

CRANFIELD UNIVERSITY

JAN BARAK

ANALYSIS OF PHOSPHORUS FLUX IN REED BEDS AT
CHEMICALLY DOSED WASTEWATER TREATMENT WORKS

SCHOOL OF APPLIED SCIENCES
DEPARTMENT OF ENVIRONMENTAL SCIENCE AND TECHNOLOGY
WATER SCIENCE INSTITUTE

MSc BY RESEARCH
Academic Year: 2013 -2014

Supervisors: Dr Gabriela Dotro and Prof Bruce Jefferson
May 2014

CRANFIELD UNIVERSITY

SCHOOL OF APPLIED SCIENCES
DEPARTMENT OF ENVIRONMENTAL SCIENCE AND TECHNOLOGY
CRANFIELD WATER SCIENCE INSTITUTE

MSc BY RESEARCH

Academic Year 2013 -2014

JAN BARAK

ANALYSIS OF PHOSPHORUS FLUX IN REED BEDS AT
CHEMICALLY DOSED WASTEWATER TREATMENT WORKS

Supervisors: Dr Gabriela Dotro and Prof Bruce Jefferson
MAY 2014

© Cranfield University 2014. All rights reserved. No part of this
publication may be reproduced without the written permission of the
copyright owner.

ABSTRACT

Phosphorus (P) as a macronutrient contributes to eutrophication. In the UK, iron (Fe) and aluminium (Al) salt dosing is a well-established wastewater treatment strategy for its removal. Small works with a population equivalent (p.e.) under 2,000 often employ horizontal subsurface flow constructed wetlands (HSSF CWs) as a means for subsequent tertiary (3°) treatment. Although these significantly improve the final effluent's quality, P release has, on occasions, been observed. This study attempts to contribute to a better understanding of P flux mechanisms in reed beds and to outline a mitigation strategy countering P release.

The literature review identified that, in given circumstances, the P concentration gradient, redox conditions, pH and Fe cycling are key potential factors governing P flux. The field survey revealed that secondary (2°) P effluent concentration negatively correlated with P release from the reed bed. In laboratory scale sludge reactor series, P concentration in wastewater was observed to be buffered by molecular diffusion driven by a concentration gradient in the sludge-wastewater interface. The instantaneous equilibrium point appeared to lie in 0.1 to 0.5 mg/L interval in the first 10 minutes, shifting to 1 mg/L in the next 8 hours and higher in the later stages. In biologically active systems, the shift of the equilibrium point seemed to be dominated by changes in redox potential linked to simultaneous microbial utilisation of oxygen (O₂) and nitrate (NO₃⁻), eventually leading to a reduction of Fe (III) and sulphate (SO₄²⁻), with subsequent P release. The start of Fe (III) reduction coincided with reductive depletion of nitrate-nitrogen (NO₃-N) below 1 mg/L. In systems with limited biological activity, P release was linked to disassociation from Fe-P compounds under decreasing pH. In an experiment assessing hydrodynamics, an increase was recorded in Fe and P flux fluctuation due to convection. Based on the findings, maintaining hydraulic residence time (HRT) under 24 hours and reed bed influent in concentrations above 0.5 mg/L total phosphorus (TP) and 15 mg/L NO₃-N is proposed as a means to prevent or delay P release.

ACKNOWLEDGEMENTS

Foremost, I would like to express my gratitude to my primary supervisor Gaby Dotro, for her all-around support and guidance throughout the project, for always doing her best and for giving me the opportunity to work on such a fascinating topic. I sincerely hope I have been of help in your quest to reedify the planet. You put your heart into your work and that certainly made it easier for me to follow.

I would also like to thank my second supervisor Bruce Jefferson. I especially appreciate your positive attitude, teaching skills in both our meetings and your lectures and your excellent guidance, particularly in later stages when analysing the results.

My thanks and respect to the technicians of Cranfield University for their technical support and training they gave me: Jane Hubble, Maria Biskupska, Jan Bingham, Paul Barton, Alan Nelson and Christine Kimpton. Especially to Richard Andrews for work with ICP analysis and instrumental training. Further thanks to everyone else at the Water Science Institute for creating such a pleasant working environment. Thanks to the Man in the Corner for his help transporting the samples.

This project would not have been possible without technical and financial support of Severn Trent Water. I enjoyed being part of the R&D team. Thank you for this interesting professional experience. It was a pleasure meeting and working with you all. I would also like to thank staff from Severn Trent Services in Tamworth, site managers and operational technicians who helped me carry out work during the field survey.

TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	v
LIST OF FIGURES	vii
LIST OF TABLES	viii
LIST OF ABBREVIATIONS.....	ix
1 INTRODUCTION	1
1.1 Aims and objectives	3
1.2 Hypothesis	3
1.3 Thesis structure	3
2 LITERATURE REVIEW	5
2.1 Phosphorus and its forms.....	5
2.2 Use of chemical precipitants in wastewater treatment	6
2.2.1 Calcium	6
2.2.2 Iron (II) and iron (III)	8
2.2.3 Aluminium	9
2.3 Factors affecting release/retention of P in sediments	10
3 MATERIALS AND METHODS	15
3.1 Field survey.....	15
3.1.1 Sites and sampling	15
3.1.2 On-site measurements	16
3.1.3 Sample processing	17
3.1.4 Data analysis.....	18
3.2 Controlled experiments	18
3.2.1 Sampling	18
3.2.2 Experiment 1: P flux in dynamic and static conditions	19
3.2.3 Experiment 2: impact of liquid phase initial P concentration	21
3.2.4 Data analysis in experiments.....	22
4 RESULTS	23
4.1 Treatment works performance.....	23
4.1.1 Overall performance of works.....	23
4.1.2 Reed bed performance	26
4.1.3 Interdependency of potential phosphorus release factors.....	29
4.2 Phosphorus and iron transformations.....	32
4.2.1 Impact of convection on P and Fe flux.....	32
4.2.2 Concentration driven transformations	36
4.2.3 Impact of competing electron acceptors on Fe and P flux.....	41
4.2.4 Impact of pH on Fe and P flux	44
5 DISCUSSION	47
6 CONCLUSIONS	53
REFERENCES	55
APPENDICES.....	65
Appendix A Photographs of the assessed reed beds	65
Appendix B Initial analysis of wastewater used in the experiments	66

Appendix C Metal content of wastewater used in the experiments	67
Appendix D Chemical content of sludge.....	68
Appendix E Phosphorus and metal content of sludge in the experiments.....	69
Appendix F Chemical content of wastewater.....	70
Appendix G Environmental conditions within reed beds	71
Appendix H Correlations of potential P release factors.....	72

LIST OF FIGURES

Figure 4.1: Wastewater constituents particle fractions percentual distribution at 3 sampling points (crude, 2° effluent, 3° effluent) across 3 sites (NA-Fe, A-Fe, NA-Al). A) total phosphorus; B) metals (iron, aluminium) dosed for phosphorus removal; C) iron (II); D) iron (III).....	26
Figure 4.2: Reed beds total phosphorus mass removal rates ($\text{g/m}^2 \cdot \text{day}$) over 3 sites..	28
Figure 4.3: Average iron (II) and iron (III) wastewater concentration change between 2° and 3° effluent at 3 sites	29
Figure 4.4: Wastewater constituents' concentrations under static vs dynamic conditions: A) total phosphorus, B) total iron, C) iron (II) and D) iron (III) ..	33
Figure 4.5: Total iron and iron (III) in liquid phase of test reactors: A) static and B) dynamic conditions.	34
Figure 4.6: Total phosphorus concentrations of the liquid phase during the experiments in reactors of distinct initial concentrations (mg/L): A) 0.1; B) 0.5 C) 1; D) 2 and E) 5 in log scale.	Chyba! Záložka není definována. 7
Figure 4.7: Total phosphorus flux rate concentration of the liquid phase in the initial stage of experiments (first 10 minutes) against the liquid phase initial TP concentration of distinct reactor series.....	39
Figure 4.8: Linear regression slope (m) of total phosphorus concentration flux change in the second stage of experiments (interval 10 th minute to 8 th hour) (A) and the third stage of experiments (from 8 th hour) (B) vs the initial liquid phase TP concentrations in reactor series (0.1, 0.5, 1, 2, 5 mg/L).	40
Figure 4.9: Concentrations of total phosphorus vs electron acceptors (dissolved oxygen, nitrate, total iron and sulphate) on a log scale over all test reactor series in Experiment 2	42
Figure 4.10: pH vs concentrations of total phosphorus, total iron and electrolytic conductivity in AIS reactor series	45
Figure 4.11: Released total phosphorus and total iron expressed in TP : Fe molar ratio in AIS reactors in the 3 rd stage of experiments (enhanced P release period) in the intervals of 8 th -24 th , 24 th -48 th and 72 nd -120 th hour.....	46
Figure A.1: Photographs of the assessed reed beds: A) NA-Fe site, B) A-Fe site, C) NA-Al site	65
Figure G.1: Spatial distribution of environmental conditions measured within the sediment of reed beds at 3 assessed sites (NA-Fe, A-Fe, NA-Al)	71

LIST OF TABLES

Table 3.1: Characteristics of wastewater treatment works assessed.....	15
Table 4.1: Environmental and wastewater characteristics across three sites at three sampling points (crude, 2° effluent, 3° effluent) with standard deviation....	24
Table 4.2: Pearson correlations of calculated TP and PO ₄ -P release to wastewater constituents and characteristics based on analyses and measurements carried out across 3 sites.....	31
Table 5.1: Overview of the most important processes possibly involved in observed phosphorus flux.	48
Table B.1: Chemical and physical characteristics of wastewater used in the experiments as measured on-site (DO, EC, Eh, pH, temperature) and in the laboratory (COD, TP, PO ₄ -P, SO ₄ ²⁻ , Fe total, Fe (II), Fe (III) and NO ₃ -N) ..	66
Table C.1: Wastewater metal concentrations in the Experiment 1 at the start and at the 72 nd hour in dynamic (D) and static (S) reactors as analysed by ICP-MS..	67
Table C.2: Wastewater metal concentrations in Experiment 2 at the start and at the 72 nd hour in the ^{0.1;0.5;1;2;5} Reactors as averages of 3 experimental replicates.	67
Table D.1: Concentrations of the chosen elements in top sludge in the inlet area of reed beds at 3 STW sites (NA-Fe, NA-Fe 2, NA-Al) as analysed by ICP-MS TotalQuant.....	68
Table E.1: Phosphorus and metal concentrations of sludge at the start and at the end of experiments and corresponding concentration change rates for dynamic (D) and static (S) conditions in Experiment 1 and ^{0.1, 0.5, 1, 2, 5} Reactors in Experiment 2	69
Table F.1: Concentrations of the chosen elements in wastewater at 3 assessed sites (NA-Fe, A-Fe, NA-Al) at 3 sampling points (crude, 2° effluent, 3° effluent) as analysed by ICP-MS TotalQuant.....	70
Table H.1: Pearson correlations of distinct factors possibly contributing to P release from reed bed based on analyses and measurements carried out across 3 sites in regards to sampling points.....	72
Table H.2: Pearson correlations of distinct factors possibly contributing to P release from reed bed based on analyses and measurements carried out across 3 sites in regards to particle fractions.....	73

LIST OF ABBREVIATIONS

2°	Secondary
3°	Tertiary
AAS	Atomic Absorption Spectroscopy
AIS	Artificial Inorganic Sludge
cBOD ₅	Carbonaceous Biochemical Oxygen Demand (5 days)
COD	Chemical Oxygen Demand
CW	Constructed Wetland
DI water	Deionised water
DO	Dissolved Oxygen
DW	Dry Weight
EC	Electrolytic Conductivity
Eh	Redox potential
HRT	Hydraulic Residence Time
HSSF	Horizontal Subsurface Flow
IC	Ion Chromatography
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
N	Total number in a statistical sample
NO ₃ -N	Nitrate-nitrogen
ORP	Orthophosphate
P	Phosphorus
PO ₄ -P	Phosphate-phosphorus
p.e.	Population equivalent
r	Pearson's correlation
rpm	Revolutions per minute
SD	Standard Deviation
SG	Specific Gravity
SOP	Standard Operating Procedure
STW	Severn Trent Water
TP	Total phosphorus
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
WwTW	Wastewater Treatment Works
*Reactor	Reactor with initial liquid phase x concentration (mg/L) of phosphorus

1 INTRODUCTION

Phosphorus is an essential macronutrient used for growth by aquatic organisms. It can limit primary production in water and its presence in elevated levels can lead to the destabilisation of ecosystems via eutrophication (Ekholm, 1998). In this context it can be considered a pollutant. The main inputs of phosphorus into rivers are from agriculture runoff and industrial and municipal wastewater effluents (Withers and Jarvie, 2008). Concerning the latter, water utilities have to ensure that their wastewater treatment works (WwTWs) final effluents meet the criteria which are set by the current legislation. In the UK, whilst current European requirements are fixed for all WwTWs serving over 10,000 population equivalent (p.e.; Council Directive 91/271/EEC), small works can be allocated a P consent if they discharge the treated water into areas identified as sensitive and are protected under the Countryside and Rights of Way Act (2000). Technologically, phosphorus removal down to 0.1 mg/L can be achieved employing micro and ultrafiltration, although such low levels could only be implemented if the increased cost of the treatment is clearly justified and reasonably balanced by ecological benefits (EA, 2012). The removal of phosphorus from wastewater is currently achieved primarily by dosing of chemicals, mainly aluminium and iron (Clark *et al.*, 1997). In the UK, when using these metal salts to remove P, the WwTWs also receive a numeric permit on the discharge of Fe and/or Al, typically between 2 – 4 mg/L Fe and 1 mg/L Al. At Severn Trent Water (STW), reed beds are used widely as a final treatment step in small works serving less than 2,000 p.e. Reed beds act as a low-energy means to polish the wastewater before it is discharged to streams. When P removal is needed at these small works, an iron or aluminium salt is added either in the crude influent, settled or secondary treated effluent, ahead of the reed beds. Occasionally, it is only during this final treatment stage that wastewater phosphorus concentrations drop below consented levels.

Reed beds are efficient in removing solids from wastewater; however, phosphorus removal appears to be more complex. Phosphorus in reed beds is in constant flux and its release and retention is controlled by the environmental

conditions present in the bed (Dunne and Reddy, 2005). These include many biological, physical and chemical factors. If the necessary conditions are not met, reed beds can consequently release phosphorus, failing to act as its permanent filter and storage (Vymazal, 2007). Ideally, reed beds should act as a permanent phosphorus sink retaining P until planned media cleaning or refurbishment is carried out.

In recent research conducted at Cranfield for Severn Trent, retention of iron and phosphorus was identified as settlement of suspended iron-bound-phosphorus particles which accumulated in the upper layers of the granular media, whereas release appeared to be related to anaerobic conditions within the beds (Prieto Fort, 2013). This link, however, could not be proven under the highly variable field conditions. Moreover, under controlled laboratory conditions, aerating the reed bed sludge resulted in higher effluent P levels than when incubating the sludge anaerobically. Aeration also slowed the release of iron from the sludge but was unable to stop it. Further research was therefore necessary in order to improve understanding of the factors affecting phosphorus cycling and propose mitigation strategies.

1.1 Aims and objectives

This thesis aims first to identify the environmental conditions in which chemically dosed reed beds retain or release wastewater phosphorus and then, based on the findings, to propose new management strategies which would prevent P and Fe release. To achieve the aforementioned aims, objectives were established and met in the following chronological order:

- Identify the possible factors that can affect phosphorus retention and release from reed beds through a critical literature review. The project hypothesis was developed based on the information collected.
- Carry out a field survey to characterise the wastewater from three STW reed bed sites with different environmental conditions, to identify the most likely factors affecting phosphorus retention and release.
- Test the effects of concentration gradients and hydrodynamic conditions on P transformations in reed bed sludge.
- Integrate all findings to propose management actions to minimise the risk of failures at dosed small WwTWs.

1.2 Hypothesis

A phosphorus concentration equilibrium between the wastewater and accumulated sludge is achieved by either release or retention of phosphorus from/to the solid phase and is driven by concentration gradients.

1.3 Thesis structure

The main chapters of the thesis - literature review, materials and methods, results, discussion and conclusions - correspond to the individual objectives. The literature review in Chapter 2 provides background information to the problem and summarises the possible P release factors highlighting only those

of greater importance. Chapter 3 describes the technical information and procedures related to the field survey and controlled experiments. The results from the experimental work are presented in chapter 4. Chapter 5 discusses the findings, by linking individual P release factors and suggesting how the results could impact on reed bed management in small works, with the main conclusions from the work found in Chapter 6. The appendices contain supplementary information on assessed reed bed sites, the correlations of potential P release factors and the chemical composition of samples used in the experiments.

2 LITERATURE REVIEW

The specific objective of the literature review was to identify environmental/chemical conditions in which phosphorus is released from/retained within reed bed sediment and if possible, define individual factors quantitatively. Some of the factors are more general than others, and there are potential interdependencies which were identified. This informed the field survey and testing of hypothesis.

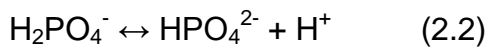
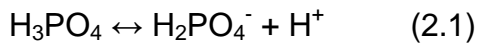
2.1 Phosphorus and its forms

Phosphorus has only one stable isotope and nearly always exists in only one oxidation state, which is phosphate (PO_4^{3-}). Phosphorus also does not have any significant atmospheric fluxes, which makes modelling its pathways much simpler than those of e.g. nitrogen (Froelich, 1988). From the perspective of analytical chemistry, PO_4^{3-} can be found in three forms: orthophosphates (ORPs); condensed phosphates in which two or more P atoms form P-O-P bonds (pyro-, meta-, and other polyphosphates); and organically bound phosphates in which P-O-C bonds are present (e.g. phosphomonoesters or phosphagens). All forms occur in solution, in particles (detritus) or in the bodies of aquatic organisms (Jenkins *et al.*, 1971; Vymazal, 2007). All three forms together are termed total phosphorus (TP).

Phosphate which does not require prior hydrolysis or oxidative digestion and still responds to colorimetric tests is termed “reactive phosphorus”. Reactive phosphorus occurs in all dissolved, colloidal and suspended forms. Almost all reactive phosphorus is actually orthophosphate, although some condensed phosphate is usually unavoidably hydrolyzed during the colorimetric procedure and is included in the results too. Dissolved, colloidal and particulate condensed phosphates are converted to dissolved orthophosphates by acid hydrolysis at 100° C. Through hydrolysis, a certain amount of PO_4^{3-} is unavoidably released from organic compounds. Nevertheless, the quantity can be minimised by applying the appropriate temperature, hydrolysis time, and acid strength. In

order to include the organic bound phosphorus in the colorimetric evaluation and hence assess the TP levels, organic matter in the sample has to be oxidised through digestion, typically using nitric acid-sulphuric acid (APHA, 1998).

Phosphate changes hydration state according to pH. In the circumneutral pH conditions present in reed beds, the most common phosphates are mono- and dibasic (Kadlec and Wallace, 2009):



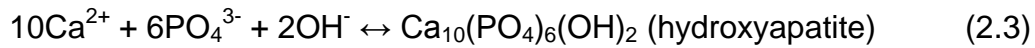
In aerobic biological wastewater treatment almost all condensed PO_4^{3-} can be hydrolysed to orthophosphate. Finstein and Hunter (1967) reported changes from 25%-85% in influent to 60%-95% in effluent. Organic forms of P are less mobile and are resistant to biological degradation and release from sediment. Some forms (nucleic acids, phospholipids, sugar phosphates) are degraded faster than others (inositol phosphate, phytin; Dunne and Reddy, 2005). In natural wetlands, they can represent a substantial fraction of TP, e.g., humic and fulvic acid associated P can account for more than 40% of TP in wetlands (Reddy and D'Angelo, 1994).

2.2 Use of chemical precipitants in wastewater treatment

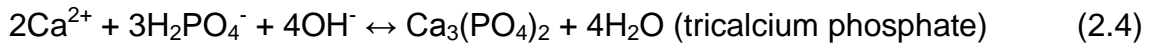
The most commonly used precipitants in wastewater treatment are aluminium, iron and calcium (Ca) based. Aluminium is considered to be the most effective, followed by iron (Clark *et al.*, 1997).

2.2.1 Calcium

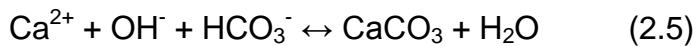
From the calcium salts, calcium oxide (CaO) and calcium hydroxide ($\text{Ca}(\text{OH})_2$) are the most widely used (EA, 2012). The reaction of calcium oxide with phosphate has two variations in wastewater treatment plant conditions (Yeoman *et al.*, 1988; Stumm and Morgan, 1970):



or



With increasing pH, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ may not be the preferred reaction product. Its formation can be prevented by Ca^{2+} reaction with carbon dioxide (CO_2), precipitating bicarbonates, which compete for hydroxide and calcium binding sites with phosphate. At pH above 9.5, they tend to form calcium carbonate, consuming the hydroxide ions (Merill and Jorden, 1975):



Only excess calcium then precipitates phosphates. Thus alkalinity of water is in direct relation to calcium P removal (Yeoman *et al.*, 1988). Phosphate precipitation is much faster than CaCO_3 precipitation, hence it prevails in lower pH. It should be noted that PO_4^{3-} can be subsequently adsorbed to a CaCO_3 precipitate (Schmid and McKinney, 1969).

Additionally, magnesium ion (Mg^{2+}) inhibits formation of CaCO_3 (Schmid and McKinney, 1969). It improves P removal at a pH range from 9 to 10, stabilizing the formation of tricalcium phosphate, although the opposite effect was observed at pH 8. In pH above 10.5, Mg^{2+} influence on P solubility disappears as it tends to form $\text{Mg}(\text{OH})_2$ instead (Ferguson and McCarty, 1971). Also Cao and Harris (2007) reported an inhibitory effect of both Mg^{2+} and HCO_3^{2-} on PO_4^{3-} removal. However, when both Mg^{2+} and HCO_3^{2-} are present in the system, they can combine as $\text{Mg}(\text{CO})_3$ (aq) and thus eliminate free activity of Mg^{2+} and HCO_3^{2-} in the solution, preventing the inhibitory effect.

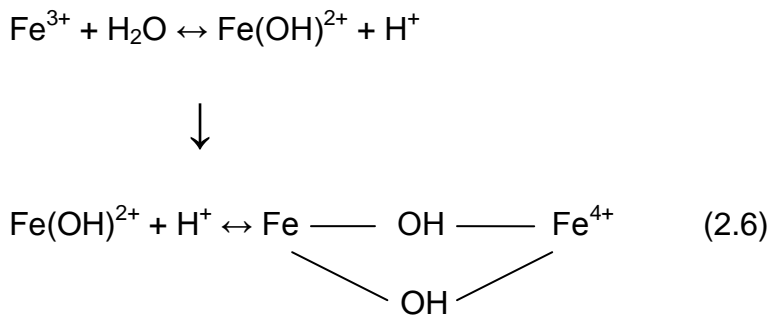
Not only alkalinity, but simultaneously carbonate (CaCO_3) hardness governs Ca mediated PO_4^{3-} precipitation. Wuhrmann (1968) reported that lime requirements were 1.5 times the carbonate hardness in a pH range of 10.5 to 11.

2.2.2 Iron (II) and iron (III)

Both iron (II) and iron (III) are widely used as precipitants, namely iron (II) sulphate (FeSO_4), iron (III) sulphate ($\text{Fe}_2(\text{SO}_4)_3$) and iron (III) chloride (FeCl_3). The pathway of Fe (II) reaction with P depends on the level of dissolved oxygen in water. In the presence of oxygen, Fe (II) is oxidised to Fe (III) which forms strong complexes with hydroxides and phosphates. Hydroxyl and phosphate ions compete for iron (III). The level of oxygen required for the reaction was reported as high as 0.15 g O_2 /g Fe (II) by Yeoman *et al.* (1988). Formation of iron hydroxides from bicarbonates with iron increases the Fe dose needed for P removal. The required Fe dose also increases with the level of colloids present in the matrix. Iron (III) forms strong associations with pyrophosphates ($\text{P}_2\text{O}_7^{4-}$) and tripolyphosphates ($\text{P}_3\text{O}_{10}^{5-}$). It is assumed that these are consequently removed during wastewater treatment by adsorption onto surfaces of iron (III) hydroxo-phosphates (Jenkins *et al.*, 1971).

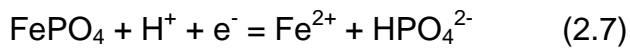
In the absence of oxygen, the oxidation of Fe (II) does not take place. Instead a fine precipitate of vivianite $\text{Fe}^{2+}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ is formed (Fox, 1991; Clark *et al.*, 1997).

Thistleton *et al.* (2002) pointed out the problem of potential reactions of iron with water molecules if phosphate is not readily available for reaction. In such cases hydrolysis might occur by reacting water with charged species of elements and metal hydroxides ($\text{Fe}(\text{OH})_2$) or oxy-hydroxides (FeOOH) may be formed instead of iron phosphates (Vymazal and Švehla, 2013). The hydrolysis-precipitation can start as follows (Dousma and de Bruyn, 1976):



Depending on the solution's ionic strengths and temperature, these products can further combine with other Fe, OH⁻ and later with also oxygen atoms and molecules to form large polymers. On introducing P into the solution, PO₄³⁻ adsorption occurs mainly at positive sites. Phosphorus binding capacity decreases with ageing of the iron-hydroxide precipitate. In the first hour of the experiment, Lijklema (1980) observed a ~50% decrease of the PO₄³⁻ binding capacity of iron (III) when 1-day-old hydroxide precipitate was used.

Previously mentioned pH and redox potential (Eh) factors also have a profound effect in relation to iron. Reduction of ferric phosphate is a principal cause of increased P solubility under acidic conditions (Dunne and Reddy, 2005):



When reduced conditions are present, insoluble crystalline ferric oxyhydroxide compounds turn into gel-like ferrous compounds which have a larger surface area and therefore more sorption sites. On the other hand, these sites have lower P bonding energy than a smaller number of sites available in oxidised substrate. Consequently, oxidised substrate adsorbs less P but retains it more strongly (Patrick and Khalid, 1974).

2.2.3 Aluminium

Precipitants used are aluminium sulphate (Al₂(SO₄)₃), aluminium chlorohydrate (Al₂(OH)₅Cl) and polyaluminium chloride (Al₂(OH)₃Cl₃; Gebbie, 2001). The aluminium sulphate reaction with phosphate can be simplified as follows (Yeoman *et al.*, 1988):



Aluminium solubility is highly dependant on pH. It ranges from soluble aluminium ions Al³⁺, Al(OH)²⁺ and Al(OH)₂⁺ at pH < 5 to soluble aluminate ion Al(OH)₄⁻ at pH 9 and it is relatively insoluble at 6 > pH < 8 as Al(OH)₃ (Batten *et al.*; Stumm and Morgan, 1996). With increasing total concentration of aluminium, Al(OH)₃ can further mineralise as gibbsite (Gensemer and Playle, 1999).

The coagulant $\text{Al}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$ added to wastewater dissociates to Al^{3+} and further hydrates to hexaaquoaluminum ion $\text{Al}(\text{H}_2\text{O})_6^{3+}$. If carbonate and bicarbonate ions represent the only source of wastewater alkalinity, $\text{Al}(\text{H}_2\text{O})_6^{3+}$ hydrolyzes to various soluble species and to solid $\text{Al}(\text{OH})_3$. This product is not only able to remove suspended solids, but also PO_4^{3-} , incorporating it into its structure through adsorption onto its surface and mechanism of co-precipitation. Phosphate ions are able to take place of -OH or -OH₂ groups of Al or Fe (III) oxides (Omoike and vanLoon, 1999). Contrary to equation 2.8, Hsu (1975) suggested that the precipitation of phosphates is more likely controlled by integration of Al-OH-Al and Al-PO₄-Al types linkages into reaction products (i.e. aluminium hydroxyphosphate), rather than by precipitation by individual phases of $\text{Al}(\text{OH})_3$ or AlPO_4 .

Hydroxide OH^- has a stronger affinity to Al^{3+} than PO_4^{3-} (Hsu, 1975). Nevertheless, significant competition from hydroxide ions was reported only in P concentrations below 10 mg/L (Stumm and Morgan, 1970).

2.3 Factors affecting release/retention of P in sediments

Phosphorus flux factors can be categorised as physical, chemical and biological. Although wastewater treatment at chemically dosed sites is dominated by chemical and physical factors, the role of biological factors in the reed bed ecosystem P flux should not be overlooked. Microbial metabolism depletes oxygen, thus decreases Eh and reduces Fe (III) to Fe (II), which induces P release (Zhou *et al.*, 2010). Also, Bates and Neafus (1980) reported that P release is higher in biologically active conditions as opposed to sterile conditions, suggesting that bacteria supply the liquid phase with PO_4^{3-} from the solid phase.

Apart from biological uptake and release, soluble P is transformed in the aquatic sediments by sorption, mineral precipitation and coprecipitation (Gainswin *et al.*, 2006). Sorption refers to abiotic retention of P in the sediment. Adsorption leads to accumulation of P on the surfaces of sediment minerals without penetrating

their structure, while absorption is the uptake mechanism when P of one phase (liquid) penetrates another phase (solid). Desorption is the release of P from mineral surfaces into surrounding porewater. Precipitation can occur where P anions react with mineral cations resulting in amorphous precipitates. The opposite mechanism is dissolution, when the precipitate solubilizes. In coprecipitation substances which would stay soluble under the absence of a precipitate are incorporated into a mixed solid (Reddy and DeLaune, 2008; Holler *et al.*, 1994). Particulate P is retained through sedimentation. Biological P storage is generally regarded as short-term, while sediment storage as rather long-term.

The weather factor which has the most prominent impact on P retention/release seems to be temperature. In laboratory conditions, when temperature increased from 2 °C to 25–35 °C, Kim *et al.* (2003) observed that P release rate rose by 500 %. This could be attributed to the increased metabolic activity of microorganisms (Reddy and D'Angelo, 1994).

The sediment characteristics also play an important role, especially particle size in relation to P retention (House *et al.*, 1995; Aminot and Andrieux, 1996; Arias *et al.*, 2001). The specific surface area for P sorption is greater if the proportion of clay particles (< 2 µm) and associated amorphous Fe and Al oxide and hydroxide coatings in the sediment is greater (Horowitz and Elrick, 1987; McGechan and Lewis, 2002). Both gravel and stone substrates and associated biofilms have an important control over the release of soluble reactive phosphorus (SRP). Gainswin *et al.* (2006) observed a rapid initial P release from greater size fractions of sediment that had well-established filamentous algae, suggesting that diffusion may not be the only driving mechanism. Fluctuation in flow is linked to advection of particulates. Bates and Neafus (1980) reported that mixing was found to enhance P release from the lake sediments, despite the fact that suspended sediment exposed greater surface area to potential ion exchange reactions. Electrical conductivity could be used as an indicator of dilution and concentration effects of wastewater due to rainfall, runoff and evapotranspiration (Kadlec and Wallace, 2009).

Phosphorus flux can be dominated by hydrostatic gradients. Molecular diffusion of dissolved P is driven by concentration gradients in: 1) porewater dissolved P concentrations within the sediment layers and 2) concentration gradients between surface sediments and the overlying water (Withers and Jarvie, 2008). Phosphorus can diffuse into the soil and adsorb/precipitate only if the floodwater P concentration is higher than that of the soil porewater P. At low loadings of P, the wetland in the study of Reddy and D'Angelo (1994) was found to release more P than was retained. Dunne *et al.* (2005) concluded from their experiments with wetland soil and wastewater spiked with 1, 5 and 15 mg/L P that P release from soil porewater occurred when P in overlying water column was low. Correspondingly, the best P retention was observed when P in overlying water was high. Moreover, as reported by Richardson (1985), wetlands have a maximum phosphorus storage capacity, which can become saturated within several years. The storage capacity seemed to be more dominated by the type of substrate rather than the actual PO_4^{3-} equilibrium concentration in the solution passing through the wetland.

Release and retention of P is significantly influenced by the solution pH. The release rate is lowest in the 7-10 range ($\sim 75 \text{ mg P/m}^2 \cdot \text{week}$) and increases fourfold at pH 2 and doubles at pH 13 (Kim *et al.*, 2003). Thistleton *et al.* (2001) in their study of P removal at iron dosed WwTW considered pH 8 the best case scenario for successful P retention.

Redox potential is considered another dominant factor in many studies influencing nutrient transformations (Reddy and D'Angelo, 1994; Petticrew and Arocena, 2001) together with dissolved oxygen (DO) levels. Phosphorus and PO_4^{3-} release increase as oxygen concentrations and redox potential decline (Petticrew and Arocena, 2001). Anoxia leads to reductive dissolution of Fe (III) hydroxide flocs to Fe (II) compounds and in effect release of soluble reactive phosphorus from bed sediments (Withers and Jarvie, 2008; Zhou *et al.*, 2010). In addition, anaerobic metabolism can oxidise the organic matter previously binding P, thus increasing the release rates of total phosphorus. This may also increase the relative TP concentrations in the sediment (Kim *et al.*, 2003).

Of importance to this study is the mineral precipitation and coprecipitation of P with various minerals, usually containing calcium, aluminium and iron. In acidic soils, P retention tends to be governed by Al and Fe, whereas in alkaline soils it is by the activity of Ca and magnesium (Mg; Reddy and D'Angelo, 1994). These processes were extensively studied (Nürnberg, 1984; Reddy and D'Angelo, 1994; Petticrew and Arocena, 2001; Vymazal 2007) and the above mentioned elements are successfully employed as dosing agents in wastewater treatment across the globe (Clark *et al.*, 1997).

Anions (especially sulphate) might act as alternative electron acceptors and inhibit P removal (Yeoman *et al.*, 1988). When sulphate is abundant, P retention by Fe (II) can be precluded by the bacteria-mediated reduction of SO_4^{2-} to hydrogen sulphide (H_2S) and the formation of ferrous sulphide (FeS ; Dunne and Reddy, 2005). However, this might not always be the case. Hsu (1975) observed improved P removal efficiency at a pH range between 4.5 and 6 when more sulphate was present in the solution, while using Al as a coagulant. It was suggested that in the acidic medium the reaction products Al-OH-PO_4 are positively charged and thus tend to remain in the solution. When sulphate is present in the reaction product, it helps to reduce its surface charge and causes precipitation. Furthermore, the same study suggested that sulphate is not able to compete with PO_4^{3-} for aluminium binding sites and it can only be integrated in the reaction product when PO_4^{3-} is deficient. Fox *et al.* (1989) observed a decrease in the extent and rate of PO_4^{3-} removal in the presence of nitrogen, although concluded that another mechanism to competition for adsorption sites was likely involved, as the nitrogen concentration was an order of magnitude higher than the P concentration.

Most of the authors focus on P uptake onto/release from static components. However, a substantial portion of P can detach from the sediment and leave in surface water runoff or in sediment macropores flow, attached to mobile suspended and colloidal material (McGechan and Lewis, 2002). Using mathematical modelling, McGechan (2002) stated that when P concentrations are 0.3 mg/L, slurry colloid passing freely through a soil matrix can adsorb as

much as 5,000 times more P in comparison to an immobile soil matrix. Such events of increased runoff typically occur due to the heavy rainfall. According to Jarvis *et al.* (1999), release of P is more often caused by rainwater impact at the soil surface rather than by its consequent sub-surface flow.

3 MATERIALS AND METHODS

3.1 Field survey

3.1.1 Sites and sampling

The field survey assessed three wastewater treatment works owned by Severn Trent Water which each apply distinct treatments (Table 3.1; Appendix A). The field survey took place from July to October 2013.

Table 3.1: Characteristics of wastewater treatment works assessed (HSSF = horizontal subsurface flow; RBC = Rotating Biological Contactor; PST = Primary Settlement Tank; cBOD₅ = carbonaceous Biochemical Oxygen Demand; source: Severn Trent Water data).

		Works (ID)		
		Non-Aerated Iron (NA-Fe)	Aerated Iron (A-Fe)	Non-Aerated Aluminium (NA-Al)
p.e.		526	282	1839
Treatment	1°	-	-	PSTs
	2°	RBC (integral: PST + RBC + humus tank)	RBC (integral: PST + RBC) and separately humus tank	trickling filters and humus tank
	3°	reed bed, HSSF	reed bed, HSSF	reed bed, HSSF
Reed bed aeration		no	yes	no
Metal dosing		iron $\text{Fe}_2(\text{SO}_4)_3$ (12.5% Fe w/w, 1.57 SG)		aluminium $\text{Al}_2(\text{SO}_4)_3 \cdot 51.8 \text{ H}_2\text{O}$ (4.2% Al w/w, 1.32 SG)
Reed bed dimensions (width x length; m)		18 x 12.5	11.5 x 36.5	12.5 x 40
NH ₄ ⁺ limit (mg/L)		N/A	15	5
cBOD ₅ limit (mg/L)		40	25	15
TSS limit (mg/L)		60	45	25
TP limit (mg/L)		2	2	2
Dosed metal limit (mg/L)		4 (Fe)	4 (Fe)	1 (Al)
Dosing point and rate		front end of biozone (settled sewage) 0.48 L/hour	front end of biozone (settled sewage) 2.1 L/hour	crude influent 5.49 L/hour

Three sampling points were used: crude influent, secondary effluent and tertiary effluent. Two sampling techniques were applied: grab sampling at crude sampling point and composite sampling at 2° and 3° effluents with the use of autosamplers ISCO 3700 (Teledyne Isco, UK) set up to collect 100 mL of sample each hour during 24 hours into one bottle.

Sampling for NA-Fe was carried out during 5 consecutive weeks as follows: 1st and 2nd week grab samples only and 3rd, 4th and 5th week composite samples for 2° and 3° effluents sampling points and grab samples for crude sampling point. Sampling for A-Fe and NA-Al was done in 3 consecutive weeks: 1st, 2nd and 3rd week by composite sampling at 2° and 3° effluents sampling points and grab sampling for crude sampling point.

Wastewater samples were stored in 1 L heavy duty polypropylene bottles.

One additional sample per site was taken in the form of sludge from the reed bed surface near the inlet channel.

Sludge samples were stored in 1L plastic opaque buckets. Samples were kept in the dark and transported to the laboratory within 2 hours and kept refrigerated at 4 °C until analysis took place.

Sludge samples were preserved by placing them in a drying cabinet at 40 °C for a minimum period of one week to achieve constant weight and stored in plastic containers at room temperature after that.

3.1.2 On-site measurements

At the time of sampling, ambient temperature and flow rate were measured on-site.

Wastewater temperature, Eh, pH, DO and electrolytic conductivity (EC) were measured at each of the designated sampling points using a HQ40d portable Meter, DO IntelliCAL LDO105 probe, pH IntelliCAL PHC10101 standard gel filled probe, gel filled ORP/Redox Probe MTC10101 and EC probe CDC40101, all manufactured by Hach (Salford, UK). For wastewater and ambient

temperature, the temperature sensor incorporated in the DO probe was used. An on-site certified flow meter was used to measure flow rate.

In addition, the above parameters were measured on a random day of the sampling campaign on-site within the reed bed in a 3 x 3 symmetrical grid of rectangles, i.e., 9 measurement points in total, at the centre of each one. Measurements were made 10-15 cm below the water surface. Because the NA-Fe site was flooded, it is anticipated that 10-15 cm below water surface corresponds to 5 cm into the ground.

Average daily flow rates were downloaded from Sever Trent Water Streamline archive database for the sampled days.

3.1.3 Sample processing

The original wastewater sample (designated “sample A”) containing all suspended, colloidal and dissolved solids was fractionated. Firstly, suspended particles were removed using 1.2 µm Whatman GF/C filter papers (70 mm diameter; GE Healthcare, UK; supplied by Fisher Scientific UK Ltd.). The remaining solution (sample B) contained only colloidal and dissolved particles. Colloidal particles were removed by passing it through low protein binding modified polyethersulfone membranes of 1 kDa pore size (76 mm diameter; PALL Life Sciences, UK; supplied by VWR International UK Ltd.), following the method described by Prieto Fort (2013) and Martin (2010), using Amicon 8400 stirred pressure cell (Millipore UK Ltd.), stirrer IKAMAG REO (MOTOR, Drehzahl Electronic) and nitrogen gas supply at the pressure of 10 psi. The remaining solution (sample C) contained only dissolved particles.

Wastewater samples were first analysed in original form (sample A), when all fractions of the particles were present. Total phosphorus, phosphate-phosphorus ($\text{PO}_4\text{-P}$), $\text{NO}_3\text{-N}$, carbonaceous biochemical oxygen demand (cBOD_5), COD, volatile suspended solids (VSS), total suspended solids (TSS), alkalinity, hardness, Mg, Ca, ammonium (NH_4^+), SO_4^{2-} , Fe total, Fe (II), Fe (III) and Al were measured. Aluminium was only measured at NA-Al. Hardness and alkalinity were only measured at the A-Fe and NA-Fe sites. Sample B and

sample C were analysed for TP, PO₄-P, Fe total, Fe (II), Fe (III) (Fe in Fe dosed sites), Al (in Al dosed site), NO₃-N and SO₄²⁻. All parameters were analysed using a DR 2800 spectrophotometer, LT 200 thermostat and cuvette test kits, all manufactured by Hach (Salford, UK), with the exception of cBOD₅, TSS and VSS measurements which were carried out following APHA (1998) Standard Methods. Suspended solids analyses were performed using Whatman GF/C filter papers with a pore size of 1.2 µm (GE Healthcare, UK; diameter 70 mm).

Sludge samples were microwave digested using *aqua regia* (HCl 1.18 SG and HNO₃ 1.42 SG in 3 : 1 ratio) and a Mars 5 Xpress (model 240/50; CEM corporation), following Cranfield University SOP/11/6064/1. Subsequently they were analysed for TP and metals by inductively coupled plasma – mass spectrometry (ICP-MS; Perkin Elmer ELAN 9000 ICP-MS system) TotalQuant following Cranfield University SOP 106/version 1. This method served as a preliminary screening, with detection limits at 0.01 mg/L.

3.1.4 Data analysis

Data were processed by both Microsoft Excel and IBM SPSS Statistics 9 (Version 21) software packages. A correlation analysis using Pearson correlation coefficient and a two-tailed test of significance was carried out for the field survey data. Total phosphorus and phosphate release was calculated by subtracting TP/PO₄-P mg/L values of 3° effluent from 2° effluent TP/PO₄-P values.

3.2 Controlled experiments

3.2.1 Sampling

Reed bed sludge and 2° effluent wastewater were collected from the NA-Fe site. Sludge was taken from the surface layer overlying granular media near the inlet area of the reed bed. Both sample types were collected into plastic drums. Containers were filled to the top to minimize contact with air and sealed

hermetically. Samples were transported to the laboratory without any special adjustment or preservation, stored in the fridge (4 °C) and used the next day.

Chemical and physical characteristics (DO, Eh, EC, pH, temperature) at the time of sampling were measured (Appendix B) using apparatus described in section 3.1.2.

3.2.2 Experiment 1: P flux in dynamic and static conditions

Small scale reactors in the form of 1L glass beakers were used to expose the sludge to the identified key conditions conducive of P and Fe release based on the field surveys. Sludge and wastewater media without adjustment were used in order to observe the changes occurring in the reactor during incubation time (wastewater metal concentrations at the beginning and during the experiments are presented in Appendix H). Phosphorus and iron concentrations of the sludge were 38.0 and 95.6 g P or Fe/kg·DW respectively (detailed chemical content of sludge in Appendix C). Both static and dynamic conditions were simulated. The impact of stirring on observed reactions was assessed by running dynamic reactors in parallel by application of programmable Jar testers PB-900 (Phipps & Bird; supplied by Camlab) set up in continuous mode at 5 rpm. After collection of the samples and on arrival to the laboratory, initial analyses of the wastewater took place: it was fractionated and analysed for TP, PO₄-P, NO₃-N, SO₄²⁻, Fe total, Fe (II) and Fe (III) in all dissolved, colloidal and suspended fractions. Additionally, unfractionated samples were analysed for COD (Appendix B).

Prior to the experiment, both sludge and wastewater were thoroughly agitated to homogenise them. Subsequently, DO, pH, temperature, Eh and EC were measured. These results were identified as “Time Initial” (TI). Sludge at TI was also sampled for analysis. Wastewater at TI was sampled and filtered using 10 ml syringe (disposable 2-part NORM-JECT®, luer needle-free sterile; supplied by VWR, UK) and 0.45 µm polypropylene non-sterile syringe filter (luer lock

inlet, male slip luer outlet; diameter 25 mm; supplied by VWR, UK) and transferred into 15 mL centrifuge tubes.

Three types of reactor were set up: test, artificial inorganic sludge (AIS) and blank.

Test reactors consisted of 0.5 L of reed bed sludge and 0.5 L of 2° effluent wastewater collected from site NA-Fe.

The AIS reactors consisted of 0.5 L of AIS and 0.5 L of 2° effluent wastewater. The AIS was made of a mixture of deionised (DI) water, Fe salt (iron (III) sulphate hydrate ($\text{Fe}_2(\text{SO}_4)_3 \cdot \text{H}_2\text{O}$); reagent grade; supplied by VWR, UK), P salt (Disodium hydrogen orthophosphate anhydrous (Na_2HPO_4); AnalaR NORMAPUR; supplied by VWR chemicals, UK) and glass beads (soda lime glass; 2 mm; supplied by VWR, UK) to make up to the target volume. The stock solutions were mixed by SSL1 orbital shaker (Stuart, UK) for 10 minutes. The final artificial product contained 6 mg/L-P and 10 mg/L-Fe with the aim of having sludge concentrations higher than they are in the overlying wastewater layer. The purpose of the artificial sludge reactor was to test P exchange with the exclusion of biota.

Blank reactors consisted of 1 L of wastewater with no sludge.

The experiment was run for one week, in triplicate, at room temperature (between 16 °C and 23 °C).

Probe measurements (DO, pH, EC, Eh) were carried out at random intervals directly in the reactors, at 0.5 cm above the sludge surface in the test and AIS reactors and approximately in the middle of the blank reactors.

Wastewater was sampled and filtered after 1, 2, 4, 6, 8 hours and 1, 3, 5 and 7 days from introduction liquid phase onto sludge in Time 0 (T0) as described earlier. Wastewater samples were analysed for TP, $\text{PO}_4\text{-P}$, SO_4^{2-} , $\text{NO}_3\text{-N}$, Fe total, Fe (II) and Fe (III) using test kits as described earlier.

Sludge from both static and dynamic reactors was sampled and analysed at the start and the end of the experiments. Samples were processed in the same way

as described in section 3.1.3 and analysed for TP following Cranfield University NR-SAS/SOP 17/Version 1 based on US EPA method 3051 and British Standard BS 7755: Section 3.13:1998 using Nicolet Evolution 100 UV-Vis spectrophotometer (Thermo Electron corporation; mode: ABS, wavelength: 880 nm; bandwidth: 2.0 nm, integration: 1s; lamp change 325 nm). The method detection limit was 0.05 mg/L. Metals (Al, Cd, Cr, Cu, Fe, Ni, Pb, Zn) were analysed using atomic absorption spectroscopy (AAS; Perkin Elmer AAnalyst 800; carrier gases: C₂H₂ & N₂O for Al analysis, C₂H₂ & air for the rest of elements) following Cranfield University SOP Analysis of Iron by Atomic Absorption Spectroscopy. The method detection limit for Al and Fe was 0.5 mg/L, for Cd was 0.1 µg/L and for the rest of the elements was 0.1 mg/L. Sludge analyses were carried out in experimental triplicates, each of which was further digested in 3 replicate subsamples.

3.2.3 Experiment 2: impact of liquid phase initial P concentration

The setup of the experiment was similar to that of Experiment 1, with only a few alterations. Only static conditions were applied to all the reactors. Phosphorus and iron concentrations of the sludge were 38.6 and 85.0 g P/kg-DW respectively (detailed chemical content of sludge in Appendix D).

Artificial sludge was made up to match the concentrations of the sludge from the reed bed. After an initial mixing of reagents as described earlier, the solution was centrifuged using Falcon 6/300 centrifuge (MSE, UK; 500 RCF for 10 minutes; acceleration 4; brake 2; temperature 19 °C), supernatant decanted to eliminate free ions and precipitant made up to target volume with DI (deionised) water. Subsequently, the procedure of initial mixing, centrifuging and decanting was repeated one more time. Environmental characteristics of the product were measured as: DO 10.86 mg/L, EC 5.61 µS/cm, pH < 2 and Eh 534.9 mV.

Five test reactors were set up, each with a different level of TP concentration applied in the overlying wastewater layer: 0.1; 0.5; 1; 2 and 5 mg/L (^{0.1,0.5,1,2,5}Reactors). These levels were chosen according to field survey results, so the range of them would cover lower as well as higher concentrations than those found typically in 2° effluent. The same concentrations were applied to

artificial sludge reactors and blanks. Disodium hydrogen orthophosphate anhydrous analytical reagent (AnalaR NORMAPUR) was used to spike the wastewater with P. On the contrary, the two lowest concentrations had to be made by dilution with DI water, because the concentration of original wastewater was higher. The ratio of sludge: wastewater in the reactor was 40:60. Sampling and filtration took place at T0 and 1, 2, 4, 8 hours and 1, 3 and 5 days after T0.

Wastewater samples were analysed for TP and metals using ICP-MS (Cranfield University SOP 106/version 1). Detection limit for the ICP-MS method used was 0.001 mg/L for Cd, Ni and Pb and 0.01 mg/L for the rest of the elements. The ICP-MS uses argon as a carrier gas and therefore Fe could not be analysed by this technique due to the interference from argon oxide (ArO^+), which has a similar molar mass as iron and is formed during the analytical run. Therefore, Fe total was analysed by AAS with the method detection limit of 0.01 mg/L. Phosphate, sulphate and nitrate was analysed by ion chromatography (IC; Dionex ICS-1600 with Dionex AS40 autosampler; column: AS 14A; suppressor: ASRS 500 4 mm; flow: 1 mL/min; injection volume: 250 μL ; column temperature: 35 °C; detector cell temperature: 35 °C; suppressor current: 43 mA). Detection limit for all the anions was 0.05 mg/L. Samples for IC were stored in the fridge (4 °C) before analysis took place.

Sludge was sampled at the start of the experiment and then at the end from all individual test reactors. Samples were processed and analysed in the same way as described in section 3.2.2.

3.2.4 Data analysis in experiments

In Experiment 1, linear regression was used to determine the relationship between dynamic and static conditions in the reactors and then in Experiment 2 to assess the slope (m) of P flux. Both Microsoft Excel and IBM SPSS Statistics (version 21) software packages were used to check for the correlations in between various characteristics and to carry out one-way analysis of variance (ANOVA) when evaluating the significance of differences between mean values.

4 RESULTS

4.1 Treatment works performance

4.1.1 Overall performance of works

All works assessed in the field survey proved to be adept at pollutant treatment, including P removal, with only some exceptions of P release (Table 4.1; detailed chemical composition of wastewater in Appendix E). Total phosphorus concentrations when entering the works were considerably higher at the A-Fe site as opposed to the other two sites, yet its levels were reduced by 82% in 2° treatment and by a further 84% in the 3° treatment, which is a comparatively better performance than the average for all sites (2°: 78% and 3°: 59%). Of the total phosphorus, phosphate-phosphorus accounted for: 66% in crude influent; 82% in 2° effluent; and 77% in 3° effluent.

Phosphorus particles distribution followed a trend from dissolved to suspended fraction between crude influent and 2° effluent (Figure 4.1A). Similarly for both TP and PO₄-P, the dissolved fraction accounted for an average 46% in crude influent and 36% in 2° effluent. Colloidal fraction was in general limited only to average 18.5% of the total fraction. Furthermore, the dissolved fraction for TP and PO₄-P increased in the 3° effluent accounting for 62% and 68% respectively. An increase of dissolved TP fraction during municipal wastewater treatment was also reported by Sophonsiri and Morgenroth (2004) when comparing primary and secondary effluents. Martin (2010) recorded change of the dissolved fraction (<1kDa) from crude influent 63% to final effluent 82% in five treatment works. Larger particles are reported to have faster settling rates and so tend to be more effectively retained (Choubert *et al.*, 2011).

Table 4.1: Environmental and wastewater characteristics across three sites at three sampling points (crude, 2° effluent, 3° effluent) with standard deviation. The highest value of each characteristic measured across all the sites is marked in red and the lowest in green.

	NA-Fe			A-Fe			NA-AI		
Ambient temperature (°C, shadow)	23.9 ±3.5			16.3 ±2.7			16.7 ±4.0		
Flow at the time of sampling (L/s)	1.49 ±0.3			1.09 ±0.64			10.2 ±7.3		
Average daily flow (L/s)	0.84 ±0.0			0.77 ±0.42			11.0 ±6.2		
	Crude	2° Eff	3° Eff	Crude	2° Eff	3° Eff	Crude	2° Eff	3° Eff
Wastewater temperature (°C)	18.8 ±0.68	18.9 ±0.86	18.5 ±1.24	17 ±0.85	16.5 ±1.6	13.6 ±1.6	14.4 ±0.7	14.5 ±1.3	13.7 ±0.7
DO (mg/L)	5.03 ±0.36	3.37 ±0.38	4.93 ±0.61	5.07 ±0.99	4.09 ±2.26	9.23 ±0.19	4.31 ±1.09	7.40 ±0.99	0.51 ±0.49
Eh (mV)	102 ±44	92.9 ±54	47.6 ±86	127 ±5.9	177 ±10	250 ±30	-36.7 ±147.3	237 ±19	240 ±16
EC (µS/cm)	1125 ±149	997 ±136	920 ±161	661 ±158	652 ±97	624 ±142	634 ±230	532 ±181	497 ±171
pH	8.0 ±0.2	8.0 ±0.3	7.9 ±0.2	7.7 ±0.5	6.6 ±0.3	7 ±0.3	8.1 ±1.4	7.2 ±0.1	7.3 ±0.1
COD (mg/L)	454 ±277	29.0 ±3.2	19.8 ±4.2	485 ±393	66.3 ±3.9	22.5 ±3.5	220 ±218	60.9 ±13	27.2 ±9
cBOD ₅ (mg/L)	135 ±61	1.41 ±0.33	0 ±0						
TSS (mg/L)	175 ±59.3	10 ±6.24	6.77 ±3.46	209 ±159	36.78 ±21.2	3.89 ±0.38	81.3 ±74.2	25.4 ±1.5	4.78 ±1.02
VSS (mg/L)	20.8 ±14.9	0.80 ±1.79	1.93 ±2.95	15.8 ±7.4	11.7 ±8.3	0.89 ±0.69	13.0 ±6.4	8.11 ±1.64	1.11 ±0.19
NO ₃ -N (mg/L)		16.7 ±4.19	7.15 ±3.71		28.0 ±10.1	34.1 ±16		36.9 ±29.4	32.0 ±21.5
NH ₄ -N (mg/L)	29.4 ±15	1.15 ±0.69	0.65 ±0.56	48.4 ±15	8.37 ±1.89	0.18 ±0.27	43.2 ±45.2	0.55 ±0.10	0 ±0.01
SO ₄ ²⁻ (mg/L)	160 ±20.3	146 ±12.1	141 ±14.8	91.4 ±47.1	133 ±11	118 ±23	75.6 ±52.4	88.8 ±32.1	83.7 ±29.8
Total P (mg/L)	5.10 ±2.44	2.02 ±1.34	1.74 ±0.67	9.42 ±9.2	2.45 ±0.35	0.66 ±0.18	5.81 ±6.39	1.19 ±0.34	0.25 ±0.07
PO ₄ -P (mg/L)	3.91 ±1.84	1.31 ±0.39	1.26 ±0.21	7.73 ±8.3	2.02 ±0.30	0.56 ±0.03	4.36 ±4.40	0.97 ±1.01	0.22 ±0.26
Al (mg/L)							0.101 ±0.074	0.106 ±0.040	0.049 ±0.036
Fe total (mg/L)	2.09 ±0.71	0.58 ±0.19	0.45 ±0.32	2.72 ±1.3	2.12 ±0.53	0.13 ±0.04	1.32 ±1.12	0.27 ±0.04	0.08 ±0.04
Fe (II) (mg/L)	1.92 ±1.10	0.065 ±0.028	0.050 ±0.025	2.58 ±1.44	0.29 ±0.03	0.15 ±0.19	1.21 ±1.13	0.15 ±0.04	0.04 ±0.04
Fe (III) (mg/L)	0.049 ±0.07	0.50 ±0.016	0.390 ±0.030	0.14 ±0.17	1.84 ±0.55	0.35 ±0.44	0.15 ±0.03	0.14 ±0.04	0.07 ±0.05
Alkalinity (mg/L as CaCO ₃)				250 ±14	16.7 ±10.4	21.7 ±5.8	247 ±81	49.2 ±19.4	49.2 ±11.8
Hardness (°dH)				11.3	10.6	15.2	13.4 ±0.9	12.3 ±3.2	11.6 ±3.9
Ca (mg/L)				60.5	52.1	81.6	62.0 ±10.2	59.8 ±15.3	55.9 ±20.7
Mg (mg/L)				12.0	14.3	16.3	20.5 ±4.7	16.8 ±4.6	16.5 ±4.4

The total iron concentrations in crude influent at both the NA-Fe and A-Fe sites were recorded at an average of 1.34 mg/L which is higher than the concentrations from the survey by Choubert *et al.* (2011) but similar to those reported by Carletti *et al.* (2008). When passing through the works, iron levels decreased by 44% between the crude influent and 2° effluent from 2.40 mg/L to 1.35 mg/L and by a further 79% to 0.29 mg/L during 3° treatment. Most of the iron leaving 2° treatment was, on average, found in the form of Fe (III), with Fe (II) : Fe (III) ratio 1 : 4.3, contrary to the domination of Fe (II) species in crude influent with Fe (II) : Fe (III) ratio 15 : 1. Iron (III) not only increased in relative amounts compared to Fe (II), but also in absolute values, from an average of 0.12 mg/L in crude influent to 0.81 mg/L in 2° effluent, which is likely due to iron addition ahead of biological treatment. Iron levels at the aluminium dosed site were generally found to be lower.

Fractionation of the wastewater revealed the total iron to be predominately associated with the suspended fraction in the crude influent and secondary effluent samples (Figure 4.1B). Furthermore, no difference was observed in relation to iron specification as both Fe (II) and Fe (III) were predominately observed in the suspended fractions (Figures 4.1C and 4.1D). However, the percentage of Fe (II) in the dissolved fraction increased from 0.9% in the crude sample to 10.5% and 11% in the 2° and 3° samples respectively, whereas the percentage of Fe (III) that was associated with the dissolved fraction decreased from 10.8% in the crude influent down to 2.9% and 3% in the secondary and tertiary samples respectively. If the Fe total in dissolved form is considered, these results largely correspond to reported 2 – 34 % range in wastewater of a wide variety of WWTWs (Choubert *et al.*, 2011).

Aluminium at the NA-Al site was observed to be predominantly associated with the dissolved fraction, with the highest dissolved content observed in the final effluent samples at 59.5% compared to 25% in the crude samples.

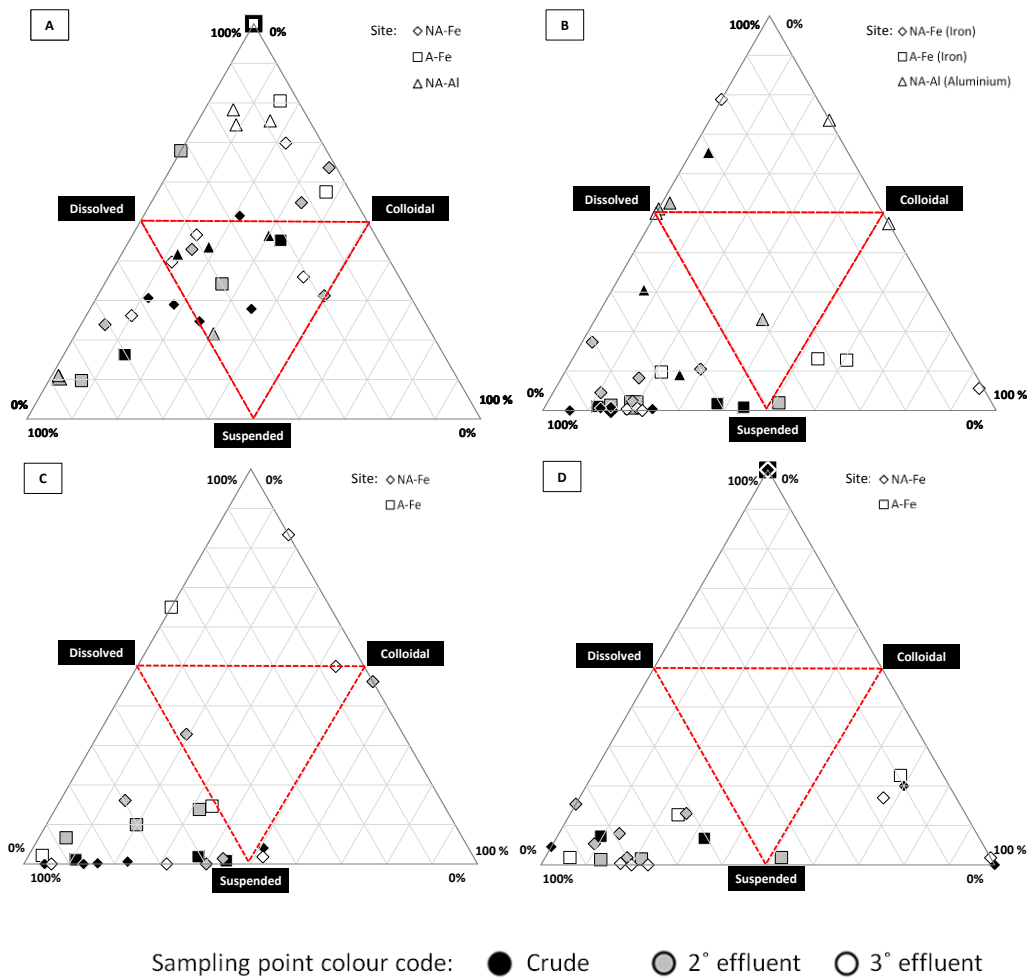


Figure 4.1: Wastewater constituents particle fractions percentual distribution at 3 sampling points (crude, 2° effluent, 3° effluent) across 3 sites (NA-Fe, A-Fe, NA-Al). A) total phosphorus; B) metals (iron, aluminium) dosed for phosphorus removal; C) iron(II); D) iron (III). Each point represents one sampling. Points outside of dotted triangle consist of more than 50 % of one of the fractions. Original Microsoft Excel design adopted from Vaughan (2010).

4.1.2 Reed bed performance

In general, the reed bed was cooler than the ambient temperature by 1.2 to 3.3 °C, with a tendency of greater differences when the ambient temperature was higher, suggesting that reed beds act as a temperature buffer of ambient daily temperature fluctuations. This is another example of the ecosystems'

ability to meet perturbations, analogous to nutrient (P) buffering capacity (Jørgensen and Johnsen, 1981).

Due to the reed beds' permanent saturation with water, anaerobic and reducing conditions were expected within the substrate. Probe measurements (Appendix F) confirmed these expectations at the NA-Fe site with an average Eh at -61 mV and DO at 0.7 mg/L. Aeration at the A-Fe site brought average DO levels up to 9.3 mg/L and Eh to 248 mV. As a consequence of high oxygen levels, transformation of NH_4^+ to nitrate is assumed, reflected in positive $\text{NH}_4\text{-N}$ and negative $\text{NO}_3\text{-N}$ removal rates of 1.39 (SD = 1.56) and -1.26 (SD = 1) $\text{g/m}^2\cdot\text{day}$ respectively. The NA-AI site showed on average levels of Eh at 211 mV and of DO at 2 mg/L. Although a non-aerated site, it was heavily flooded on two occasions during the sampling, which most probably contributed to aeration of the sludge and dilution of the overlying wastewater.

All non-aerated reed beds removed nitrate, COD and solids within the range reported for other HSSF beds at 1.98 $\text{g/m}^2\cdot\text{day}$, 18.2 $\text{g/m}^2\cdot\text{day}$ and 13.2 $\text{g/m}^2\cdot\text{day}$, respectively (Prieto Fort, 2013; Vymazal and Kröpfelová, 2009; Vymazal, 2011).

During the sampling, two cases of phosphorus release were observed with corresponding removal rates of -0.148 and -0.427 $\text{g TP/m}^2\cdot\text{day}$ (Figure 4.2). In all other cases mass removal rates were positive with total phosphorus remaining below 2 mg/L when leaving WwTW at all the sites, with removal rates of 0.1 to 3.5 $\text{g TP/m}^2\cdot\text{day}$. To compare, the average removal rate for the same reed bed in 2012 was recorded at -0.09 $\text{g TP/m}^2\cdot\text{day}$ (N = 5; SD = 0.06), close to the removal rate of another two HSSF reed beds at iron dosed STW sites at -0.04 (N = 5; SD = 0.15) and 0.00 $\text{g TP/m}^2\cdot\text{day}$ (N = 7; SD = 0.03; Prieto Fort, 2013). Based on 104 wetlands in 15 countries, the average total phosphorous removal in HSSF reed beds was reported at 0.123 $\text{g/m}^2\cdot\text{day}$ (Vymazal, 2007). Similarly, a review of 23 HSSF systems in the USA revealed a range between 0.03 to 1.48 $\text{g/m}^2\cdot\text{day}$ (WPCF, 1990).

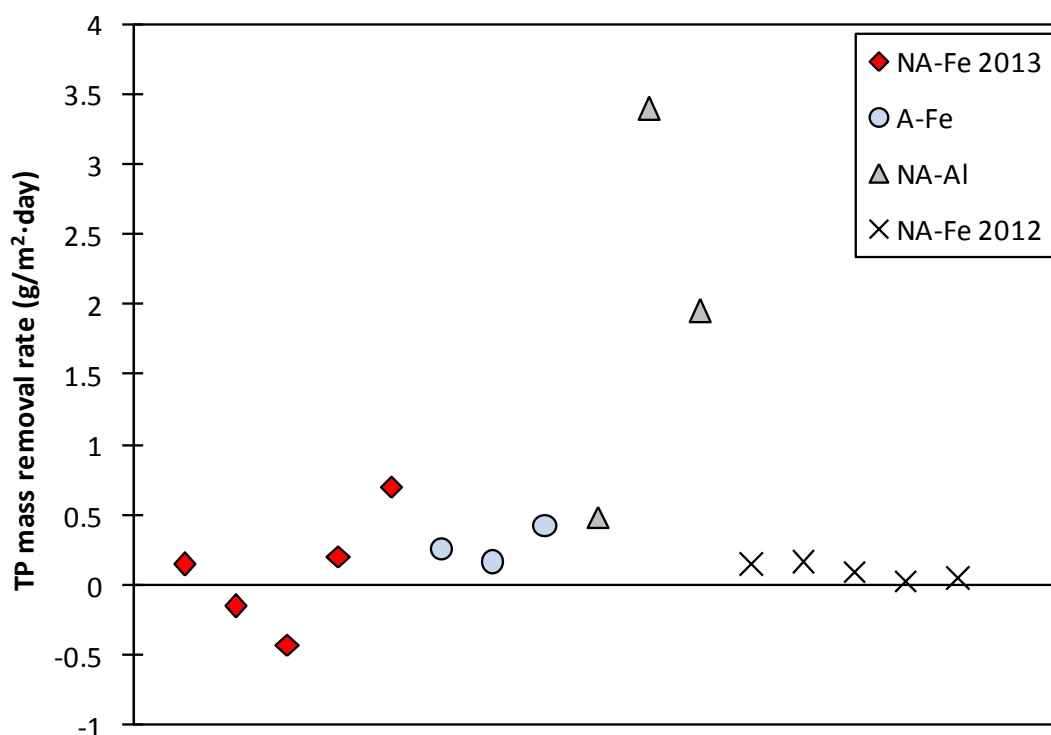


Figure 4.2: Reed beds total phosphorus mass removal rates ($\text{g/m}^2\cdot\text{day}$) over 3 sites. Site NA-Fe was analysed in two consecutive years. Positive values signify P retention, negative values signify P release.

Iron was also retained within the wetlands with average removal rates for total iron at $0.20 \text{ g/m}^2\cdot\text{day}$ ($\text{SD} = 0.21$) with corresponding rates for Fe (II) of $0.07 \text{ g/m}^2\cdot\text{day}$ ($\text{SD} = 0.11$) and Fe (III) of $0.12 \text{ g/m}^2\cdot\text{day}$ ($\text{SD} = 0.16$). Reduction was observed across both iron species although proportionally greater in terms of Fe (III) consistent with its relative abundance (Figure 4.3). Although the data showed no indications of iron reductive transformations, intermittent cycling of iron species within the bed is suspected. Iron and especially iron (II) removal was the lowest at the NA-Fe site, where episodes of P release occurred. Indeed, a low Fe (II) mass removal rate of $0.005 \text{ g/m}^2\cdot\text{day}$ ($\text{SD} = 0.007$) coincided with a low or negative TP mass removal rate $0.04 \text{ g/m}^2\cdot\text{day}$ ($\text{SD} = 0.11$). Total phosphorus and iron mass removal rates were strongly positively

correlated ($r = 0.906$, $p < 0.01$) over all sites. Significantly higher mass removal rates were found in the aerated reed bed site.

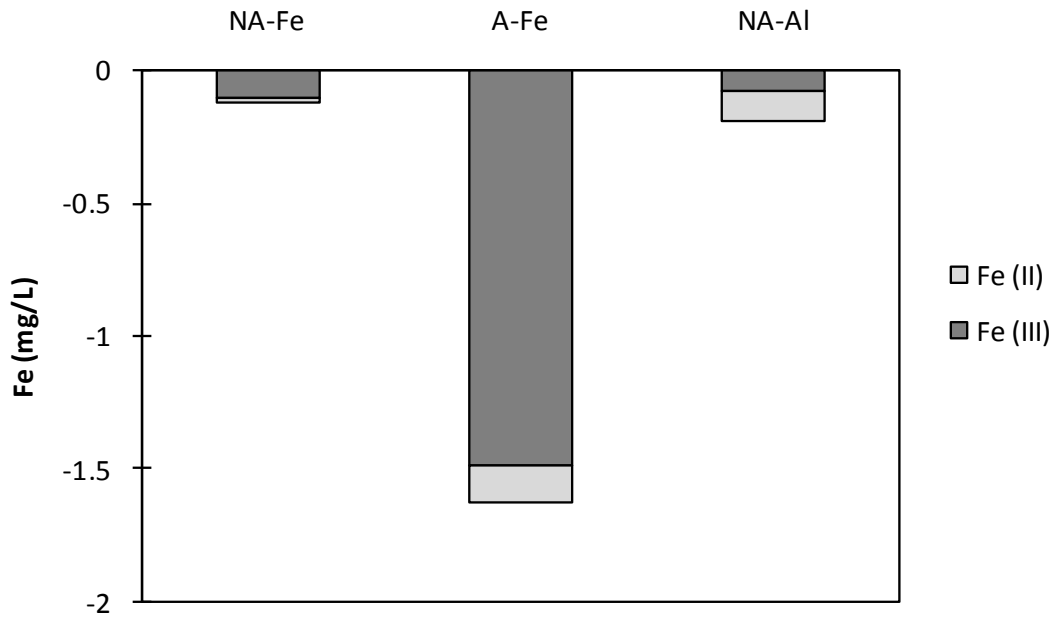


Figure 4.3: Average iron (II) and iron (III) wastewater concentration change between 2° and 3° effluent at 3 sites ($N_{NA-Fe} = 5$; $N_{A-Fe} = 3$; $N_{NA-AI} = 3$).

4.1.3 Interdependency of potential phosphorus release factors

Across all three sites and considering all sampling points together ($N = 33$; $p < 0.05$; $-0.7 > r > 0.7$; Appendix H.1), positive correlations of TP and PO_4 -P concentrations were found with COD, $cBOD_5$, TSS and VSS. Additionally, these four factors, with some exceptions, were also consistently correlated with each other - possibly indicating that a higher amount of P is simply caused by higher loading of organic material in the form of suspended solids in the crude influent. Such an explanation is supported by the fact that TP/ PO_4 -P and concentrations of the above mentioned factors were additionally correlated when considering crude sampling points individually, but not when individual 2° and 3° effluents were taken into account.

Both TP and PO₄-P concentrations correlated positively with Fe (II), with PO₄-P also correlating with Fe total.

In the 3° effluent, PO₄-P concentrations in wastewater negatively correlated with redox potential, linking the increase of PO₄-P in the liquid phase to reducing conditions. The 3° effluent TP and/or PO₄-P concentrations also positively correlated with wastewater temperature, ambient temperature, EC, pH and SO₄²⁻. Temperature can accelerate the metabolism of iron reducers in hydric soils (Rabenhorst and Castenson, 2005). Phosphates in 3° effluent were negatively correlated to NO₃-N concentration ($r = -0.65$), supporting the assumption that nitrate is able to limit the reduction of iron and subsequent P release by carbon competition (Lovley 1991; Hume *et al.* 2002).

Regarding TP release and PO₄-P release, negative correlations were found with TP and PO₄-P concentrations in the 2° effluent (Table 4.2) i.e., the higher the TP/PO₄-P in wastewater entering the reed bed, the lower the TP/PO₄-P release from the reed bed. This suggests a potential role of concentration gradients.

A negative correlation was found between effluent Eh and TP release ($r = -0.77$), supporting the expected link between P release and reduced conditions in the environment. Total iron, Fe (II) and Fe (III) in 2° effluent negatively correlated with PO₄-P release ($r = -0.90$, -0.82 and -0.89 respectively), suggesting that increased iron inflow to the reed bed enhanced P retention.

Phosphate and TP correlations of distinct fractions did not match the correlations of overall PO₄-P and TP correlations (Appendix H.2), which suggests that the flux in between individual fractions is not significantly related to concentrations of TP/PO₄-P in the wastewater. Additionally hardness and magnesium concentration was positively correlated with TP release of suspended fraction.

Table 4.2: Pearson correlations (sig. 2-tailed) of calculated TP and PO₄-P release to wastewater constituents and characteristics based on analyses and measurements carried out across 3 sites. Larger fields express correlations of all sampling points in general. Smaller fields each represent an individual sampling point: crude (top), secondary effluent (middle) and tertiary effluent (bottom). Correlations of only $-0.7 \geq r \geq 0.7$ are included. N of all sampling points correlations = 33; N of individual sampling points correlations = 11; $p < 0.05$.

	TP release (mg/L)	PO ₄ -P release (mg/L)
Eh (mV)		
	-.77	
pH		
		.71
TSS (mg/L)		
	.75	
NH ₄ -N (mg/L)		
		-.86
Total P (mg/L)		
	-.70	
PO ₄ -P (mg/L)		
		-.93
Fe total (mg/L)		
		-.90
Fe (II) (mg/L)		
		-.82
Fe (III) (mg/L)		
		-.89
Mg (mg/L)	0.72	

4.2 Phosphorus and iron transformations

4.2.1 Impact of convection on P and Fe flux

In order to assess the impact of hydrodynamics on flux rates of P and Fe, results from reactors in dynamic and static conditions were compared. While in the static reactors all the changes during the experiment can be attributed to molecular diffusion, dynamic conditions are driven by both convection and diffusion factors. The difference in flux between static and dynamic conditions can then be attributed to the influence of convection:

$$\frac{1}{K} = \frac{1}{k_{diffusion}} + \frac{1}{k_{convection}} \quad (4.1)$$

A significant linear relationship was observed when comparing TP concentrations observed in dynamic and static test reactors ($r = 0.532$; $p < 0.01$; $N = 27$) and dynamic and static AIS reactors ($r = 0.679$; $p < 0.01$; $N = 27$; Figure 4.4). The AIS reactors were found to consistently correlate throughout the entire duration of experiments, whereas in the test reactors a significant difference was observed in the first 4 hours when increased convection in dynamic conditions resulted in a higher P sludge uptake. Total phosphorus concentrations in the test reactors decreased in the first 4 hours of the experiment, reaching a minimum at the 1st hour (0.36 mg/L) in dynamic reactors and at the 4th hour (0.49 mg/L) in static reactors. Beyond that time period, however, an increase of TP concentrations took place, reaching the highest levels at the 72nd hour (1.17 mg/L) in dynamic reactors and at the 24th hour (0.93 mg/L) in static reactors. High variability between replicates was observed, with a range as high as 1.27 in test reactors and 0.28 in AIS reactors. In general, greater fluctuation between sampling times was found between concentrations of dynamic and static conditions in test reactors (range: static 1.36; dynamic 1.65) than in AIS reactors (range: static 0.24; dynamic 0.22). It can be concluded that biologically active sludge is more affected by agitation than inorganic sludge in terms of P mass transfer. Phosphate levels followed a similar pattern as TP and will not be further discussed here.

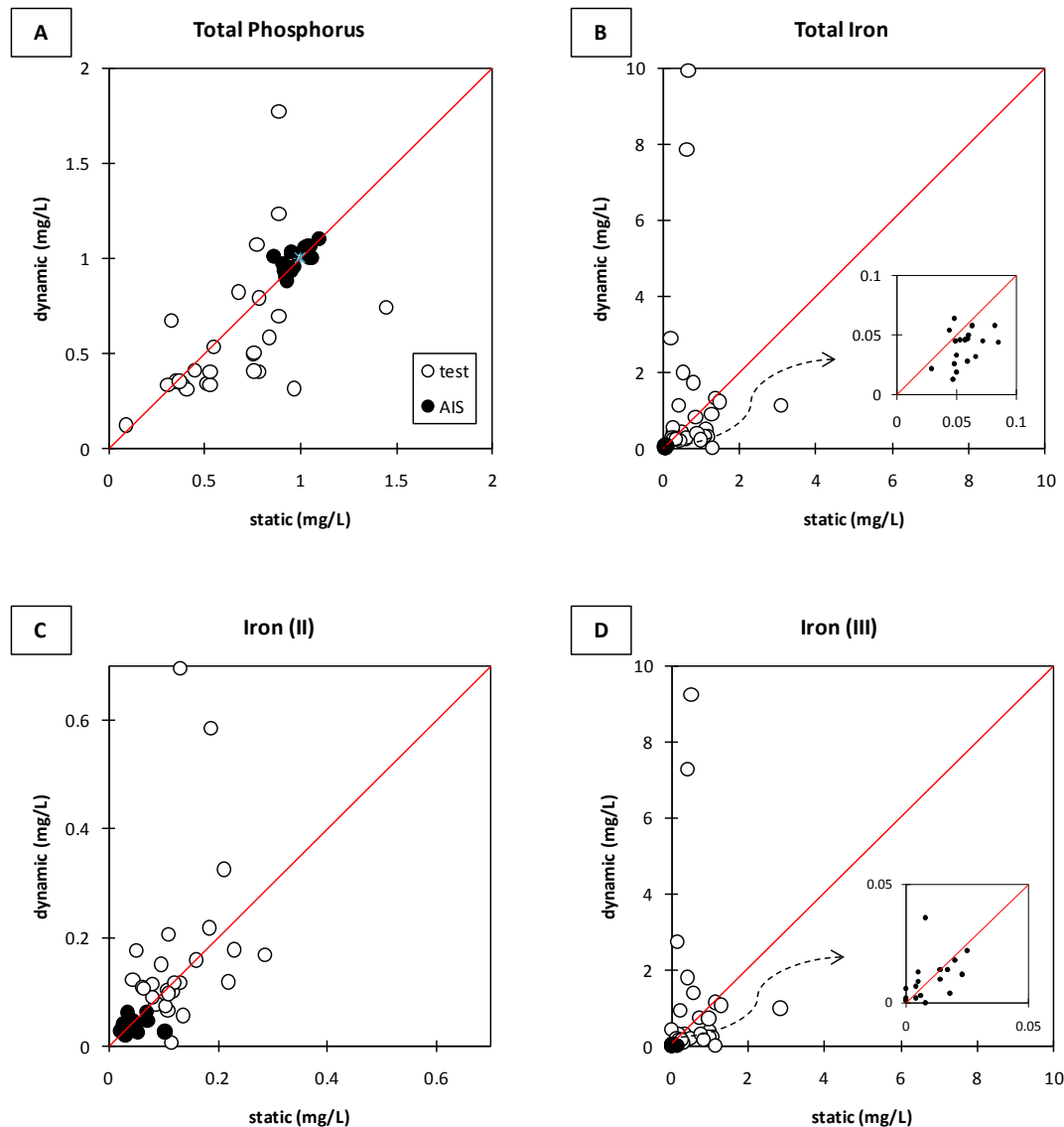


Figure 4.4: Wastewater constituents' concentrations under static vs dynamic conditions: A) total phosphorus, B) total iron, C) iron (II) and D) iron (III). Enlargements are used to show values close to zero. The red parity line of perfect fit represents a scenario of no difference between static and dynamic.

Trends in iron proved to poorly correlate, especially in a biological active environment. Total iron linear regression ($N = 27$) was not significantly correlated for both test ($r = 0.018$; $p > 0.05$) and AIS reactors ($r = 0.493$; $p > 0.05$). Similarly for Fe (II) ($N = 27$) in test reactors ($r = 0.307$) and in AIS reactors ($r = 0.077$) and Fe (III) ($N = 27$) in test reactors ($r = 0.056$) and in AIS reactors ($r = 0.194$), all with $p > 0.05$.

Total iron, Fe (II) and Fe (III) in AIS reactors remained at low levels during the experiment, with Fe total not exceeding 0.06 mg/L. In contrast, the Fe total concentrations in test reactors increased from an initial value of 0.16 mg/L 25-fold during the dynamic conditions and 7-fold during the static conditions across the first 5 days (Figure 4.5). Iron (II) and iron (III) concentrations followed similar trends over the time with a progressive fluctuating release. Mean proportions of Fe total : Fe (II) : Fe (III) were similar between dynamic and static conditions in all the reactors, when differences between Fe total-Fe (II) and Fe total-Fe (III) were considered as independent variables ($p > 0.05$).

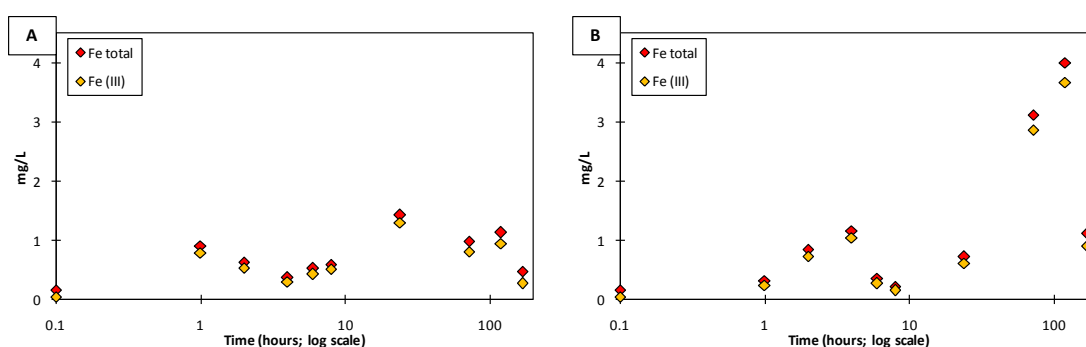


Figure 4.5: Total iron and iron (III) in liquid phase of test reactors: A) static and B) dynamic conditions.

Evidence of biological activity was found upon analysis of DO, Eh and chemical composition of the wastewater during the experiments. While DO in the AIS reactors remained at between 7 and 9 mg/L, oxygen in the test reactors depleted with DO dropping to under 1 mg/L within 1.5 hours. After 120 hours DO dropped to less than 0.2 mg/L and the Eh to below -200 mV. Iron release started when the Eh values changed from -50 to -100 mV, i.e. inside the acknowledged range -100 to +100 mV of biological reduction of Fe (III) oxides in wetland soils (Reddy and DeLaune, 2008). In the liquid phase iron (III) was predominately observed which reflects the potential reoxidation of Fe (II) during sampling (Manahan, 2001).

Nitrate levels, similarly to DO in the test reactors, dropped dramatically during the first 8 hours, from an initial 14.3 mg/L to 0.32 mg/L in the dynamic tests and

to 1.72 in the static tests and stayed near zero levels until the end of experiments. In contrast, the level of nitrate in the AIS reactors remained at 14.5 mg/L. Sulphate concentrations had a similar pattern of progress over time when compared to nitrate, only the decrease in test reactors was slower and lagged. It resulted, however, in a drop from an initial 112 mg/L to 3.6 mg/L in static and 19.8 mg/L in dynamic reactors measured in the 72nd hour, maintaining around 5 mg/L until the end of the experiments. In contrast, SO_4^{2-} in AIS reactors increased for the whole period, reaching the level of 127 mg/L in static and 115 mg/L in dynamic conditions. The impact of electron acceptor depletion on P release will be described in more detail in section 4.2.3.

Release of ions as a result of decomposition of organic matter affected the EC of the liquid phase, which increased during the experiments in all the reactors, but especially in the case of the test reactors: the EC started around the same level as in the blank reactors at 800 to 1000 $\mu\text{S}/\text{cm}$ and then doubled to 2000 $\mu\text{S}/\text{cm}$.

The reed bed's hydraulic residence time (HRT) should be taken into account when considering the impact of convection. Various authors used the simplest model described by Kadlec and Wallace (2009) of HRT calculated from the volume of water in the reed bed divided by influent flow rate. Caselles-Osorio and Garcia (2007) used HRT in the range from 2 to 6 days in their pilot experiments with SSF constructed wetlands. Tertiary reed beds in STW usually have a lower residence time: of around one day (Prieto Fort, 2013; Butterworth, 2014). Hydraulic residence time of the NA-Fe site in the period of sampling was 1.85 days. Bearing this in mind, the first two days of the experimental run are of key importance. Both P flux and especially Fe flux were significantly affected by convection in this period as demonstrated above.

4.2.2 Concentration driven transformations

The blank reactor TP concentrations remained constant during the entire length of experiments. The test and AIS reactors had common TP flux dynamics, with three distinct stages. The first stage occurred in the first 10 minutes of the experiments and can be described as a very fast initial flux of P (uptake into sludge, except from ^{0.1}Reactors). The second stage occurred during approximately the first 8 hours and can be characterised as a lag phase - a stage when the rate of P flux is relatively slow. In this stage, different trends were found between reactors with different initial concentrations, however, no difference was found between biologically active and inorganic conditions. The third stage can be described as a period of increased rate of P release into the liquid phase and lasted until the end of experiments. Overall, biologically active and AIS reactors followed similar trends at a similar rate (Figure 4.6). Phosphate concentrations developed in a congenerous manner to TP concentrations, thus they will not be discussed further here.

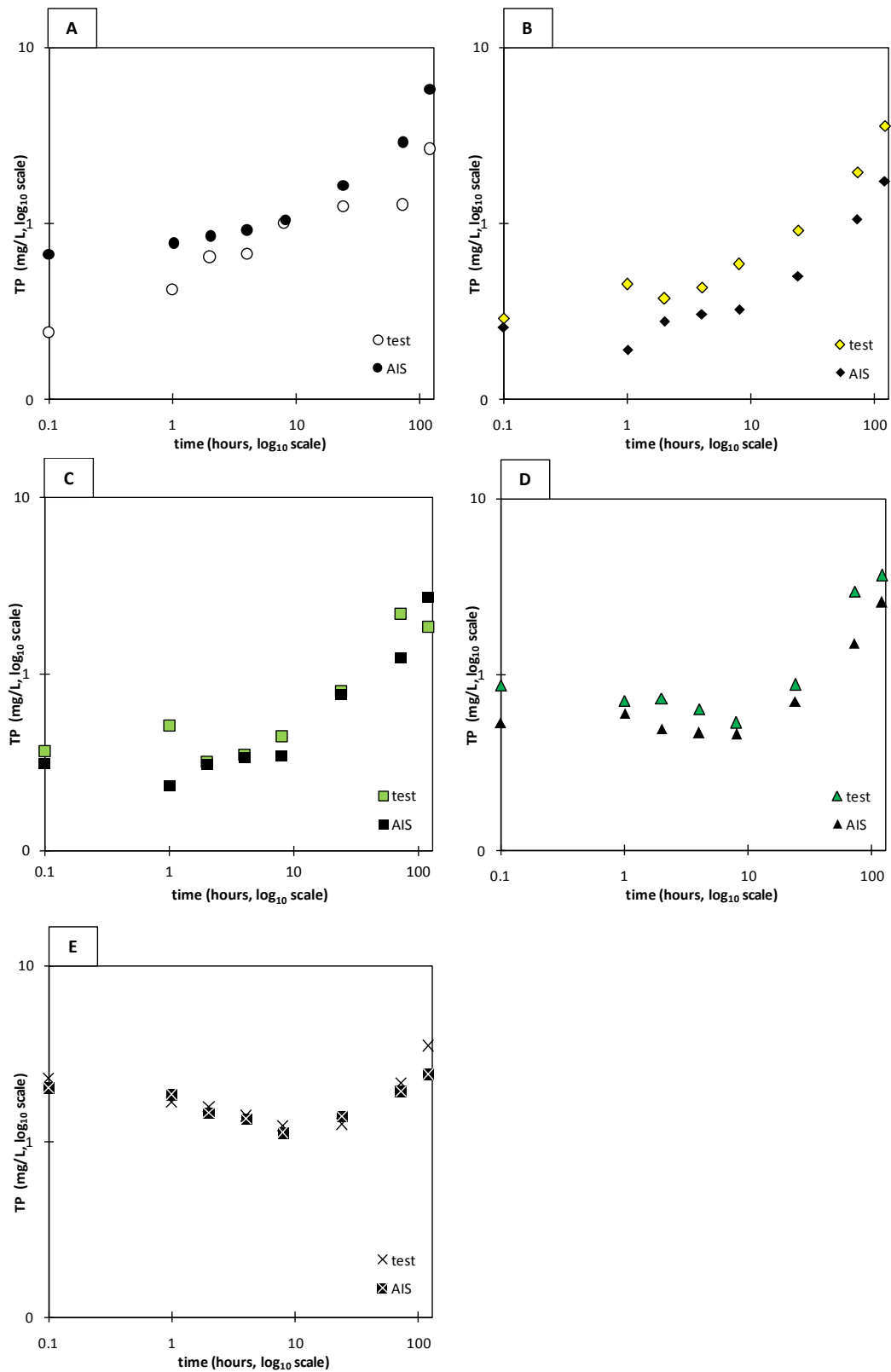


Figure 4.6: Total phosphorus concentrations of the liquid phase during the experiments in reactors of distinct initial concentrations (mg/L): A) 0.1; B) 0.5 C) 1; D) 2 and E) 5 in log scale.

The first stage of fast P flux is characterised by balancing the concentrations between different reactor series. All reactors except ^{0.1}Reactor series saw a decrease in the liquid phase P concentration (Figure 4.7). On the contrary, phosphorus in ^{0.1}Reactors during the same time increased. This suggests that all the reactor series moved towards a common equilibrium point, although never reaching it. It should also be noted that the equilibrium point may be common only theoretically, as it depends on the instantaneous TP concentration of sludge, which always differs to some extent between reactors. The equilibrium point in the 10th minute at the end of stage 1 was estimated to be between 0.1 mg/L and 0.5 mg/L.

The flux rate of this stage also derives from the initial liquid phase TP concentration in the reactors. The initial TP flux rate increased depending on the distance between the initial TP concentration and the hypothetical instantaneous equilibrium point.

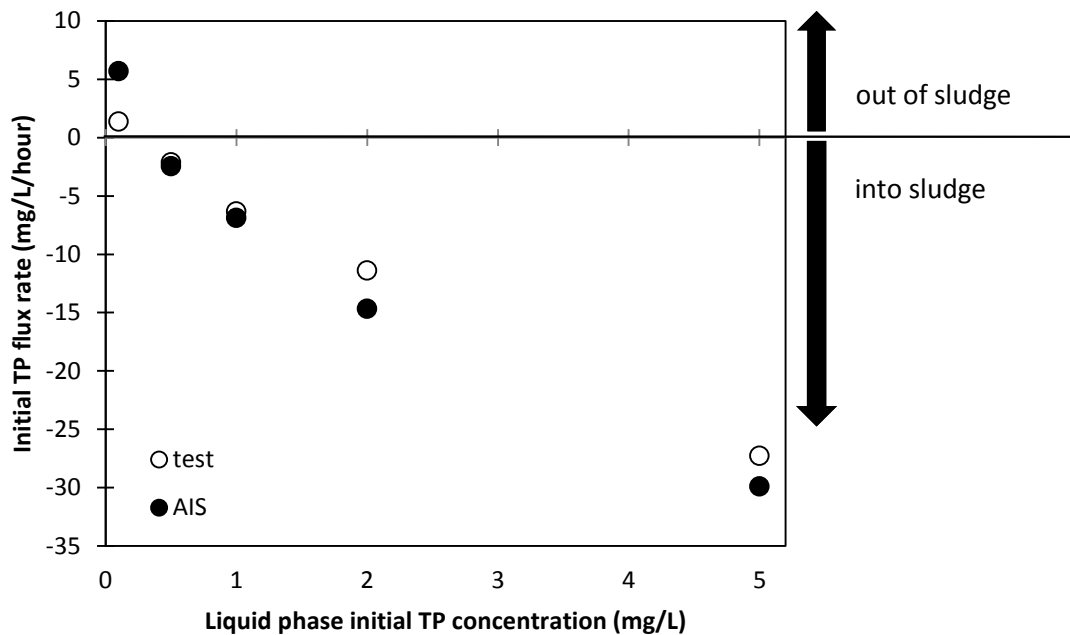


Figure 4.7: Total phosphorus flux rate concentration of the liquid phase in the initial stage of experiments (first 10 minutes) against the liquid phase initial TP concentration of distinct reactor series.

In the second stage of the process, the instantaneous concentration gradient between liquid and solid still dominated the trend. This was evidenced by comparing the slope of linear regression of TP change during the second stage plotted against the initial liquid phase TP concentration in the reactors (Figure 4.8A), where concentrations decreased in ⁵Reactors and ²Reactors, were in constant flux in ¹Reactors and ^{0.5}Reactors and increased in ^{0.1}Reactors.

The ⁵Reactors' total phosphorus flux saw the highest uptake into sludge, while ^{0.1}Reactors' TP concentration saw the highest release out of sludge, suggesting that the hypothetical equilibrium point was close to 1 mg/L. Differences between test reactors and AIS reactors were again minimal.

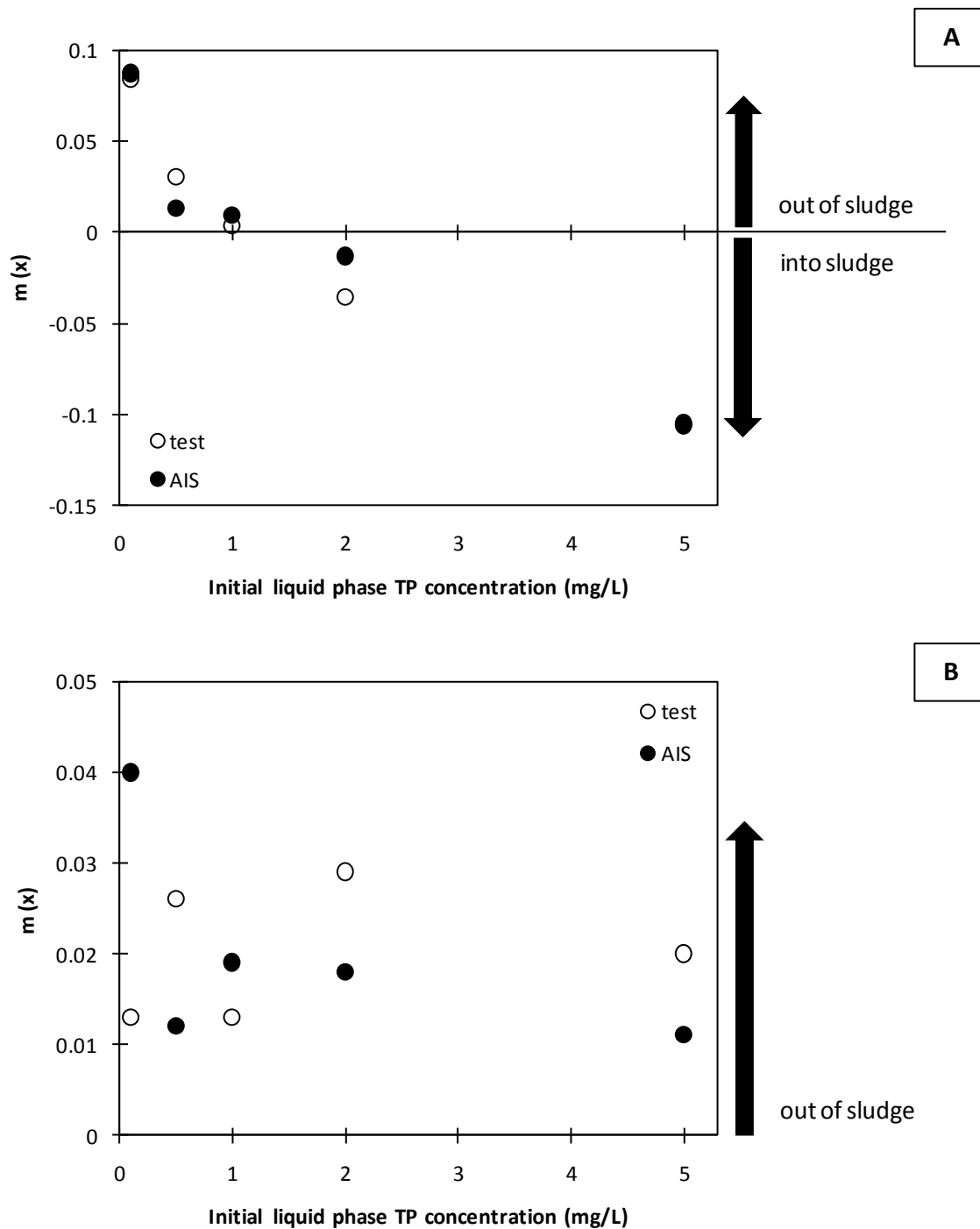


Figure 4.8: Linear regression slope (m) of total phosphorus concentration flux change in the second stage of experiments (interval 10th minute to 8th hour) (A) and the third stage of experiments (from 8th hour) (B) vs the initial liquid phase TP concentrations in reactor series (0.1, 0.5, 1, 2, 5 mg/L).

During the third stage of experiments, from the 8th hour onwards, no uptake into sludge took place and the assumed common equilibrium point moved above the level of all the instantaneous concentrations in the reactors. The slopes of linear regression of TP change showed no particular differences between reactor series nor patterns (Figure 4.8B). The average flux rate at the end of the experiment was 0.048 mg/L·hour for test reactors and 0.050 mg/L·hour in AIS reactors, the highest being in ^{0.1}AIS reactors at 0.099 mg/L·hour.

The TP : PO₄-P ratio in the test reactors varied between sampling times and also between reactor series. In general, contribution of PO₄-P to TP concentrations rose from an initial 29% to 54% and 60% in the 8th and the 24th hour respectively, which is the beginning of the identified 3rd phase of P flux, when net P release took place. After that point relative PO₄-P concentration slowly decreased again. Phosphate released between the 8th and 24th hour possibly formed bonds with organic matter present in the liquid phase, which was reflected in its relative decrease in the later stage. The concentrations in distinct reactor series fluctuated less than the TP concentrations over time.

4.2.3 Impact of competing electron acceptors on Fe and P flux

Typical Eh intervals (mV) for reduction of electron acceptors in wetlands have been reported to be > +300 for O₂, +100 to +300 for NO₃⁻, -100 to +100 for Fe (III) and < -100 for SO₄²⁻ (Reddy and DeLaune, 2008). These indicators were monitored in order to assess microbial respiration in the reactors (Figure 4.9). The concentrations of all electron acceptors and change indicators were stable throughout the experiment for the blank reactors. In general, all of them were found to occur in lower Eh levels than those stated above. Dissolved oxygen in the test reactors decreased from an average of 9.4 mg/L at the start of experiments to 1.2 mg/L measured after 3.5 hours. When concentrations dropped below 1 mg/L, there was an observable impact on iron and phosphorus release. At that stage, Eh conditions had decreased from an initial value of 170 mV to -90 mV.

Such a trend was not observed in the AIS reactors where DO conditions remained stable at around 9 mg/L. In contrast to the test reactors, a progressive increase in Eh was observed to an average of 478 mV at the 120th hour. As AIS reactors were not seeded, biological activity was expected to be minimal; thus, the changes in Eh observed in the AIS reactors were governed completely by chemical and physical factors. This also supports that the Eh decrease in the test reactors was biologically mediated. The redox potential in the blank reactors remained relatively stable during the experiments, mostly in the range of +150 to +200 mV.

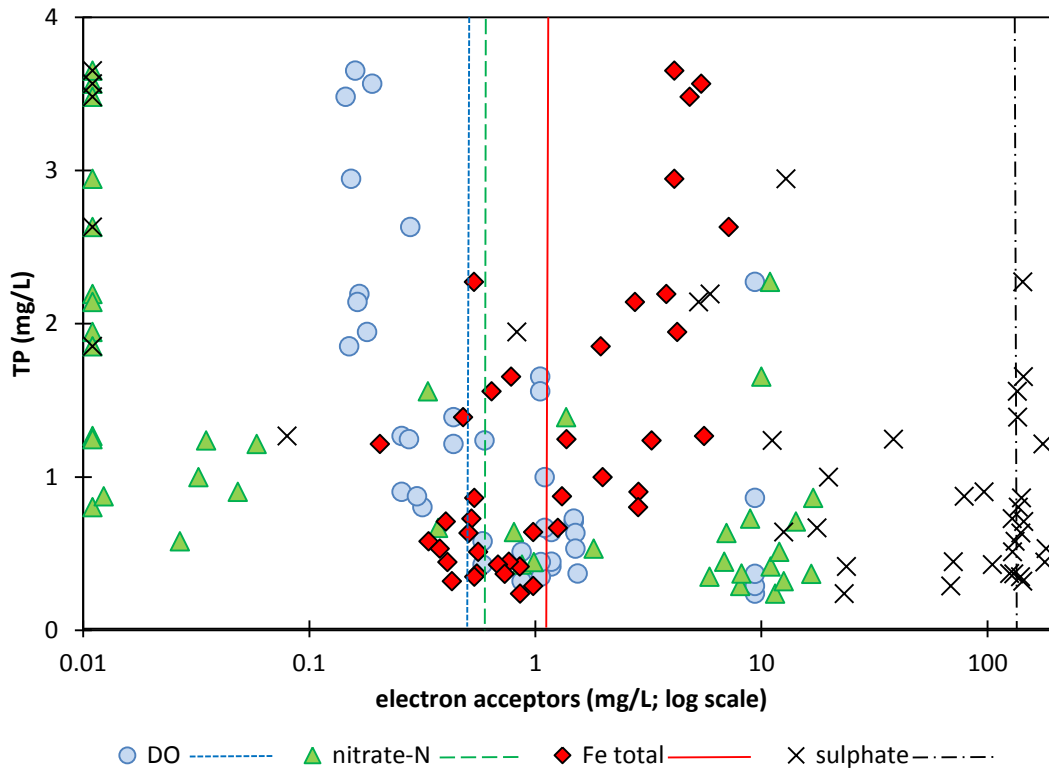


Figure 4.9: Concentrations of total phosphorus vs electron acceptors (dissolved oxygen, nitrate, total iron and sulphate) on a log scale over all test reactor series in Experiment 2. Each point represents a pair of one averaged triplicate measurement. The lines represent a critical point, when simultaneous DO and NO_3^- depletion, Fe release and SO_4^{2-} decrease start to have impact on P release.

Nitrate concentrations in all series of test reactors decreased from an initial average of 12.8 mg/L during the first 8 hours below 1 mg/L and continued to decrease to non-detectable levels. A nitrate trigger level below which the consistent release of iron and phosphorus occurred was observed at around 0.58 mg/L (Figure 4.9). Nitrate depletion occurred in parallel with DO depletion, in agreement with the findings of Laanbroek (1990).

In terms of Fe total, the test reactors showed a small decrease of concentrations within the first 8 hours, after which they had an increasing trend. When the Fe total values in the liquid phase rose above 1 mg/L, P release was observed. Simultaneous P and Fe release in test reactors after the 8th hour is assumed to be the result of microbially mediated Fe (III) oxides reduction, which releases free Fe (II) into the liquid phase (Nevin and Lovley, 2002). As a consequence of this transformation, phosphorus present in iron-oxo-hydroxide complexes may be released.

The phosphorus and iron liquid phase concentrations' correlation was checked for significance, however the only significant correlation was found in ²Reactors (Pearson; N = 9, p < 0.05; r = 0.764) and ⁵Reactors (N = 9; p < 0.05; r = 0.794).

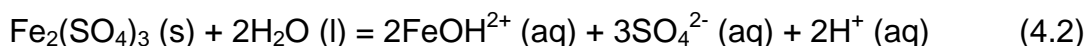
Sulphate concentrations in test reactors remained at a stable level around 150 mg/L until the 8th hour, then started to slowly decrease, and reached zero levels at 120th hour. The 8th hour corresponded to the same time point when nitrogen concentrations reached the level of severe depletion and it is anticipated that as NO₃⁻, also SO₄²⁻ was consumed in biological respiration, producing sulphide, and consequently increasing pH and further decreasing Eh. Simultaneous Fe release, TP release and SO₄²⁻ uptake was first observed at -150 mV. Redox potential continued to decrease until the end of experiments to an average of -232 mV at the 120th hour. Dissolved ions in the test reactors raised EC of the liquid phase from an average of 825 µS/cm at the 8th hour by 69% compared to the 120th hour.

In AIS reactors, SO₄²⁻ increased sharply after 24 hours, never dropping below 500 mg/L.

4.2.4 Impact of pH on Fe and P flux

The pH conditions in test reactors remained in the range between pH 7 and 8, consistent with the results recorded in the field survey.

On the contrary, conditions in the AIS reactors became increasingly acidic during the experiments, falling to an average of pH 2 at the 120th hour. This is an important difference, as whilst the P flux had a very similar trend to the test reactors, the drivers of change could be entirely different, principally based on hydrolysis. This was reflected by an increase of ions in the liquid phase (Figure 4.10). Part of the hydrolytic reactions believed to be the contribution of yet unreacted Fe₂(SO₄)₃ hydrolysis:



Upon reaction of Fe₂(SO₄)₃ with water, sulphate as an anion of a strong acid stayed dissociated, while Fe (II) attached to OH⁻ of a water molecule and released H⁺ into the solution, thus increasing pH. This is in agreement with the SO₄²⁻ trend and the EC changes. A general release of the dissolved particles in the liquid phase was reflected by dramatic EC changes in all the reactor series, progressively and steadily increasing during the whole experiment. From an average of 782 μS/cm at the 8th hour it rose by 204% to an average of 2379 μS/cm at the 120th hour. It is assumed that Na⁺, H⁺ and SO₄²⁻ were the main contributors, while Fe²⁺ and PO₄³⁻ had a comparatively minor influence.

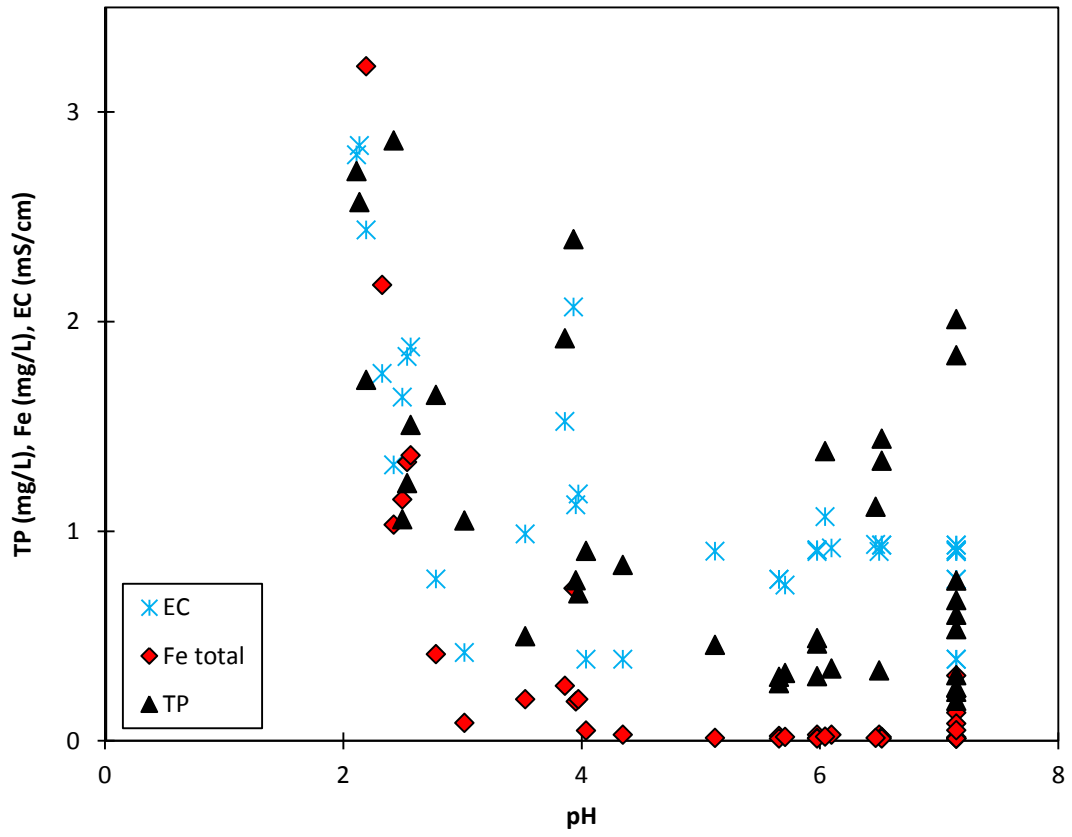
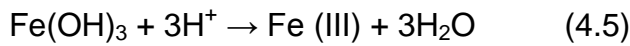
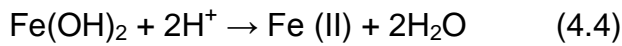
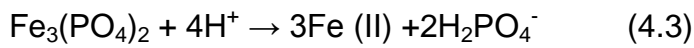


Figure 4.10: pH vs concentrations of total phosphorus, total iron and electrolytic conductivity in AIS reactor series. Each point represents a pair of one averaged triplicate measurement.

Iron concentrations in the liquid phase were negligible compared to sulphate, however, they are assumed to have a major impact on P flux. As dissasociation of FePO_4 (equation 2.7), $\text{Fe}_3(\text{PO}_4)_2$ and various iron hydroxides in reducing pH took place, dissolved fraction of both elements in the liquid phase increased (Levlin and Hultman, 2004):



The solubility of both FePO_4 and iron hydroxides increases together with pH if below pH 5 (Jenkins *et al.*, 1971). Nevertheless, P adsorbed to $\text{Fe}(\text{OH})_3$ was found to increase with decreasing pH when tested in the interval from 5 to 8 (Lijklema, 1980). Concentration values in the AIS reactors remained near 0 during the first 4 hours, then increased 6.4-fold from the 8th to the 24th hour and continued irregularly increasing until the end of experiments. It can be concluded that a relationship between iron and P release existed, independent of biological activity and linked to pH (Stumm and Morgan, 1996).

The molar ratios of TP : Fe released in AIS reactors from the 8th to 24th hour above 1 indicate that P was dissociated from adsorption sites of oxo-hydroxide complexes, supposedly without dissolution of the iron compounds (Figure 4.11). Later the ratios were found to be closer to 1 in most of the reactors, suggesting dissolution of P-Fe compounds. The opposite trend in ^{0.1}Reactors may be the consequence of less P being adsorbed to the solid phase in the first and second stages of the experiment.

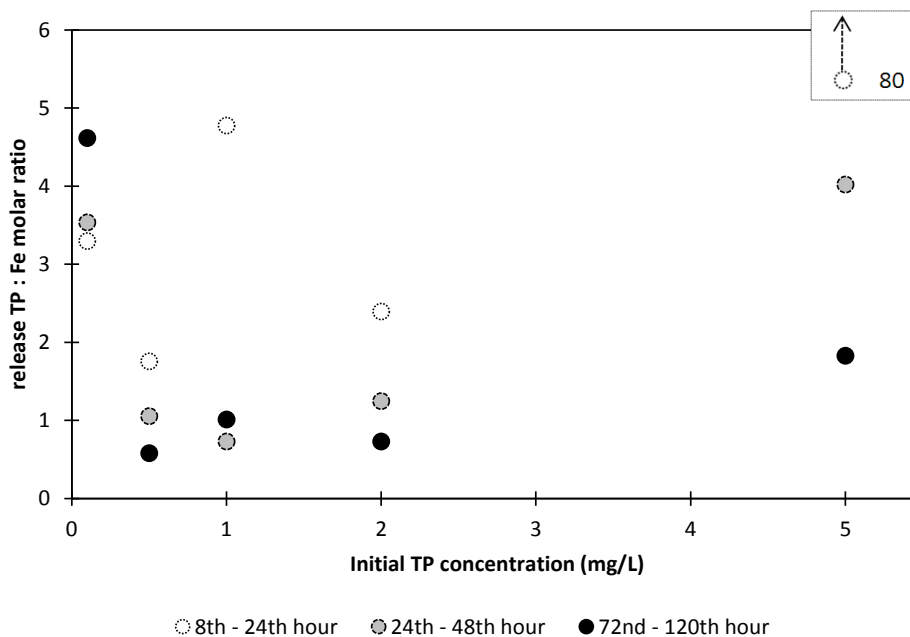


Figure 4.11: Released total phosphorus and total iron expressed in TP : Fe molar ratio in AIS reactors in the 3rd stage of experiments (enhanced P release period) in the intervals of 8th-24th, 24th-48th and 72nd-120th hour. Molar ratio of 5 mg/L data point in 8th-24th interval reached 80 (separated dotted frame).

5 DISCUSSION

A key observation from the work relates to the ecosystem's tendency to maintain a P balance between the liquid and solid phases (Figure 4.6). This was demonstrated in the field survey in that the level of phosphorus released from the bed decreased when higher concentrations of P existed in the liquid phase entering the reed beds. Consequently, if the equilibrium point can be established for a given moment and providing the P concentration of the influent liquid phase exceeds it, the solid phase will retain excessive amounts. Analogously, if the P concentration of the influent liquid phase is below the equilibrium point, the solid phase will then release phosphorus.

The work outlined within the thesis indicates that three main pathways are responsible for P flux within a reed bed:

- 1) adsorption of phosphorus to iron oxo-hydroxide complexes;
- 2) complexation of P with FePO_4 ;
- 3) assimilation of P by microorganisms (living or dead).

Various mechanisms may be involved in relation to each of these sinks. In general, in each of them, biological activity, directly or indirectly, can play a role, however some of the mechanisms occur on a purely chemical basis. Certain mechanisms are directly part of the flux based on the concentration gradient itself. Others, both chemical and biological, such as hydrolysis or microbial reduction of electron acceptors, contribute either to P release or P uptake which consequently trigger additional P flux based on the concentration gradient in the water-sediment interface at a given moment, i.e. they switch the instantaneous equilibrium point. The influence of the concentration gradients on those indirect mechanisms is anticipated, nevertheless, the influence of initial P concentrations in the given range was not observed or monitored (Table 5.1).

Table 5.1: Overview of the most important processes possibly involved in observed phosphorus flux.

P sink	P flux mechanism*	What would that look like?**	Impact of (initial) liquid phase P concentration	How to mitigate P release?
Adsorbed to solid phase surface (iron oxo-hydroxides)	Electrostatic adsorption/desorption	Dissolved $\text{PO}_4\text{-P}$ (l) \uparrow/\downarrow according to instantaneous concentration in liquid and solid phase	Balancing PO_4^{3-} levels towards equilibrium point; fast: mainly first 10 minutes	Influent TP > 0.5 mg/L; HRT < 8 hours
	Bio mediated Fe (III) reduction Possibly reinforced by SO_4^{2-} reduction	Preceded or coupled with: pH \uparrow ; DO \downarrow ; $\text{NO}_3\text{-N}$ \downarrow ; Potentially also SO_4^{2-} \downarrow ; Resulting in: Fe (III) (s, l) \downarrow ; Fe (II) (s, l) \uparrow ; easily monitored by dissolved Fe (II) (l) $\uparrow \rightarrow \text{PO}_4\text{-P}$ (l) \uparrow	No impact observed	O_2 supply or $\text{NO}_3\text{-N}$ in excess of 15 mg/L; avoid flooding
Complexed with Fe (iron oxo-hydroxides, iron phosphate)	P concentration gradient driven precipitation/ dissolution of FePO_4	Simultaneous \uparrow/\downarrow of dissolved Fe (II) (l) and $\text{PO}_4\text{-P}$ (l) in close to 1 : 1 ratio Dissolution additionally: colloidal and suspended Fe total (l) \uparrow and/or TP (l) \uparrow if larger molecules released	Balancing PO_4^{3-} levels towards equilibrium point	Influent TP > 0.5 mg/L; HRT < 24 hours
	P concentration gradient driven precipitation/ dissolution of oxo-hydroxide complexes	Simultaneous \uparrow/\downarrow of dissolved Fe (II) (l) and $\text{PO}_4\text{-P}$ (l) in uneven ratio Dissolution additionally: colloidal and suspended Fe total (l) \uparrow and/or TP (l) \uparrow if larger molecules released	Balancing PO_4^{3-} levels towards equilibrium point; slow: hours, days	Flow control, even P supply; influent TP > 0.5 mg/L; HRT < 24 hours
	Hydrolysis and dissolution of iron compounds in low pH; FePO_4 lowest solubility at pH 5 – 6 and oxo-hydroxide solubility increase with pH decrease	pH \downarrow ; Eh \uparrow ; DO stable; EC \uparrow ; ions (l) \uparrow ; SO_4^{2-} (l) \uparrow observed after 8 hours; Fe (l) increase observed after 1 day	No impact observed	Maintain pH > 5

	Bio mediated Fe (III) oxides to Fe (II) hydroxides reduction and FePO ₄ reduction	Simultaneous ↑ of dissolved Fe (II) (l) and PO ₄ -P (l) in uneven ratio in case of oxo-hydroxides or close to 1 : 1 ratio in case of FePO ₄ Additionally: colloidal and suspended Fe total (l) ↑ and/ or TP (l) ↑ if larger molecules released Observed starting at DO < 1 mg/L, NO ₃ -N < 1 mg/L, Eh < -90 mV	No impact observed	O ₂ supply or NO ₃ -N in excess of 1 mg/L; avoid flooding
Biologically bound	Release following death of organisms	TP (l) ↑; Colloids (l) & SS (l) ↑ as dead matter detach from the substrate	Not monitored: extent unknown but believed to be minimal	Unavoidable
	Uptake during metabolism of organisms	PO ₄ -P (l) ↓	Potential increase with P (l) increase if P is limiting nutrient, but not monitored	Desirable
	Controlled excess P release when supply of P is limiting	PO ₄ -P ↑ from near-zero levels	Balancing PO ₄ ³⁻ levels up to biological equilibrium point; not monitored	Flow control ensuring even P supply

* biologically based mechanisms in green

** (l) = liquid phase; (s) = solid phase; ↑ = increase; ↓ = decrease

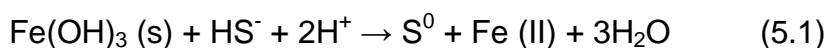
Every type of wetland bed media has a certain P saturation point (Pant *et al.*, 2001; Del Bubba *et al.*, 2003). When it is reached, no retention of P takes place, regardless of the P concentration of wastewater such that negative P fluxes may occur when the overlaying liquid phase is low in phosphorus.

One of the key mechanisms behind such ecosystem phosphorus buffering is suggested to be related to an ion exchange pathway where PO_4^{3-} molecules are attracted to the positively charged sites on solid surfaces, including iron hydroxide. Consequently, the pathway includes a pH dependency through the amphoteric nature of iron hydroxide with the switch in the charge of the surface depicted through the isoelectric point, pH 8.5 (Parks, 1965). It is anticipated that most of the adsorption reaction takes place in the initial stages of contact, as illustrated through the fast initial uptake in both test and AIS reactors (Figure 4.7). In addition, penetration into the solid phase and the formation of multilayer complexes (precipitates) of iron oxo-hydroxides and FePO_4 need to be considered. It is suggested that such pathways are more likely when the driving force is maximised and hence when the liquid phase P concentrations are higher. Given that dissolution of P precipitates occurs at a slower rate than PO_4^{3-} desorption, the latter is assumed to dominate during release.

However, phosphorus equilibrium is not necessarily controlled only by chemical means. Bacteria are also known to be capable of buffering P concentrations, releasing excess cellular P, when the external P supply into the liquid phase is not sufficient (Manahan, 2001). In addition, many microorganisms can use the particles associated with P in the reed bed, e.g., organic matter complexes, iron hydroxide complexes and ferric phosphate precipitates, as a source of energy. The progressive use of different electron acceptors was observed in the experiments in the following order: DO together with NO_3^- followed by Fe together with SO_4^{2-} . In the former both occurred despite differences in the required Eh conditions ($> +300$ mV and $+100$ to $+300$ mV for DO and NO_3^- respectively; Reddy and DeLaunne, 2008) which is consistent with previous reports of the simultaneous use of both nitrate and oxygen (Kuenen and

Robertson, 1994; Matsuzaka *et al.*, 2003; Yao *et al.*, 2013). Similarly, simultaneous iron and sulphate reduction can take place, observed to start at an Eh of approximately -90 mV (Figure 4.9), although typical Eh values reported in the literature for the two species are +100 mV and -100 mV for iron and sulphate respectively (Reddy and DeLaunne, 2008).

As discussed earlier, reduction of iron compounds may lead to breakage of its bonds with phosphorus and manifests itself as a release of Fe (II) and PO₄³⁻ into the liquid phase. Importantly, the potential for simultaneous Fe (II) and PO₄³⁻ release into the liquid phase was identified during the field survey through a negative correlation between Eh in 3rd effluent and TP release, although no increase of liquid phase Fe (II) was observed during the trials. The likely pathways for such reductions can be attributed to catabolic (i.e. dissimilatory reduction) and, to a lesser extent, to anabolic processes (i.e. assimilatory reduction) with the excreted metabolic products able to reduce iron (Lovley, 1991). The latter was not seen in the current work, as the pathway is known to be inhibited under neutral to slightly basic environments, as seen in the field survey. Furthermore, reduced sulphate, in the HS⁻ form can also act as a reducing agent (Kwon *et al.*, 2014):



Reduced iron could then form Fe-S phase (e.g. siderite or vivianite) or alternatively FeS:



It is known that sulphate becomes the dominant electron acceptor when the redox conditions fall under -100 mV (Reddy and DeLaunne, 2008), which was in agreement with the 24th hour in biologically active reactors, when Eh reached between -100 to -200 mV. Consequently, simultaneous iron and sulphate release can occur during partial utilisation of the available sulphate species. However, the dominant reduction pathways remain related to DO and nitrate such that sulphate pathways are only likely to be significant when the retention

time is elongated. Given that typical HRTs in reed beds remain under 24 hours it can be anticipated that sulphate pathways under the $\text{NO}_3\text{-N}$ concentrations observed in the field remain relatively minor.

The timing of the events in the different stages of the experiments is important if the HRT of reed beds is considered. In the first two stages of the process observed, i.e. in the first 8 hours of residence time, the buffering of P concentration rather than P release is characteristic. After this period P release is more likely to happen due to a shift of the concentration balance between the solid and liquid phases.

Preventive measures for wastewater treatment concluded from the above observations may be summarized into addressing three of the following areas:

- Although counterintuitive, it appears to be beneficial to feed the reed bed with concentrations of TP > 0.5 mg/L. This, together with maintaining residence times of < 8 hours would ensure that the flux rate remains within the first and second stages (i.e., uptake and lag). The risk of P release is increased during episodes of wastewater dilution in rainy seasons. Furthermore, long-term reed bed flooding should be avoided as it also enhances reduced conditions within substrate and on its surface.
- Artificially aerating the bed and/ or ensuring nitrate concentrations in excess of 15 mg/L when compared to Fe at the reed bed inlet would minimise or delay iron reduction, provided HRT does not exceed 24 hours.
- Avoid decreases in pH below 5. Although events of this kind are unlikely, it should be acknowledged that it accelerates P release.

6 CONCLUSIONS

This study identified the concentration gradient at the liquid-solid interface as a driving force buffering the P level within reed bed. The most prominent changes were recorded in the first 10 minutes upon liquid phase contact with the sludge, when weak adsorption bonds are likely to be involved in the retention and release. The following 8 hours were defined as a lag phase, when P concentrations seemed to continue acting as the main driver of P flux, although buffering occurred at a slower rate. The 8th hour was identified as a threshold of crucial importance. However, it should be remembered that it was proven only in laboratory conditions and cannot be applied as a general rule. After the lag phase other factors were observed to take over and shift the P flux regardless of the initial wastewater P level.

The reduction of iron in the biologically active system seemed to be the main mechanism triggering P release beyond the lag phase, coinciding with oxygen and nitrate-nitrogen depletion below 1 mg/L. Sulphate presence is suspected to enhance iron reduction. Thus, depletion of both oxygen and nitrate in wetlands is best avoided. If the reed bed is not aerated, a supplement of nitrate is a possible solution, provided HRT in the bed leave a residual of 1 mg NO₃-N/L. Furthermore, a flow rate optimization in order to decrease HRT will lessen the chance of nitrate depletion.

Convection was found to increase the fluctuation of Fe and P concentrations, however, it did not increase net P release. This suggests that faster flow rates can be safely applied, although further testing is needed to quantify the limits.

In regards to the above, flooding of reed beds appears to be the worst case scenario, where several risk factors are combined: dilution of the wastewater; enhancement of anoxic and reducing conditions; easy detachment of particles from the submerged substrate and subsequent P advection; and disturbance of microbial community, potentially leading to die off. The latter two need further research to be confirmed.

REFERENCES

Aminot, A. & Andrieux, F. (1996). Concept and determination of exchangeable phosphate in aquatic sediments. *Water Research*, 30(11), 2805-2811.

APHA, (1998). Standard methods for the examination of water and wastewater. *American Public Health Association*. Washington, DC.

Arias, C. A., Del Bubba, M. & Brix, H. (2001). Phosphorus removal by sands for use as media in subsurface flow constructed reed beds. *Water Research*, 35(5), 1159-1168.

Bates, M. H. & Neafus, N. J. E. (1980). Phosphorus release from sediments from lake Carl Blackwell, Oklahoma. *Water Research*, 14(10), 1477-1481.

Batten Jr., G. L. & Lafayette, G. K. Aluminum oxide, soluble aluminum, and coral toxicity. Seachem Library, <http://www.seachem.com/Library/Downloads.html> (accessed 26th February 2014).

Butterworth, E. (2014). The use of artificial aeration in horizontal sub-surface flow constructed wetlands for tertiary nitrification. PhD Thesis. School of Applied Sciences. Cranfield University.

Cao, X. & Harris, W. (2007). Carbonate and magnesium interactive effect on calcium phosphate precipitation. *Environmental Science & Technology*, 42(2), 436-442.

Carletti, G., Fatone, F., Bolzonella, D. & Cecchi, F. (2008). Occurrence and fate of heavy metals in large wastewater treatment plants treating municipal and industrial wastewaters. *Water Science & Technology*, 57(9).

Caselles-Osorio, A. & Garcia, J. (2007). Effect of physico-chemical pretreatment on the removal efficiency of horizontal subsurface-flow constructed wetlands. *Environmental Pollution*, 146(1), 55-63.

Choubert, J. M., Pomiès, M., Coquery, M. & Ruel, S. M. (2011). Influent concentrations and removal performances of metals through municipal wastewater treatment processes. *Water Science & Technology*, 63(9).

Clark, T. Stephenson, T. & Pearce, P. A. (1997). Phosphorus removal by chemical precipitation in a biological aerated filter. *Water Research*, 31(10), 2557-2563.

Council Directive 91/271/EEC (1991). Council Directive 91/271/EEC of 21 May 1991 concerning urban waste water treatment (Urban Waste Water Treatment Directive). *Official Journal of the European Communities*, Belgium, 21 May 1991.

Del Bubba, M., Arias, C. A. & Brix, H. (2003). Phosphorus adsorption maximum of sands for use as media in subsurface flow constructed reed beds as measured by the Langmuir isotherm. *Water Research*, 37(14), 3390-3400.

Dousma, J. & de Bruyn, P. L. (1976). Hydrolysis-precipitation studies of iron solutions. I. Model for hydrolysis and precipitation from Fe (III) nitrate solutions. *Journal of Colloid and Interface Science*, 56(3), 527-539.

Dunne, E. J. & Reddy, K. R. (2005). Phosphorus biogeochemistry of wetlands in agricultural watersheds. In: Dunne, E.J., Reddy, K.R., Carton, O.T. (Eds.), *Nutrient management in agricultural watersheds: A wetland solution*. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 105-119.

Dunne, E. J., Culleton, N., O'Donovan, G., Harrington, R. & Daly, K. (2005).

Phosphorus retention and sorption by constructed wetland soils in Southeast Ireland. *Water Research*, 39(18), 4355-4362.

Ekholm, P. (1998). Algal-available phosphorus originating from agriculture and municipalities. *Monographs of the Boreal Environment Research*, 11. Finnish Environment Institute, Helsinki, Finland.

Environment Agency (EA) (2012). Review of best practice in treatment and reuse/recycling of phosphorus at wastewater treatment works. Environment Agency, Bristol, UK.

Ferguson, J. F. & McCarty, P. L. (1971). Effects of carbonate and magnesium on calcium phosphate precipitation. *Environmental Science & Technology*, 5(6), 534-540.

Finstein, M. S. & Hunter, J. V. (1967). Hydrolysis of condensed phosphates during aerobic biological sewage treatment. *Water Research*, 1(4), 247-254.

Fox, I., Malati, M. A. & Perry, R. (1989). The adsorption and release of phosphate from sediments of a river receiving sewage effluent. *Water Research*, 23(6), 725-732.

Fox, L. E. (1991). Phosphorus chemistry in the tidal Hudson River. *Geochimica et Cosmochimica Acta*, 55(6), 1529-1538.

Froelich, P. N. (1988). Kinetic control of dissolved phosphate in natural rivers and estuaries: A primer on the phosphate buffer mechanism. *Limnology and Oceanography*, 649-668.

Gainswin, B. E., House, W. A., Leadbeater, B. S. C., Armitage, P. D. & Patten, J. (2006). The effects of sediment size fraction and associated algal biofilms on the kinetics of phosphorus release. *Science of the Total Environment*,

360(1), 142-157.

Gebbie, P. (2001). Using polyaluminium coagulants in water treatment. In: *Conference Proceedings Edition: 64th Victorian Water Industry Engineers and Operator's Conference*, All Seasons International Hotel, Bendigo, Victoria, Australia, 5 and 6 September, 2001, pp. 39-47.

Gensemer, R. W. & Playle, R. C. (1999). The bioavailability and toxicity of aluminum in aquatic environments. *Critical Reviews in Environmental Science and Technology*, 29(4), 315-450.

Holler, J. F., Skoog, D. A. & West, D. M. (1994). *Analytical Chemistry: An Introduction*. Saunders College Publishing, USA.

Horowitz, A. J. & Elrick, K. A. (1987). The relation of stream sediment surface area, grain size and composition to trace element chemistry. *Applied Geochemistry*, 2(4), 437-451.

House, W. A., Denison, F. H. & Armitage, P. D. (1995). Comparison of the uptake of inorganic phosphorus to a suspended and stream bed-sediment. *Water Research*, 29(3), 767-779.

Hsu, P. H. (1975). Precipitation of phosphate from solution using aluminum salt. *Water Research*, 9(12), 1155-1161.

Hubble, J. (2011). Sample preparation for metal analysis. Standard Operating Procedure reference SOP/11/6064/1. School of Applied Sciences. Cranfield University, UK.

Hume, N. P., Fleming, M. S. & Horne, A. J. (2002). Denitrification potential and carbon quality of four aquatic plants in wetland microcosms. *Soil Science Society of America Journal*, 66(5), 1706-1712.

Jarvis, N. J., Villholth, K. G. & Ulen, B. (1999). Modelling particle mobilization and leaching in macroporous soil. *European Journal of Soil Science*, 50(4), 621-632.

Jenkins, D., Ferguson, J. F. & Menar, A. B. (1971). Chemical processes for phosphate removal. *Water Research*, 5(7), 369-389.

Jørgensen, S. E. & Johnsen, I. (1981). Principles of environmental science and technology. *Studies in Environmental Science*, 14. Elsevier.

Kadlec, R. H. & Wallace, S. (2009). *Treatment wetlands*. CRC press, Taylor and Francis Group, Boca Raton, Florida.

Kim, L. H., Choi, E. & Stenstrom, M. K. (2003). Sediment characteristics, phosphorus types and phosphorus release rates between river and lake sediments. *Chemosphere*, 50(1), 53-61.

Kuenen, J. G. & Robertson, L. A. (1994). Combined nitrification-denitrification processes. *FEMS Microbiology Reviews*, 15(2-3), 109-117.

Kwon, M.J., Boyanov, M.I., Antonopoulos, D.A., Brulc, J.M., Johnston, E.R., Skinner, K.A., Kemner, K.M. & O'Loughlin, E.J. (2014). *Geochimica et Cosmochimica Acta* 129, 177–190.

Laanbroek, H. J. (1990). Bacterial cycling of minerals that affect plant growth in waterlogged soils: a review. *Aquatic Botany*, 38(1), 109-125.

Levlin, E. & Hultman, B. (2004). Phosphorus recovery from sewage sludge - Ideas for further studies to improve leaching. In: Plaza, E., Levlin, E. (Eds.), *Report no. 12. Proceedings of Polish-Swedish Seminars. Integration and Optimisation of Urban Sanitation System*, Stockholm, June 6-8, 2004, pp. 61-70.

Lijklema, L. (1980). Interaction of orthophosphate with iron (III) and aluminum hydroxides. *Environmental Science & Technology*, 14(5), 537-541.

Lovley, D. R. (1991). Dissimilatory Fe (III) and Mn (IV) reduction. *Microbiological Reviews*, 55(2), 259.

Manahan, S. E. (2001). *Fundamentals of Environmental Chemistry*. Lewis Publishers Ltd., Boca Raton, Florida.

Martin, B.D. (2010). Removal and recovery of phosphorus from municipal wastewaters using a ferric nanoparticle adsorbent. PhD Thesis. School of Applied Sciences. Cranfield University, UK.

Matsuzaka, E., Nomura, N., Nakajima-Kambe, T., Okada, N. & Nakahara, T. (2003). A simple screening procedure for heterotrophic nitrifying bacteria with oxygen-tolerant denitrification activity. *Journal of Bioscience and Bioengineering*, 95(4), 409-411.

McGechan, M. B. & Lewis, D. R. (2002). SW - Soil and Water: Sorption of phosphorus by soil, Part 1: principles, equations and models. *Biosystems Engineering*, 82(1), 1-24.

McGechan, M. B. (2002). SW - Soil and Water: Sorption of phosphorus by soil, Part 2: measurement methods, results and model parameter values. *Biosystems Engineering*, 82(2), 115-130.

Merill, D. T. & Jorden, R. M. (1975). Lime-induced reactions in municipal wastewaters. *Journal (Water Pollution Control Federation)*, 47(12), 2783-2808.

Nevin, K. P. & Lovley, D. R. (2002). Mechanisms for accessing insoluble Fe (III) oxide during dissimilatory Fe (III) reduction by *Geothrix fermentans*.

Applied and Environmental Microbiology, 68(5), 2294-2299.

Nürnberg, G. (1984). Iron and hydrogen sulfide interference in the analysis of soluble reactive phosphorus in anoxic waters. *Water Research*, 18(3), 369-377.

Omoike, A. I. & vanLoon, G. W. (1999). Removal of phosphorus and organic matter removal by alum during wastewater treatment. *Water Research*, 33(17), 3617-3627.

Pant, H. K., Reddy, K. R. & Lemon, E. (2001). Phosphorus retention capacity of root bed media of sub-surface flow constructed wetlands. *Ecological Engineering*, 17(4), 345-355.

Parks, G. A. (1965). The isoelectric points of solid oxides, solid hydroxides, and aqueous hydroxo complex systems. *Chemical Reviews*, 65(2), 177-198.

Patrick, W. H. & Khalid, R. A. (1974). Phosphate release and sorption by soils and sediments: effect of aerobic and anaerobic conditions. *Science*, 186(4158), 53-55.

Petticrew, E. L. & Arocena, J. M. (2001). Evaluation of iron-phosphate as a source of internal lake phosphorus loadings. *Science of the Total Environment*, 266(1), 87-93.

Prieto Fort, R. (2013). Assessment of tertiary reed beds in chemically-dosed wastewater treatment plants for phosphorus removal. MRes thesis. School of Applied Sciences. Cranfield University, UK.

Rabenhorst, M. C. & Castenson, K. L. (2005). Temperature effects on iron reduction in a hydric soil. *Soil Science*, 170(9), 734-742.

Reddy, K. R. & D'Angelo, E. M. (1994). Soil processes regulating water quality in wetlands. In: Mitsch, W.J. (Ed.), *Global wetlands: old world and new*. Elsevier, Amsterdam, The Netherlands, pp. 309–24.

Reddy, K. R. & DeLaune, R. D. (2008). *Biochemistry of Wetlands - Sciences and Applications*. CRC Press.

Richardson, C. J. (1985). Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science (Washington)*, 228(4706), 1424-1426.

Schmid, L. A. & McKinney, R. E. (1969). Phosphate removal by a lime-biological treatment scheme. *Journal (Water Pollution Control Federation)*, 41(7) 1259-1276.

Sophonsiri, C. & Morgenroth, E. (2004). Chemical composition associated with different particle size fractions in municipal, industrial, and agricultural wastewaters. *Chemosphere*, 55(5), 691-703.

Stumm, W. & Morgan, J. J. (1970). *Aquatic chemistry. Inter-science*. New York.

Stumm, W. & Morgan, J. J. (1996). *Aquatic chemistry: chemical equilibria and rates in natural waters. Environmental Science and Technology*. John Wiley and Sons, Inc., New York.

Thistleton, J., Berry, T. A., Pearce, P. & Parsons, S. A. (2002). Mechanisms of chemical phosphorus removal II - iron (III) salts. *Process Safety and Environmental Protection*, 80(5), 265-269.

Thistleton, J., Clark, T., Pearce, P. & Parsons, S. A. (2001). Mechanisms of Chemical Phosphorus Removal 1 - Iron (II) Salts. *Process Safety and*

Environmental Protection, 79(6), 339-344.

Vaughan, W. personal webpage (2010). Ternary plots.
<http://wwaughan.org/ternaryplots.html> (accessed 24th January 2014).

Vymazal, J. & Kröpfelová, L. (2009). Removal of organics in constructed wetlands with horizontal sub-surface flow: a review of the field experience. *Science of the Total Environment*, 407(13), 3911-3922.

Vymazal, J. & Švehla, J. (2013). Iron and manganese in sediments of constructed wetlands with horizontal subsurface flow treating municipal sewage. *Ecological Engineering*, 50, 69-75.

Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment*, 380(1), 48-65.

Vymazal, J. (2011). Long-term performance of constructed wetlands with horizontal sub-surface flow: Ten case studies from the Czech Republic. *Ecological Engineering*, 37(1), 54-63.

Water Pollution Control Federation (WPCF) (1990). *Natural systems for wastewater treatment (Manual of practice FD-16)*. Imperial printing.

Withers, P. J. A. & Jarvie, H. P. (2008). Delivery and cycling of phosphorus in rivers: A review. *Science of the Total Environment*, 400(1), 379-395.

Wuhrmann, K. (1968). Objectives, technology, and results of nitrogen and phosphorus removal processes. In: Gloyna, E. F. & Eckenfelder, W. W. (Eds.), *Advances in Water Quality Improvement*. Center for Research in Water Resources, University of Texas Press, Austin, pp. 21-48.

Yao, S., Ni, J., Ma, T. & Li, C. (2013). Heterotrophic nitrification and aerobic

denitrification at low temperature by a newly isolated bacterium, *Acinetobacter* sp. HA2. *Bioresource Technology*, 139, 80-86.

Yeoman, S., Stephenson, T., Lester, J. N. & Perry, R. (1988). The removal of phosphorus during wastewater treatment: a review. *Environmental Pollution*, 49(3), 183-233.

Zhou, X., Shan, B. & Zhang, H. (2010). Phosphorus release: A biogeochemical insight from a restored lakeside wetland in the Yangtze-Huaihe region, China. *Journal of Environmental Sciences*, 22(3), 347-354.

APPENDICES

Appendix A Photographs of the assessed reed beds



A



B



C

Figure A.1: Photographs of the assessed reed beds: A) NA-Fe site, B) A-Fe site, C) NA-Al site (reed bed on the left assessed only).

Appendix B Initial analysis of wastewater used in the experiments

Table B.1: Chemical and physical characteristics of wastewater used in the experiments as measured on-site (DO, EC, Eh, pH, temperature) and in the laboratory (COD, TP, PO₄-P, SO₄²⁻, Fe total, Fe (II), Fe (III) and NO₃-N). The concentrations of individual particle fractions are included.

Experiment 1				
	Particle fraction			
	Total	Suspended	Colloidal	Dissolved
COD (mg/L)	71.1			
TP (mg/L)	1.49	0.61	0.31	0.58
PO ₄ -P (mg/L)	1.03	0.25	0.16	0.62
SO ₄ ²⁻ (mg/L)	106	7.40	7.50	91.1
Fe total (mg/L)	1.14	1.01	0.13	0
Fe (II) (mg/L)	0.32	0.28	0.04	0
Fe (III) (mg/L)	0.82	0.72	0.10	0
NO ₃ -N (mg/L)	14.9	-	0.50	14.4
DO (mg/L)	7.91			
EC (µS/cm)	804			
Eh (mV)	78.9			
pH	7.6			
Temperature (°C)	9.5			
Experiment 2				
COD (mg/L)	21.4			
TP (mg/L)	0.60	0.08	0.06	0.46
PO ₄ -P (mg/L)	0.28	0.09	0.02	0.17
SO ₄ ²⁻ (mg/L)	106	0	1.30	104.7
Fe total (mg/L)	1.11	0.99	0.11	0.01
Fe (II) (mg/L)	0.11	0.08	0.02	0.01
Fe (III) (mg/L)	1.00	0.91	0.08	0.01
NO ₃ -N (mg/L)	14.9	-	0	14.9
DO (mg/L)	7.87			
EC (µS/cm)	812			
Eh (mV)	46.3			
pH	7.6			
Temperature (°C)	8.6			

Appendix C Metal content of wastewater used in the experiments

Table C.1: Wastewater metal concentrations in the Experiment 1 at the start and at the 72nd hour in dynamic (D) and static (S) reactors as analysed by ICP-MS.

Experiment 1					
	µg/L				
	Time 0	72 th hour : blank		72 th hour : test	
		D	S	D	S
Al	5.12	2.02	2.70	14.8	6.87
Cr	10.1	11.0	11.9	32.8	33.0
Ni	2.57	5.40	2.94	5.80	5.47
Cu	3.57	4.88	4.52	3.27	0.86
Zn	14.9	9.90	12.1	1.24	0.62
Cd	0.022	0.008	0.009	0.044	0.019
Pb	0.17	0.093	0.11	0.24	0.056

Table C.2: Wastewater metal concentrations in Experiment 2 at the start and at the 72nd hour in the ^{0.1;0.5;1;2;5}Reactors as averages of 3 experimental replicates.

Experiment 2										
	blanks; µg/L									
	0.1		0.5		1		2		5	
	time 0	72 th hour	time 0	72 th hour	time 0	72 th hour	time 0	72 th hour	time 0	72 th hour
Al	0.66	0.91	2.56	1.63	3.11	1.48	2.89	1.47	2.96	1.22
Cr	1.76	2.49	7.67	8.66	9.92	10.1	9.64	10.0	9.94	10.1
Ni	0.66	0.83	2.39	2.91	3.11	3.53	3.02	3.51	3.05	3.47
Cu	0.62	0.85	2.52	3.15	3.18	3.79	3.15	3.77	3.27	3.69
Zn	4.62	5.95	11.1	12.5	16.9	15.7	15.7	15.5	12.6	8.63
Cd	0.0065	0.011	0.021	0.022	0.011	0.012	0.0081	0.0088	0.0078	0.0073
Pb	0.044	0.086	0.20	0.073	0.44	0.062	0.35	0.09	0.21	0.43

test reactors; µg/L										
	0.1		0.5		1		2		5	
	time 0	72 th hour	time 0	72 th hour	time 0	72 th hour	time 0	72 th hour	time 0	72 th hour
Al	2.55	7.06	4.65	7.24	3.84	7.14	3.44	7.93	2.03	5.91
Cr	6.93	26.33	12.34	33.60	13.67	34.3	13.69	36.6	15.24	35.3
Ni	2.25	5.80	3.79	5.69	3.79	5.43	3.79	6.08	4.01	5.43
Cu	0.71	0.70	2.04	0.89	2.39	0.86	2.61	0.94	2.41	1.03
Zn	2.43	1.99	6.5	1.6	7.5	2.3	7.7	2.4	7.7	1.74
Cd	0.0025	0.003	0.001	0.003	0.003	0.004	0.0007	0.0062	0.0007	0.0064
Pb	0.089	0.095	0.11	0.059	0.04	0.048	0.01	0.26	0.05	0.15

Appendix D Chemical content of sludge

Table D.1: Concentrations of the chosen elements in top sludge in the inlet area of reed beds at 3 STW sites (NA-Fe, NA-Fe 2, NA-AI) as analysed by ICP-MS TotalQuant. No sludge was present at the A-Fe site. Instead, results for another iron dosed site of 795 p.e. are included (NA-Fe 2). This analysis was carried out in order to obtain approximate figures only. Average of 3 replicate samples.

	mg/kg DW		
	NA-Fe	NA-Fe 2	NA-AI
Ag	5.75	7.08	4.09
Al	5644	10091	
As	14.9	12.4	12
Ba	556	264	297
Bi	3.03	4.34	1.47
Br	36.5	7.95	43
Ca	26939	17110	9081
Cd	0.54	0.69	0.37
Ce	4.47	6.49	11.3
Co	5.60	10.1	8.4
Cr	20.1	27.1	56.8
Cu	183	220	89
Fe	94329	101893	10904
Ge	0	0	0
Hg	0.146	0.257	0.230
In	0.040	0.010	0.006
K	1968	2572	1185
Li	4.44	10.4	8.69
Mg	4754	4706	1867
Mn	255	338	929
Na	1414	700	648
Ni	17.6	28.7	17.8
P	29728	27535	29135
Pb	37.1	38.5	43.5
Re	0.002	0.002	0.001
Sb	2.44	3.60	1.57
Sc	1.03	1.84	1.87
Se	0	0	0
Si	967	1030	735
Sn	42.7	57.8	28.5
Sr	1170	629	69
Ti	371	496	238
Tl	0.038	0.105	0.045
U	1.56	2	3.35
Zn	1531	624	351
Zr	4.28	4.27	4.71

Appendix E Phosphorus and metal content of sludge in the experiments

Table E.1: Phosphorus and metal concentrations of sludge at the start and at the end of experiments and corresponding concentration change rates for dynamic (D) and static (S) conditions in Experiment 1 and ^{0.1, 0.5, 1, 2, 5}Reactors in Experiment 2. All values are averages of 3 experimental replicates, each of which was further digested in 3 replicate subsamples. Concentrations in mg/kg were converted to µg/L on the basis of calculated ratio between sludge volume and weight. Phosphorus was analysed spectrophotometrically. Metals were analysed by AAS.

Experiment 1					
	mg/kg-DW			concentration change rate (µg/L-hour)	
	start	D end	S end	D	S
Al	4470	4412	4460	-394	-72.4
Cd	0.90	0.82	0.84	-0.548	-0.402
Cr	28.0	28.9	24.5	5.90	-24.0
Cu	259	219	214	-277.1	-310.2
Fe	95589	98221	82993	17890	-85589
Ni	30.2	34.0	34.5	25.8	29.1
P	37978	33831	33722	-28179	-28922
Pb	75.8	63.2	61.5	-85.4	-97.3
Zn	1381	1505	1463	839.4	557.3

Experiment 2											
	mg/kg-DW						concentration change rate (µg/L-hour)				
	start	0.1 end	0.5 end	1 end	2 end	5 end	0.1	0.5	1	2	5
Al	4929	5452	5519	5914	5877	6085	51.6	58.2	97.2	93.6	114
Cd	0.871	0.827	0.819	0.788	0.816	0.806	-0.0043	-0.0051	-0.0081	-0.0054	-0.0064
Cr	26.7	81.7	102	88.6	133	71.4	5.43	7.42	6.10	10.5	4.41
Cu	248	255	244	255	260	253	0.70	-0.37	0.76	1.21	0.54
Fe	85029	83231	79587	85406	87612	84431	-177	-537	37.2	255	-59.0
Ni	35.3	72.8	92.9	81.8	103	62.3	3.70	5.67	4.58	6.69	2.66
P	36859	37292	36176	35584	35343	36432	42.7	-67.4	-126	-150	-42.2
Pb	66.3	80.0	75.2	79.6	81.4	78.0	1.34	0.87	1.31	1.49	1.15
Zn	1788	1809	1739	1814	1932	1815	2.13	-4.86	2.61	14.2	2.72

Appendix F Chemical content of wastewater

Table F.1: Concentrations of the chosen elements in wastewater at 3 assessed sites (NA-Fe, A-Fe, NA-Al) at 3 sampling points (crude, 2° effluent, 3° effluent) as analysed by ICP-MS TotalQuant. This analysis was carried out in order to obtain approximate figures only. Average of $N^{NA-Fe} = 5$, $N^{A-Fe} = 2$, $N^{NA-Al} = 3$.

	$\mu\text{g/L}$								
	NA-Fe			A-Fe			NA-Al		
	Crude	2° Eff	3° Eff	Crude	2° Eff	3° Eff	Crude	2° Eff	3° Eff
Ag	7.18	7.18	0.87	0.11	0.50	0.82	0.42	0.23	0.13
As	2.87	2.87	2.82	0.51	1.08	1.10	1.20	0.67	0.43
Ba	23.2	23.2	103	0	57.6	0	0	14.4	0
Bi	0	0	0.16	0	0.17	0.30	0.05	0.06	0.02
Br	365	365	242	92.4	107.3	92.6	36.2	59.3	37.9
Ca	78891	78891	53892	17131	17218	15478	14350	14036	8639
Cd	0	0	0.01	0.04	0	0.02	0	0	0
Ce	0	0	0.07	0	0.23	0.29	0.33	0.11	0.10
Co	0.98	0.98	0	0	2.17	0	0	0.54	0
Cr	0.99	0.99	1.41	0	0.42	1.67	0.15	0.11	0
Cu	0.34	0.34	23.2	0	12.7	17	8.95	4.75	2.99
Ge	0.07	0.08	0.06	0.02	0.03	0.03	0.05	0.03	0.03
Hg	0	0	0	0	0	0.03	0.08	0.09	0.14
In	0	0	0	0	0	0	0	0	0
K	10824	10824	0	10744	10415	8344	1338	3489	0
Li	30.9	30.9	21.4	0.88	3.10	2.94	7.71	7.98	7.04
Mg	37213	37213	26995	3469	5562	5417	5563	4863	2849
Mn	59.2	59.2	5.60	103	136	14.9	0	67.1	10.5
Na	85319	85319	59495			40822	25746	32419	33537
Ni	3.52	3.52	2.14	37.9	4.91	1.87	0	1.23	0
Pb	0	0	1.97	0.17	3.18	3.35	3.87	1.25	0.90
Re	0	0.01	0	0	0	0	0	0	0
Sb	0.66	0.66	22.4	0.09	0.44	0.57	0.48	0.56	0.62
Sc	1.10	1.10	0.61	0.20	0.46	0.48	0.56	0.56	0.33
Se	5.14	5.14	4.38	0.42	0	0	0	0	0
Si	6040	6040	3727	467	822	931	1287	1288	1151
Sn	0.94	1.04	5.96	0.15	2.75	4.37	0	0.69	0
Sr	1639	1639	1380	32.3	53.8	42.9	88.9	71.1	58.3
Ti	140	140	80.1	33.4	44.2	43.1	30.7	21.3	11.8
Tl	0	0	0	0	0.01	0.01	0	0	0
U	1.08	1.08	1.01	0	0.17	0.20	0.30	0.07	0.03
Zn	852	852	0	419	798	556	0	200	0
Zr	0.44	0.44	0.64	0.07	0.63	0.98	0.19	0.19	0.13

Appendix G Environmental conditions within reed beds

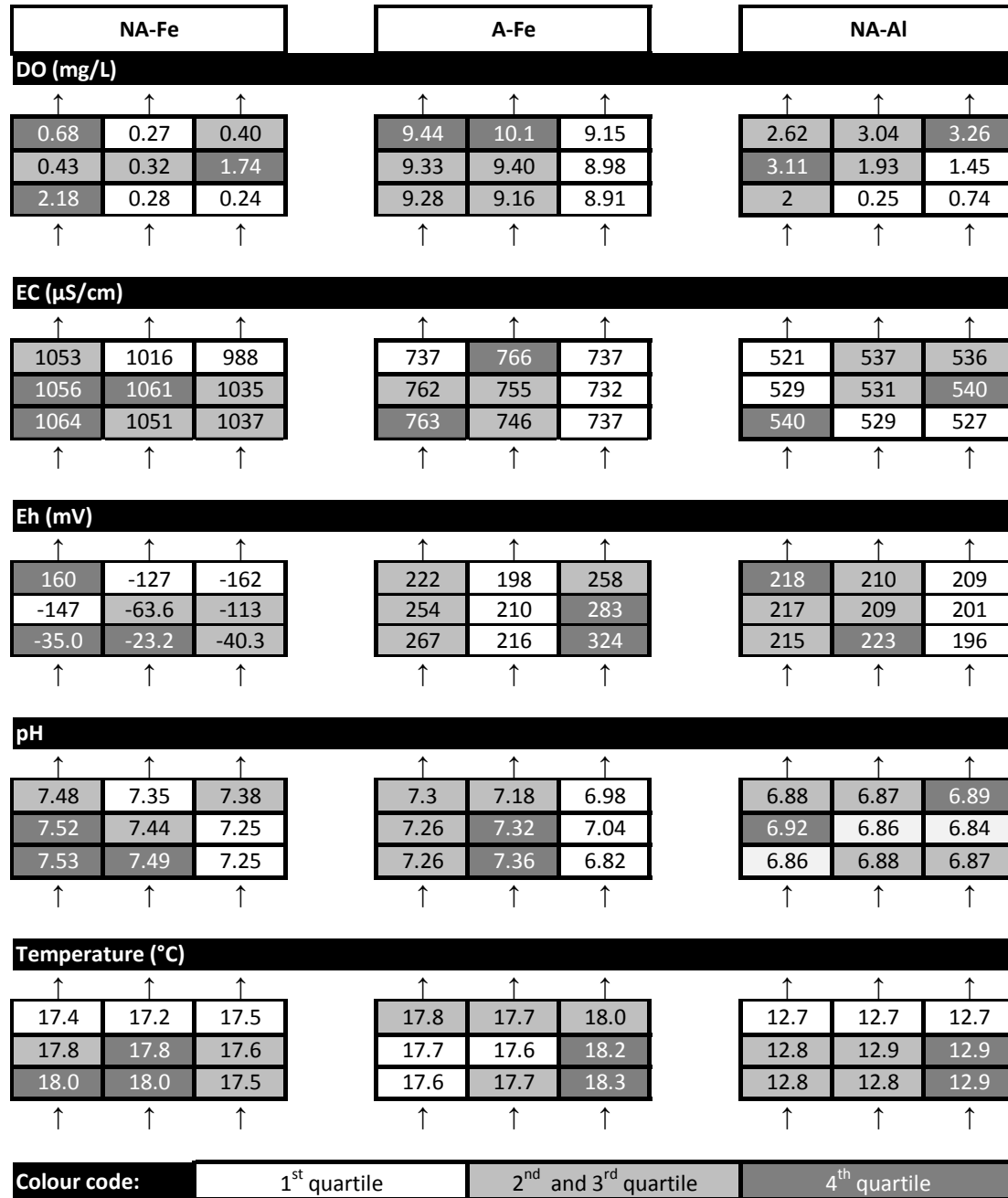


Figure G.1: Spatial distribution of environmental conditions measured within the sediment of reed beds at 3 assessed sites (NA-Fe, A-Fe, NA-AI). The rectangle represents a reed bed divided into a grid of 3 x 3 identically sized areas. The arrows indicate the wastewater inlet and outlet. Nine measurement values within one bed were divided into quartiles: white fields correspond to 1st quartile, dark grey to 4th quartile.

Appendix H Correlations of potential P release factors

H.1 Correlations in regards to sampling points

Table H.1: Pearson correlations (sig. 2-tailed) of distinct factors possibly contributing to P release from reed bed based on analyses and measurements carried out across 3 sites in regards to sampling points. Larger fields express correlations of all sampling points in general. Smaller fields each represent individual sampling points: crude influent, secondary effluent and tertiary effluent. Correlations of only r higher than 0.7 or lower than -0.7 are included. N of all sampling points correlations = 33; N of individual sampling points correlations = 11, $p < 0.05$.

	Temperature (°C)	DO (mg/L)	Eh (mV)	EC (µS/cm)	Flow (L/s)	Average Daily Flow (L/s)	COD (mg/L)	eBOD ₅ (mg/L)	TSS (mg/L)	VSS (mg/L)	NO ₃ -N (mg/L)	NH ₄ -N (mg/L)	SO ₄ ²⁻ (mg/L)	Total P (mg/L)	PO ₄ -P (mg/L)	Al (mg/L)	Fe total (mg/L)	Fe (II) (mg/L)	Fe (III) (mg/L)	Alkalinity	Hardness (°dH)	Ca (mg/L)	Mg (mg/L)
Temperature (°C)																							
DO (mg/L)																							
Eh (mV)																							
EC (µS/cm)																							
Flow (L/s)																							
Average Daily Flow (L/s)																							
COD (mg/L)																							
eBOD ₅ (mg/L)																							
TSS (mg/L)																							
VSS (mg/L)																							
NO ₃ -N (mg/L)																							
NH ₄ -N (mg/L)																							
SO ₄ ²⁻ (mg/L)																							
Total P (mg/L)																							
PO ₄ -P (mg/L)																							
Al (mg/L)																							
Fe total (mg/L)																							
Fe (II) (mg/L)																							
Fe (III) (mg/L)																							
Alkalinity																							
Hardness (°dH)																							
Ca (mg/L)																							
Mg (mg/L)																							

Legend:

negative correlations

positive correlations

All sampling points

Crude2° effluent3° effluent

H.2 Correlations in regards to particle fractions

Table H.2: Pearson correlations (sig. 2-tailed) of distinct factors possibly contributing to P release from reed bed based on analyses and measurements carried out across 3 sites in regards to particle fractions. Larger fields express correlations of all particle fractions in general. Smaller fields each represent individual particle fractions: suspended, colloidal and dissolved. Correlations of only r higher than 0.7 or lower than -0.7 are included. $N = 33$; $p < 0.05$. Rows not included in this table would have identical values as the ones in Table H.1.

	Temperature (°C)	Ambient Temperature (°C)	DO (mg/L)	Eh (mV)	EC (µS/cm)	pH	Flow (L/s)	Average Daily Flow (L/s)	COD (mg/L)	eBO ₅ (mg/L)	TSS (mg/L)	VSS (mg/L)	NO ₃ -N (mg/L)	NH ₄ -N (mg/L)	SO ₄ ²⁻ (mg/L)	Total P (mg/L)	PO ₄ -P (mg/L)	Al (mg/L)	Fe total (mg/L)	Fe (II) (mg/L)	Fe (III) (mg/L)	Alkalinity	Hardness (°dH)	Ca (mg/L)	Mg (mg/L)
NO ₃ -N (mg/L)																									
Total P (mg/L)																									
PO ₄ -P (mg/L)																									
Al (mg/L)																									
Fe total (mg/L)																									
Fe (II) (mg/L)																									
Fe (III) (mg/L)																									

Legend:

- negative correlations
- positive correlations

Total
Suspended
Colloidal
Dissolved