic sulphide minerals such as arsenopyrite and orpiment favors arsenic mobilization but in planted wetland W1 and W3, a highly stable and immobilized arsenic was observed despite an outflow mean concentration of 0.015 ± 0.05, 0.01 ± 0.05 mg As l^{-1} respectively (see Fig 9.1). Arsenic concentrations were at all time at or near the detection limit in the inflow of all three corresponding wetland beds. In the unplanted control W2, re-mobilisation of arsenic due to oxidative dissolution of sulphur bounded arsenic or re-oxidation of reduced sulphur contributed to the arsenic release and a

maximum of 0.097 mg As l^{-1} was recorded in the outflow, which was nearly 7-fold higher than the planted beds.

Despite the presumable sulphur re-oxidation and arsenic release in the transition of anoxic to oxic conditions, presence of plants and associated root activity (provide oxygen into the rhizosphere) along with microbial biomass within the root vicinity assisted potentially strong arsenic immobilization in planted beds in comparison to the unplanted W2. Dynamics of arsenic in the middle section showed more release of arsenic than the outflow which might be due to high re-oxidation of reduced sulphur and arsenic sulphur precipitates in the first half of wetland segment, accumulating more reduced sulphur and more organic matter and arsenic bounded sulphur. Similarly, area specific removal rates showed a substantially high immobilisation of arsenic in both planted wetlands W1 and W3 but a maximum of 2.9 mg As m^{-2} d^{-1} was remobilised in the unplanted bed W2 prior to achieve stable condition again in the same phase C (see Fig 9.2).

Addition of arsenic (0.2 mg As $l^{-1)}$ with sufficient organic carbon (COD ~ 340 mg l^{-1}) as similar to the running condition in experimental phase B was performed in experimental phase D, only exception was the trace amount of sulphate-sulphur (0.2 mg S l^{-1}) in phase D instead of a high sulphate-sulphur (50 mg S l^{-1}) in the model wastewater inflow. Depletion of dissolved oxygen by microbial anaerobic oxidation of organic matter contributed towards a rapid transition from oxic to anoxic conditions (see Fig 9.8) in all three wetland beds. In the whole experimental period (corresponding to days 372 – 463), mean arsenic removal efficiencies reached to 66%, 57% and 62% with mean outflow concentration of 0.06 ± 0.03, 0.08 ± 0.02 and 0.06 ± 0.02 mg As l^{-1} in beds W1, W2 and W3 respectively.

Comparatively low removal of 48%, 29% and 52% in the middle section was observed. In comparison to phase B with high sulphate-sulphur content under C-surplus conditions, nearly 20% more arsenic removal attained under very low sulphate-sulphur concentration in experimental phase D of both planted wetlands W1 and W3. The higher the sulphate content, better the sulphide formation and more toxic effects were exhibited by the plants along with arsenic under reducing condition in phase B, whereas with a low sulphate content and already immobilized sulphur along with arsenic in phase D showed healthier plant shoots (no toxic effects) and subsequent evidences of better arsenic removal. No substantial changes took place in unplanted control W2 in terms of arsenic removal between these two phases B and D, which clearly demonstrated that plants were playing an

important role for a better performance of arsenic removal despite the low sulphate loading under C-surplus and reducing conditions. Area specific removal rate datas were also supporting this behavior with a higher mean arsenic retention of 77%, 62% and 69% in this particular phase in W1, W2 and W3 respectively.

The dynamics of already immobilized arsenic was investigated in the last experimental phase E (corresponding of days 463 - 491) by canceling sulphate-sulphur and arsenic from the inflow wastewater. Organic carbon (COD ~ 340 mg l^{-1}) and plant nutrients were the only constituents of the model wastewater inflow feeding in order to continue stimulating microbial activity alongside with the growth of plant biomass in this particular phase. The obtained results showed a sharp downfall of outflow curves from the previous phase but still a slight evidence of arsenic re-mobilisation was observed in all three wetland beds (see Fig 9.1). A mean value of 0.053 ± 0.02, 0.058 ± 0.03 and 0.04 ± 0.01 mg As l^{-1} was recorded in the outflow of W1, W2 and W3 respectively. Arsenic levels are elevated only where sulphate content is low, as previously noted (Holm, 1995; Holm et al. 2004; Warner, 2001) was consistent with the investigation in this particular phase. Moreover, re-oxidation of already immobilised sulphur and arsenic bounded sulphur due to oxygen leakage by the plant roots might lead to a mobilisation of sulphur and subsequent release of arsenic in both planted wetlands. Another reason might be the rapid water loss due to plant transpiration caused the increment of total arsenic concentration in the outflow.

Area specific inflow loading and removal rate diagram (see Fig 9.2) showed that after cancellation of sulphate loading, remobilisation of arsenic exhibited as the immediate impact of stopping sulphate loading and became stable and immobilised again as day progressed. Similar trend of outflow arsenic dynamics was seen in unplanted control bed W2. An extended operation period might have been necessary before making any concluding remarks on this particular phase.

4.2.2   Transformation and dynamics arsenic species

The bioavailability and mobility of arsenic is dependent on the parent mineral form, oxidation state and mobilization mechanisms. As(III) is generally found under reduced conditions and near-neutral pH, while arsenate is predominant under well-oxidized conditions (Fendorf et al., 1997; Manning and Goldberg, 1997; Manning et al., 1998; Raven et al., 1998; La Force et al., 2000). Moreover, in a reducing environment, reductive dissolution of arsenic containing iron oxides and hydroxides can also result in increased

concentrations of dissolved arsenic (Pierce and Moore, 1982; Nickson et al., 2000; Meng et al., 2001). These are all well experienced and with the agreement with this study with laboratory-scale horizontal subsurface-flow constructed wetlands under redox conditions and prevailing gradients (both micro- and macro) within the systems.

Under oxic conditions in phase A, as expected, more As(V) was observed than As(III) in the outflow of all three wetland beds. Arsenic solubility was low because As(V) was the predominant species in this phase and strongly adsorbed onto or forms minerals with Fe(III)oxyhydroxide (Mok and Wai. 1994). Figure 9.3 illustrates the dynamics of arsenic species in different experimental phases in all three wetland beds. Mean concentration of As(III) was substantially lower than As(V) and transformation into As(III) under oxic condition in phase A was less than 2% of total As in all three beds. More than 75%, 81% and 72% of total arsenic was remained as As(V) in W1, W2 and W3 respectively since reduction from As(V) to As(III) was inhibited under persistent oxic conditions. Lack of electron donor inhibited dissimilatory sulphate-reduction despite very high sulphate-sulphur (~50 mg S l^{-1}) in all systems ensured that arsenic removal accomplished other than arsenic(III) sulphide precipitation.

On the other hand, it is known that reducing conditions lead to the mobilization of arsenic as As(III) goes into the liquid phase (Mok and Wai, 1994) and this was clearly demonstrated in experimental phase B, where reducing condition resulted an increase in dissolved total arsenic concentration due to reductive dissolution of Fe(III)-oxyhydroxides and also chemically and microbially mediated reduction of As(V) to more soluble As(III) in the aqueous phase. It also revealed that an elevated concentration of As(III) was observed in both planted wetlands (W1 and W3), which was almost 76% of the total outflow arsenic, whereas a low concentration (only 28% of total arsenic) of As(III) in the unplanted control W2 suggested that planted wetlands are more efficient in arsenic transformation from As(V) to As(III) and subsequent precipitation under C-surplus and reducing conditions than the unplanted wetlands. Redox conditions and root-near environment of the rhizosphere are presumably providing the growth of huge microbial biomass which play a big role for the reduction of As(V) to As(III) and also transformation to other methylated polar species. Only traces (2-3 µg l^{-1}) of DMA were observed with the spatial variation in both planted wetlands (W1 and W3).
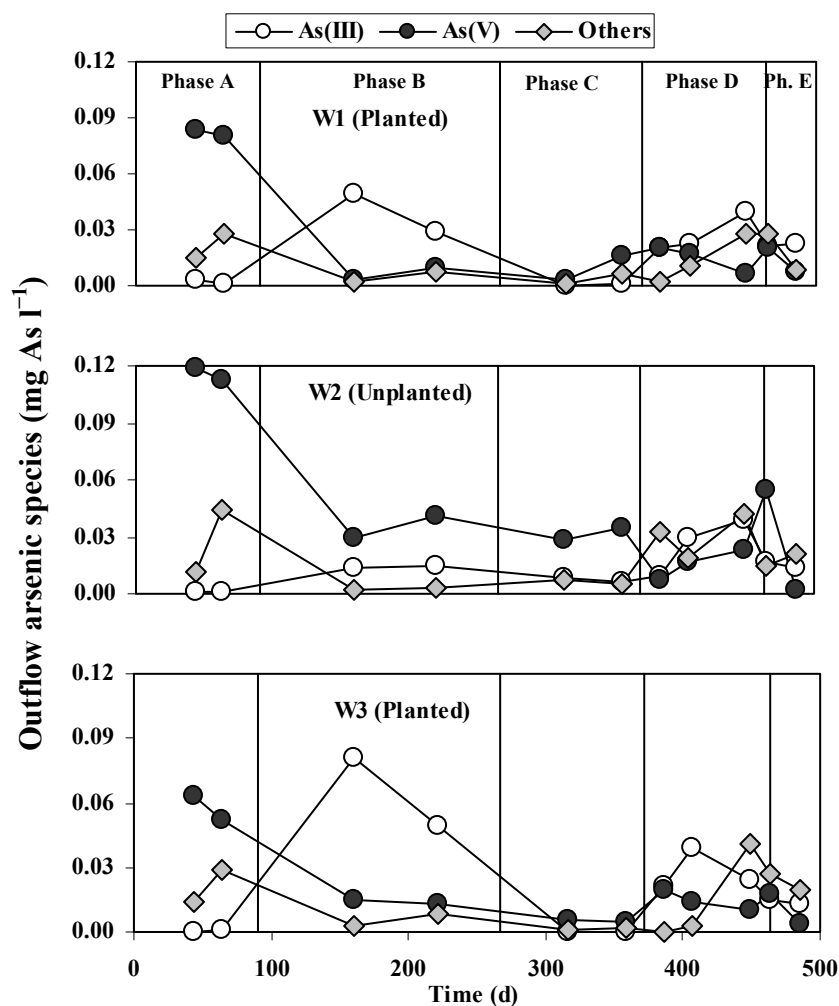
Figure 9.3 Concentrations of As(III), As(V) and other methylated compounds in different experimental phases in laboratory-scale horizontal subsurface-flow constructed wetlands

Due to shifted oxic conditions in phase C where organic carbon, arsenic and high sulphate-sulphur feeding was limited, a wide variation of As(V) concentration was observed in between both planted and unplanted control wetland. Re-dissolution and re-mobilisaion of arsenic as As(V) with a mean concentration of 0.031 ± 0.01 mg As l^{-1} was obtained in unplanted control bed than a stable and immobilized As(V) in both planted wetlands (see Fig 9.3). No substantial As(III) was amounted in any wetland beds under this prevailing oxic conditions.

After addition of electron donor in phase D, reducing condition achieved very shortly (see Fig 9.8) and a slight re-mobilization of arsenic at the transition of oxic to anoxic was observed and a relatively higher As(III) concentration than As(V) was obtained in both planted wetlands (W1 and W3) due to changes in redox potential and plant root activity as

compared to unplanted wetland W2. Transformation rate varied with no obvious tendency in experimental phase E and no particular trend of As(V) reduction was monitored in the transformation dynamics at the middle section (first half of the flow path) for all corresponding wetland beds.

4.2.3 Sulphur removal

Dynamics of sulphate-sulphur removal and formation of sulphide within the wetland systems have a major influence on arsenic removal. Under C-deficient oxic condition in phase A (see Fig 9.8), an elevated SO_4^{2-}-S concentration in the outflow even than the inflow in both planted wetland (W1 and W3) and no striking variation in unplanted W2 was observed. The mean inflow SO_4^{2-}-S concentration was 45.6 ± 3.1 mg S l^{-1} and subsequent mean outflow concentration was amounted as 57 ± 11.2, 47.2 ± 3.2 and 57.9 ± 8 mg S l^{-1} in W1, W2 and W3 respectively (see Fig 9.4). Prevailing oxic condition and lack of electron donor inhibited microbial sulphate reduction and likely became concentrated with the formation of a S-pool in both planted wetlands presumably due to high EVT rate. Despite sulphate reduction and substantial sulphide formation (Fig 9.4), high arsenic removal in this phase ensured that arsenic removal was accomplished by other processes than arsenic-sulphide precipitation.

After addition of organic carbon (COD ~ 340 mg l^{-1}) in experimental phase B (see Table 8), changes in dynamic redox conditions resulted in rapid sulphate reduction in all corresponding wetlands. Outflow SO_4^{2-}-S concentration decreased down to a mean value of 17 ± 11.2, 9.3 ± 8.5 and 13.5 ± 8 mg S l^{-1} which resulted a mean removal of 61%, 79% and 69% in corresponding W1, W2 and W3 respectively. Interestingly, better sulphate reduction was observed in the dynamics of middle section (half of the flow path) in each wetland.

Strict and persistent reducing conditions fostered anaerobic microbial sulphate reduction and subsequent gradually increasing sulphide production was observed. Sulphide found in the outflow remained in excess and mean concentrations of 2.4 ± 0.8, 2.9 ± 0.4 and 2.8 ± 0.6 mg S l^{-1} were obtained in the corresponding wetlands. Consistent reduction of As(V) to more soluble As(III) under this reducing conditions and concomitant precipitation with sulphide propelled probably better arsenic removal in this high sulphate reducing environment. It was evident that sulphate removal rate was higher in unplanted systems than in both planted wetlands, due to the greater reducing conditions in the system without

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plants. This high sulphate removal also attributed to the better arsenic removal in unplanted wetland than the planted under reducing conditions and presence of available electron donor for the respiration of sulphate reducers. Doubling the organic carbon (COD ~ 680 mg l^{-1}) in planted W3 did not show any significant changes both in sulphate and arsenic removal.
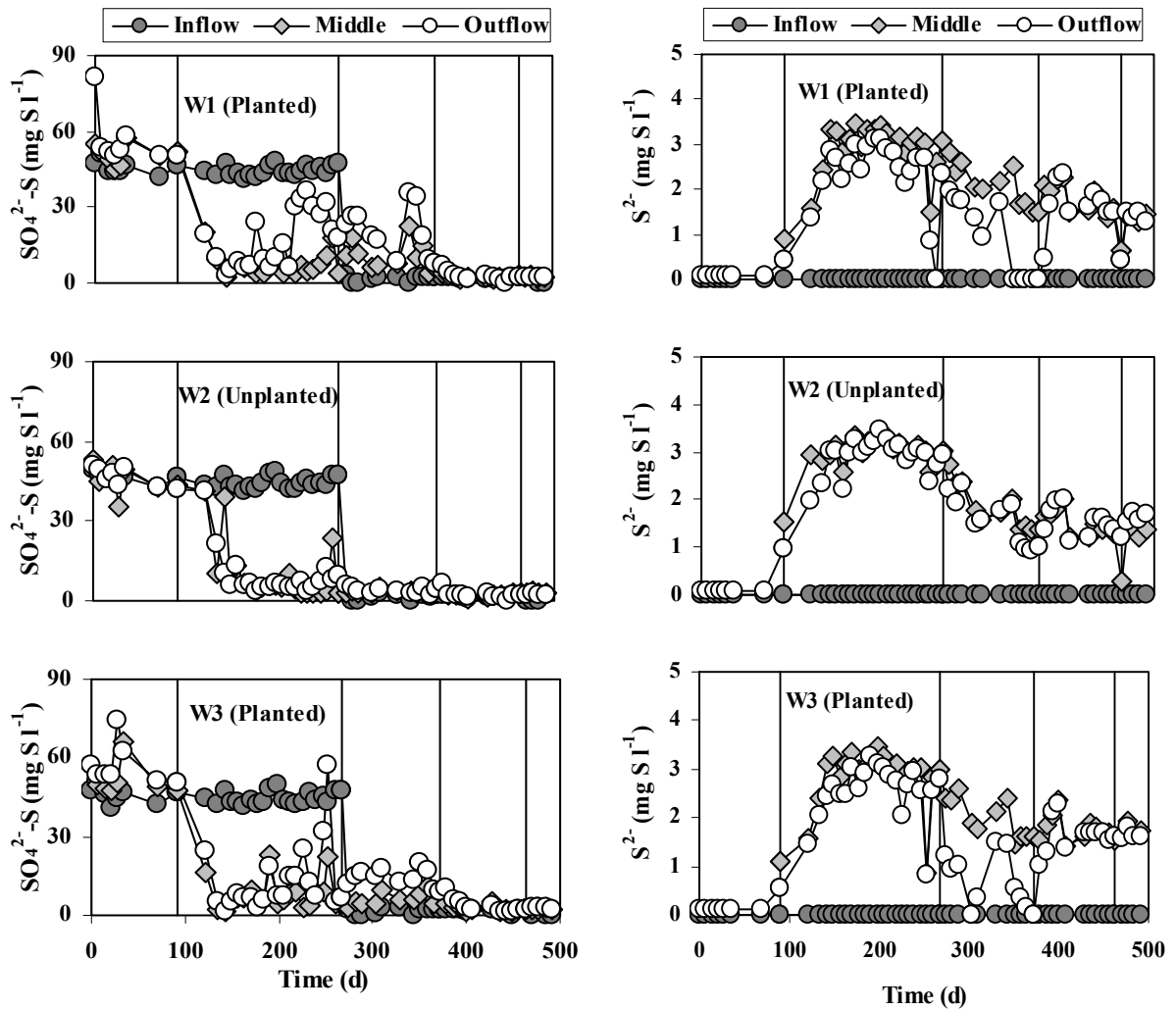

Figure 9.4 Specific loading rate and removal rate of total sulphur of the subsurface horizontal laboratory-scale constructed wetlands.

Corresponding area specific mass loading rate revealed no wide variation with a mean removal rate of SO_4^{2-}-S as 1.04 ± 0.2, 1.09 ± 0.3 and 1.07 ± 0.3 g S $m^{-2} d^{-1}$ which resulted in a mean SO_4^{2-}-S mass retention nearly as 76%, 80% and 78% in W1, W2 and W3 respectively. Mbuligwe (2004) reported in horizontal subsurface wetlands systems in the treatment of anaerobically pre-treated domestic wastewater (UASB reactor) retention of 0.94 g S $m^{-2}d^{-1}$ for unplanted bed and 1.46 and 1.56 g S $m^{-2}d^{-1}$ for *Typha*, and *Colocasia*

units, respectively. As dealing with *Juncus effusus* and arsenic in this study, sulphur removal results were closely in agreement with other previous studies.

But plants exhibited toxic effects (decreasing water loss in terms of plant transpiration and less green shoots, see Fig 9.7) probably due to sulphide toxicity along with available more toxic inorganic arsenic species. Plants physiological inhibition of several helophytes was shown for S^{2-} concentrations of approximately 10 to 50 mg l^{-1} (Armstrong et al., 1996; Chambers et al., 1998; Fürtig et al., 1996). But measured S^{2-} concentrations in this study were always <3.5 mg l^{-1} in the outflow of all experimental wetlands.

Exhibited toxic effects and rapid plant death in phase B was limited by the cancellation of organic carbon, arsenic and high sulphate loading in experimental phase C. Only a traces of SO_4^{2-}-S (0.2 mg S l^{-1}) was added in the inflow feeding (see Table 8) and outflow concentrations revealed that SO_4^{2-}-S values were getting higher with a mean value of 21.8 ± 9.2 and 15 ± 3.1 mg S l^{-1} in planted wetland W1 and W3 respectively and flushing out of the systems. Contrarily, better sulphate removal was observed in unplanted W2 (Fig 9.4). Oxic condition with high redox potential values in planted beds (see Fig 9.8) apparently caused by re-oxidation of reduced sulphur and thus increased sulphate concentration. Lack of sulphate reduction (low sulphide values in the outflow) and water loss by plant transpirations (see Fig 9.7) might also be the reasons for elevated sulphate concentration in both planted beds. This tendency for sulphate to be conserved and concentrated has been observed by other authors in natural wetlands and is clearly correlated with a decrease in the activity of sulphate-reducing bacteria (King, 1988; Choi, 2006).

It is important to note that oxidation of sulphides produces oxidised sulphur species (i.e. S^0, SO_4^{2-}) and may release associated metals or metalloids to the water column (Simpson et al. 1998). Similarly, an oxidative dissolution of already precipitated insoluble As_2S_3 resulted in an increment of sulphur concentrations and subsequent mobilisation of arsenic. Instead, a better arsenic immobilisation reflected a better stability in planted beds. Therefore, plants are playing a huge role for better stabilisation of immobilised arsenic under oxic conditions despite sulphur re-oxidation. On the contrary, better sulphate reduction in unplanted bed did not generate any significant sulphur re-oxidation but a clear re-mobilisation of arsenic (see Fig 9.1) in this phase demonstrated the instability of immobilised arsenic due to the absence of plants.

Reduced sulphur re-oxidation and concomitant low level sulphide in planted beds support the concept of the existence of multi-gradients within the rhizosphere of treatment

wetlands; meaning there are oxic and anaerobic micro-zones within the same system at the same time (Bezbaruah and Zhang, 2004; Wiessner et al., 2005b). Better sulphate reduction was observed in the later phases (D and E) of all wetlands and no wide variations could be found in between both planted and unplanted wetland (see Fig 9.4).

4.2.4 Nitrogen removal

The data of the total nitrogen concentrations in the inflow and outflow area and consequent outflow nitrogen species concentration of the model wetlands are shown in Figure 9.5. Under C-deficient and oxic conditions in experimental phase A (see Table 8 and Fig 9.8), highly efficient total nitrogen removal revealed that almost all NH_4^+-N in both planted beds (W1 and W3) was removed, while in the unplanted bed (W2) total nitrogen concentration decreased only about 8% of the inflow (5 mg N l^{-1}).

There were remarkable differences concerning total nitrogen removal in both planted beds in comparison to the unplanted control bed (W2). Nitrification, K^+ uptake and release of H^+ by the plants might be responsible for a slightly low pH value in planted wetlands than the unplanted. This oxic condition in phase A facilitated better adsorption of As(V) onto and co-precipitated with Fe(III) oxyhydroxides.

Nearly a 6-fold increment of the inflow total nitrogen to 30.8 mg N l^{-1} (exclusively as NH_4^+) and under anaerobic and C-surplus conditions in phase B (see Table 8), more than 60% total nitrogen removal was observed in both planted wetlands whereas only 27% reduction was attained in unplanted bed. Despite low nitrogen removal efficiency, unplanted wetland showed comparatively better sulphate-sulphur and arsenic removal under reducing condition in phase B (see Fig 9.1). Highly efficient sulphate reduction by sulphate reducers might restrict the nitrifiers and thus resulted a poor nitrification under reducing environment. Wiessner et al. (2005 a, b) also reported this inverse relationship coincides with high concentrations of organic matter and sulphate in the influent wastewater, and indicated that reduced sulphur compounds, such as hydrogen sulphide, are known to be potent inhibitors of plant growth and certain microbial activities, including nitrification. In fact, these authors observed an exponential decrease in ammonium removal from 75 to 35% in conjunction with an increase in sulphate removal.
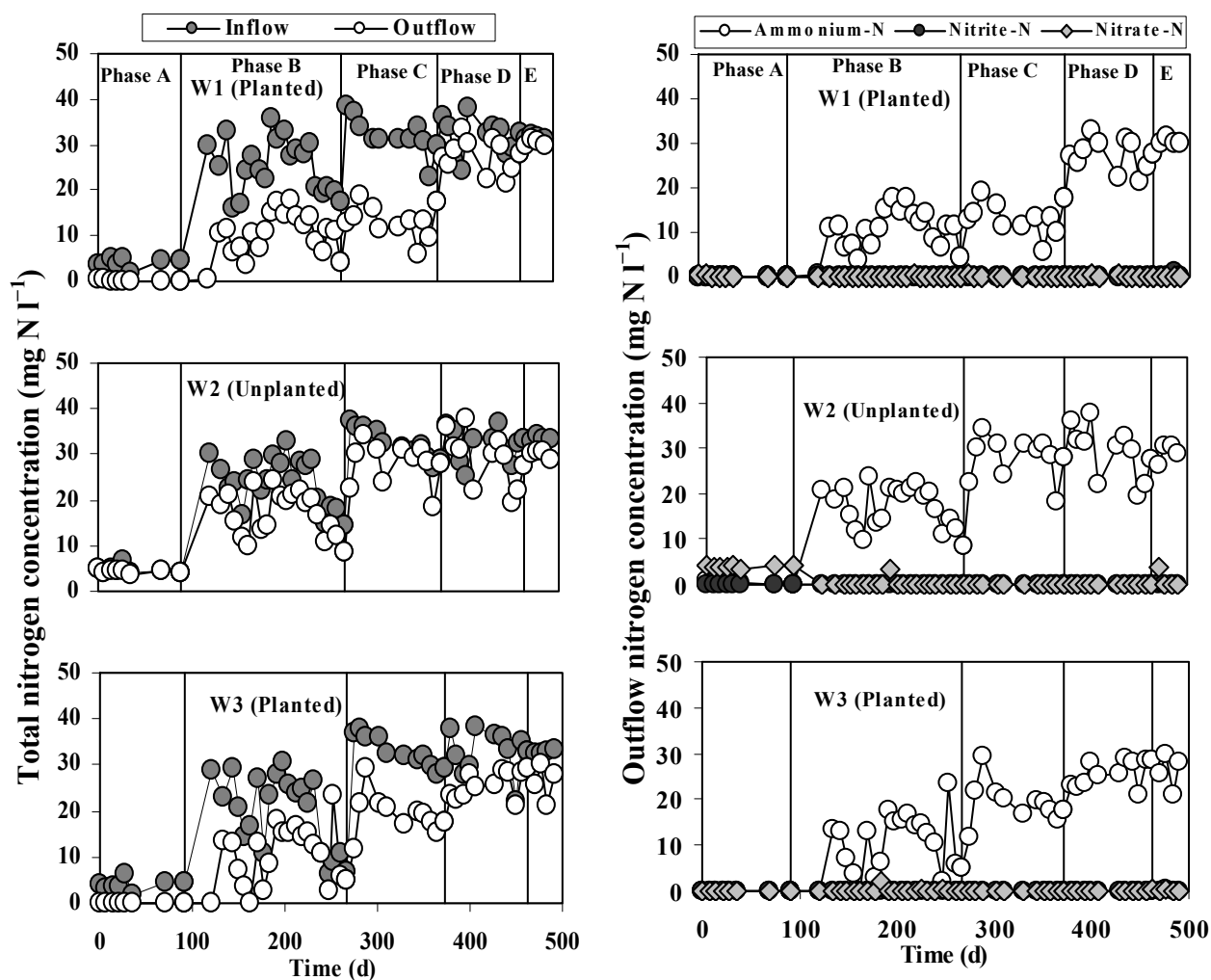
Figure 9.5 Inflow and outflow total nitrogen and outflow nitrogen species in planted (W1 and W3) and unplanted horizontal subsurface-flow constructed wetlands

More than 60% of inflow nitrogen was also removed from both the planted bed (W1 and W3) in highly oxic condition in phase C while in unplanted bed (W2) the outflow concentration was closely to the inflow. Still existing slightly reducing condition (Eh ~ 75 mV, see Fig 9.8) along with arsenic toxicity might be responsible for a poor nitrogen removal in unplanted wetland.

Ammonia volatilisation within treatment wetlands can provide a removal pathway for nitrogen; however the reaction is pH dependent. The pH values in these experiments (see Fig 9.8) were well below the pH where substantial ammonia volatilisation can occur (Reddy and Patrick, 1984). No significant concentrations of nitrite and nitrate in the outflow (see Fig 9.5) indicating highly efficient denitrification was the major process

taking place for nitrogen removal in all experimental phases. No clear evidences of arsenic toxicity responsible for the inhibition of nitrification and denitrification were observed in this study.

The plantation showed a clear stimulating effect on the nitrogen removal rate. In the unplanted control bed (W2), area specific removal rate in the range of $180 - 270$ mg N m^{-2} d^{-1} and within the planted beds in a broader range of $400 - 750$ mg N m^{-2} d^{-1} was observed. The mean removal efficiencies were therefore greater in the planted systems than the unplanted control. These removal rates are in the range reported in the literatures for subsurface flow constructed wetlands (Sikora et al., 1995; Kuschk et al., 2003). Because of aerobic zones near the plant roots by continuous oxygen transport and anaerobic zones more distant from the root surface, simultaneous nitrification and denitrification can occur in the "same environment" of the rhizosphere.

4.2.5   Carbon removal

Fig. 9.6 illustrates the changes of the TOC values over time in the influent and effluent of W1, W2 and W3. It was evident that inflow TOC concentration was slightly lower in the wetlands due to microbial activities in the container used for the inflow feeding process. In phase B, outflow TOC concentration showed a sharp downfall at the beginning and removed efficiently at the end in planted W1 whereas unplanted wetland W2 exhibited a smooth and steady outflow path. Doubling the organic carbon loading in planted W3 (see Table 8) did not show any remarkable performance in terms of carbon removal (Fig 9.6). Mean TOC removal in this particular phase was amounted to 85%, 68% and only 29% in corresponding W1, W2 and W3 respectively.

It was evident that higher outflow concentration of organic carbon appeared at the beginning of phase B while re-dissolution and re-mobilisation of arsenic also exhibited higher arenic concentration in the outflow (see Fig 9.1). Planted wetland W1 showed slightly better in organic carbon removal and subsequent less arsenic remobilisation than the unplanted bed W2. Planted W3 exhibited even higher arsenic remobilisation due to a very low carbon removal at the beginning of phase B. It can be concluded that in the transition of oxic to anoxic conditions in constructed wetlands, rate of arsenic release greatly afftected organic carbon removal within the systems. Similar trend was also seen at the beginning of phase D where addition of organic carbon also initiated slight re-mobilisation of arsenic and low outflow TOC values in all corresponding wetlands. No

significant differences in the outflow concentration in phase D and E was observed in both planted and unplanted wetlands.
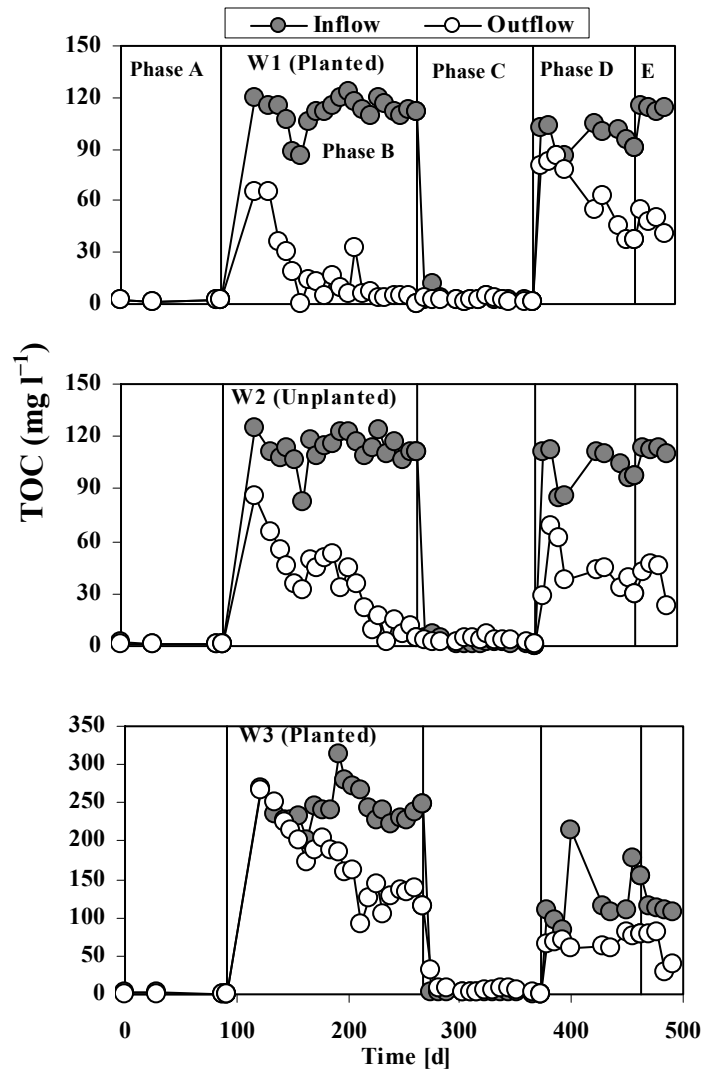

Fig 9.6 Inflow and outflow concentrations of total organic carbon (TOC) during different experimental phases in horizontal subsurface-flow constructed wetlands.

In a recent study on sulphate reduction in subsurface flow constructed wetlands, it was observed that when influent concentrations were above 75 mg SO_4^{2-} l^{-1}, the organic matter removal decreased in a 20% and it was concluded that this may be related to sulphide toxicity (Wiessner et al., 2005). Note that in this study, the mean influent sulphate concentration was greater than 150 mg SO_4^{2-} l^{-1} during phases A and B. In subsurface-flow wetlands (SSWs), influent organic matter is believed to be removed primarily by anaerobic microbial metabolism, with some aerobic metabolism near roots and at the gravel bed surface (USEPA, 1993, 2000). In SSW, sulphate reduction and methanogenesis seemed to

be the most effective metabolic pathways for removing organic matter (Wiessner et al., 2005, Garcia et al., 2006)

TOC mass removal rates observed with a mean value of 3.02 ± 0.9 and 2.7 ± 1.2 g m^{-2} d^{-1} at the end of experimental phase B with the same inflow loading in planted W1 and unplanted W2 respectively (data not shown). These results indicated that there was a clear improvement of organic matter removal in planted bed since planted wetland operated in more oxidised conditions. Despite a relatively low carbon removal in unplanted W2, high sulphate removal and subsequent more sulphide formation resulted in a better arsenic removal in this particular phase under high organic carbon and sulphate concentrations. It should be noted here that the outflow TOC concentrations in phase D and E were rather high in both planted wetlands (in comparison to the results observed in previous phases A, B and C) and were related with the high evapo-transpiration rates observed in these two experimental phases D and E.

Heavy organic loading in planted W3 (only in phase B, see Table 8) was particularly causing odor problem and potential reason for clogging the outflow tubes and outlet structures. However, it has to be noted that relatively extreme variations in the carbon load did not influence the organic carbon and arsenic removal efficiency to a greater extent, whereas the intensity of sulphate reduction changed immediately in the other planted bed W3 with the rapid changes in carbon load.

4.2.6 Further parameters (shoot density, EVT, Eh and pH, CO$_2$ and CH$_4$)

4.2.6.1 Growth of plant biomass (shoot density) and water loss (EVT)

Water loss in terms of evapo-transpiration (EVT) was taken into account in the calculation of removal rates, which in fact, were mass removal loading rates. This was particularly important to distinguish between planted and unplanted wetlands as because the rate of water loss due to the presence of plant biomass in planted wetlands hugely differed with the wetlands without plants.

The growth status of the plant biomass in terms of total number of green and healthy shoots and therefore the variation in water loss via EVT is shown in Fig 9.7. By the end of experimental phase A and B, corresponding to 267 days of operation, shoot density was repeatedly decreased with a consistent 30% and 27%, from an initial 9987 and 9400 m^{-2} to 7007 and 6820 m^{-2} in planted wetland W1 and W3 respectively.
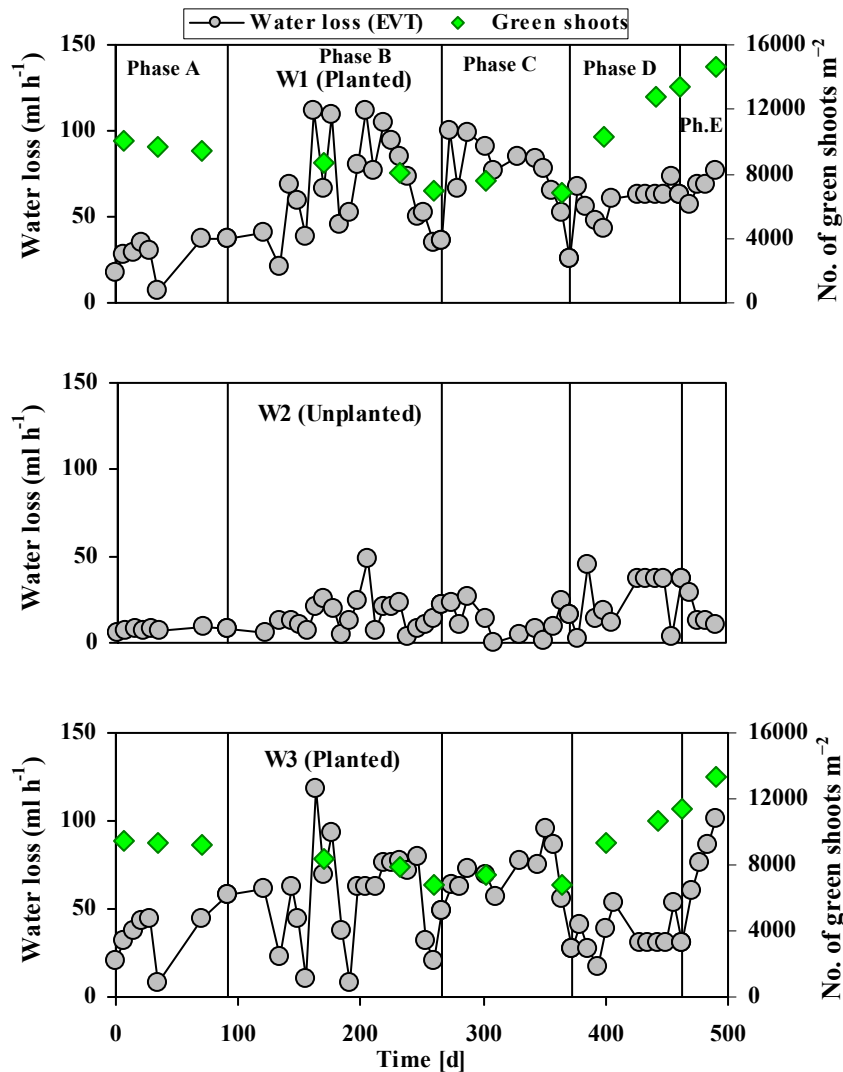
Fig 9.7 Number of green shoots and corresponding water loss via eapo-transpiration in both planted (W1 and W3) and unplanted W2 laboratory-scale horizontal subsurface flow constructed wetlands

In general, plants were nutritionally stressed and perhaps damaged by high sulphide formation due to high sulphate loading (see Fig 9.4) and arsenic accumulation into their biomass (in W1) and additionally high organic carbon (doubled than W1) loading in wetland W3. High organic and sulphate loading can become strictly anaerobic (see Fig 9.8) and As(V) in the system may shift to soluble toxic forms of As(III) and rapid sulphide formation within the anaerobic root zone might caused mortality of wetland plants. In fact, shoot density started to decline in both W1 and W3 even in oxic condition in phase A where sulphate reduction was widely unfavored due to highly oxic conditions, clearly suggested that plants exhibit toxic effects in presence of arsenic regardless of the redox conditions within the systems.

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After the stressful loading conditions in phase B, shoot density became stable and started increasing positively in phase C, led by the cancellation of all probable toxic components like organic carbon, arsenic and high sulphate loading in both planted W1 and W3. At the end of the study period on day 491, shoot density increased remarkably to the highest 14693 and 13353 m^{-2} from 7007 and 6820 m^{-2} (end of phase B) in W1 and W3 respectively. Continuous organic carbon loading along with low level arsenic under persistent anaerobic conditions might not work as limiting factor for rapid plant growth as long as sulphate loading is lowered down to trace levels or even cancelled at the later phases in both wetland systems. It appeared very important to notify that number of green shoots in the inflow segment (0-25 cm from inflow) exhibited severe stress and shoot density decreased rapidly from an initial 2394 m^{-2} to only 780 m^{-2} at the end of experimental phase B in planted bed W1.

Plant shoot density in the inflow region was declined rapidly in response to the prolonged exposure of arsenic along with sulphide. The plants of W3, to a lesser extent, exhibited a similar response in the inflow region. Additionally, the roots appeared more stable over time and exhibited a more consistent biomass along the distance from inflow. Tanner (1996) indicated that *Juncus effusus* showed the highest mean shoot density (4534 shoots m^{-2}) of the eight tested species. The results from this study, in particular arsenic alongside with *J. effusus*, shows a higher density of the plants that previously reported by Tanner (1996) under field conditions with less effects.

In general, higher plant shoot density contributed to an increment of water loss via plant transpirations and hence an obvious decreasing tendency of water loss in experimental phase B with an elevated arsenic and sulphide concentration could be observed (see Fig 9.7). Plant transpiration decreased from a maximum 110 and 118 ml h^{-1} (nearly 53 and 56% of the inflow) to 35 and 21 ml h^{-1} (nearly 17 and 10% of the inflow) at the end of high loading conditions of experimental phase B in planted W1 and W3 respectively. Corresponding area specific EVT rates amounted to a similar declination from 18 and 19 L $m^{-2}d^{-1}$ to nearly 6 and 4 L $m^{-2}d^{-1}$ at the end of phase B in those two respective planted wetlands. Transpiration rate increased rapidly in experimental phase C with no particular contaminants in the inflow feeding and decreased again in the prevailing anaerobic condition in phase D (with trace sulphate loading). Very sharp increase in transpiration rate via highly dense and dark green shoots was observed at the end phase E after complete withdrawal of sulphate and arsenic loading from the systems. Therefore, it can be

concluded that sulphate loading along with moderately low arsenic under persistent anaerobic condition repress plant physiological activity and inhibits plant transpiration rate. At the day of termination of this study (day 491), area specific EVT rate was recorded as 12 and 16 L $m^{-2}d^{-1}$ in planted beds W1 and W3 respectively.

Water loss varied within a range of 6 – 48 ml h^{-1} (corresponding to specific EVT rate of 1 – 8 L $m^{-2}d^{-1}$) with no obvious changes or drastic fluctuations (Figure 5.7b) in unplanted control W2, where evaporation from the surface of the wetland bed to the atmosphere was the only way for water loss due to the absence of plants and subsequent plant transpirations.

The effect of the plants on the removal efficiency of arsenic, organic carbon, total sulphur and total nitrogen was studied in all experimental phases of planted W1 and W3 as compared to unplanted control W2 and in all cases. It was observed that planted wetlands considerably enhanced the retention of total arsenic and organic matter than the unplanted ones. The presence of plants substantially improved the total nitrogen and total sulphur retention within the wetland beds. Since plant roots serve as carriers for attached microbial growth, transfer oxygen and release exudates into the root zone, it leads to an efficient contaminant removal than in constructed wetlands without plants.

4.2.6.2 Redox potential (E_h) and pH

Figure 9.8 illustrates the temporal variations in both inflow and outflow redox potential values as experienced in both planted (W1 and W3) and unplanted bed W2. Mean inflow values did not show any sudden fluctuations throughout all experimental phases in all wetland beds and measured as + 424 mV at the beginning of inflow feeding zone. But the outflow redox dynamics showed a sharp decreasing tendency as day progressed until the end of experimental phase B in all bed reactors. During the initial experimental phase A under carbon deficient condition, very high redox potential to a maximum value of 590, 375 and 400 mV were obtained in W1, W2 and W3 respectively (see Fig 9.8) which clearly indicated an aerobic conditions with the wetland beds. Adsorption and co-precipitation of predominant As(V) with Fe(III) oxyhydroxides were governed by this persistent oxic conditions. Redox potential never measured below +100 mV in this phase which suggested an unfavorable sulphate-reducing environment despite high sulphate loading via tap water was present in the systems.

Addition of electron donor in experimental phase B (see Table 8) remarkably changed the

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redox dynamics due to depletion of dissolved oxygen by microbial degradation of organic matter and facilitated a rapid decrease in redox values to a minimum of -139, -189 and -162 mV in the three respective wetlands. This particular changes in redox dynamics from oxic to anoxic probably accelerated reductive dissolution of Fe(III) oxyhydroxides and subsequent release of arsenic increased total arsenic concentration at the beginning of this experimental phase B. Strictly reduced conditions could have also favored microbial reduction from As(V) to more soluble As(III) and dissimilatory sulphate reduction to form dissolved sulphide which contributed towards arsenic sulphide precipitation (likely as As_2S_3) under C-surplus conditions.
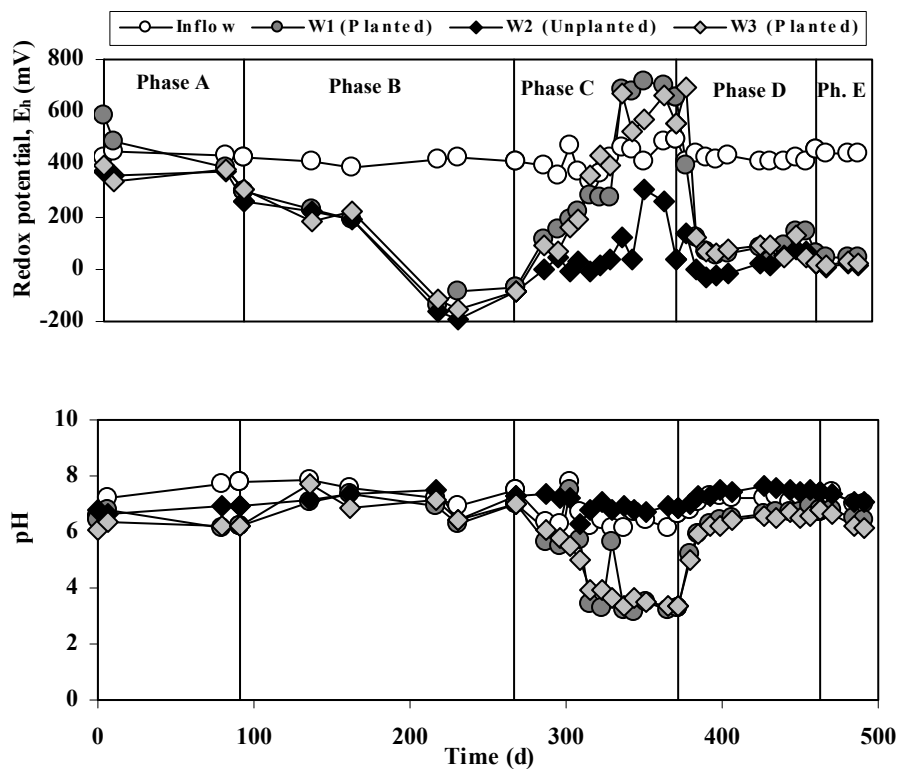

Fig 9.8 Temporal variation of redox potential and pH during different experimental phases in laboratory-scale horizontal subsurface-flow wetlands

A substantially high difference of about 300 mV between unplanted (W2) and both planted wetlands (W1 and W3) was observed in experimental phase C after the cancellation of organic carbon, arsenic and high sulphate loading in the model wastewater (see Fig 9.8). This progressively elevated E_h values clearly indicated that planted wetlands exhibited more oxidized conditions than the unplanted control wetland since plants are directly involved to transport oxygen through their roots into the wetland systems. Highly oxic conditions in both planted wetlands resulted better immobilisation of arsenic whereas still

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existing reducing conditions presumably responsible for slight arsenic remobilisation in unplanted control W2 (see Figure 9.1). Higher redox potential values also favored better ammonium-N removal via nitification in both planted beds compared to the unplanted ones with slightly reduced levels of sulphate-sulphur removal. This inverse relationship between ammonium and sulphate removal has been described in other studies (García et al., 2004, 2005; Wiessner et al., 2005 a, b).

Redox values dropped down drastically again in all three wetlands after addition of organic carbon in experimental phase D (Fig 9.8). Mean values of +120, +28 and +141 mV were obtained in W1, W2 and W3 respectively. Consistent low redox potential values were appeared and maintained also in next experimental phase E despite the cancellation of sulphate-sulphur from the inflow. Better arsenic immobilization under persistent low redox conditions were monitored in these later experimental phases.

In fact, the binding mechanisms of arsenic are dependent on the pH and redox potential of the environment. Adsorption affinity for As(V) is higher at low pH and for As(III), at higher pH values (Masscheleyn et al., 1991; Yang et al., 2002). In this study, mean inflow pH value was always in the range of 6.5 to 7.8 during the entire experimental period both in planted (W1 and W3) and unplanted wetland W2 (see Fig 9.8). Throughout the sampling period in phase A, outflow pH values showed typical of secondary domestic wastewater effluent with no major oscillations or drastic changes. No significant changes in outflow pH due to plant activity or rhizosphere acidification by nitrification was observed in planted wetlands W1 and W3 as compared to the unplanted W2. After addition of organic carbon in wetland systems in experimental phase B, low and constant pH should appear related to decomposition of organic matter, which produces organic acid metabolites but the pH were well buffered at or slightly below 7.0 due to alkalinity production by microbial sulphate reduction and also caused by efficient denitrification with the consumption of protons (Tiedje et al., 1984; Küsel and Alewell, 2004), which may subsequently favor As methylation (Bissen and Frimmel, 2003).

Similar to inflow, the pH in the unplanted bed (W2) exhibited relatively unchanged in the range of 6.3 − 7.6 during all experimental phases. But in experimental phase C (without organic carbon, arsenic and high sulphate-sulphur loading in the inflow), the outflow pH of both planted wetlands was significantly lower in the range of 3.2 − 5.6 and 3.3 − 5.7 in planted bed W1 and W3 respectively (see Fig 9.8). The lower pH level in both planted wetlands compared to the unplanted in this phase is probably caused by better ammonium

oxidation (see Fig 9.5), uptake of K^+, release of H^+ by the plants and by sulphide oxidation (Raven and Scrimgeour, 1997). This lowered pH value highly favored predominant As(V) adsorption and co-precipitaion with Fe(III) oxyhydroxides and hence better immobilisation was observed in both planted wetlands as compared to the unplanted control wetland.

Addition of organic carbon in experimental phase D resulted in an increment of pH in both planted wetlands and maintained within the same range till the end of experimental phase E. But average pH in unplanted bed (7.4 ± 0.18) was at least 1.0 pH unit higher than the planted beds (6.4 ± 0.5 and 6.3 ± 0.3) under persistent anaerobic conditions (see Fig 9.8). Plant root activity and low level ammonium oxidation might be responsible for a slight declination of rhizosphere pH than the unplanted control bed.

4.2.6.3 Production of CO_2 and CH_4 gas

Greenhouse-gas concentrations in the pore water of the wetland beds ranged from 40 – 90 mg l^{-1} and 7 – 12 mg l^{-1} as CO_2 in both planted beds (W1 and W3) and unplanted bed W2 respectively. In terms of CH_4, gas concentrations ranged from 0 – 14 mg l^{-1} in all respective wetland beds (see Fig 9.9).

The rates of aerobic and anaerobic CO_2 production by heterotrophic microbial processes were higher in planted beds indicated more decomposition of organic matter may be due to the fact that plants were well established/developed and there was a layer of accumulated organic matter in the root-near environment of the rhizosphere.

Consequently, CH_4 flux enhanced in unplanted wetland (W2) upto a maximum concentration of 13.3 mg l^{-1} (57% saturation) whereas a maximum concentration of 10 mg l^{-1} (43% saturation) and 8.7 mg l^{-1} (37% saturation) was obtained in planted wetlands (W1 and W3) respectively. Both CO_2 and CH_4 were not measured in aerobic environment in phase A and beginning part of phase B. But we can see a higher trend of CH_4 production in both planted beds when plants were hugely stressed (declination of transpiration rate, see Fig 9.7) in phase B due to heavy organic carbon and sulphate loading along with continuous arsenic inflow feeding.
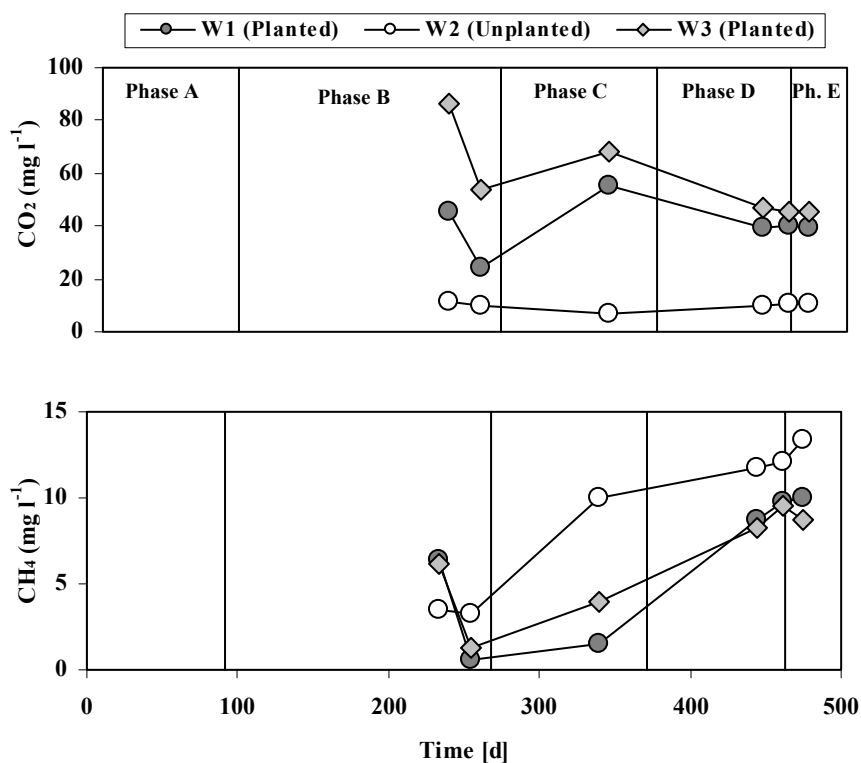
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Fig 9.9 Concentration of CO_2 and CH_4 in the pore water during different experimental phases in the horizontal subsurface-flow constructed wetlands.

Concentration of CH_4 was obviously enriched in unplanted wetland due to the absence of plant mediated oxygen in the later phases (D and E) of the experiment which suggested that anaerobic degradation predominated in the unplanted bed. Whereas, in both of the planted beds, plant growth was very dense and more leakage of oxygen through their roots inhibited CH_4 production (see Fig 9.9). Over production of acid by fermenting bacteria and plant uptake of K^+ in exchange to H^+ might rapidly result in low pH in both planted wetlands in comparison to the unplanted ones and because of the acid-sensitivity by the methane-formers, CH_4 production was comparatively less in both planted wetlands due to slightly low pH and plant root oxidation of the pore water which presumably associated with better nitrification processes.

Co-existence of methanogenesis and sulphidogenesis in natural and technical ecosystems is well known (Liesack et al., 2000). Nevertheless, for natural wetlands it was shown that sulphate can substantially reduce the methane formation (Gauci et al., 2004). Therefore, a very low level of sulphate loading (only traces in phase D and cancelled in phase E) might be another reason for comparatively higher CH_4 production in both planted and unplanted wetlands. Comparison between CH_4 concentration in the pore waters of all wetland beds in

phase B (with high sulphate loading) and phase D (low sulphate) in Figure 9.9 can be able to justify this finding.

No clear indication of inhibitory effect of arsenic on the extent of CH_4 concentration was observed in our model wetland systems. A few literatures suggest that exposure to relatively higher concentration of soluble arsenic inhibits methanogenic activity and decreases CO_2 and CH_4 production. With a low inflow concentration of total arsenic in this study, apparently no sensitivity was displayed by methanogenesis.

4.2.7   Bioaccumulation of arsenic in plant biomass

Special attention was paid in this study to investigate arsenic bioaccumulation in plant biomass (into shoots and roots of *Juncus effusus*) which was then attributed to a rough mass-balance calculation. After 491 days of operation, plant shoots and roots were collected from different segments of the planted beds and analyed for total arsenic content and the results are represented in Figure 9.10.
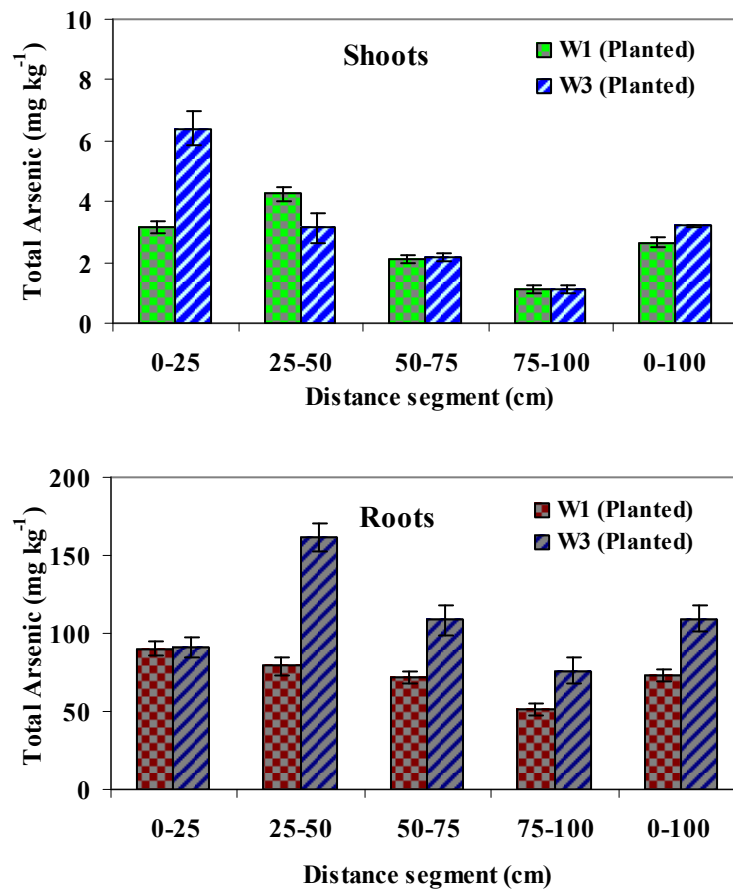

Fig 9.10 Concentration of total arsenic in shoots a) and roots b) in both planted (W1 and

W3) horizontal subsurface-flow constructed wetlands.

At the end of the experimental phase E, the total fresh weight of plant shoots were 0.29, 0.72, 0.67, 0.64 kg and corresponding root fresh weights were 1.56, 1.57, 1.52, and 1.46 kg in wetland segments $0-25$, $25-50$, $50-75$ and $75-100$ cm respectively in planted bed W1. Mean total arsenic concentration was measured as 3.15 ± 0.22, 4.25 ± 0.21, 2.1 ± 0.14 and 1.1 ± 0.14 mg kg^{-1} (dry weight) in shoots and 90 ± 4, 79 ± 6, 72 ± 4 and 52 ± 5 mg kg^{-1} (dry weight) in roots of planted W1. It was shown that there was a substantial increase in arsenic level in the inflow regions (first half of the bed) and down the wetland gradient at third quarter and in the outflow regions, declination of arsenic concentration level was more pronounced than the inflow regions. The response of plant shoots As-level in planted W3 to concentrations and wetland locations was very similar to that of planted W1. Therefore, more than 50% of arsenic accumulation was translocated into the shoots located in the first half of wetland beds.

In general, higher As-level was exhibited by the roots than the shoots in both planted beds (W1 and W3). This was also reported by other authors elsewhere (Buddhawong, 2005). At the end of the whole operation period, mean arsenic concentrations in the roots of planted W1 were obtained as 90 ± 4, 79 ± 6, 72 ± 4 and 52 ± 3 mg kg^{-1} in respective four segments. Comparatively higher concentration was also obtained in the inflow zone than down the length of the wetland. Similar trend was also observed in the planted bed W3 but significantly higher concentration was amounted in W3 than the roots of W1. Higher organic carbon loading in planted wetland W3 might be a probable reason for the increment of arsenic uptake in comparison to the wetland bed W1.

4.2.8 Arsenic in sludge sediment

After the termination of model experiments, sediments were collected from the wetland beds and analytical results showed wide variations of As-level among the wetland beds and also in different segments (Figure 9.11). Total arsenic concentrations in the sediment were significantly affected by the position along the flow path of the wetland beds (inflow zone, middle segments and outflow zone). Clear evidence of high arsenic adsorption and precipitation in the sludge sediment of first half (50 cm from the inflow) of all wetland beds were observed. Concentration amounted in the sediments of $75-100$ cm segment (end segment, outflow zone) was less than 10%, 27% and 41% of the inflow segment or zone of W1, W2 and W3 respectively.
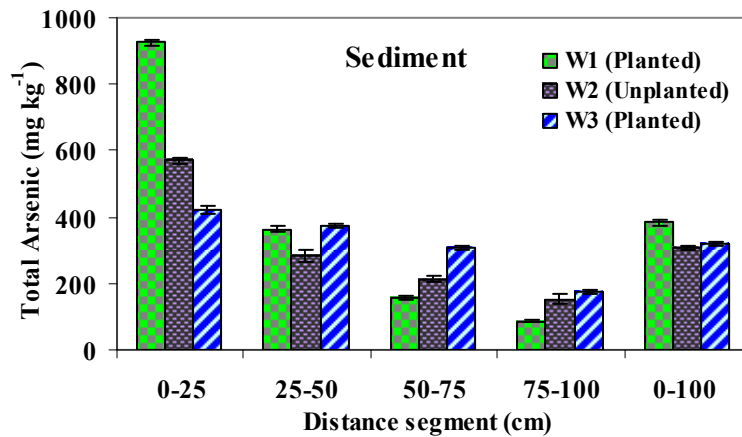
Fig 9.11 Concentration of total arsenic accumulated in the sludge sediment of planted (W1 and W3) and unplanted (W2) horizontal subsurface-flow constructed wetlands.

Therefore, concentrations of total arsenic were elevated close to the inlet area and decreased down the wetland gradient more rapidly. Vymazal (2003), Vymazal and Krása (2003), and Obarska-Pempkowiak and Klimkowska (1999) also reported a decrease of metals in the sediment of a constructed wetland treating municipal wastewater. Sulphides were observed in an elevated level within the first 25 cm (inflow zone) of the wetland beds and precipitation as arsenic-sulphide complexes was thought to be an important removal process for arsenic within the first 25 cm of the wetland beds. Mean total concentration measured as 383 ± 8, 305 ± 7 and 320 ± 4 mg kg^{-1} in respective wetlands (see Fig 9.11).

4.2.9   As-mass balance

There might be four possible fates for the contaminants removal from the model horizontal subsurface-flow wetlands: (1) chelation or complexation by organic matter, biofilm and thus sequester into the sediments, (2) bioaccumulation into plant biomass (shoots, roots etc.) (3) retain in the standing water at the termination of the experiment, and (4) volatilise into the atmosphere. The remainder of the contaminants flushed out of the wetlands in the outflow where it was collected.

Figure 9.12 demonstrates total amount of As-mass inflow and retention in all respective wetlands and also quantitative As-mass balance resulting from various amounts of arsenic retained in individual compartments to various extents. A total of 335.2, 335.7 and 323.2 mg inflow As-loading and significantly high 196.4, 146.04 and 195.8 mg As-mass was retained in W1, W2 and W3 respectively which resulted a 58%, 43% and 61% retention in respective wetlands.

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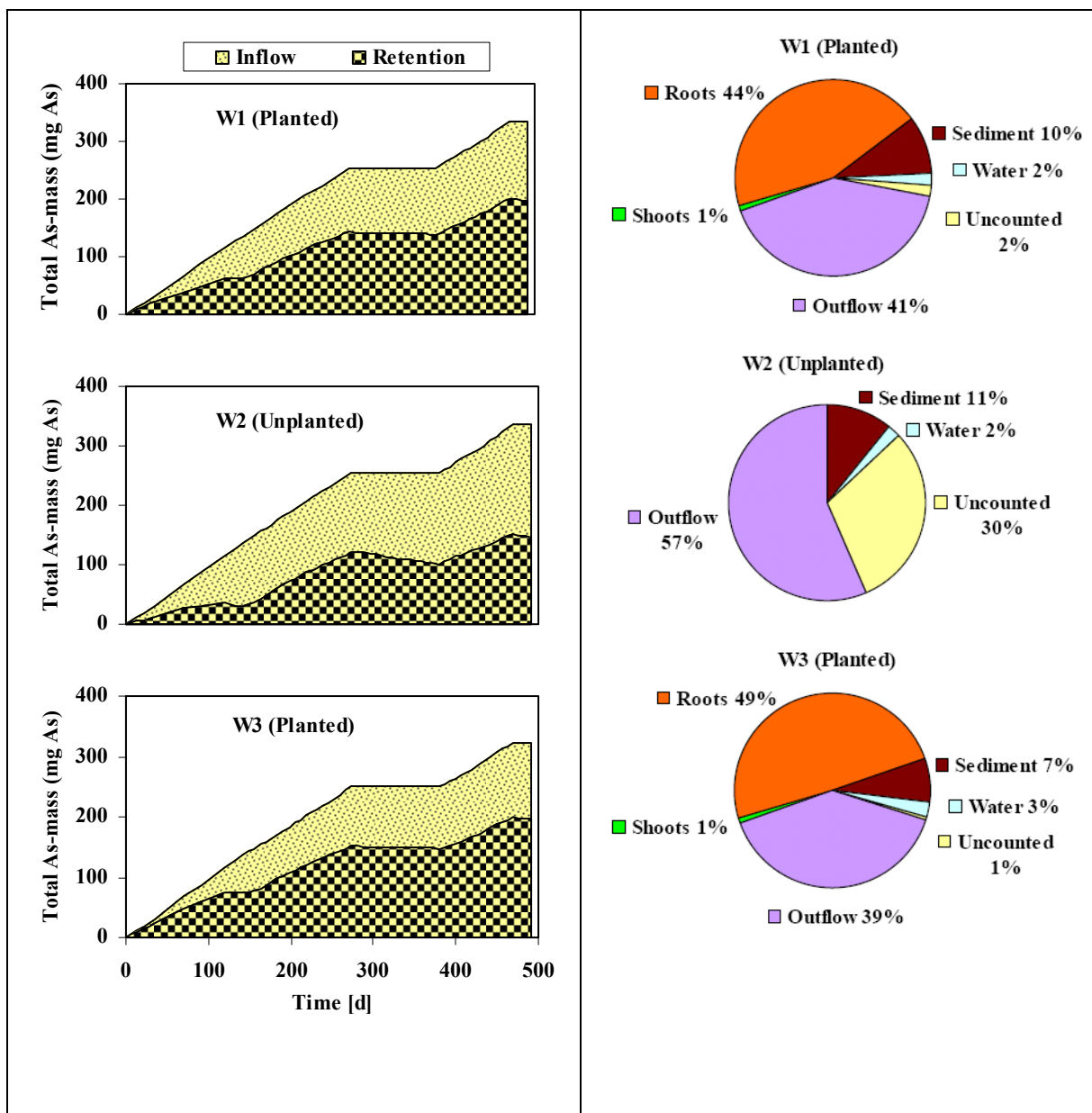
Fig 9.12 Cumulative total As-mass inflow and retention during the whole experimental period and As-mass balance estimated as percentage of inflow loading in horizontal subsurface-flow constructed wetlands

Based on the concentration of arsenic in the plant shoots, roots and sludge sediments of both wetland beds, it was calculated that 43% and 49% of total inflow arsenic loading into the wetlands were accumulated in the roots and 10% and 11% were sequestered or deposited within planted W1 an W3 respectively (Figure 9.12). Consequently, total arsenic accumulation in plant shoots had a small contribution to the mass balance. Only 1% of total arsenic loaded into the reactors were translocated into the plant shoots (see Fig 9.12).

This demonstrated the minor role of plant shoots in terms of arsenic uptake and very low

translocation efficiency from roots-to-shoots (ranges only 4 - 6 %) in both planted wetlands, which is a common finding in studies of treatment wetlands (Batty and Younger, 2002). Substantially higher amount of arsenic was accumulated in roots and sediments of inflow zone (0-25 cm) in planted W1 as 37% (55 mg) and 68% (22 mg) of the total 149.14 and 32.46 mg respectively. This trend was also seen in other planted W3 and also total As-mass in the sediment of unplanted bed W2.

Standing water accounted for not more than 3% of total arsenic inflow loading in all three respective wetlands. Only traces (2-3 μg l^{-1}) of inorganic volatile arsine (AsH_3) were measured a few times in different locations in this study which contributed to a small amount of total arsenic left out of the systems in terms of phyto-volatilisation. Nearly 2% of total arsenic in both planted beds was uncounted, which resembled a better arsenic mass-balance in both planted beds. Contrarily, in unplanted wetland W2, more than 30% of inflow arsenic was accumulated into uncounted sink which could be due to even more volatlisation. A total of 41%, 57%, and 39% of total arsenic were passed through the wetlands W1, W2 and W3 respectively and were collected in the outflow.

For long-term operation, special attention must be paid to arsenic accumulation in the constructed wetland beds in order to avoid potential toxic effects the accumulated arsenic could pose to the wetland plants, sediments and nearby environment. A suitable and cost-effective technique should be implemented for a meaningful post-treatment hazardous biosolid handling and management strategy.

4.2.10 Outlined results and principle remarks

The results of the experiments substantiate the suitability of arsenic contaminated secondary domestic effluents in horizontal subsurface-flow constructed wetlands under the specified conditions. Based on the results of this study, important observations and interpretations can be summarised as follows:

- This wetland study has important implications for arsenic immobilisation associated with different organic C and S-loading under oxidising and reducing environments. In general, higher dynamics of arsenic transformations with concomitant higher arsenic removal was observed in planted wetlands as compared to unplanted wetlands. Under C-deficient and oxic conditions, substantially high

removal efficiency ranged from 65-75% on average in planted wetlands and a relatively low 38% attains in unplanted wetland. Oxic conditions favored better arsenic adsorption and precipitation on Fe(III) oxyhydroxides regardless of sulphate loading and plants provided multi-gradient (both micro- and macro) in the root-near environment of the rhizosphere which proved beneficial for better arsenic retention as compared to unplanted wetland.

- Thermodynamically more stable As(V) was predominant in oxic conditions and more mobile and toxic As(III) was generally found in the aqueous phase under reduced conditions within the wetland systems. Consequently, reductive dissolution of arsenic containing Fe(III)oxyhydroxides resulted in an increasing concentration of dissolved arsenic in the transition of oxic to anoxic conditions regardless of the plantation on the wetland beds.

- From the standpoint of arsenic removal under C-surplus and reducing conditions, arsenic-sulphate combination appeared to be more efficient in constructed wetland treatment systems. The higher the amount of sulphate, the better should be the removal of arsenic likely as As_2S_3 precipitation after microbial sulphate-reduction and sulphide formation. But plants exhibited toxic effects and afterwards caused their mortality presumably due to high organic carbon loading and sulphide toxicity along with more soluble and toxic forms of arsenic as As(III) within the planted beds. On the other hand, better sulphate removal promoted in a slightly higher arsenic removal in unplanted bed as compared to planted beds. Therefore, for the treatment of arsenic contaminated secondary domestic effluent with high organic carbon and sulphate loading, unplanted wetland will be much more favorable and efficient than planted beds.

- When the sulphate loading lowered down to trace levels, planted wetland showed far better arsenic removal (>70%) efficiencies with healthy plant shoots than wetlands with high sulphate loading.

- Doubling the organic carbon in planted wetland did not necessarily improve arsenic removal efficiencies with a greater extent. It is worth noting that most of the removals for all parameters of concern occurred within the first half of wetland beds (50 cm from the inflow, middle section)

- Plant biomass near the inflow region (25 cm from the inflow) of the wetlands exhibited the densest, most robust standing biomass which might have been due to higher availability of inflow wastewater macro- and micro-nutrients. Eventually, plants were adversely impacted at that specific inflow zone due to more pronounced toxicity impact by the overloading of higher organic C- and S-loading along with arsenic feeding and presumable bioaccumulation into plant biomass.

- Despite the fact of reduced sulphur oxidation and presumable arsenic release by oxidative dissolution of As_2S_3, planted wetlands showed high stability on already immobilized arsenic under oxic conditions as compared to unplanted wetland, with a substantial arsenic re-dissollution and re-mobilisation. So, high binding capacity of arsenic with sulphur and also immobilization with organic matter in the presence of plants and associated root activity along with microbial biomass within the root vicinity assisted potentially strong arsenic immobilisation in planted beds as compared to unplanted ones. Therefore, the presence of plants proved to be an important factor when it comes to arsenic removal in constructed wetlands.

- The analytical results in this study indicated that there were significant and pronounced differences between the unplanted and planted experimental wetlands concerning arsenic removal rates. While less pronounced differences between experimental phases in planted beds was observed. Tanner (2002) has also observed that planted wetland beds exhibit overall improved performance compared to unplanted wetlands beds. Based on studies carried out by Kaseva et al. (2002) have observed as well that planted subsurface flow wetlands perform better that an unplanted one when treating anaerobically pre-treated domestic wastewater.

- Total retention of As-mass in planted wetland was high at the end of the study, with the mass retention of nearly 60%. On the other hand, the retention capacity of unplanted bed was substantially lower as compared to planted beds and amounted to only 43%.

- As expected, less amount of As-mass was retained in shoots than roots and sediments of planted wetlands and on average, only 1% of the inflow As-mass was retained in shoots while more than 55% was sequestered in roots and sediments. However, the retention varied widely among each segments.

- Substantially more As-mass was retained in the roots (37% of total root retention) and sediments (68% of total sediment retention) of first segment (0 – 25 cm, inflow zone) than the other segments along the planted wetland gradient. Similarly, 53% of total sediment retention was calculated in the first segment in unplanted wetland.

- The presence of plant biomass created a more oxidized conditions in comparison with wetlands without plants, and this in turn enhanced the removal of TOC (on average 87% compared with 68%) as C-fixation and ammonium removal (on average 74% compared with 33%).

- Briefly, the obtained results allowed to state that horizontal subsurface-flow constructed wetlands seemed to be viable alternatives for effectively eliminate elevated arsenic along with organic matter and high sulphate content from secondary domestic wastewater, but not being able to tolerate high sulphide toxicity exhibited by the wetland plants.

- The overall experimental results demonstrated the feasibility of applying horizontal subsurface-flow wetlands in a strategic pilot-scale basis for a long-term investigation and afterwards full-scale operation units to treat arsenic contaminated secondary domestic effluents prior to disposal to the nearby water body (rivers, lakes etc.) or application to the agricultural field for irrigation purposes.

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- Future studies intended to elucidate i) an in-depth understanding the effects and mechanisms of sulphur transformation processes on the arsenic transformations and removal efficiencies in constructed wetlands, particularly in terms of biotical or abiotical oxidation of reduced sulphur compounds, ii) potential interactions and influences of sulphur species like elemental sulphur, sulphite and thiosulphates on arsenic removal and transformation by varying organic C and S loadings, iii) functional pathways of microbially driven redox transformations, distributions and bio-availability within planted and unplanted systems, iv) exploring the patterns in microbial ecology and associated arsenic toxicity on plants and microbial biomass under dynamic redox conditions v) potential inhibition of arsenic on nitrification-denitrification, sulphidogenesis, methanogenesis under multi-gradient conditions, vi) more intensive and more complete analytical methods for methylated polar and volatile arsenic compounds.

5 Conclusions

5.1 General introduction

Energy saving low-cost technologies for wastewater treatments are highly needed in developing as well as in industrialized countries. Especially the anaerobic methanogenic fermentation technology for wastewater offers a high energy saving potential – even a useful biogas is produced. So, already in many countries, especially in the tropics, domestic sewage treatment is often realised by an anaerobic fermentation step such as a septic tank, an anaerobic filter etc. By such a treatment step the biochemical oxygen demand (BOD) of the wastewater is considerably reduced, but it contains still relatively high residual organic matter, ammonia and sulphide formed by the bacterial dissimilatory sulphate reduction. For this post treatment constructed wetlands – also a technology of low energy need - are suitable. Generally, constructed wetlands are increasingly being used, especially for the treatment of domestic wastewater. The main advantages of this technology are lower costs in comparison to conventional techniques; constructed wetlands are partly solar powered "technical"ecosystems with the rhizosphere as their "main reactor".

Several internal and external conditions determine the activity, the interaction and finally the efficiency of the various redox processes for removal inside the "reactor" rhizosphere, such as nitrification, denitrification, transformation of organic and inorganic carbon compounds, oxidation and reduction of different sulphur compounds, arsenic and heavy metals, biotransformation and immobilization of arsenic etc. The complexity and variability of these redox transformation processes needs to be understood in order to be able to create and operate treatment wetlands for the post-treatment of anaerobic reactor effluents as effectively as possible.

5.2 Concluding remarks

The results in this study showed that the processes of arsenic biotransformation and immobilization, reduced sulphur oxidation, nitrification, denitrification and dissimilatory sulphate reduction can occur simultaneously in the rhizosphere of treatment wetlands caused by dynamic redox gradients (aerobic-anaerobic) conditions. For a detailed understanding, the effects of sulphur species on the removal performance of arsenic and transformation of arsenic to various methylated polar and even volatile compounds in

constructed wetlands should be investigated in future experiments, particularly in terms of biotical or abiotical of oxidation of reduced sulphur compounds, competition for oxygen due to oxidation of reduced species, changes of micro-environmental conditions in the rhizosphere due to redox potentials and arsenic bounded sulphur deposits, nutrient mobilization or immobilization, and biofilm formation.

The principle of the planted fixed bed reactor ensures macro-gradient-free conditions for investigating redox processes inside the rhizosphere. This was a part of basic research in order to investigate arsenic biotransformation and fixation processes in a continuously flow model wetland systems and thus disturbing the concentration gradient to achieve better results. The main focus of our objective in this investigation has been fulfilled after achieving better arsenic removal results under distinct experimental conditions. But in horizontal subsurface-flow wetlands which are more realistic and near to practice design, results due to different redox reactions clearly demonstrate the differences in arsenic removal and other physico-chemical processes between these two wetland systems.

Presence of multi-gradient (both micro- and macro) plays a dominant role contaminant removal in planted wetlands than the unplanted and hence it ensures the importance of plants on wetland beds. Nearly all arsenic mass retention can be calculated in a quantitative mass-balance in planted beds also encourage investigating arsenic removal processes in a pilot-scale constructed wetland system. Results from this study and near future pilot-scale can also be used to construct a computer model and influences of different physico-chemical parameters that are playing a significant role for better arsenic removal can be utilized to optimize flow model.

Constructed wetland biosolids handling and management might be a challenge for further treatment when dealing with arsenic in the secondary domestic wastewater effluent. But when it comes to arsenic removal from wastewater as the first priority, then constructed wetland can be a suitable option especially for tropical countries where land availability might not be a major issue to face with. Better strategy needs to be investigated and implement to treat arsenic contaminated hazardous wastes (like plant shoots, roots, gravel matrix etc.) in order to guarantee a far better environment which is also one of the major concern.

In general, the results of the experiments substantiate the suitability of post-treatment of anaerobic reactor effluents in subsurface horizontal flow constructed wetlands.

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