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SCIENZE E TECNOLOGIE PER L'AMBIENTE ED IL TERRITORIO

The Significance of Resorption During Anoxic Mobilization of Phosphorus

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Abstract

Phosphorus has a fundamental role in the regulation of biotic cycles in both aquatic and terrestrial environments. Phosphorus is in fact the limiting nutrient for plants in the majority of the cases. Phosphorus has been historically used in huge amounts to improve agricultural crops production, and this use has had some implications on environment health. Eutrophication processes are evident and can be seen in many rivers, lakes and costal waters all around the world. Even small concentration of phosphorus (0.005 mg/L) can cause eutrophication. Phosphorus is commonly present in the soil as phosphate ion. Phosphate binds to many soil components such as calcium, iron oxides, aluminium oxides and aluminium silicate minerals. In general the majority of these bounds is very stable. Sorption with iron oxides in fact might be unstable. Under anaerobic conditions, iron, present as Fe(III), is used by microorganisms as electron acceptor and therefore reduced to the Fe(II) form. Fe(II) is soluble and all phosphate bound to it gets consequently solubilized as well. Once in solution, Phosphate can easily migrate from agricultural fields to the closest stream or lake. Particular attention has then to be paid on riparian zones, as they represent the last chance to stop phosphorus migration, filtrating the water passing through them. It could be hypothesized to resorb soluble phosphate thanks to addition of non-soluble sorbents to soils in the riparian zones. The main objective of this thesis work is to test the efficiency in phosphate resorption of sorbents containing aluminium. Gibbsite, an aluminium-oxide as also known as hydrargillite, was added at different concentrations to two Danish soils and its phosphorus sorption activity was investigated during the first four weeks of soils reduction process. Soil incubations were carried out inside a *glove box* in order to work in a deep anaerobic environment. Fe(II) was determined as indicator of soils reduction state and phosphate ion in solution was measured as it is an index of phosphorus available to plants. Results clearly show how phosphate resorption took place in both of the soils. The role of gibbsite is the resorption process is totally less relevant than the role of non-reduced iron oxides. In fact, a large number of iron(III) oxides were still present in both of the soils at the end of the experiments, as soils were not totally reduced.

Riassunto

Il fosforo ha un ruolo fondamentale nella regolazione dei cicli biotici sia in ambiente acquatico che terrestre. Per le piante il fosforo è, infatti, nella maggior parte dei casi l'elemento che ne limita la crescita. Per incrementare la produzione agricola, il fosforo è stato storicamente usato come fertilizzante in grandi quantità tanto da creare alcuni effetti collaterali all'ambiente. Gli effetti sull'ambiente sono osservabili con processi di eutrofizzazione dei bacini acquatici quali laghi, fiumi e acque costiere anche a concentrazioni molto modeste (0.05 mg/L). Nel suolo, solitamente presente come ione orto fosfato, il fosforo reagisce legandosi o complessandosi, con molti composti, quali calcio, ossidi di ferro, ossidi di alluminio e minerali contenenti alluminio.Tutti questi legami sono in genere molto stabili e rendono insolubili i fosfati tranne che nel caso del legame tra fosforo e ossidi di ferro che in determinate condizioni diventa instabile. Infatti, in condizioni anaerobiche, il ferro, presente in forma ferrica, è usato dai microorganismi come accettore di elettroni e quindi ridotto alla forma ferrosa. In questo caso gli ossidi di ferro diventano solubili e con loro anche i fosfati. Una volta in soluzione i fosfati possono facilmente essere trasportati dai terreni agricoli ai corpi idrici superficiali. Particolare attenzione quindi deve essere prestata alle cosiddette fasce tampone, quali ultimo possibile filtro tra i suoli agricoli e i corpi idrici. È, infatti, ipotizzabile la ri-cattura dei fosfati alla fase non solubile, mediante l'applicazione di sorbenti non solubili nei terreni delle fasce tampone. Questo lavoro di tesi ha lo scopo di testare l'efficacia di composti contenenti alluminio in grado di riassorbire i fosfati resi solubili dalle condizioni anaerobiche. La gibbsite, un ossido di alluminio conosciuto anche come idrargillite, è stata aggiunta a varie concentrazioni a due suoli danesi e la sua attività di adsorbimento del fosforo è stata investiga durante la fase iniziale del processo di riduzione dei suoli stessi. Le incubazioni dei suoli sono avvenute all'interno di una *glove box* in completa assenza di ossigeno. La produzione di Fe(II) è stata analizzata quale indice dello stato di riduzione dei suoli e lo ione orto fosfato in soluzione misurato perché specchio del fosforo reso bio-disponibile. I risultati mostrano un chiaro riassorbimento dei fosfati da parte dei suoli. Il ruolo della gibbsite è però da ritenersi secondario rispetto a quello degli ossidi di ferro non ridotti. Entrambi suoli, non essendo completamente ossidati al termine degli esperimenti,

contenevano, infatti, un largo numero di ossidi di ferro(III) che hanno riassorbito il fosforo mobilizzato.

Key words

Phosphorus. Phosphate. Iron oxide. Gibbsite. Sorption. Mobilization. Anoxic. Anaerobic.

Abbreviations

P: inorganic phosphorous. PAC: phosphorus adsorption capacity. Ortho-P: inorganic phosphorus present as orthophosphate ion. ZPC: zero point charge.

1.Introduction

1.1.Aims of the study

Inorganic phosphorus (P) is between all others, the most common nutrient limiting vegetative production in lakes, other fresh water systems and some costal waters, especially in lagoons and estuaries (Bridgham et al. 2001; Peretyazhko and Sposito 2005). Phosphorus is necessary for plants because of its role in biochemical reactions. It is also a component of nucleic acids and nucleoside triphosphates, the basis of enzyme synthesis and energy transfer systems at cellular level (Pant and Reddy 2001). On the other hand, when P concentrations rise above a certain threshold, algae growth becomes vigorous (algae bloom starts at P concentration often as low as 0.05 mg/L), generating eutrophication (Pant and Reddy 2001; Wright et al. 2001) with all its undesired effects, such as: shadow effects and sedimentation of dead algae, consuming oxygen in lake bottom sediments which can cause fish death. In fact, P has been the main cause of excessive and harmful fertilization of lakes for many years (Syers et al. 1973). Hence in Europe and in North America, there is much focus on decreasing the P export to lakes in order to have less eutrofied waters. Historically, point sources from households and industries were, together with non point sources from agricultural soils, a major source of P to lake water, due to the high P content in everyday-products such as soaps and detergents , especially in softeners, where polyphosphates were used to sequester calcium ion, which is in high concentrations in hard-waters (vanLoon and Duffy 2005). Today the P content in wastewater is much lower than in the past due to the high efficiency of wastewater cleaning, and the fact that most of the P exports from households to lakes have been closed down. As a consequence of this, diffuse (non-point) sources from arable soils have now become the only major contributor to P balances of lakes and water bodies in general. As an example, phosphorous deriving from agricultural runoff from seasonally flooded soils, was demonstrated to be a leading cause of water quality degradation of Lake Champlain Basin in the State of New York (Young and Ross 2001). In the last years, phosphorous content in agricultural soils has increased progressively, reaching saturation of soil sorption capacities as a consequence of long-term and recurrent application of fertilizers and livestock waste (Kleinman et al. 1999; Young and Ross 2001; Ajmone-

Marsan et al. 2006). Therefore, many agricultural soils are now considered to be a potential diffuse source of phosphorus to surface waters (Scalenghe et al. 2002; Murray and Hesterberg 2006), moreover, the use of fertilizers and/or animal waste, when surface-applied, lead to accumulation of soil P that can be easily carried away by floodwater from the fields to the closest waterbody, in significant amounts (Sallade and Sims 1997; Wright et al. 2001). As a consequence of this, currently there is much focus on how to decrease the amount of P from agriculture leaching into rivers and ending up in lakes.

Drainage water from arable soils often passes through tracts of wetland soils along rivers (or riparian soils) before reaching the river. It is thought that constructed and natural isolated wetlands (Dunne et al. 2005; Dunne et al. 2006) and riparian soils can act as a trap for the P leached from the arable soils. In the riparian soils, soluble P compounds can sorb to iron(III) and aluminium oxides very specifically, and particulate P-forms can be retained by sedimentation. Despite that, wetlands constructed on high fertilized or manure impacted soils and riparian areas have been proved to lead to an important solubilisation of P stored in those soils and release it into surface water body systems (Pant and Reddy 2003; Surridge et al. 2007). In autumn and winter, these soils often become anoxic; that is to say that all the gas fraction in the soil disappears because water replaces it. In anoxic soil environment (no oxygen present) iron(III) oxides are reduced by bacteria to iron(II) and become soluble and with them all their load of sorbed phosphate, as it will be explained further on. Hence, anoxic conditions can cause a dramatic increase of soluble P (Pratt 2006). Not all the P solubilized, as in above-mentioned reaction, would leach to water bodies. A part of it can be sorbed by other redox-stable sorbents such as aluminium oxides (e.g. gibbsite, $Al(OH)_3$) and clay silicates (e.g. kaolinite, $Al_2Si_2O_5(OH)_4$). In other cases, phosphates can precipitate as calcium phosphates or as iron(II)-phosphate (e.g. vivianite, $Fe_3(PO_4)_2*8H_2O$). Therefore, these secondary reactions can be important for the retention potential of wetland soils and riparian soils and can effectively limit the maximum P concentration that can be reached during anoxic conditions in flooded soils. Chemical treatments acting on this resorption processes have been used as control of point and non-point pollution sources for more than 30 years, and they are nowadays an established technology, but the efficiency of different chemical amendments is yet to be well investigated especially for non point sources (Ann et al. 2000).

Nowadays the concept of sustainability in the use of resources is more and more accepted. It has been seen, as mentioned before, that eutrophication can be reduced or totally avoided if the causing agents (N and P) are properly managed. As many countries have been doing in the last decades, the Danish government made some decision about water environments quality and so far three Water Action Plans have been implemented. Danish water system is very fragile: ground water is not

separated by different layers of non-permeable materials (e.g. clays) thus all soil and atmospheric pollution entering any kind of water environment is consequently affecting the quality of drinking water. In the first two Water Action Plans the Danish authorities stressed a lot of attention on the effects of nitrate on water. Therefore agricultural practises were finally regulated and decisions were made about decreasing the use of fertilizers and increasing the number of wetlands all over the country. With the third Water Action Plan (2003) a lot of attention was put on phosphorus. At that time there was no clear idea on how to reduce P leaching into water systems (Mijøministeriet 2003). Several projects were therefore financed in order to gain sufficient knowledge to manage the use of phosphorus in agriculture and knowledge on phosphorous chemistry in soil and water environments. This master thesis is part of the Buffalo-P Project, one of those above-mentioned projects financed by the third Danish Water Action Plan.

A part from the eutrophication problem, there's another very important reason why P use in agriculture should be managed in a sustainable way; it's in fact well known that phosphorous is a finite resource. According to later studies (Robetrs and Stewart 2002), P ore reserves will be exploitable for the next 25 years only (about 100 years in the most optimistic estimations). It is thus very important to reduce any leach of phosphate to ground water and it would be very important to find a way to immobilize P in order to store it in accessible sites where at the same times it does not harm environment.

This study will therefore focus on the significance of phosphate resorption to different sorbents: gibbsite ($Al(OH)_3$) and kaolinite ($Al_2Si_2O_5(OH)_4$). Different agricultural soils from the region of Jutland in Denmark will be incubated under anoxic conditions, and P release in absence and presence of different amounts of added sorbents will be studied to examine the efficiency of the above-mentioned sorbents, in a low-cost and wide scope soil remediation policy point of view.

1.2 Phosphorus

1.2.1 Phosphorus biogeochemical cycle

As mentioned in the previous paragraph, phosphorus is essential for life in both terrestrial and aquatic environments. Phosphorus is present throughout the lithosphere, hydrosphere and biosphere. Phosphorus moves slowly from deposits in soils and sediments to living organisms such as plants, algae and phytoplankton. Phosphorus can move then to upper trophic levels when plant and other

primary producers are eaten. Phosphorus then moves back into the soil and water sediment when living organisms die or when they excrete it as sewage. Phosphorus can be then very slowly transformed into a mineral or simply be back in the cycle. Historically phosphate minerals have been a sink of the element. Phosphorus precipitates and slowly forms the so-called phosphate rock. Since human started to use inorganic fertilizers, a lot of P has been brought back to the cycle. Phosphorus is mined and fertilizers are produced and then P is reintroduced to the cycle through agricultural soils. Obviously, fertilizers application alters natural equilibriums in the cycle. Plants in the crops do not totally up take all the phosphorus applied by human; a big part of it is retained along the cycle. Most of it stays in non-soluble forms in the soil and moves then, when solubilized to waterbodies creating imbalance on the local P cycle. This aspect is really of environmental concern: when there is a surplus of nutrients there are consequences throughout all the food chain impacting also the ecosystems where those organisms live.

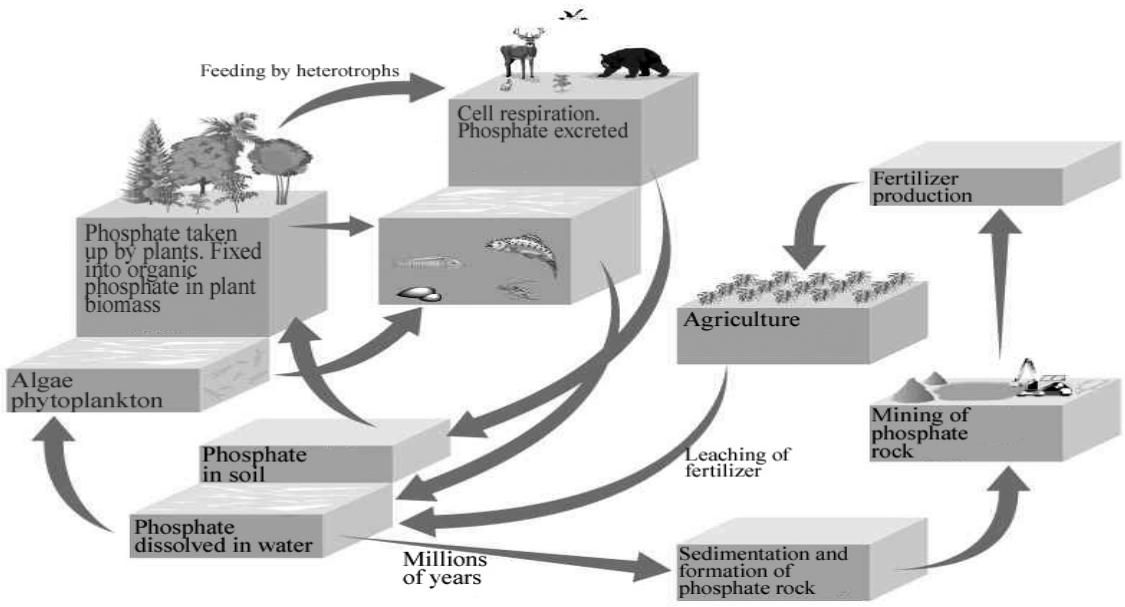


Figure 1.a **The phosphorus cycle**. Phosphorus is naturally used in plants, animals and other living organisms metabolism and then it goes back to soil or aquatic environment. Formation of phosphorus minerals and rocks is to be considered to be a loss of the element in the cycle, while human activities take back all the phosphorus immobilized and stored in soils. (Edited by the author, original from http://arnica.csustan.edu/carosella/Biol4050W03/figures/phosphorus_cycle.htm).

1.2.2 Immobilization of phosphorus

Once the path of phosphorus in its own cycle is known, it is important to have an overview on the processes that are involved in its immobilization in soil environments.

Phosphorous is not present in any common gaseous form; however, it can be found in soluble and non-soluble forms. Pure elemental phosphorus is very rare to be found.

In water environments (as well as in the soil solution) phosphorus is usually stable as phosphate ($X_nPO_4^{n-3}$, where X could for example be H, Na or K). Depending on which kind of compounds the phosphate ion binds to, organic or inorganic phosphates are formed. In the aquatic environment both organic (associated with an organic molecule) and inorganic phosphate can be found. In soils, even though organic phosphates can be found in organic matter and plant tissues, most of the phosphate is associated with minerals (vanLoon and Duffy 2005). To understand phosphate's properties in soil solution it is necessary to have a look at how phosphate is generated from dissociation of phosphoric acid. Figure 1.b shows pH effects the distribution of phosphate ions in solution.

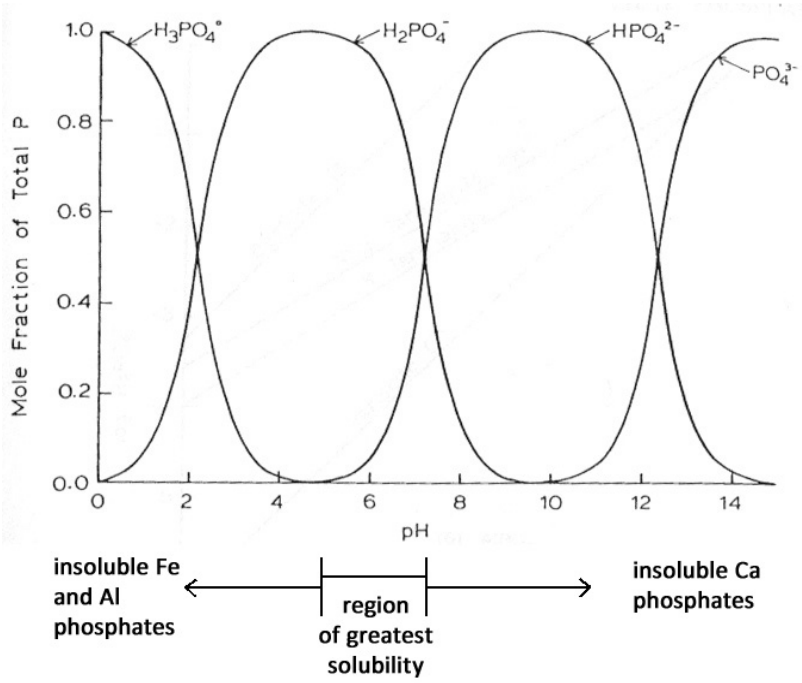


Figure 1.b **Distribution and common forms of phosphate in solution depending on pH.** (Lindsay 1979; vanLoon and Duffy 2005)

Where pH is on the slightly acid side (values between 5 and 7), phosphorus has its maximum solubility, and the predominant species under these conditions is $H_2PO_4^-$ (vanLoon and Duffy 2005).

In soil, phosphate ions have most frequently reactions with cations (e.g. iron, aluminium, calcium, magnesium, potassium and manganese) that are more abundant than P itself and that control its solubility forming stable minerals. Most of the phosphate present in soils are calcium, iron,

aluminium phosphates (Lindsay 1979). Acid environments promote the formation iron and aluminium phosphate, while calcium phosphates are formed under alkaline conditions. Each of these metals forms insoluble phosphates. The most common mineral forming with calcium is apatite ($Ca_5(PO_4)_3(F,Cl,OH)$). With iron, phosphorus can form different minerals such as $FePO_4$, strengite ($FePO_4*2H_2O$) and vivianite ($Fe_3(PO_4)_2*8H_2O$), that are usually very stable and non-soluble at common natural pH and E_H. Aluminium-phosphate minerals are also insoluble and must be considered as an important controller of P solubility. Common Al phosphates are: berlinite ($AlPO_4$), variscite ($AlPO_4*2H_2O$) and K or NH_4-taranakites ($H_6(K_3/(NH_4)_3)Al_5(PO_4)_8*18H_2O$) (Lindsay 1979). All the reactions forming these minerals involve phosphate ions together with elemental metal ions. Moreover, these minerals are not of environmental interest because they are very stable and it is difficult that they release phosphorus to the solution at normal pHs and E_Hs, although they buffer soil solution. Reactions that phosphate ions have iron and aluminium are sorption reactions, meaning that phosphate accumulates on the surface of the oxides. Phosphate sorption to iron and aluminium oxides will be explained in the following paragraphs.

Plants also are important for P immobilization, as phosphorus is an important plant macronutrient, making up about 0.2% of a plant's dry weight. Most studies on the pH dependence of P uptake in higher plants have found that uptake rates are highest between pH 5.0 and 6.0, which suggests that P is taken up as the monovalent form (Schachtman et al. 1998).

1.3 Iron oxides in soils

1.3.1 A brief description

The basic structural unit of iron oxides is FeO_6 or $Fe(O, OH)_6$ octahedron with the oxygen atoms arranged around the iron atom in hexagonal α forms, or cubic γ forms (Klein and Hurlbut Jr 1993). Depending on how the octahedrons link with each other, structure and properties of the iron oxides change, forming thus different kinds of minerals. Soil iron oxides can origin from the parent material during weathering of iron minerals. These are basically the most important adsorbents of phosphate (Borggaard 1990). Normally iron oxides are formed by small particles and they mix very well with clay fractions. They can easily be found well distributed and mixed up all over the soil but sometimes they can be found concentrated in certain horizons or packed up in nodules or grains. It is possible that impurities such as atoms or ions other than Fe, O and H are present in the mineral

structure as a result of isomorphous substitution, e.g. Al for Fe substitution and Mn for Fe substitution (Borggaard 1990).

There are mainly three types of iron oxides: basic oxides (Fe_aO_b, α-Fe_aO_b), hydroxides ($Fe_a(OH)_b$) and oxide-hydroxides (α-FeOOH). Let us now have a look at the most common iron oxide minerals. Goethite (α-FeOOH) can be found in almost all soil types all around the world. Hematite (α-Fe_2O_3) often occurs with goethite and it can be found in reddish tropical and subtropical soils. Maghemite (γ-Fe_2O_3) can be seen, in similar soils but located in more temperate areas. Lepidocrocite (γ-FeOOH) can be found in hydromorphic soils, often associated with goethite. Iron hydroxides are basically represented only by ferrihydrite (Fe_5HO_8*$4H_2O$, also written as $5Fe_2O_3$*$9H_2O$ or as Fe_2O_3*2FeOOH*$2.6H_2O$) (Jambor and Dutrizac 1998; Hansel et al. 2003). Ferrihydrite is known to be a precursor of other iron oxide soil minerals like hematite and goethite (Stanjek and Weilder 1992). Ferrihydrite is a very reactive mineral that can interact either by surface adsorption or by co-precipitation, with a number of environmentally important chemical species, including phosphates (Jambor and Dutrizac 1998).

Iron oxides are, together with aluminium oxides and 1:1 layer silicates, the most important inorganic variable charge constituents in soils, while organic matter has a variable negative charge (Theng 1980). Iron oxides can develop variable charges as the composition of the soil solution changes, due to e.g. pH variations. Thus, they can react with ions and adsorb them. Therefore, they are very important in the control of pollution and in the mobility and availability of nutrients.

Adsorption can be of two kinds: ions (adsorbate) can be bound to the oxide surface (adsorbent) with no solvent molecules interposed in the case of specific adsorption with formation of covalent bonds and also electrostatic reactions; or they can bind to the adsorbent, with the help of a solvent in the case of non specific adsorption. Analyzing only the concentration decrease of anions and cations in a solution, where the adsorbent is present, it is not possible to distinguish between adsorption and soil precipitation (Stanforth 2000). Precipitation involves the formation of multiple layers of the ion over the adsorbing oxide, and not only a single layer as in the case of specific adsorption. The ions complexed on the surface, behave differently from the precipitated ones in terms of mobility and desorption (Stanforth 2000). In this work, the term sorption will be used when impossible to distinguish between proper adsorption and soil precipitation.

To describe phosphate adsorption, as well as other anions and cation adsorption the Langmuir equation is often used (Bolan et al. 1985). This kind of adsorption model, also called isotherm as this sorption relationship applies only at constant temperature, is very useful to calculate sorption of any compound over its fraction in solution, and to known the point of maximum adsorption. This

model describes the perfect sorbent, which means that its surface is supposed to be uniform. All the adsorption sites should be equivalent and at the point of maximum adsorption, only a monolayer should form. These conditions are unrealistic as surface precipitation occurs very often. Langmuir equation as well as Freundlich equation may, however be useful to summarize information (Bolan et al. 1985) and to compare different adsorbents with each others.

As (Borggaard 1990) explains, only singly coordinated hydroxyl groups (-OH, A-type) are thought to bind anions and cations. A-type hydroxyl groups are bound to one Fe atom only, while B-type and C-type are coordinated to more Fe atoms at the same time. In goethite, A-type hydroxyl groups have been calculated to be 3.3 per square nm. In contact with water, every iron oxide is hydroxylated, meaning that according to pH, the hydroxyl groups gains or loses protons:

$$Fe\text{-}OH + H^+ \rightleftharpoons Fe\text{-}OH_2^+ \quad (1)$$

$$Fe\text{-}OH \rightleftharpoons Fe\text{-}O^- + H^+ \quad (2)$$

Obviously, at a certain pH, the amount of positive and negative charges are equal; this point, called the zero point charge (ZPC) seems to be close to pH 7 for various pure iron oxides (Borggaard 1990).

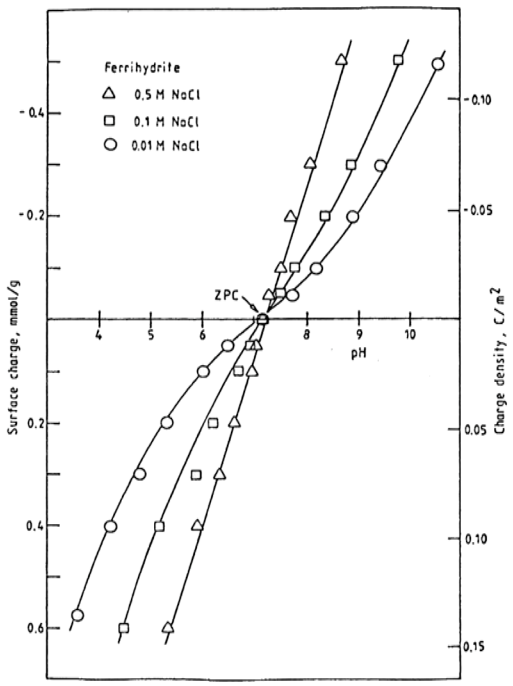


Figure 1.c **Titration curves for synthetic ferrihydrite** (Borggaard 1990). The figure shows what the ZPC is for ferrihydrite in a solution buffered with NaCl at different concentrations. With pH > ZPC then ferrihydrite surface is negatively charged while it is positively charged with pH < ZPC.

Iron oxides are positively charged when pH < ZPC, and attract anions. This kind of attraction, solely driven by coulombic forces, is non-specific. Anyhow, in many soils, in particular cultivated ones, pH is close to or above the ZPC leaving no positive charges on the oxides. Beside this, positive charges are neutralized by other soil components, naturally in the anion form such as organic matter and silicates; reducing thus the non-specific sorption power of iron oxides. That is why non-specific adsorption is relevant only in acid soils very rich in iron oxides (Borggaard 1990). As Bolan et al. (1985) describe, specific adsorption of anions takes place in much greater proportions than their presence in soil solution, meaning that the ratio between specifically adsorbed anions on anions in solution would be much bigger than one. Specifically adsorbable anions (e.g. silicates or phosphates) are adsorbed at every pH value, even under alkaline conditions, where iron oxides are negatively charged.

1.3.2 Interactions between phosphates and iron oxides

There is a very close relationship between phosphates and iron oxides in soils. When iron oxides are hydroxilated, an inner-sphere complex with phosphate is formed, where phosphate is bound in one (rare) or two of its oxygen ions to a Fe atom (Borggaard 1990; Auerswaldt et al. 1997). As said before, only A-type -OH groups take part in adsorption. Knowing their density, and specific surface area for every face of the crystal, it is possible to estimate the phosphate adsorption capacity (PAC).

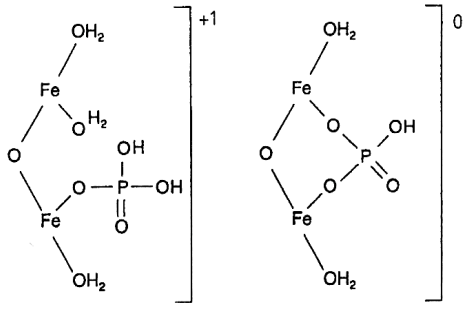


Figure 1.d **Mononuclear (left) and binuclear (right) phosphate-iron oxide surface complexes** (Auerswaldt et al. 1997).

Lots of experiments have been obtaining a PAC value for different iron oxides in the last years. Natural goethite phosphate adsorption capacity, depending on crystal type, is assessed to be from 77.5 to 210 $\mu g/m^2$, while 77.5 $\mu g/m^2$ can be adopted as a mean value for most Fe oxides (Auerswaldt et al. 1997). Adsorption studies with phosphate show that synthetic goethite can bind

61-186 μg P/m^2, depending on pH excursions (Borggaard 1983). As a study by Kosmulski et al. (1004) shows, goethite has a specific surface of circa 51 m^2/g. With an easy calculation it is possible to se that goethite can sorb 3.8-6.5 $mg_P/g_{GOETHITE}$. Next, phosphates adsorption is, due to PAC concept and definition, independent from adsorbents' surface area. However, iron oxides with a big crystal structure are slower phosphate exchangers than the smaller ones (Borggaard 1990). Table 1.1 shows the PAC for some commonly occurring soil iron oxides.

This property can also be extended to other oxides, e.g. aluminium oxides, and this is the reason why the finest gibbsite on the market was chosen for this project. Further details about this are given in paragraph 3.3 "Sorbents characterisation".

Phosphate adsorption by iron oxides over time is very fast in the beginning then it slows down, as sorbed P gets closer to the oxide PAC. Sometimes, the concentration of phosphate in soils is lower than PAC value; in this case, the sorption over time follows the described dynamic, and the phosphate is totally adsorbed after a significant period of time (Auerswaldt et al. 1997).

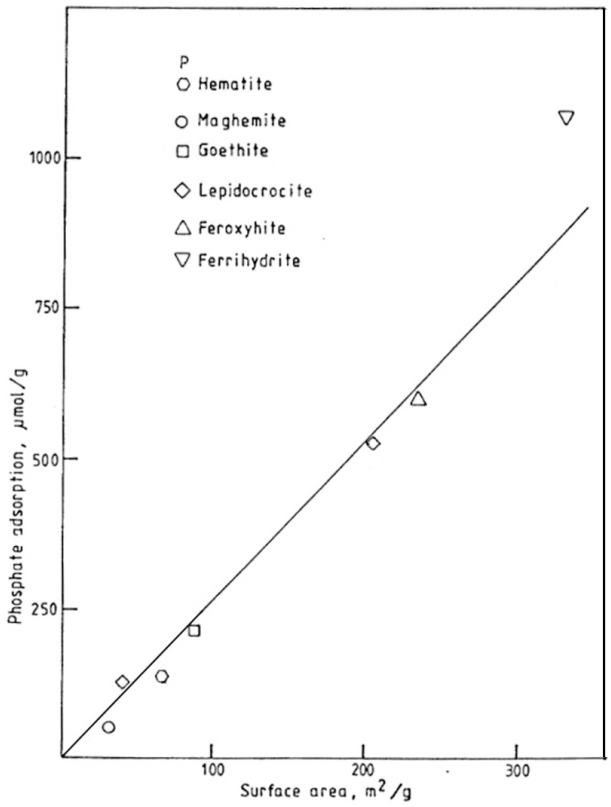


Figure 1.e **Amount of phosphate adsorbed by various synthetic iron oxides plotted against their specific surface area** (Borggaard 1990). Figure shows the positive linear correlations between specific surface area and total adsorbable phosphate.

Table 1.1 **Phosphate adsorption capacity for various iron oxides** (Auerswaldt et al. 1997). All references are given in the original table present in Auerswaldt's book. PAC values have been edited to format them with this project results-giving layout (g unit instead of mol). To calculate the PAC values in $mg_P/g_{IRON\ OXIDE}$ this values have been used: ferrihydrite, 200 m^2/g (Weidler 1997); goethite, 51.2 m^2/g (Kosmulski et al. 2004); hematite, 45 m^2/g (Hwang and Lenhart 2008); lepidocrocite, 100 m^2/g (Subrt et al. 1981); maghemite, 18.6 m^2/g (Watanabe and Seto 1993); akaganeite 100 m^2/g (Xiong et al. 2008).

Mineral	Origin	PAC		Observations	Equilibrium pH	Reference
		mg P / g	μg P / m^2			
Ferrihydrite [a]	Synthetic	15.5	77.4		7.0	Lijklema, 1980
Ferrihydrite [b]	Synthetic	19.2	96.0		7.0	Borggaard, 1983a
Ferrihydrite	Soils	14.9	74.3	Mean, 18 samples	7.0	Borggaard, 1983a
Ferrihydrite [c]	Synthetic	24.2	120.8		4.0	Willett et al., 1988
Ferrihydrite [d]	Synthetic	19.2	96.0		6.0	Guzmán et al., 1994
Goethite	Synthetic	4.0	77.4	Mean, 8 samples	3.0-4.0	Atkinson et al., 1972
Goethite	Synthetic	3.8	74.3		5.7	Cabrera et al., 1977
Goethite	Synthetic	3.8	74.3		7.0	Bowden et al., 1980
Goethite	Synthetic	4.1	80.5		7.0	Borggaard, 1983a
Goethite	Synthetic	4.4	86.7	Mean, 31 samples	6.0	Torrent et al., 1990
Goethite	Soils, ferricretes	3.8	74.3	Mean, 10 samples	5.0	Torrent et al., 1992
Goethite	Synthetic	6.5	127.0	Mean, 4 samples	6.5	Fontes et al., 1992
Hematite	Synthetic	4.2	92.9		7.0	Breeuwsma, 1973
Hematite	Synthetic	2.2	49.6		7.6	Cabrera et al., 1977
Hematite	Synthetic	3.3	74.3		7.0	Borggaard, 1983a
Hematite	Synthetic (alominous)	2.5	55.8	Mean, 43 samples	6.0	Barrón et al., 1988
Hematite	Synthetic	3.3	74.3	Mean, 30 samples	6.0	Colombo et al., 1994
Hematite	Soils, nodules, ferricretes	3.5	77.4	Mean, 14 samples	5.5	Torrent et al, 1994
Lepidocrocite	Synthetic	4.6	46.5		6.4	Cabrera et al., 1977
Lepidocrocite	Synthetic	7.4	74.3		7.0	Borggaard, 1983a
Lepidocrocite	Synthetic	8.1	80.5		7.0	Borggaard, 1983a
Maghemite	Synthetic	1.7	92.9		7.0	Borggaard, 1983a
Maghemite	Synthetic	1.0	55.8		7.0	Borggaard, 1983a
Akaganeite	Synthetic	9.6	96.0		6.0	McLaughling et al., 1981
Akaganeite	Synthetic	19.2	192.0		7.0	Borggaard, 1983a
Feroxyhite	Synthetic	N.D.*	77.4		7.0	Borggaard, 1983a

[a] Under experimental conditions where increasing the P concentration in solution does not result in significantly increasing of adsorbed P.

[b] This ferrihydrite was not dried. Calculation was made on the basis of a specific surface area of 600 m2/g.

[c] 2-line ferrihydrite dried at low temperature (< 340 K)

[d] From goethite-humic acid complexes.

[*] N.D.=non-determined. Any value for feroxyhite specific surface could be found.

Phosphate concentration may also be well above the isotopically exchangeable (specific-monolayer adsorption) P upper limit (Figure 1.f); that is when surface precipitation occurs and the Fe phosphate precipitate is formed (Auerswaldt et al. 1997; Stanforth 2000). It is still unclear how Fe phosphates form, it probably happens because part of the first adsorbed layer binds Fe in a new way, as a result of interactions from the extra P layers deposited on it. In fact, in contradiction to

what was commonly thought before, Stanforth (2000) shows how surface precipitate - secondly adsorbed - is the one involved with exchange reactions with other anions, thus in equilibrium with phosphate in solution; rather than the surface complex – firstly adsorbed - , that seems to be very strongly bound and will not desorb at all.

Phosphate desorption, regardless of its high significance for the environment and agriculture (e.g. rice cropping), has historically not received much attention. Adsorbed P was believed to be irreversibly bound to iron oxides, in particular when adsorbed to non-accessible crystal walls, where exchange has a hard time occurring; also, as said before, precipitation was believed to be totally irreversible. In fact, when the soil environment is oxic, P adsorbed to soil particles is very stable. Problems arise when all the oxygen in the soil solution is consumed and/or replaced by water, and bacteria reduce the iron oxides from non soluble Fe(III) to soluble Fe(II).

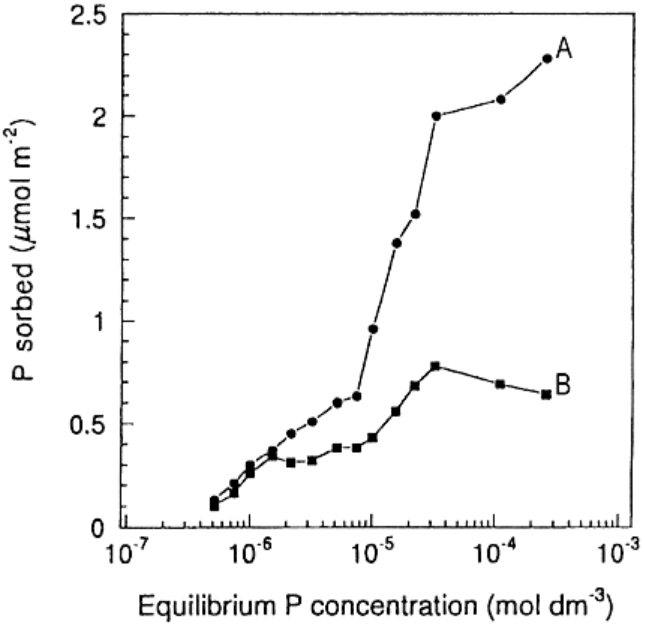


Figure 1.f **Total phosphate sorbed (A) and isotopically exchangeable phosphorus (B) on a hematite as a function of the equilibrium phosphate concentration**. (Auerswaldt et al. 1997)

It's not clear how phosphate is solubilized after iron reduction. The classical model, firstly developed by (Mortimer 1941) and describing phosphate cycle in lakes sediments, points the formation of soluble iron(II) phosphates as the main reason for P solubility. In anoxic environments iron(II) phosphates will interact with sulphides and originate iron(II) sulphides releasing thus phosphate ions in solution (Pratt 2006). Some authors suggest that bacteria could have also a very important role in sorbing and releasing phosphorus (Gächter et al. 1988). Bacteria are though to release phosphates once they reduce iron oxides as part of their metabolism pathway.

1.3.3 Dissimilatory Fe(III) reduction.

It is well known that phosphate solution concentration in soils tends to increase under anoxic conditions (Murray and Hesterberg 2006), when soils are flooded a lot of P is released into the soil solution and somehow have beneficial effects on crop production, as it has with rice. As reported by many studies (Patrick Jr and Khalid 1974; Ann et al. 2000; Young and Ross 2001; Scalenghe et al. 2002; Zhang et al. 2003; Peretyazhko and Sposito 2005; Shenker et al. 2005; Murray and Hesterberg 2006), P release under anaerobic state is thought to be strictly related to iron reduction. Fe(II) is in fact water-soluble. At pH lower natural water's pH Fe(II) is totally soluble, while Fe(III) is very stable in the solid phase and it solubilizes only at very extremes pH values such as 2 or less (Atkins 1996; Strandberg and LLC 2001). Regardless to the reason of P mobilization, that is not yet completely clear, its consequence is indeed very relevant. It is also clear that when P is released it would be very important to have it sorbed back to a solid non-soluble sorbent. It is well known also that Fe oxides are not the perfect binders just because they get soluble when reduced and if bacteria were responsible for P sorption, they wouldn't sorb it back due to the redox conditions of soils completely reduced. It is therefore very important to find a non-soluble and stable sorbent working also in anoxic conditions.

Iron (III) is used as an electron acceptor by organisms, which get energy for their metabolism oxidising completely organic compounds to the simple CO_2 (Gächter et al. 1988; Lovley 1991; Hansel et al. 2003; Kim et al. 2005). Let us now have a look to the most common types, and actors of Fe(III) reduction and to the reactions describing the process. Dissimilatory Fe(III) reduction can be defined as the use of Fe as an external electron acceptor in the metabolism of a wide variety of bacteria and fungi. Having the highly insoluble Fe(III) as an internal, thus assimilated, electron acceptor would be too energy consuming at a point that the entire reaction would not result energetically convenient for the microorganisms (Di Christina et al. 2002). Therefore microorganisms developed this way of expelling proteins working as "electron shuttles" out of their cell membrane and reduce Fe(III) in a secondary external reaction (Di Christina et al. 2002). Microorganisms with a primary fermentative metabolism, e.g *Esterichia coli, Lactobacillus lactis,* other bacteria and also some fungi, were the first ones showing an Fe(III) dissimilatory reduction activity as fermentative reducers (Lovley 1991). These organisms reduce Fe(III) while metabolizing fermentable sugars and amino acids. As reported in the same article, *Bacillus polymyxa* was found to reduce 24 mol of Fe(III) for every 100 mol of glucose it consumed, going from the sugar molecule to carbon dioxide/carbonic acid as equation 3 shows. As it can be deduced from the

equation, Fe is not the only electron acceptor for these organisms, even if this is not the most efficient reduction process amongst them all it is still considered to be one of the most significant, due to the rate of the reaction (Lovley 1991).

$$C_6H_{12}O_6 + 24Fe(III) + 12H_2O \rightarrow 6HCO_3^- + 24Fe(II) + 30H^+ \quad (3)$$

Sulfur-oxidizing Fe(III) reducers, e.g. *Thiobacillus ferrooxidans* and *Sulfolobus acidocaldarius* use elemental S - as a electron donor - and Fe(III) to support their metabolism, without necessarily obtaining any energy from this redox reaction (Lovley 1991), as follows:

$$S^0 + 6Fe(III) + 4H_2O \rightarrow HSO_4^- + 6Fe(II) + 7H^+ \quad (4)$$

Also sulphur-oxidizing Fe(III) reducers do not use iron as the sole electron acceptor, their activity in this sense is although very relevant. In a rich media other kind of organisms such as *Pseudomonas sp.* and *Shewanella putrefaciens* can grow, and use Fe(III) as the sole electron acceptor while oxidizing H_2. The growth of these bacteria depends on Fe(III) abundance and the reaction has a rate of H oxidized on Fe(III) reduced equal to one (Lovley 1991).

$$H_2 + 2Fe(III) \rightarrow 2H^+ + 2Fe(II) \quad (5)$$

Another important group of Fe(III) reducers is the one of organic-acid-oxidizing Fe(III) reducers. These microorganisms (e.g. *Geobacter sp*) are able to oxidize completely organic compounds to carbon dioxide, using only Fe(III) as the electron acceptor when the environment is strictly anaerobic according to the following equation for acetate (Lovley 1991)

$$CH_3COO^- + 8Fe(III) + 4H_2O \rightarrow 2HCO_3^- + 8Fe(II) + 9H^+ \quad (6)$$

Also aromatic compounds can be completely oxidized anaerobically by Fe(III) reducers, but it is a minor process of significance, due to their low concentration in natural environments.
In general, according to all the studies cited in this paragraph, a simple model for the oxidation of organic matter with Fe(III) serving as the sole electron acceptor can be made where sugars and amino acids are the first ones to be metabolized, producing fermentation acids and hydrogen, and then elemental S or other atoms as side products. The whole process could roughly be shown as presented in Figure 1.g.

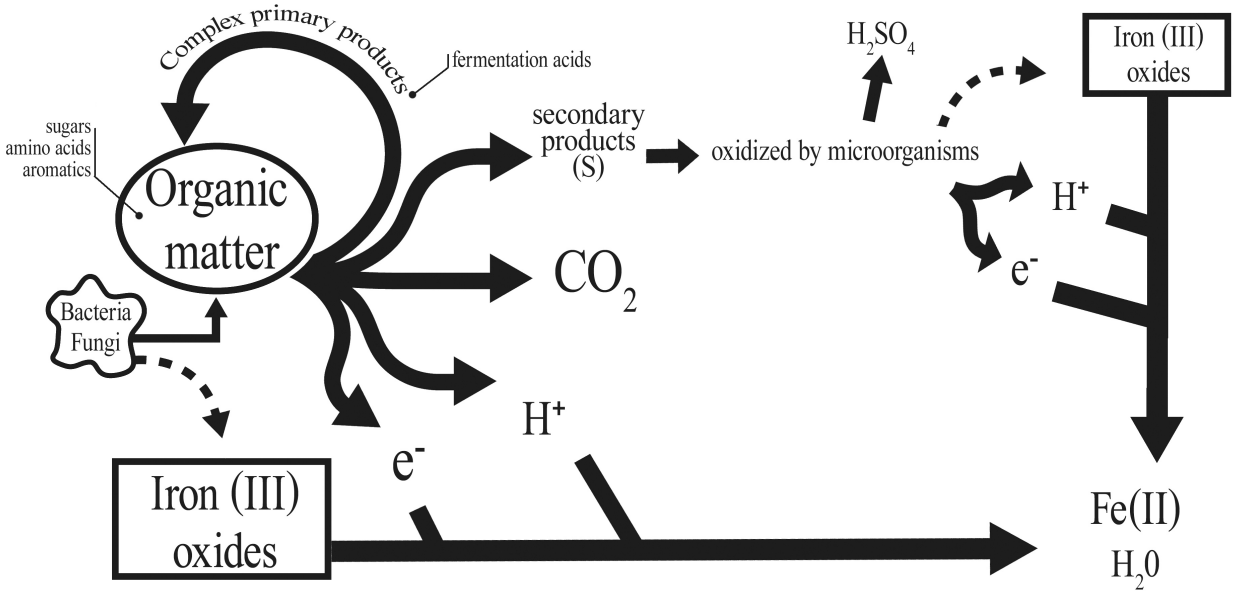


Figure 1.g **Overview on microbial Fe(III) reduction process.** The final step of the process can be reassumed with the following reaction: $e^- + 3H^+ + Fe(OH)_3 \rightarrow Fe^{2+} + 3H_2O$. (by the author).

The Fe(II) produced by reductive dissolution undergoes complex secondary chemical transformations. Along with aqueous Fe(II) complex formation and Fe^{2+} adsorption on oxide surfaces and bacterial cells, Fe(II) secondary minerals precipitate (siderite ($FeCO_3$), vivianite ($Fe_3(PO_4)_2$ * $8H_2O$) and magnetite (Fe_3O_4) (Hansel et al. 2003). Formation of these Fe(II) secondary phases is influenced by the presence of soluble P, atmospheric composition, pH, temperature, time, and the species of bacteria present.

When Fe(III) is reduced to Fe(II), most of the phosphate bound to the oxide becomes then soluble as well (Mortimer 1941; Lovley 1991). This process was suggested more than 60 years ago for the first time and was widely accepted in few years (Patrick Jr and Khalid 1974; Ann et al. 2000; Young and Ross 2001; Scalenghe et al. 2002; Zhang et al. 2003; Peretyazhko and Sposito 2005; Shenker et al. 2005; Murray and Hesterberg 2006). Some scientists (Gächter et al. 1988) anyways, suggested that release of P occurs with Fe(III) reduction in sediments under anoxic conditions just as a coincidence. In fact they could not find any relationship between the amount of P released and the Fe(II) dissolved in solution. They suggested that phosphate released in solution is not totally coupled with iron oxides but could instead be stored by sediment microorganisms under aerobic conditions and then released from the intracellular space to the outside solution as soon as the environment becomes anoxic. Further studies on trace metals (Mackin et al. 1988; Lovley 1991), also adsorbed by iron(III) oxides demonstrated that there is a strict relation between Fe(III) reduced and sorbed compounds released to the water solution. Probably Gächter and his team did not take in

consideration surface precipitation of P on iron oxides, or the combined action of other oxides, e.g. aluminium oxides, on P dissolution.

1.4 Aluminium oxides in soils

Aluminium can be normally found in soils coupled with oxygen. It can form oxides (Al_2O_3) hydroxides ($Al(OH)_3$) and oxide-hydroxides (AlO(OH). Gibbsite (γ-$Al(OH)_3$) is the most common aluminium oxide found in soils. It comes in double layers of hydroxyl groups where aluminium ions occupying two-thirds of the octahedral holes between the two layers (Klein and Hurlbut Jr 1993). Aluminium can be also found as a common substitute of Si in silicate minerals, such as kaolinite ($Al_2Si_2O_5(OH)_4$). Kaolinite is a clay-silicate mineral with one tetrahedral sheet bound through oxygen atoms to one octahedral sheet of aluminium octahedrons (Klein and Hurlbut Jr 1993). For what concerns with the ability of soil aluminium to sorb cations the reader is invited to refer to the previous paragraph about iron oxides in soils. Aluminium properties do not differ from the ones explained about iron. The only difference that is relevant about P sorption and desorption is that Al is always non-soluble (Atkins 1996; Kopacek et al. 2000)4.

Because iron oxides and the phosphate bound to them are solubilized when Fe(III) is reduced to Fe(II) under anaerobic conditions, which occur in soils when flooded for long time or in anoxic sediments; it looks reasonable that aluminium oxides might be an important agent for dissolved P retention. The valence state of Al does not change with the variation of redox potentials, and anoxic environment does not cause the solubilization of aluminium-phosphate complexes (Darke and Walbridge 2000). Soil aluminium oxides thus should be less affected by flooding than iron oxides.

However, the fact that Al concentrations are often highly correlated with P sorption capacity in wetland soils (Richardson 1985) suggests that there may be some mechanism that favours aluminium oxides formation and/or persistence in these soils (Darke and Walbridge 2000). As anaerobic conditions associated with flooding slow down its decomposition, organic matter also tends to accumulate in wetland soils and free Al(III) in solution can bind with organic matter to form an organic matter-aluminium complex that is very efficient in sorbing P (Darke and Walbridge 2000; Hogan et al. 2004; Giesler et al. 2005). Organic anions also can reduce phosphate sorption capacity of existing oxides by competing with phosphate ions for binding sites (Easterwood and Sartain 1990). Finally, organic matter can inhibit the crystallization of pre-existing amorphous aluminium and iron oxide minerals (Kodama & Schnitzer 1979, 1980), enhancing phosphate

sorption capacity (Borggaard et al. 1990; Borggaard et al. 2005). In sandy Danish soils, Borggaard et al. (1990) found that organic matter inhibited the crystallization of soil aluminium oxides much more than it did on iron oxides; the resulting poorly crystalline aluminium oxides adsorbed nearly twice as much phosphate than the iron oxides present in those soils. Moreover, results found by Darke and Walbridge (2000) suggest that Al biogeochemistry is strongly influenced by complexation reactions with organic matter, while iron oxides are more significantly influenced by flooding and consequent anoxic conditions, suggesting the importance of redox reactions.
Aluminium is thus the ideal P sorbent to choose having objectives similar to the ones that this project has.

1.5 About eutrophication

Depending on content of nutrients, a waterbody can be classified as oligotrophic, mesotrophic or eutrophic. A waterbody with low primary production and low content of nutrients it is called oligotrophic. Oppositely, the European Community, in its Urban Waste Water Treatment Directive (1991), defined eutrophication as:

> *"the enrichment of water by nutrients especially compounds of nitrogen and phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms and the quality of the water concerned"*.

Finally, a mesotrophic waterbody has moderate primary production, less than a eutrophic one but more than an oligotrophic.
Eutrophication was recognized as a pollution problem in European and North American lakes and reservoirs around 1950s. From that time, it has become more and more a global problem. Surveys showed that most of the lakes all around the world are eutrophic: 54% of lakes in Asia, 53%in Europe, 48% in North America, 41% in South America and 28% in Africa (ILEC-Lake-Biwa-Research-Institute 1994)
In lakes and slow streams nutrients enrichment stimulates phytoplankton, as micro-algae and cyanobacteria grow much faster than bigger algae and plants (Tett 2003), that adsorbs light and generate shadow effect on benthic organisms. In faster flowing water, attached plant growth is stimulated, as phytoplankton is usually carried away downstream. As a common species growing in eutrophic waters water hyacinth (*Eichhornia crassipes*), duckweed (*Lemna minor*) and the water fern (*Azolla filiculoides)* may be seen (Tett 2003). Eutrophic waters can be recognized because of

their greenish colour, cloudiness and low content of oxygen. Eutrophication may be a natural event in environments that accumulate nutrients but it is most frequently a consequence of nutrient pollution such as the release of sewage effluent and fertilizers runoff into waterbodies. Human activities usually accelerate the rate at which nutrients enter ecosystems. Runoff from agriculture and other human-related activities increase the flux of both inorganic nutrients and organic substances into terrestrial, and aquatic ecosystems. As said before, phosphorous is usually the limiting nutrient for plant growth (Bridgham et al. 2001; Peretyazhko and Sposito 2005) and thus it is the element applied at higher concentrations compared with actual plant demands. Humankind has increased the rate of phosphorus cycling on Earth by four times, mainly due to agricultural fertilizer production and application. Between 1950 and 1995, 600 millions tonnes of phosphorus were applied to Earth's surface, primarily on croplands (Carpenter et al. 1989). As a consequence phosphorus results to be the main responsible of eutrophication in rivers and lakes. A control on point sources of phosphorus have been done in the last years, optimizing water cleaning and industrial processes and resulted in a rapid control of eutrophication events caused by them.

Nitrogen also is a very common nutrient causing eutrophication. Nitrogen can however be the limiting nutrient only in rare cases in marine water (e.g. costal waters and estuaries). In fact the Redfield ratio (molecular ratio for phytoplankton's health growth of N:P) in a normal freshwater body is 28:1 (Tett 2003) while in marine waters it is 16:1. Nitrogen is the most common gas in atmosphere, but plants do not usually assimilate it as N_2, they need it in other forms such as nitrate (NO_3^-). Terrestrial nitrogen fixing microorganisms convert N_2 into nitrates so higher plants can uptake it. Massive fixation by these bacteria and anthropogenic inputs contribute to saturate soils with N. When nitrogen is present in higher amounts than plants could uptake, it easily leaches into waterbodies causing eutrophication. Phosphorus is, on the other hand, much less soluble than nitrogen, and it is consequently more important as a limiting nutrient in aquatic systems. Moreover, Schindler (1977) found that N concentration depends most of times on dissolved P availability, as he observed that nitrogen contents in lakes increased when phosphate inputs increased, even when N was no added at all with fertilizers. Schindler explains that nitrogen was provided with the mineralization to nitrate of dead N_2 fixating microorganisms.

Table 1.1 gives a list of the most common point and non-point sources of nutrients causing eutrophication. To give a brief definition, a point source is a localized and stationary pollution source. In a mathematical model it could be represented as a simple point. Contrarily, non-point sources are represented with an area, as they are diffuse and widespread sources.

Table 1.2 **Source of nutrients causing eutrophication** according to Carpenter et al. (1989).

Point Sources	Nonpoint Sources
• Wastewater effluent (municipal and industrial) • Runoff and leach from waste disposal systems • Runoff and infiltration from animal feedlots • Runoff from mines, oil fields, industrial sites • Overflows of combined storm and sanitary sewers • Runoff from construction sites <20,000 m² • Septic tank leachate	• Runoff from agriculture/irrigation • Runoff from pasture and range • Urban runoff • Runoff from construction sites >20,000 m² • Atmospheric deposition over a water surface • Other land activities generating contaminants

As said, non point sources are nowadays the most relevant ones in relation to eutrophication. Between all, agricultural runoff is the most common, frequent and important for both phosphorus and nitrogen (Young and Ross 2001; Ajmone-Marsan et al. 2006). Nutrients are transported to waterbodies via surface floodwater or leached through groundwater. Nitrogen can also be deposited from the atmosphere. Especially in highly industrialized regions, it can be introduced to water ecosystems via acid rains in the form of nitric acid (pKa 1.4), which then dissociates into nitrate.

As a consequence of enhancement of primary productivity, many problems for the environment can arise dealing with decrease of biodiversity, invasion by new species and presence of toxic compounds in water. Biodiversity has a fragile equilibrium in most of the natural ecosystems, where inputs and outputs balance each other. When primary productivity increase rapidly and to a big extent in eutrophic waters, surface water is filled with algae reducing thus the amount of sunlight reaching the lower levels of the waterbody. An increased population of algae also deal to a rapid decrease of dissolved oxygen availability because it is consumed by algae and by those microorganisms that feed on the dead algae. As a consequence, fishes and shellfishes die, impacting on all the food chain. Changing biodiversity's equilibrium and nutrient composition of the ecosystem, new species can move in and become dominant or relevant. Those new species resulted in many cases to be dangerous for their toxic products that heavily affect water quality with effects also on human health. Some algae bloom produce toxins that kills directly organisms that feed on them or just accumulate in the food chain (Chorus and Bartram 1999). High nitrogen content in water can also be harmful to human and animal health. The Blue Baby syndrome is a clear example of that. Furthermore, in a wider point of view, economic aspects such as recreation, fishing, hunting

and aesthetic enjoyment are negatively impacted by eutrophication, lowering then the economical and social value of rivers, lakes and estuaries.

From the 1960s ecologists started considering chlorophyll concentration in water as the indicator of primary productivity (Tett 2003) and found a linear correlation between P concentration in water and chlorophyll production. After a study on the lakes at the Experimental Lakes Area (ELA) in North West Ontario, Schindler (1977) concluded that there was a very precise relationship between P concentration in solution and the total chlorophyll produced by phytoplankton and algae, which is commonly 1:1 (every microgram of P taken out from the lake, decreased of 1 mg the amount of chlorophyll produced). Because of this very relationship, parameters can be settled to describe the trophic state of a lake and other waterbodies.

Table 1.3 **Definitions of lake trophy** (TN, total nitrogen; TP, total phosphorus; chl a, chlorophyll a; SD, Secchi disk transparency)

	Trophic state	TN (mg/m^3)	TP (mg/m^3)	chl a (mg/m^3)	SD (m)
Lakes*	Oligotrophic	< 350	< 10	< 3.5	> 4
	Mesotrophic	350-650	10-30	3.5-9	2-4
	Eutrophic	> 650	>30	> 9	< 2
				Suspended chl a (mg/m^3)	Benthic chl a (mg/m^3)
Streams*	Oligotrophic	< 700	< 25	< 10	< 20
	Mesotrophic	700-1500	25-75	10-23	20-70
	Eutrophic	> 1500	> 75	>30	>70
				chl a (mg/m^3)	SD (m)
Marine*	Oligotrophic	< 260	< 10	< 1	> 6
	Mesotrophic	260-350	10-30	1-3	3-6
	Eutrophic	> 350	> 30	> 3	< 3
Lakes#	Oligotrophic	-	< 10	<8	> 3
	Mesotrophic	-	10-35	8-25	1.5-3
	Eutrophic	-	> 35	>25	<1.5

*data from (Smith et al. 1999).

#data from Organization for Economic Co-operation and Development. Eutrophication of Waters, Monitoring, Assessment and Control 1982 Report.(Tett 2003)

A wide list of examples on how to remediate eutrophic waters is given by Smith et al. (1999). Most of the polluted events were solved simply eliminating or well managing the point sources of nutrients, while a different strategy has to be followed with non-point sources. In the last years fertilizers have been optimized, and their use has been strictly regulated. Finally, riparian buffer zones have been indicated as possible sink for nutrients in their path from fields to surface waterbodies.

1.6 With respect to reductive mobilization of phosphorus

The author is now going to guide the reader through some studies that have already been published in the last years by various authors. Understanding these papers, the aim of the authors, their results and conclusion will help to understand this work, knowing what similar researches found out and what was known at the time this project begun. Four papers are going to be presented, in such a order to give firstly an overview on the Fe(III) reduction and consequent P dissolution (first two papers) and later on to focus on the role of aluminium on P resorption under anoxic environment.

-"Phosphate release from seasonally flooded soils: A laboratory microcosm study" by E.O. Young and D.S. Ross, published in *Journal of Environmental Quality* in 2001;

-"Iron(III) reduction and phosphorous solubilization in humid tropical forest soils" by T. Peretyazhko and G. Sposito, published in *Geochimica et Cosmochimica Acta* in 2005;

-"Phosphorus inactivation by aluminum in the water column and sediments: Lowering of in-lake phosphorus availability in an acidified watershed-lake ecosystem" by J. Kopacek, J. Hejzlar, J. Borovec, P. Porcal and I. Kotorova, published in *Limnology and Oceanography* in 2000.

-"Iron and phosphate dissolution during abiotic reduction of ferrihydrite-boehmite mixtures." By G.C. Murray and D. Hesterberg, published in *Soil Science Society of America Journal* in 2006.

1.6.1 "Phosphate release from seasonally flooded soils: A laboratory microcosm study"

Young and Ross are two researchers from the Department of Plant and Soil Sciences at the University of Vermont in Burlington and in 2001 they had published this study on mobility of

agricultural P into Lake Champlain, situated in the State of New York. Since the seventies, spot eutrophication of Lake Champlain became apparent and Phosphorus deriving from agricultural activities has been pointed as the leading cause for this impoverishment of water quality. This paper was one of the first works that have been trying to investigate on the fate of phosphates once they are released to soil solution and then to overlying water under anoxic conditions that occur with flooding. Author's specific objectives were to (i) find a correlation between amount of P dissolved over time and differences of phosphate fertility of the flooded soils, (ii) determine relationship between phosphorus release and soil characteristics (e.g. extractable P, Fe and Al content) and (iii) find out if Fe(III) to Fe(II) transformations affect P cycling in the test soils. Young and Ross collected 12 agricultural soils and 2 wetland soils of varying drainage, flooding regime, and fertility from a research farm located among the lakeshore. These soils were different for drainage, flooding regime and fertility proprieties. Soil from the first 5 cm was taken and incubated with NH_4-acetate (pH 4.8, 1.25 M) and stored in a glove box. Fe(II), pH, E_H, acid ammonium oxalate extractable Al and Fe, soluble reactive P and total P were determined. As the authors report: the 90 days flooded incubation induced significant P release to soil solution. Porewater P increased as much as 27.0 times the initial phosphate concentration, while floodwater phosphate could just reach 3.6 times the initial concentration, showing that phosphate migrates out from the soil to the porewater very slowly.

The authors concluded that the higher the soil fertility, the higher is the amount of P released into porewater. The majority of solubilized phosphate was not mobilized to floodwater. Generally, no precise correlation between amount of total extractable P and amount of P found in porewater was found, even though phosphate in floodwater and porewater tended to reach a common equilibrium in all soils. Young and Ross hypothesized that differences could have been due: to oversaturation of P in some soils (over the PAC), to a slow down in the Fe(III) reduction process or to iron ability to precipitate and resorb it. The presence of non-soluble aluminium oxides was shown to be irrelevant as all the soils contained approximately the same amount of aluminium. Thus, P solubility and mobility depends mainly on redox conditions and also on soil properties.

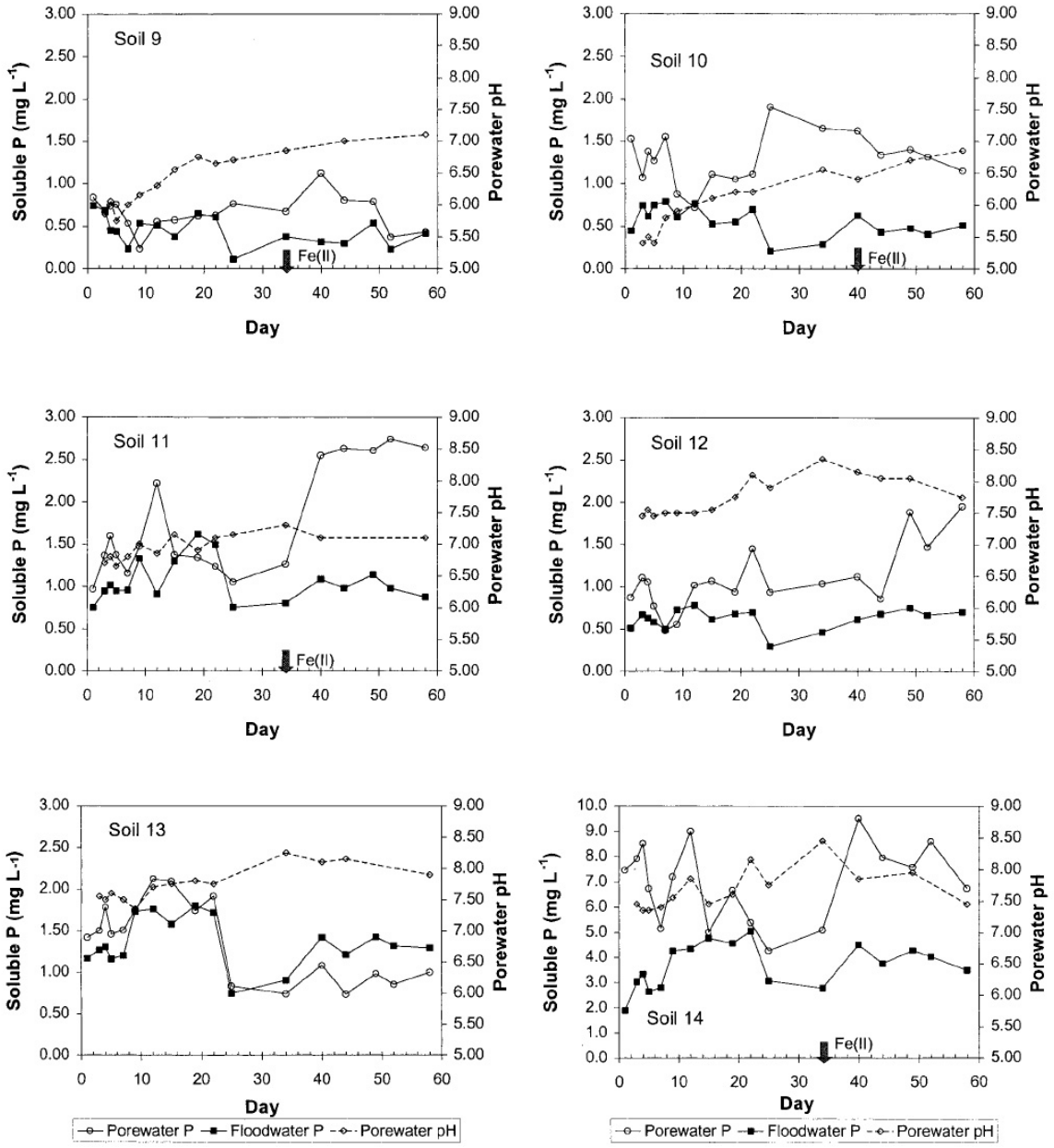


Figure 1.h **Changes in porewater phosphate and floodwater phosphate and porewater pH for the 6 soils with higher P content** (Young and Ross 2001). The arrow indicates the day of the first qualitative appearance of Fe(II). Note the different scale for soluble P in Soil 14.

1.6.2 "Iron(III) reduction and phosphorous solubilization in humid tropical forest soils"

In 2005 Peretyanzhko and Sposito, from the Division of Ecosystem Sciences at the University of California in Berkley, had published this very relevant work on tropical forest topsoil. The two authors were interested on the chemistry of iron oxides and related phosphorus mobility in soils that

are under a wet moisture regime for most part of the year. The forest Ultisol they used for this study was taken in Porto Rico, in a region where the annual rainfall is around 3000-4000 mm. The aim of this study was to determine the relationship between Fe(III) reduction and P solubilisation in a tropical soil. To make a good description of this process the authors decided then to focus on three objectives: (i) to quantify the ratio of Fe(III) reduced on P solubilized, (ii) to examine the influence of the electron shuttle anthraquinone-2,6-disulfonate (AQDS), (iii) to characterize any form of P an Fe(II) originated in the process, both with and without AQDS.

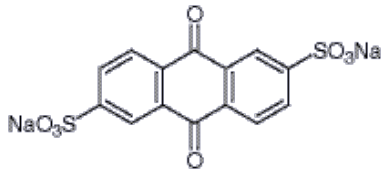


Figure 1.i **Disodium anthraquinone-2,6-disulfonate.**
(from Tokyo Chemical Industry Europe Co. website. url:http://www.tcieurope.eu/en/common/img-structure/A0308.gif)

Disodium anthraquinone-2,6-disulfonate is a model compound used frequently as a probe to study Fe(III) oxides reduction process by electron shuttling, it contains semiquinones that are believed to act as the semiquinones in humic matter do. Microorganisms transfer two electrons to AQDS, transforming it in anthrahydroquinone-2,6-disulfonate (AHDS) which combines with Fe(III) and reduces it to Fe(II).

Donating the electrons to iron, AHDS goes back to the oxidized form AQDS. This compound has been used to promote reduction of natural and synthetic, crystalline and amorphous iron(III) oxides. Peretyanzhko and Sposito were, however, the first ones using it with native microbial communities in natural soils.

The authors made two series of soil incubations, one without AQDS and another one with AQDS, added at the beginning of the experiment in a concentration of 7.5 $mmol/kg_{AIR\ DRIED\ SOIL}$. The soil was a homogenised selection of >2mm size fraction taken from the first 20 cm of soil horizon containing poorly crystalline iron(III) oxides. All the experiment was carried out, in strict absence of oxygen, sampling inside a glove box with a 95% CO_2 and 5% H_2 atmosphere. Production of Fe(II), total Fe [Fe(III) + Fe(II)], inorganic and organic P, pH, E_H (inside the glove box) and biogenetic gases (CO_2, H_2, CH_4) production (outside the glove box) were investigated.

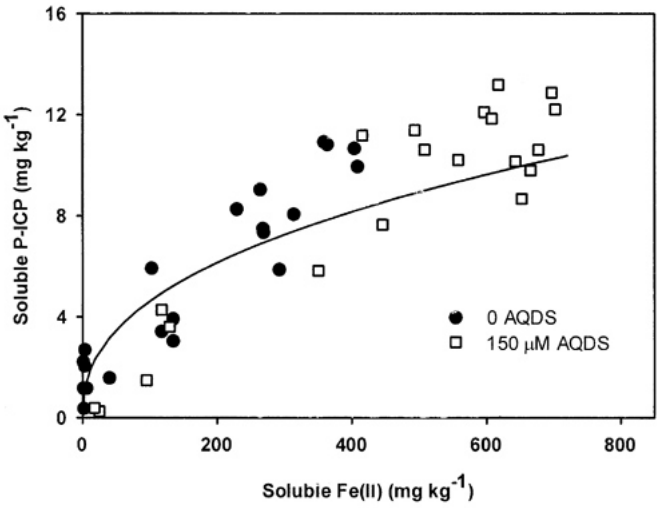


Figure 1.j **Soluble P-ICP** (defined by the authors as: "filterable inorganic P + organic P in supernatant solution") **concentration Vs. soluble Fe(II) concentration measured during the soil incubation**. The power-law regression through the data is described by equation: $[P\text{-}ICP] = 0.7(\pm 0.3)[Fe(II)]^{0.4\pm0.1}$. R^2=0.62. (Peretyazhko and Sposito 2005)

Peretyanzhko and Sposito founded a positive power-law correlation between soluble P release and soluble Fe(II) production under anoxic conditions, implying that reduction of iron(III) oxides solubilizes the phosphate bound to it. Most of the Fe(II) formed accumulated in particulate form, probably due to the formation of Fe(II) solid phases; vivianite and siderite were pointed as the likely candidates after thermodynamic calculations were made. Hydrogen gas, produced in the bacterial fermentation process, dropped sharply, while Fe(II) and CH_4 increased correspondingly, showing that the soil contained the above described (in paragraph 1.3.3) hydrogen-oxidizing Fe(lII) reducers. Carbon dioxide rose quickly in the beginning of the incubation until it reached a constant level; at that time siderite began to form. An overview on the whole process indicates that Fe(III) reduction goes together with H_2 consumption and C oxidation in the soil. Finally, the AQDS was proved to be a good electron shuttle enhancing bacteria activity. Its function, however, was only catalytic. In fact, no significant difference between pH, E_H and Fe(II)-dissolved P ratio were found between the two experimental series.

This paper shows how the control of iron oxides is important when we want to study P mobility in the environment. It also showed the importance of bacteria activity in the process. Moreover, the fact that a soil is more "catalyzed" than one other e.g. containing more humic matter, thus semiquinones, does not in the end make a difference in the amount of P that can be released per amount of iron(III) oxides reduced; it just makes a difference on the time this process takes to occur.

1.6.3 "Phosphorus inactivation by aluminium in the water column and sediments: Lowering of in-lake phosphorus availability in an acidified watershed-lake ecosystem"

These four researchers from the Faculty of Biological Sciences at the University of South Bohemia in Czech Republic noticed that, in an acidified lake, water chemistry of the anoxic zone did not develop in the typical way. In fact, Plešné Lake's anoxia that occurs above the bottom during winter and summer stratification periods implied, as commonly expected, depletion of dissolved Oxygen, decrease in nitrate and sulphate, and increase of ammonium, Fe(II) concentrations and pH increase; on the other hand, dissolved reactive P stayed always at the same constant concentration (<1μg/L) above the sediment. Plešné Lake is a dystrophic and dimictic lake, situated in the Bohemian Forest with a pH of 4.5-4-9 and a total P content in the bottom sediment of circa 10 μg/L. The term dystrophic defines lakes with brown-coloured waters with the colour being the result of humic substances and organic acids suspended in the water. The term dimictic describes the characteristic way in which some lakes have their water mixed from top to bottom twice a year; in spring and in fall the water in these kind of lakes will have a uniform temperature and density from top to bottom, allowing the lake waters to mix completely; while in winter and in summer times the epilimnion (surface waters) is separated from the hypolimnion (bottom waters), where anoxic conditions can occur due to the lack in oxygen supply. Its acidity and the typical brown-water colour are due to high concentrations of humic substances and organic acids suspended in the water. The aim of this study was to understand why P solubilization was inactivated after Fe(III) reduction.

The authors noticed that in the upper sediment layer, P was not released at all consequently to Iron(III) oxides reduction. The presence of fresh colloidal aluminium oxides floc originated in the water column was pointed as the cause of this event. This aluminium had origins from terrestrial losses caused by strong acidification of soils surrounding the lake. Together with Al^{n+}, also NO_3^- and SO_4^{2-} were leached into the lake but were removed by bacterial activity, contributing thus to alkalinity generation and the increase of water pH. At higher pH, ionic Al species hydrolyze and form colloidal aluminium (hydro)oxides especially in the hypolimnion. Settling, during stratification periods, increase the P sorption capacity of the sediment. The high content of aluminium oxides in all the sediment layers shows that the process has been going on for several years and that the oxides generated are very stable. The example of Plešné Lake is clearly showing the effect of amorphous virgin aluminium oxides on the capture of soluble P when the iron oxides adsorbing power is inactivated by reduction. This P-inactivation process has already been used in

lake restoration as described in many papers (authors cite a work by Cooke et al. 1993: Restoration and management of lakes and reservoirs. Lewis editor).

1.6.4 "Iron and phosphate dissolution during abiotic reduction of ferrihydrite-boehmite mixtures"

The last paper presented here describes a work very similar for goals to this thesis work. In a series of laboratory experiments carried out at the North Carolina State University, Murray and Hesterberg tried to assess the combined effect of synthetic ferric hydroxide ($Fe(OH)_3$) and microcrystalline boehmite (α-AlOOH) on mobility of added PO_4. The authors decided to make a complete abiotic set up to investigate this process in the simplest way and to set the basis for further studies where microorganisms and organic matter take part, as a more realistic model for soil dynamics. An aqueous suspension was thus made, containing 0.5 g ferrihydrite/$Kg_{SOLUTION}$, monopotassium phosphate (KH_2PO_4) added at 750 mmol/$kg_{FERRHYDRITE}$ and boehmite at different concentrations (up to 0.7 g/$kg_{SOLUTION}$). Ferrihydrite was abiotically reduced at pH 6.0 for 72 hours using H_2 gas in presence of a catalyst as Brennan and Lindsay (1998) and other scientists already did and described. The following reaction took place:

$$\tfrac{1}{2}\, H_{2(g)} + Fe(OH)_{3(s)} + 2H^+_{(aq)} \xleftrightarrow{catalyst} Fe^{2+}_{(aq)} + 3H_2O_{(l)} \qquad (7)$$

Net dissolution of PO_4 occurred over time in the control series, where boehmite concentration was equal to zero. As boehmite was added the author noticed a net uptake of P. Additional experiments showed that Al(III) dissolved from boehmite decreased Fe(III) reduction sorbing to the surface of the iron oxide and thus blocking electron transfer, directly proportionably to the amount of boehmite added. The effect of Al(III) on the decrease of P content in solution could so be due to two different reasons: (i) free P sorption on boehmite and (ii) deficiency of the Fe(III) reduction and consequent P desorption. In some experiments ($\leq$0.008 g boehmite/kg series), the authors operated with PO_4 well above the PAC for boehmite and realized that P was taken up in excess of the maximum boehmite sorption capacity for boehmite. These results suggested them the formation of Al-phosphate or an Al(III)-PO_4 surface-complex on ferrihydrite.

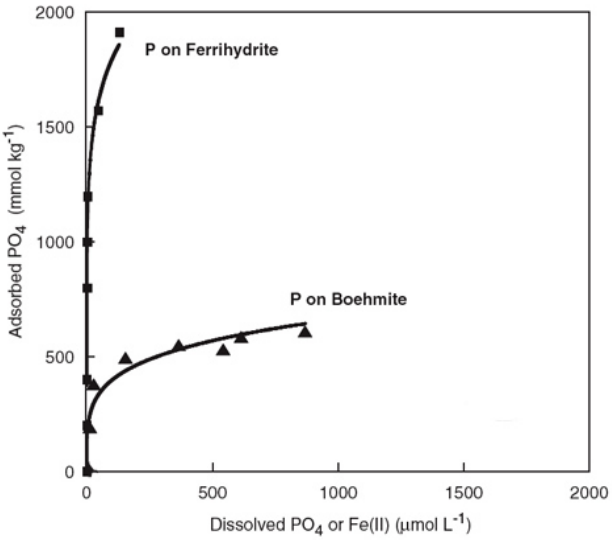


Figure 1.k **Adsorption isotherms for PO_4 on ferrihydrite or boehmite**. Smooth curves are Freundlich model fits to the data. (Murray and Hesterberg 2006)

Clearly, this is a very interesting work that shows a positive efficiency of one aluminium oxide on immobilization of P under anoxic conditions. This model, anyways, does not study the effect of added aluminium oxides in a environment where all the P is already sorbed by the iron oxides as this project has been planned to do, but it studies an environment where P is added at the two different oxides at the same time. Finally, a major difference between the work by Murray & Hesterberg and this work is that they did not made a soil experiment. This can be considered both a weak and a strong point. From one side it does not represent real soil dynamics that include variables: bacteria and other soil microorganisms' activity, unstable pH and EH, combined action of different iron oxides present at the same time, organic matter presence and the different activity that a real soil aluminium or iron oxides has. From another side this simplification is very useful to understand the basic trend-effects on P mobility that the different types of oxides create under anoxic conditions. Again, however, the presence of Al-phosphate or Al(III)-PO_4 surface-complex and Fe(III) reduction inhibition by excess of aluminium oxides in solution do not help to make a clear and easy model. Moreover it underlines how complex this all matter is about.

1.7 The Buffalo-P Project

This thesis work is part of a big research project on phosphorous titled "Best Management of Stream Banks, Buffer zones and Floodplains for reducing Agricultural Phosphorus Losses", also

called Buffalo-P project. Many Danish institutions participate: the University of Copenhagen, Faculty of Life Sciences, Department of Basic Sciences and Environment, in Frederiksberg C; the National Environmental Research Institute, Department of Freshwater Ecology, in Silkeborg; the Danish Institute of Agricultural Sciences, Department of Agro-ecology, in Tjele; and the University of Southern Denmark, Department of Biology, in Odense. The project started in 2006 and it is going to finish at the end of 2009, after 4 years. "The project has these main objectives: (i) to quantify bank erosion and bank failure rates and losses of phosphorus forms with bank material in natural and regulated Danish stream types and describe the main factors influencing the bank erosion process for development of a decision support tool supporting end users in applying Best Management Practice (BMP); (ii) **to determine the phosphorus retention efficiency of differently managed buffer strips receiving runoff from agricultural land and to devise rules for their management and placement in landscapes;** (iii) to quantify and model the spatial and temporary net deposition of sediment and attached phosphorus forms on natural and restored floodplains and investigate the content of phosphorus in floodplain soils and the risk for phosphorus mobilization along gradients in phosphorus content, duration of inundation and redox conditions in floodplain soils; (iv) to risk assess Danish riparian soil types with respect to in situ phosphorus mobilization following drainage termination, identify the controlling factors for phosphorus release or retention, evaluate their ability to retain tile drainage water phosphorus and develop guidelines for Best Management Practice (BMP)".

The Buffalo-P projects has been developing and testing various tools to help the management of streams, buffer zones and floodplains with the scope of reducing the agricultural losses of phosphorus to the aquatic environments helping to obtain a good ecological quality as required in the EU Water Framework Directive. The research strategy chosen was meant to investigate and increase the knowledge on the diverse mechanisms responsible for sorption and desorption of phosphorus in those areas directly connected to waterbodies and thus responsible for most of the agricultural loss in Denmark. About 400,000 ha of cultivated low-lying soils and all the riparian areas along the 65,000 km of national watercourses belong to this area type.

One PhD-student and one Post Doc were committed with this project and several Bachelor and Master thesis have been financed as well.

2 Materials and Methods

2.1 Overview of the soil anoxic incubation experiments

During this project many experiments have been carried out, many of them were done with the goal of describing the soil and sorbents, optimizing the set up and assessing some variables related to the experiment itself (e.g. bacterial reductive activity).

Three big experiments are the core of this work: the experiments in which soils were incubated inside a glove box under anoxic conditions with a sorbent added to test its ability to sorb P, when iron reduction occurs. Two Al containing sorbents were pointed out as usable for project's aim: gibbsite and kaolinite. Due to lack of time only gibbsite has been used in the incubations even though preliminary analysis for both compound, such as sorption isotherms determination, have been carried out. The three experiments are named: Lydum-Gibbsite #1, Lydum Gibbsite #2 and Vedersø-Gibbsite #1; where the first name refers to the soil used and the second name to the sorbent used. Please see paragraph 2.3 "sorbents characterisation" for more information on the two sorbents.

In order to test the efficiency of the sole gibbsite as binder for soluble phosphates in anoxic environments, two different Danish agricultural soils were incubated. Ten grams of dry soil were put inside a 500 mL glass flask with 500 mL of Type I purified (TI) water and a series of 5 different gibbsite concentrations; 0.05, 0.1, 0.2, 1.0 and 2.0 g/L of gibbsite plus a control, where no gibbsite was added, were made (flasks and data from the various gibbsite concentration are from now on called: gibbsite 1, gibbsite 2, gibbsite 3, gibbsite 4, gibbsite 5 and control respectively). Type I purified water has the following characteristics: ions resistivity (at 25 °C) >18.0 MΩ*cm; ions conductivity (at 25 °C) < 0.056 μS/cm; total organic content <10 ppb; particulates (diameter) <0.2 particulates (diameter) <0.2 μm; colloids (silicia) <10 ppb and bacteria <1 CFU/ml. In order to enhance microbial reductive activity, sodium acetate with a pH buffered at circa 6, was added in order to have a total concentration of 0.5 mM in the Lydum-Gibbsite #1 and Vedersø-Gibbsite #1 experiments while in the Lydum Gibbsite #2 experiment, acetate was added to have a total

concentration of 1 mM. All solutions were made with TI water, fluxed for at least 3 hours with Ar gas (99.9% pure) in order to get rid of the oxygen inside it. Triplicates were made.

Soil incubation was started inside a glove box, when the TI water and the acetate were added to the soil already present in the flasks. Flasks have been stored inside the glove box for all the duration of the experiments.

For the Lydum-Gibbsite #1 and Vedersø-Gibbsite #1 experiments, incubations were carried out inside a glove box produced by MBRAUN (M. Braun Inertgas-Systeme GmbH, Head-Office Germany • Dieselstr. 31, D-85748 Garching. www.mbraun.de), model "Labstar 50". Useful information about this glove box workstation can be found in the producer's website, in particular at the url: http://www.mbraun.com/pdf/mb-labstar_v3.1.pdf.

For the Lydum-Gibbsite #2 experiment a different glove box workstation was used, a 2 person vinyl glove box produced by COY Laboratory products inc (14500 Coy Drive; Grass Lake, MI 49240; United States; http://www.coylab.com).

The two glove boxes had an inner atmosphere of nitrogen gas (99.5%) and hydrogen gas (0.5%). Presence of oxygen was checked (see paragraph 2.4 "Sampling and analysis procedure" to read more about how oxygen presence was checked) every morning and evening and before opening the flasks. Flasks were wrapped with Al foil and stored inside a box in order to reduce photolysis.

Samples were taken approximately every 50 hours from the beginning of the incubation including day zero and ending after 4 weeks. Fe(II), dissolved ortho-P, pH value were analysed at each sampling. Total P should also have been analysed, thus samples for total P were taken, but in the end it was decided not to proceed with this analysis; the reason of this choice will be explained in paragraph 2.4.3: "total P determination procedure". The temperature was checked every day, once in the morning and once in the evening. Temperature was stable at 27±2° C for the whole experimental period. All of plastic and glassware used in the project were carefully acid washed with a solution of HNO_3 for at least one hour and then rinsed with DI and TI water, in order to eliminate possible sources of P contamination, which could have interfered with the analysis and results of the experiments.

The objectives of this thesis are: (i) to confirm that an anoxic incubation of a flooded soil will lead to reduction of iron(III) oxides and consequently to the solubilisation of iron(II); (ii) to confirm that significant amounts of phosphorus are released once soil iron(III) oxides are reduced; (iii) confirm the importance of aluminium oxides as sorbents for soluble P; and (iv) to demonstrate that a positive correlation exists between the amount of P resorbed and the amount of aluminium oxides added in the soils (taking into consideration that soils already contain natural aluminium oxides).

All the experiments where carried on at the Department of Basic Sciences and Environment, Faculty of Life Sciences, University of Copenhagen; Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark from March 2008 to January 2009.

2.2 Site and soil characterization

From the Buffalo-P project soils database, two sampling localities were chosen: Vedersø (n°2063) and Lydum Å (n°1146). These are sites in Western Jutland, Denmark. Their economy is based on agriculture; hence arable fields occupy most of the areas. Samples from the two localities had already been taken previously and were called Vedersø (I) and (II) and Lydum Å, Sønder Bork (I) and (II). New samples were finally taken for this project on the 5th of February 2008, and named Vedersø (II) and Lydum Å, Sønder Bork (III).

Vedersø (II) (32-U-447709-6233290) is a sandy topsoil (0-25 cm) very poor in iron and very wet at the time of sampling.

Lydum Å, Sønder Bork (III) (32-U-0457652-6184066) is a sandy topsoil (0-25 cm) extremely rich in iron and also high in phosphorus. Lydum Å looked very heterogeneous, with bands of iron rich and iron poor material.

Table 2.1 **Soils textures.** Values are given in percentage. Definitions for different particle sizes are given; d is particle diameter.

Soil sample	% Gross sand 2000 μm > d > 200 μm	% Fine sand 200 μm > d > 20 μm	% Clay 20 μm > d > 2 μm	% Silt 2 μm > d > 0.2 μm	Total
Lydum Å (III)	54.3	35.3	6.2	4.2	100
Vedersø (II)	90	8	1	1	100

In June 2008 some chemical tests were done on the two soils to define some of their properties. After having the soils air dried, oxalate extractable Fe and Al according to Schvertmann (1964). Citrate-Bicarbonate-Dithionite (CBD) extractable Fe and Al according to Jensen and Thamdrup (1993) and soil pH were measured. Total organic carbon content was also detected (by dry combustion) as well as Olsen-P. Olsen-P analysis is used to estimate inorganic P availability to plants in soils (Torrent and Horta 2007).

Table 2.2 gives an overview of the soil properties. It is clearly seen that the two soils differ from each other inn their iron oxide content. Lydum Å (III) is very rich in iron, while Vedersø (II) is contains only little iron.

Values of total organic carbon (TOC) indicate that the two soils are very poor in organic matter and that Lydum Å (III) contains an amount of humic matter about three times as big as Vedersø (II). Humic matter have implications in sorption of soluble Fe(II) and of amorphous Al compounds (Darke and Walbridge 2000) and thus influence Fe(II) detection analysis, as the method used in this project detects only the free soluble Fe(II).

Thanks to the given value of Olsen-P it is possible to make a P/Fe ratio and, after some comparison with published values (presented in paragraph 2.3.2 "Interactions between phosphates and iron oxides"), also estimate whether iron oxides are or are not saturated with P, as typical value of PAC are known. The P/Fe now presented refers to the ratio between Olsen-P and CBD extractable Fe, with data expressed in $mol/kg_{DRY\text{-}SOIL}$. For Lydum Å (III) this ratio would be approximately $5*10^{-4}$ while for Vedersø (II) it would be approximately $2.4*10^{-3}$. To estimate the grade of saturation in P of the two soils it is necessary to make an assumption: as said before (paragraph 2.3.2 "Interactions between phosphates and iron oxides"), 77.5 $\mu g/m^2$ can be taken as a mean PAC value for most of the iron oxides. Table 1.1 gives mean values of specific surface area for the most common soil iron oxides. It is assumed that iron oxides present in the two soils are a homogeneous mixture of all the common oxides present in Table 1.1: thus a value for the specific surface of iron oxides of Lydum Å (III) and Vedersø (II) can be calculated as the average value from all the specific surfaces presented in Table1.1. With a mean value of 85.8 $m^2/g_{IRON\ OXIDE}$, Lydum Å has a PAC of:

$$88.513\ g_{Fe}/kg_{DRY\text{-}SOIL} * 85.8\ m^2/g_{IRON\ OXIDE} * 77.5\ \mu g_P/m^2 * 0.001 = 588.6\ mg_P/kg_{DRY\ SOIL} \quad (8)$$

The above equation shows that this soil has only been sorbing around 4.09% of its PAC.

Degree of soil saturation:

$$\frac{24.08\ mg_P/kg_{dry\text{-}soil}}{588.6\ mg_P/kg_{dry\text{-}soil}} * 100 = 4.09\% \quad (9)$$

Vedersø (II) has a PAC of:

$$1.817\ g_{Fe}/kg_{DRY\text{-}SOIL} * 85.8\ m^2/g_{IRON\ OXIDE} * 77.5\ \mu g_P/m^2 * 0.001 = 12.1\ mg_P/kg_{DRY\text{-}SOIL} \quad (10)$$

Vedersø (II) is therefore a bit more saturated with P compared to Lydum Å (III). Degree of soil saturation:

$$\frac{2.406\ mg_P/kg_{dry\text{-}soil}}{12.1\ mg_P/kg_{dry\text{-}soil}} * 100 = 19.9\% \quad (11)$$

These calculations show how both of the soils are not very rich in P.

Each soil sample was taken using a spade and the whole depth (0-25 cm) was sampled. Only one hole was made for each locality, from where 5 to 8 kg of soil was sampled and stored in buckets.

In order to become ready for the experiments, the soils had to be homogenized and mixed. The soils were brought to the soil milling room at the Faculty of Life Sciences, Copenhagen University and taken out of the buckets. Stones, roots and rain worms were removed from the soils with care, to avoid pollution of them. Once homogenized, the soils were put back in the buckets, while about 500 g were left in the soil milling room to air dry for all those soil chemical tests, determining different Fe, Al and P fractions as well as pH, and total-organic C above discussed. Hereafter, the buckets were stored inside a cooling room at a constant temperature of about 5° C. Hans Bruun Christian Hansen, Lisa Heiberg and Elia Scudiero did the samplings and homogenization the soils.

Table 2.2 **Results from preliminary tests on dry soil.** Note that Olsen-P data is given in mg P/kg of dry soil, where oxalate and CBD data for Fe and Al are given in mg/kg of dry soil. TOC stands for total organic carbon.

Soil sample	Oxalate Fe g Fe / kg dry soil	CBD Fe g Fe /kg dry soil	Oxalate Al g Al /kg dry soil	CBD Al g Al /kg dry soil	Olsen-P mg P /kg dry soil	TOC g C /kg dry soil	pH in water
Lydum Å (III)	13.506	88.513	0.441	0.535	24.080	1.583	4.845
Vedersø (II)	0.128	1.817	1.316	0.094	2.406	0.542	4.415

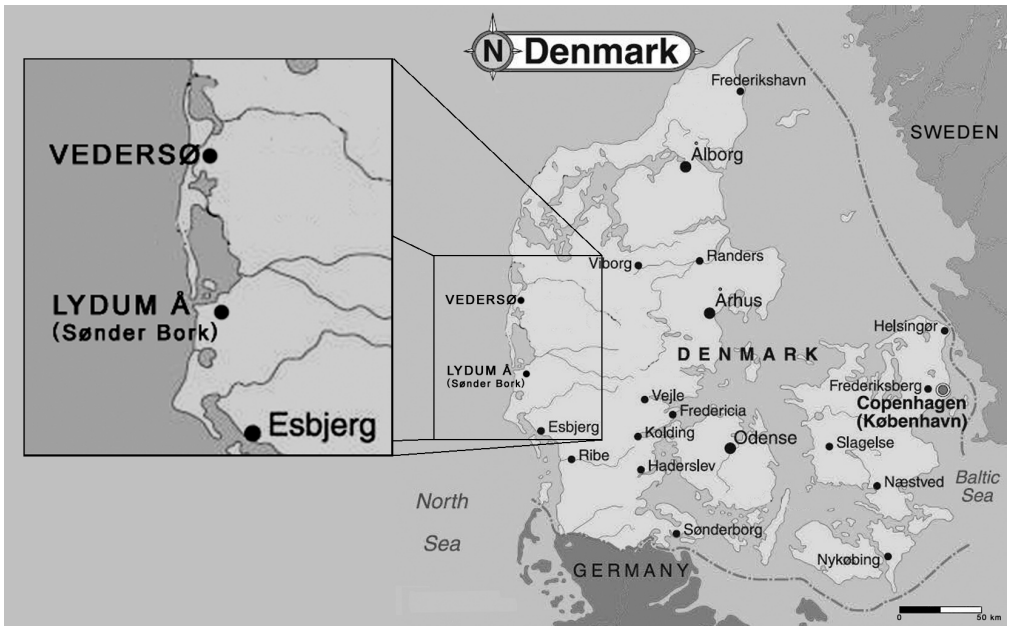


Image 2.a **Map of Denmark with a highlight on Vedersø and Lydum Å**. (edited by the author, taken from www.worldatlas.com)

2.3 Sorbents characterization

As mentioned before, two sorbents containing aluminium were chosen for this project: gibbsite ($Al(OH)_3$) and kaolinite ($Al_2Si_2O_5(OH)_4$) .

Gibbsite, also known as aluminium hydroxide or hydrargillite, is one of the three main phases that constitute the ore Al-rock bauxite (Klein and Hurlbut Jr 1993). Gibbsite was bought from Acros Organics (New Jersey, USA: 1-800-ACROS-01. Code: 219135000. CAS: 21645-51-2. EC: 244-492-7. Lot: A0249686) and as indicated by the seller, it comes as extra pure powder, with a molecular weight of 78.00 g/mol and a pH of 8.5 to 10 (5% aq. suspension). Product specifications can be found on the website of the seller at the url: http://www.acros.com/DesktopModules/Acros_Search_Results/Acros_Search_Results.aspx?search_type=Specifications&SearchString=21913.

With some rough calculation the amount of gibbsite to be used for the experiments was chosen. Lydum soil, being the one richest in P was chosen for the calculation. About 30 mg of P per Kg soil were expected to be mobilized. Ten grams of soil should then contain around 6 mg of P, which could be mobilized with a complete reduction of the soils. Borggaard et al. (2005) stated that natural gibbsite has a specific surface area of minimum 10 m^2/g and a PAC of approximately 125 $\mu g/m^2$. This means that 1 gram of gibbsite could sorb 1.25 mg P per 10 g of soil; which correspond to 125 mg P per kg of dry soil, a value that is clearly well above the expected 30 mg. The reader needs also to consider that this project planned to examine reduction processes at their very beginning, implicating that the amount of P would then be much smaller than the expected 30 mg. After these calculations and considerations, it was decided to use 1 g of gibbsite as the maximum amount to add. Gibbsite was then added according to Table 2.3. This table shows the amounts of gibbsite added to the soil incubations during the experiments.

Table 2.3 **Amounts of gibbsite added in the Lydum-Gibbsite #1, Lydum Gibbsite #2 and Vedersø-Gibbsite #1**.

Flask Name	Gibbsite		
	g	g/L TI water	g/kg dry-soil
Control	0	0	0
Gibbsite 1	0.0025	0.005	2.5
Gibbsite 2	0.05	0.1	5
Gibbsite 3	0.1	0.2	10
Gibbsite 4	0.5	1	50
Gibbsite 5	1	2	100

Kaolinite is a naturally occurring hydrous aluminium silicate mineral with one tetrahedral sheet linked through oxygen atoms to one octahedral sheet of Al (Klein and Hurlbut Jr 1993). Kaolinite

was bought from Ward's Natural Science Establishment Inc (Rochester, NY, USA. Code: 46E0995. Cas: CAS: 1332-58-7. China clay). It is delivered as fine white powder and it is nearly insoluble in water. Due to lack of time kaolinite could not be tested.
Sorption isotherms were performed.

2.3.1 Sorption isotherms

Sorption isotherms have been calculated for both of the above described sorbents. Sorption isotherms were made from data from different amounts of P added, as shown in Table 2.4. Aqueous solutions were prepared in 500 mL flasks with 5 g of pure sorbent and buffered with 0.5 mM sodium acetate at pH 6 (Figure 2.b, section a) and with 1 mM sodium acetate at pH 5 (Figure 2.b, section b and c). The flasks were kept on a shaking table and after 48 hours samples were taken to determine P sorption to the sorbents.

Table 2.4 **Initial concentrations of Phosphorus**. Phosphorus was added as KH_2PO_4; values in mg/L refer to the sole phosphorus.

Sorbent	P (start concentration) mg/L										
Gibbsite pH 5	0.00	0.15	0.31	0.77	1.24			3.10	4.65	7.74	10.84
Gibbsite pH 6	0.00	0.15	0.31	0.77	1.24			3.10	4.65	7.74	10.84
Kaolinite pH 5	0.00	0.15	0.31	0.93	1.24	1.70	2.32	3.10			

Sorption isotherms indicate how much P is sorbed by gibbsite (or kaolinite) once the amount of P in solution is known. This relationship is valid when only gibbsite or kaolinite is present in solution. In the soil incubation experiments, not only these sorbents are likely to bind P, in fact iron oxides will play an important role on the mobility of P, as soils are not completely saturated with it. Sorption isotherms will then help to estimate the amount P expected to be sorbed to the added sorbent.
Data on the sorption of P to gibbsite and kaolinite were fitted with a logarithmic curve ($y = k * \ln(x) + a$); formulas are shown in Figure 2.b. Mathematically, logarithmic curves are characterized by a very rapid increase of the dependent variable (y, in this case "sorbed P") with small values of the independent variable (x, in this case "solution P"); later on, as the P in solution increases, the ratio of sorbed P on P in solution decreases. Logarithmic curves also do not have any upper bound, meaning that the more P is added, the more P would be sorbed by the sorbent. This actually goes against the concept of PAC, as a finite amount of sorbent has a finite number of sorption sites. The amount of P solubilized in the experiments was not expected to exceed 2 mg/L, thus the logarithmic

isotherms can be used as at low concentrations of P in solution, it fits the real sorption process with a R^2 bigger than 0.95 in each one of the three cases.

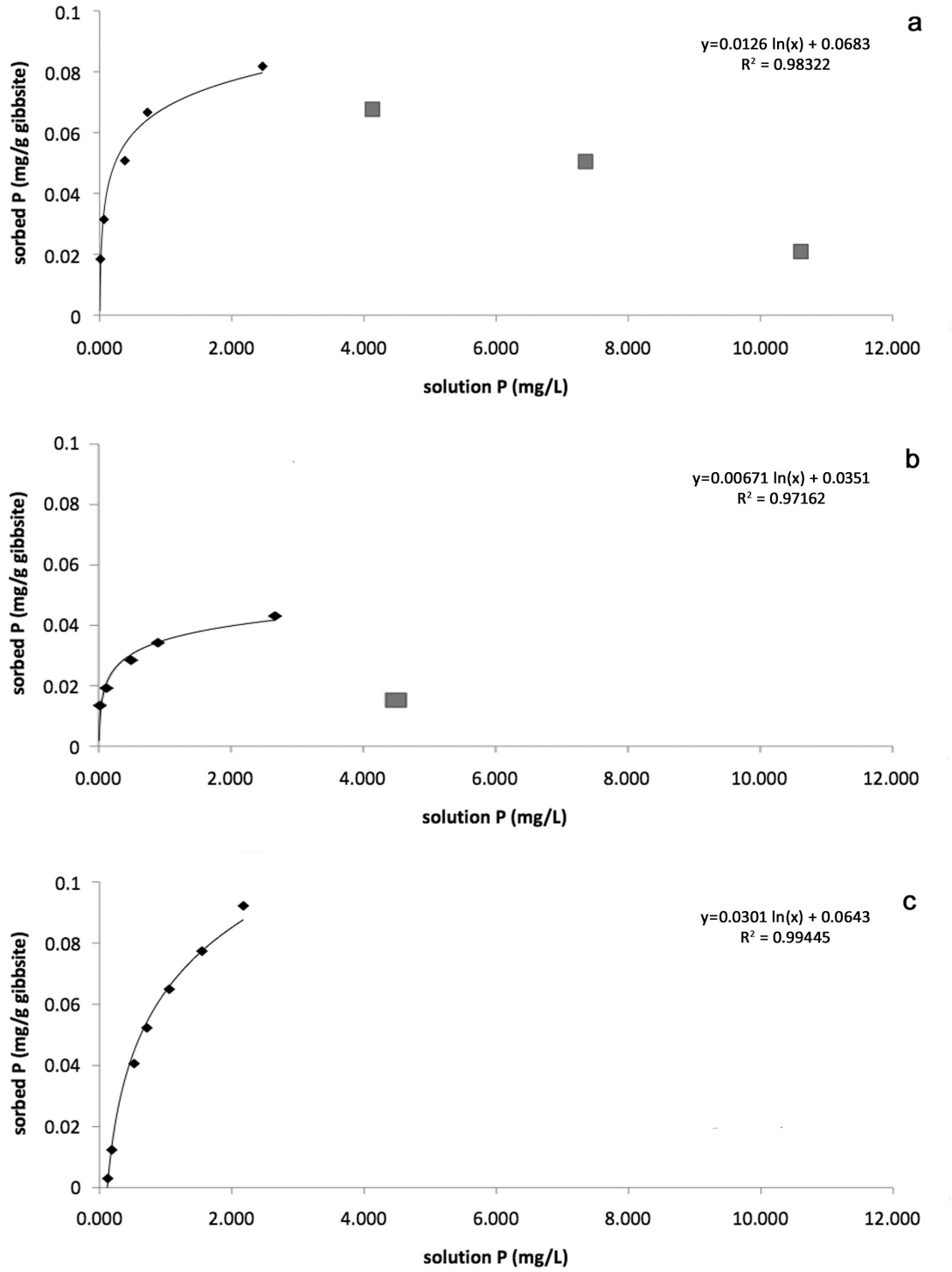


Figure 2.b **Sorption isotherms for gibbsite and kaolinite at different pH values**. Gibbsite was tested at pH=5 (a) and pH=6 (b); kaolinite was only tested at pH=5 (c).

For gibbsite trend curves could only be fitted for values of added P smaller than 3 mg_P/L (cf. "sorption isotherms" in appendix B); in fact, the sorption power of gibbsite decreases drastically with added amounts bigger than 3.10 mg_P/L (see Table 2.4). Negative values are not shown in the graphs.

Both of the sorbents have a low sorption power; the sorption isotherms indicate that the amount of P in solution is much bigger than the sorbed fraction unless the sorbents are present in very high amounts.

Gibbsite loses a lot of its sorption power if the solution is at pH = 5 instead of pH = 6.

More considerations about the sorption power of gibbsite will be given in the discussion part. Moreover, from now on, kaolinite will not appear anymore in the text as, due to lack of time, the studies on this sorbent are limited to this preliminary experiment on its sorption capacity.

2.4 Sampling and analysis procedures

In order to detect the amount of Fe(II) produced as result of soil reduction, the orthophosphate content and total P solution was sampled from each flask with a 10 ml syringe and filtrated with a 0.20 μm and a 0.45 μm filter mounted together on the syringe. Filters were bought at Mikrolab Aarhus and were made of cellulose. About 10 ml of solution was sampled every time and were enough to run iron(II) analysis and the other two other analyses as well. Samples were taken approximately every 50 hours in the four weeks after the beginning of the incubation. The whole sampling procedure took place inside the glove box and the test tubes were taken out as described below. Before sampling, the flasks where shaken turning them upside-down and up again, five times and then weighed.

In order to analyse Fe(II) the soil solution was diluted twice in 0.1 M HCl. 2 ml were taken with a pipette from the filtrate and put into another test tube with 2 ml of 0.1 M HCl (with Lydum soil dilution ratio was changed on the second and third week, respectively 10 and 20 times diluted); the test tube was then taken out of the glove box (tap was put on outside) where 0.4 ml of ferrozine and 0.6 ml of Na-acetate buffer were added in order to have the samples ready to be run on an UV-visible spectrophotometer (Shimadzu UV - 1601) at λ=562 nm. Iron(II) is not stable in aerobic conditions because it becomes oxidized to Fe(III) very easily and quickly. Oxidation cannot be avoided unless solution pH is very low. In fact Fe(II) follows the chemical-physical law:

$$\frac{d[Fe^{2+}]}{dt} = k \bullet [OH^-]^2 \bullet [O_2] \quad (12)$$

The equation implies that the lower the pH, the lower the oxidation rate. This is why filtrated sample solution was mixed with HCl, creating a solution with pH around 2 (measured).

Of the remaining sampled solution, 2 ml were taken and transferred to a test tube with 0.5 ml of 0.1M H_2SO_4 to determine the soluble phosphate content. Hereafter, the test tubes were shaken and brought outside the glove box where 0.5 ml of reagent (50% H_2SO_4 0.1M, 15% NH_4-molybdate, 30% ascorbic acid and, as the last chemical added, 5% antimony-potassium-tartrate -1 mg Sb/ml-) was added. The samples were then analysed with a spectrophotometer with the same procedure used for Fe(II) analysis but at λ=882 nm.

To quantify total P content, 1.2 ml of solution were mixed with 0.3 ml of 0.1 M H_2SO_4, test tubes were then put in a freezer and should have been analysed at the end of the experiments.

The rest of the sampled soil solution (~2 ml) was not filtrated and used to determine pH inside the glove box with a pH-meter. The pH-meter was calibrated before every measure with buffers at pH 4 and 7.

Every day, temperature was measured in the morning and in evening as well as during pH measurements.

To check if oxygen was present inside the glove box used in the Lydum-Gibbsite #1 and Vedersø-Gibbsite #1 experiments, a light bulb with a hole in it was used. The light bulb was switched on for approximately half a minute every time before and after entering things in the glove box, before opening the flasks, at the end of the sampling procedure and at the end of the day. In the Lydum-Gibbsite #2 experiment, with the other glove box, an oxygen tester produced by Coy Laboratory products inc. (*model 10*), which could detect oxygen concentrations as low as 1 ppm, was used to check the atmosphere inside the glove box.

2.4.1 Iron(II) determination

By measuring the concentration of Fe(II) present in solution it is possible to estimate at which extent the incubated soils are reduced. The analytical procedure used in this project allows to know the concentration of free Fe(II) in solution, as the samples were filtrated and no acid was used to extract all the Fe(II) bound to dissolved organic matter and dissolved clays. At constant pH values

the ratio (13) remains constant, therefore Fe(II) in solution analysis can be used as indicator of soil reduction. In fact the total Fe(II) produced because of bacterial reduction can be approximated to the sum of the sorbed and soluble Fe(II) fractions, as showed in equation 23. Please note that a K_d ratio for Vedersø (II) and Lydum Å (III) is given in paragraph 3.2 "Soil reduction". The unit of K_d is L/kg and it is based on sorbed [Fe(II)] measured as mg/kg and solution concentration of Fe(II) measured as mg/L.

$$K_d = \frac{[Fe(II)]_{sorbed}}{[Fe(II)]_{solution}} \tag{13}$$

Iron(II) was measured with the ferrozine method (Stookey 1970). Samples (4 mL) were mixed with ferrozine reagent (0.4 mL) and with 0.6 mL of acetic acid buffer at pH 5 and 5.40 M. When Fe(II) reacts with ferrozine, a stable magenta complex forms, which is very soluble in water and can be used for direct determination of Fe(II) in water (Stookey 1970). The ferrozine-Fe(II) complex forms completely only in aqueous solutions with pH values between 4 and 9. The colour adsorption obeys the Beer-Lambert law to approximately 4 mg/L of Fe(II).

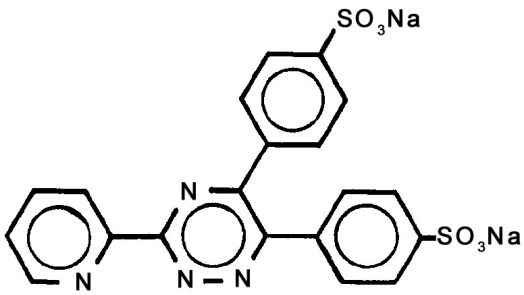


Figure 2.c **Disodium salt of 3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine, known as ferrozine** (Stookey 1970).

Samples were analysed with a UV-spectrophotometer half an hour after the ferrozine and acetate buffer were added. Absorbance was measured at 562 nm, 1cm wide cuvettes were used.

2.4.2 Soluble ortho-P determination

In order to determine molybdate reactive phosphate according to Murphy and Riley (1958; 1962) and Stephens (1963), a reagent containing H_2SO_4, $(NH_4)MoO_4$, ascorbic acid, and antimony potassium tartrate was prepared before every analysis. In an acid solution ammonium molybdate

forms a yellow complex of phosphorus molybdate, which is reduced to a blue complex with ascorbic acid. Antimony is added in order to accelerate the reduction. The colour adsorption obeys the Lambert-Beer law up to approximately 1.3 mg/L (in this case 50 µg PO_4-P/sample), and the colour remains stable for 24 hours.

The soil solution in the incubation flasks contained Fe(II) and hence it was important to avoid aerial oxidation of the Fe(II) during sampling and before analysis as this would have given rise to precipitation of iron(III) (hydr)oxides and subsequent adsorption of phosphate causing erroneous determination of phosphate. Oxidation was avoided by acidifying the sample, as iron(II) oxidises very slowly under acid conditions (equation 13). H_2SO_4 was added in a concentration of 0.02 M in the test tube when the test tubes were still inside the glove box.

Table 2.4 **Components ratio for reagent mixture**. Note that KSb was added last.

H_2SO_4	**NH_4-molybdate**	**Ascorbic acid**	**KSb solution**
50%	15%	30%	5%

Before each analysis a standard solution was made following the following table (Table 2.5). Analysis were carried out 15 minutes after the reagent was added, as the solution needed some time to colour up. Only if the R^2 value for the respective standard curve was higher than 0.975, analyses were carried out.

Table 2.5 **Standard solutions prepared for P determination**. Total volume of the solution = 3ml.

	0	**1**	**2**	**3**	**4**	**5**	**6**
H_2SO_4 (0.02 M)	2.5	2.47	2.35	2.2	2.05	1.9	1.3
(P)1 mg/L stock solution	0	0.03	0.150	0.300	0.450	0.600	1.200
Reagent	0.5	0.5	0.5	0.5	0.5	0.5	0.5
P conc. (mg/L)	0	0.01	0.05	0.1	0.15	0.2	0.4

Reagent mixture was then pipetted into the sample test tubes and mixed thoroughly. Absorbance at 882 nm was then measured using 1cm cuvettes for the first two experiments (Lydum-Gibbsite #1 and Vedersø-Gibbsite #1), later on, with the last experiment, 5 cm cuvettes were used in order to have more precise analysis with the low amounts of P mobilised in the incubations.

2.4.3 Total P determination

As shown in the result part, ortho-P analysis provided many results under or right above detection limit, and with big standard deviations. With such results it was decided not to proceed with total P analysis. In fact, the method used for ortho-P determination could not be made working reliable at low P concentrations. Concentrations of total P were expected to be much lower compared to the ones of ortho-P, as much less solution (1.2 mL) compared to the procedure for orho-P determination (2 mL) was sample to carry these analyses out.
Total P analysis should have carried out according to Menzel and Corwin (1965). Samples wold have been oxidized by peroxydisulphate and total P determined as ortho-P.

2.5 Sodium acetate buffer preparation

Experiments from other projects related to the Buffalo-P project showed how soil samples from Lydum Å, S.B. and Vedersø had their iron(III) oxides completely reduced to soluble Fe(II) in about 2 months from the beginning of the incubation without any external addition of substrate. For this project, it was decided to reduce the time frame of each single experiment to no more than 4 weeks. In order to have the same reduction in a shorter time, bacterial activity needed to be promoted. Sodium acetate was chosen as the ideal substrate because it showed very good results in previous studies dealing with anaerobic metabolism enhancement (McFarland et al. 1996; Hori et al. 2007; Chang et al. 2008) and, as a waterfall effect, with Fe(III) reduction promotion (Chidthaisong and Conrad 2000; He and Sanford 2003). Sodium acetate is a soluble organic substrate that occurs naturally in many soils and is easily used as an electron donor and completely reduced to carbon dioxide (equation 6) by a large range of anaerobic microorganisms such as Fe(III), nitrate, nitrite, and fumarate reducers. Hori et al. (2007) explain how sodium acetate utilisation depends much on the presence of electron acceptors: e.g. the more Fe(III) in the soil the more acetate ion is consumed.
In the most extreme sodium acetate additions it can be seen how up-to 20 mM of acetate can lead to very fast and well efficient reduction of Fe(III). Chidthaisong and Conrad (2000) found out that a solution containing up to 28 g Fe(III)/kg dry soil was totally reduced in less than 10 days adding Na acetate to the solution at 5 mM. Chang et al. (2008) added sodium acetate and other substrates to remediate via bacterial oxidation a site polluted with phenanthrene and pyrene and noticed how the

optimum bacterial growth occurred with a 20 mM acetate concentration.

In order to promote bacterial Fe(III) reduction and not to create an unnatural environment with unusual high sodium acetate concentration, and in order to study phosphate mobilization dynamics in a time frame in which is common to have naturally flooded soils; it was decided to have a 1 mM sodium acetate soil solution.

A 1 M solution was prepared and buffered at pH 4.95 with the addition of acetic acid in order to have a value close to the values of pure soil solution (see Table 2.2). Five ml of this solution were added to each flask at the beginning of the incubation, just before adding the TI water. The final concentration of sodium in each flask hence was 1 mM.

To prepare the acetate buffer at a defined pH, acetic acid and sodium acetate have to be mixed according to the Hendersen-Hasselbach equation (Atkins 1996):

$$pH = pK_a + \log\frac{[Ac^-]}{[HAc]}; \tag{14}$$

In the equation, Ac^- is an acetate anion and HAc is acetic acid (pK_a= 4.76). This calculation is now shown for preparation of sodium acetate at pH 6 and concentration 0.5 M (buffer used in the Lydum-Gibbsite #1 and Vedersø-Gibbsite #1 experiments). The calculation for the preparation of sodium acetate at pH 5 and 1 M were done in an analog way.

$$\log\frac{[\mathrm{Ac}^-]}{[\mathrm{HAc}]} = p\mathrm{H} - p\mathrm{K}_a = 6.0 - 4.76 = 1.24 \tag{15}$$

$$\frac{[\mathrm{Ac}^-]}{[\mathrm{HAc}]} = 17.38 \tag{16}$$

$$[\mathrm{HAc}] + [\mathrm{Ac}^-] = 0.5 \text{ mol/L} \tag{17}$$

$$[\mathrm{HAc}] + 17.38 * [\mathrm{HAc}] = 0.5 \tag{18}$$

$$[\mathrm{HAc}] = 0.0272 \text{ mol/L} \quad ; \quad [\mathrm{Ac}^-] = 0.47 \text{ mol/L} \tag{19}$$

One litre of solution was prepared mixing 63.96 grams of sodium acetate tri-hydrate salt and approximately 2 mL of concentrated (99.9%) acetic acid.

Sodium acetate was chosen instead of other nutrients such as glucose or lactate since microorganisms degrade it easily and no toxic or dangerous products (towards microorganisms activity) form. In fact the entire degradation pathway of other nutrients like pyruvate anion, lactate anion, glucose and malate form much more carboxylic acid when used as electron acceptors in the

Fe(III) reducing process (Lovley 1991). Also, oxidation of glucose and malate often lead to the formation and accumulation of side products such as ethanol, 2,3-butanediol and formic acid that can possibly harm microorganisms and thus inhibit their reductive activity (Jones et al. 1984; Lovley 1991).

3 Results

3.1 Data management and calculations

After having obtained all the raw data from laboratory analysis, all the data were processed with Microsoft Excel. All results from triplicates were put together in a mean value and this value was used for calculations and graph making. Standard deviation was also calculated in order to assess data quality. All the results from the three experiments can be seen in Appendix B. Due to author's choice only graphs are going to be shown in this chapter. In any case, referring to particular data, a reference to the proper data sheet will be given.

Iron(II) production and solubilization is a consequence of bacterial oxidation of soil nutrients substrate. For each mole of substrate a quantitative amount of Fe(III) is reduced. Thus Fe(II) production over time can be described with an equation that describes the increase of bacterial activity over time. Such a model could be the logistic one, described by equation 20 and image 3.a (Schmidt et al. 1985).

$$[Fe(II)]_{soluble_t} = \frac{\max [Fe(II)]_{soluble}}{(1 + [(\max [Fe(II)]_{soluble} - [Fe(II)]_{soluble_0})/[Fe(II)]_{soluble_0}] * e^{rt})} \quad (20)$$

Equation 20 express the amount of Fe(II) in solution over time as a function of the maximum Fe(II) that can be solubilized in a certain amount of soil at a certain pH ("max [Fe(II)] soluble"), and as a function of the specific bacterial Fe(III) reduction in a particular and stable environment ("r"). Logistic models can be approximated with linear methods in order to simplify calculations (Schmidt et al. 1985).

For concentration of Fe(II) and phosphorous (mobilized as a consequence of Fe(III) reduction) in solution over time a trendline with equation 21 was fit. This equation represents a line: y and x are variables; x is the independent variable while y is the dependent variable; the constant *m* determines

the slope or gradient of that line; and the constant term q determines the point at which the line crosses the y-axis.

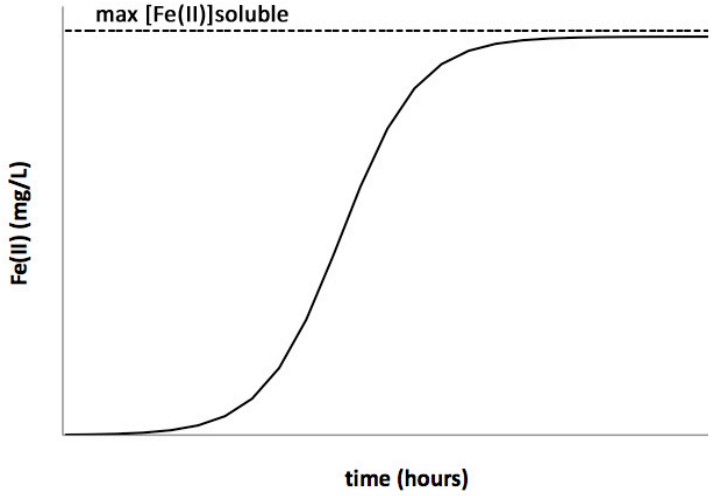


Figure 3.a **Logistic production of soluble Fe(II) over time** according to equation 20.

Trendlines for Fe(II) solubilization were set to intercept the origin of axes because soils reduction is believed to start from point zero. This condition should in fact be representative of a non-saturated soil that comes to be flooded all in a sudden (moment coinciding with experiments starting point). For ortho-P the intercept with the x-axis was not fixed as it can be assumed that some free phosphate could be present in the soils be solubilized as soon as the soil is flooded. Where a coefficient of determination (R^2) > 0.750 was found, the equation describing the correlation is shown. This coefficient provides a measure on how well the linear correlation model fits the data (Garretto 2002).

$$y = mx + q \tag{21}$$

The relationship between solubilized P and Fe(II) in solution is also shown in this chapter. Data were fit with a linear correlation model. This time no intercept for the trendline was set. As previously done with the other results just presented, where the R^2 value was bigger than 0.750 the equation describing the correlation is shown.

For results regarding pH values of the incubated soils, simple graphs showing pH value over time were made. A table with temperature for every pH measurement value is also shown, as pH value is dependent on temperature (Atkins 1996).

Graphs for Fe(II) in solution, soluble P, and pH come with their standard deviation. The standard deviation is shown as positive/negative error bars ending with a cap and are parallel to y-axis. The three major experiments (Lydum-Gibbsite #1 & #2 and Vedersø-Gibbsite #1) were carried out in triplicates and data differ from one replica to another. Standard deviation (σ^2) is then used to measure data dispersion from their mean value. All numeric values for σ^2 can be found in Appendix B together with calculated mean value.

Results are given in mg/L to show which concentrations the experiment was carried out at; more meaningful measures in $mg/kg_{DRY\text{-}SOIL}$ can be obtained by multiplying each result for a factor equal to 50 (0.020 $kg_{DRY\text{-}SOIL}$ was used for each litre of solution). Only results in paragraph 3.4 (Ortho phosphate ratio on Fe(II)) are plotted with the mmol system, as the ratio there presented is stoichiometric.

3.2 Soil reduction

Figures 3.c, 3.d and 3.e give a graphic representation of the Fe(II) solubilization in the Lydum-Gibbsite #1, Lydum-Gibbsite #2 and Vedersø-Gibbsite #1 experiments respectively. Iron(III) oxides are reduced to iron(II) oxides as a consequence of soil reduction and can be a way of representing this process as the total amount of Fe(II) produced can be calculated easily thanks to equation 23.

Heiberg et al. (2009) have been studying phosphate sorption under oxic and anoxic conditions in Danish lowland soils. Their project was financed by the Buffalo P project and part of their research has been taking place at the same department where the author carried out this research. Heiberg and her team have been working with several soils, including Lydum Å (III) and Vedersø (II). Equation 21 shows the relation between K_d (equation 13) and pH of the soil solution that Heiberg and her team found this relation to be valid for all the soil that they have been working with (thus including Lydum Å (III) and Vedersø (II)). Despite the fact that soils are very different in composition between each other, Heiberg et al. found this equation to be good enough to describe Fe(II) speciation in all of the soils. Heiberg and her team concluded that pH is controlling the sorption of Fe(II), irrespectively of the available sorbing surface. Surface precipitation could take place in case in case sorbing surfaces are not sufficient to sorb all the Fe(II) that is supposed to be sorbed at a certain pH.

$$\log K_d = 0.68pH - 1.62 \quad (22)$$

$$K_d = \frac{[Fe(II)]_{sorbed\ (mg/kg\ soil)}}{[Fe(II)]_{solution\ (mg/L)}} = 10^{0.68pH-1.62}{}_{(L/kg)} \quad (23)$$

In 1 L of soil solution only 20 g of soil are present. The K_d ratio described by equation 23 results then to be 50 higher the real K_d ratio (from now on called K_d^L).The K_d^L ratio is dimensionless, while the K_d ratio by Heiberg et al. is expressed in L/kg.

$$K_d^L = \frac{[Fe(II)]_{sorbed\ (mg/kg\ soil)} * 0.02\ (kg/L)}{[Fe(II)]_{solution\ (mg/L)}} = \frac{K_d}{50} \quad (24)$$

The amount of Fe(II) in solution and of Fe(II) sorbed to the soil are pointed as the main fractions of Fe(II) in the soil solution. Amount of Iron metabolized by microorganisms during assimilatory Fe(III) reduction is not believed to be significant. Therefore, the whole amount of Fe(III) reduced to Fe(II) in 1 L of solution (and 20g of soil) can be calculated as shown in equation 25.

$$Fe(II)_{total} = Fe(II)_{sorbed} + Fe(II)_{solution} \quad (25)$$

The ratio between Fe(II) sorbed by 20 grams of soil and the total Fe(II) present in 1 L of soil solution can be then calculated with equation 26.

$$\frac{Fe(II)\ sorbed}{Fe(II)\ total} = \frac{K_d}{50 + K_d} \quad (26)$$

The K_d ratio does not depend only on the pH of the soil solution, as said before; but also on the soil-water ratio of the soil solution, especially when the soil solution is acid. Thanks to equation 26 and data about soluble Fe(II) concentrations (given in this paragraph and in section B.3 “Main experiments” of appendix B “Data tables”) it is possible to estimate total Fe(II) and thus the amount of Fe(III) that has been reduced in 1 L of soil solution. This value cannot be used for calculations in the real soil, as the soil-water ratio in nature is different. Nevertheless, the K_d ratio given in equation 23 allows to know total Fe(II) in any soil solution once the soil-water ratio and concentration of Fe(II) in solution are known.

Figure 3.b shows the percentage of total Fe(II) produced that is sorbed by soil sorbing surfaces (e.g. organic matter, clay minerals) at different pH. The curve is described by equation 26. During the

experiments pH was regularly measured during every sampling as indicated in paragraph 2.4 "Sampling and analysis procedures"; results are shown in paragraph 3.5 "Variation in pH value" and in section B.3 "Main experiments" of appendix B "Data tables".

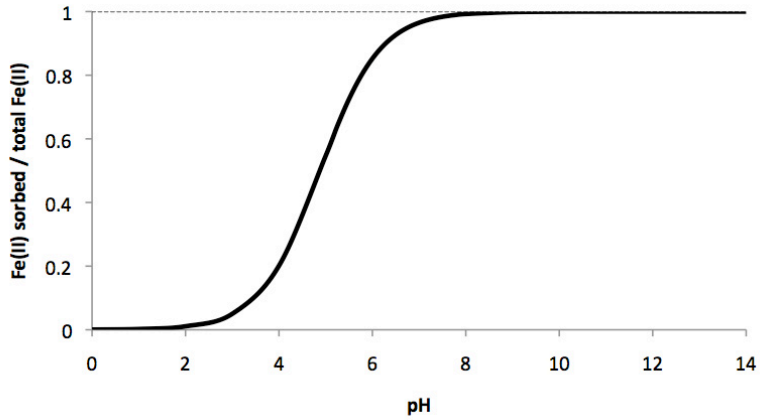


Figure 3.b **Sorbed Fe(II) on total Fe(II) ratio calculated for 1 L of soil solution.**

In the Lydum-Gibbsite #1 experiment the soil started to be reduced by microorganisms rather quickly: with a Fe(II) production of 0.902 ± 0.15 $mg_{Fe(II)}$/L per day. With a small calculation is possible to see that in the 20 grams of soil present in 1 litre of solution around 22.6 mg of Fe(II) are solubilized after 600 hours as a mean value for all the 6 different series. At 600 hours pH was approximately 7.4 in all the flasks. Thanks to equation 25 the sorbed Fe(II) on total Fe(II) ratio can be calculated, and consequently total Fe(II) produced in 20 grams of soil (equation 25) can be estimated to be approximately 1107.4 mg.

In 20 grams of soil, 270.2 of oxalate extractable Fe 1770.2 mg of CBD extractable iron. Therefore, all of the amorphous iron(III) oxides have been reduced (oxalate extracted iron). The 62.6% of total CBD extractable Fe has been reduced to Fe(II). The trend is thus positive, indicating that a reduction of the soil has been taking place.

To check if the different curves could be considered to be the same curve, Student's t-test was carried out. The test had the aim of checking the differences between the angular coefficients (slopes) of the different curves (Garretto 2002). The t-test was carried out with the hypothesis $m_a=m_b=m_c=\ldots$ Control (section a) and gibbsite 1 are significantly different from the other four series. Thus it is possible to say that the gibbsite 2, gibbsite 3, gibbsite 4 and the gibbsite 5 series have been producing and solubilising Fe(II) at the same extent, while the control series have been producing and solubilising less Fe(II) and the gibbsite 1 series have been producing and solubilising more Fe(II).

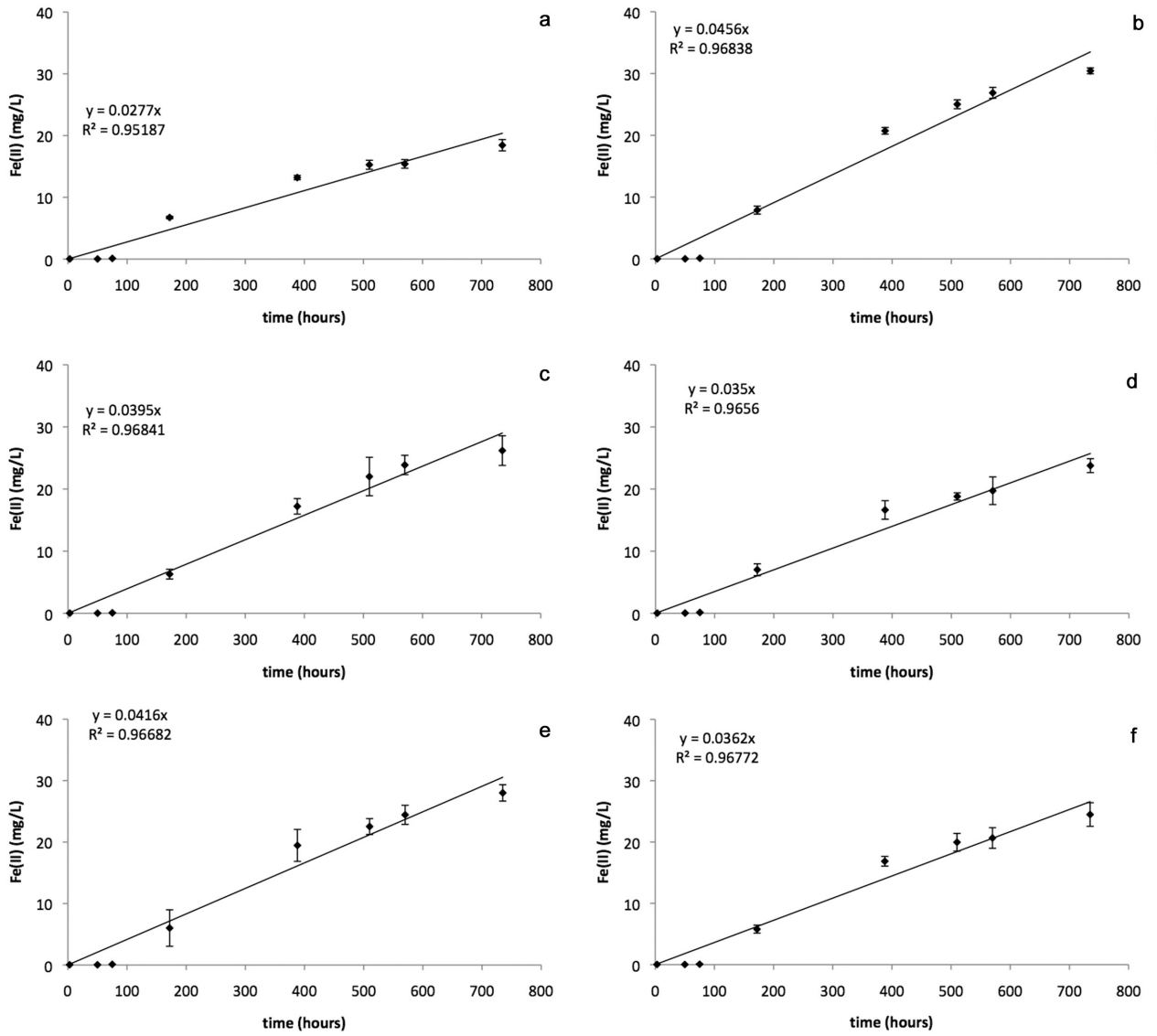


figure 3.c **Iron(II) dissolution over time for the Lydum-Gibbsite #1 experiment.** The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite 5. Bars refer to standard deviation.

In the Lydum-Gibbsite #2 experiment, microorganisms have been starting to reduce the soil in a much slower pace. Even though being clearly positive, the trend shows a Fe production of 0.254 ± 0.02 $mg_{Fe(II)}/L$ per day. At the end of the experiment (after 600 hours, at pH=5.1), 15.5 mg of Fe(II) are produced in 20 grams of soil, thus only the 0.05% of the total CBD extractable iron present in the soil has been reduced to Fe(II). As a result of Student's t-test, the trend lines of all gibbsite series can be considered as having the same slope.

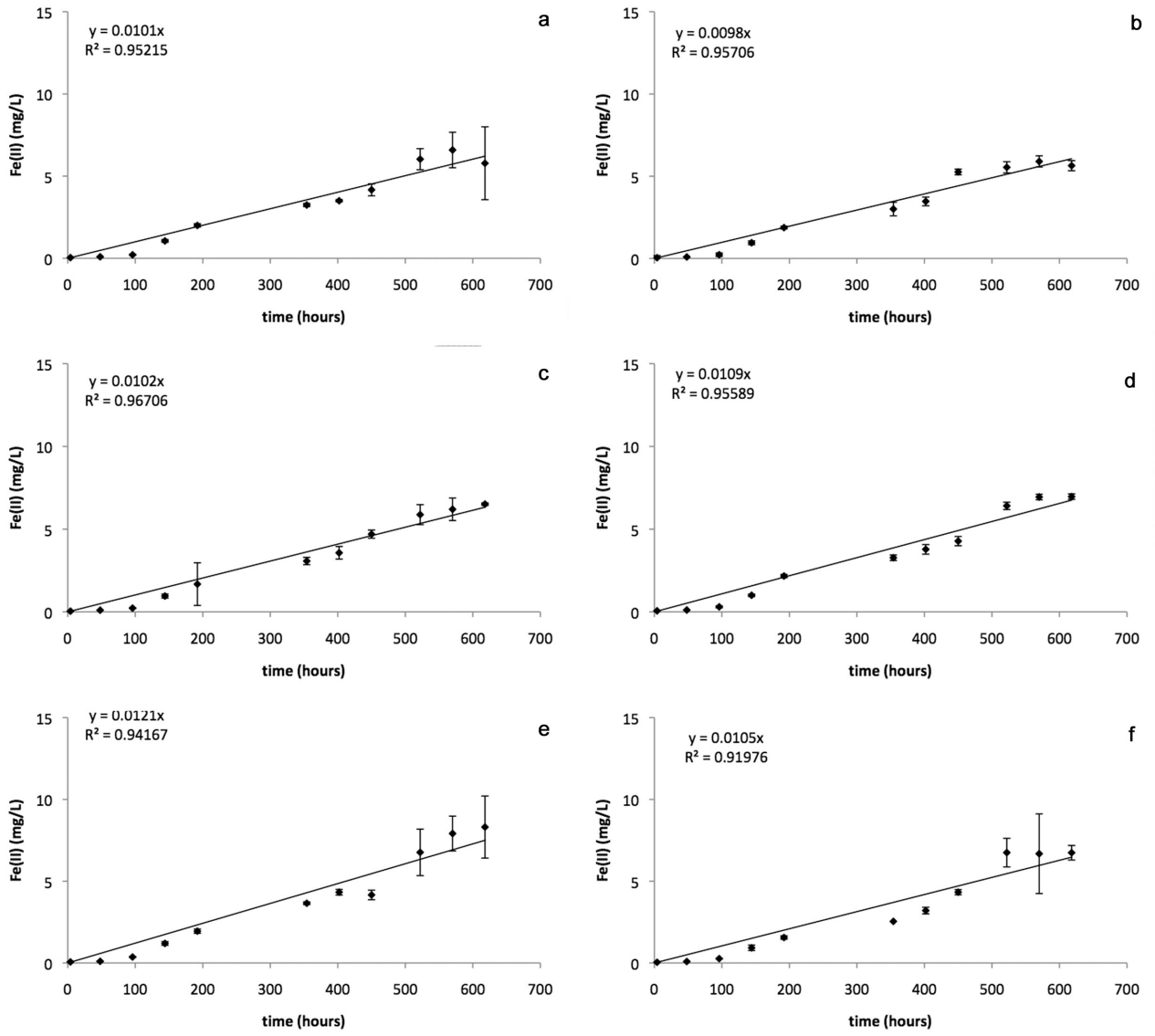


Figure 3.d **Iron(II) dissolution over time for the Lydum-Gibbsite #2 experiment.** The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite 5. Bars refer to standard deviation.

Formation of Fe(II) could not be fit with a straight line for Vedersø-Gibbsite #1 experiment as it could be with the previous ones (Figures 3.c and 3.d). Microorganisms have clearly been reducing the soil iron(III) oxides, but Fe(II) concentration clearly dropped after the 4th sampling (170 hours) in all of the six series. The first four points in the graphs are perfectly correlated with a R^2-value larger than 0.750.

Even if the objective of the experiment was to investigate the activity of gibbsite in a longer time frame, for this experiment only the first four points will be considered. As a consequence, results for this experiment will be much stronger but considerably less relevant. Figure 3.f shows the new graphs for Fe(II) production in the Vedersø-Gibbsite #1 experiment.

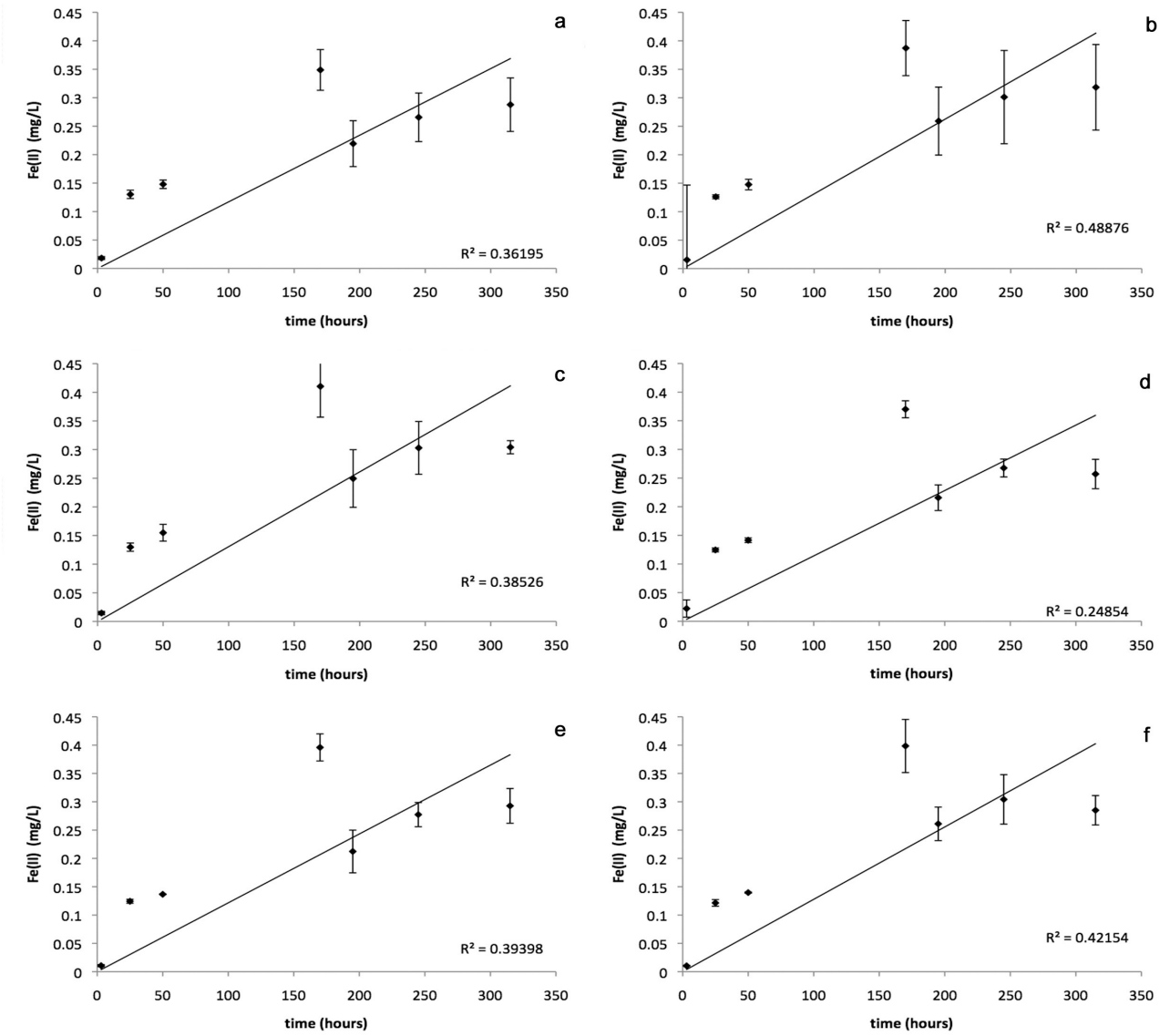


Figure 3.e **Iron(II) dissolution over time for the Vedersø-Gibbsite experiment.** The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite 5. Bars refer to standard deviation.

Soil reduction trends for the first 172 hours are clearly positive and a Fe(II) production rate for the six series can be calculated and is equal to 0.057 ± 0.002 $mg_{Fe(II)}$/L per day. After 170 hours (pH=6.1), 3.15 mg of Fe(II) are produced in 20 g of soil. Therefore, the 8.7% of the CBD extractable iron in the soil has been reduced to Fe(II). If this reduction rate had been stable for 600 or more hours, like it occurred in the other experiments, the CBD extractable iron present in Vedersø (II) would have been reduced by the 30.3%. This speculation shows how Vedersø soil becomes reduced much faster than Lydum soil. As a result fro the t-test, it is possible to say that all the series are very similar to each other; no difference could be found between the different lines, hence the soil is reduced to the same extent in all of the gibbsite series.

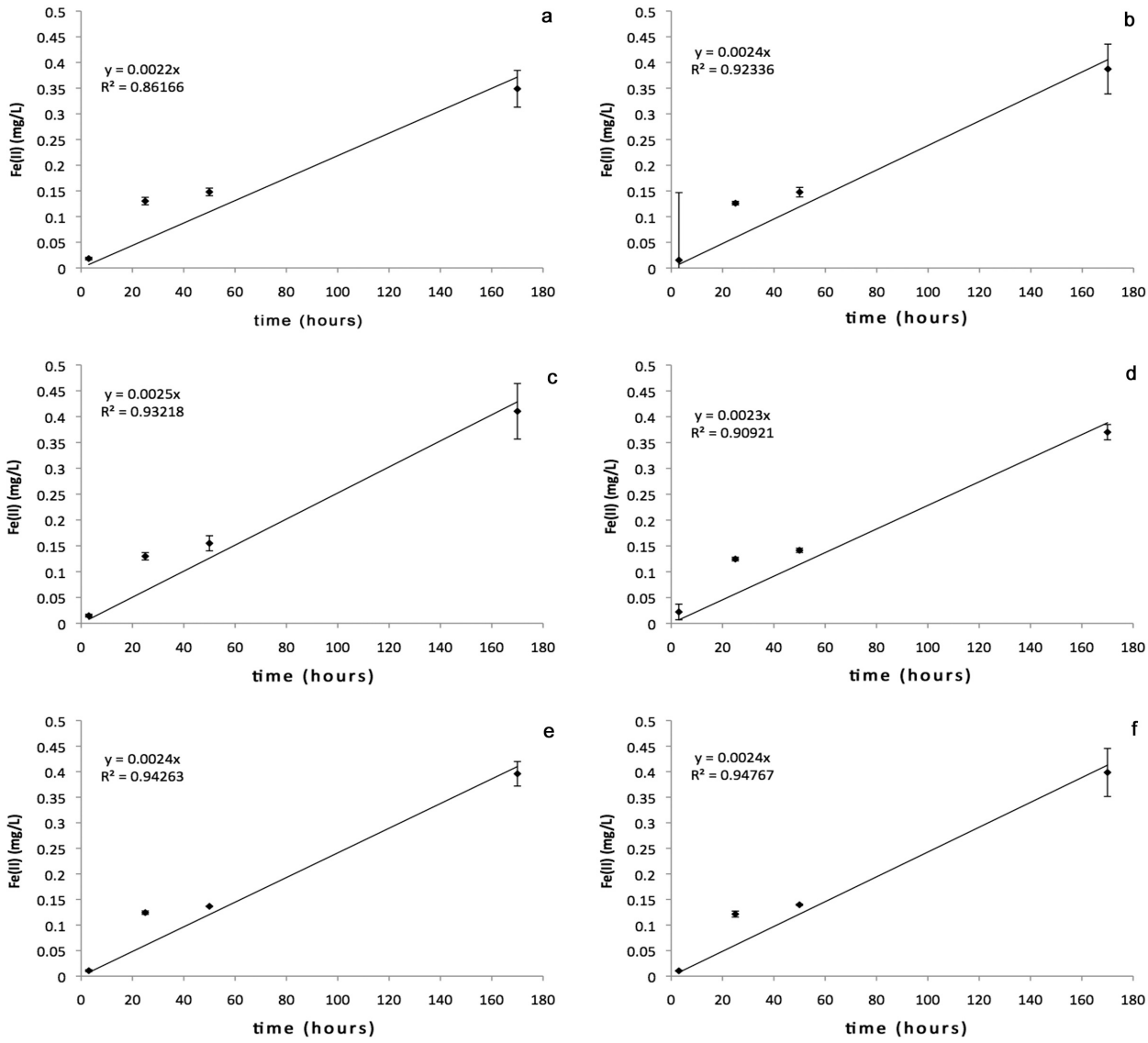


Figure 3.f **Iron(II) dissolution over time for the Vedersø-Gibbsite experiment showing only the first four samplings.** The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite 5. Bars refer to standard deviation.

Table 3.1 shows all the slopes and R^2 values for soluble Fe(II) production over time. For the Vedersø-Gibbsite #1 experiment only the firs four sampling were taken in account.

Table 3.1 **Equations for the linear correlations of soluble Fe(II) over time.**

Experiment Name	Series Name	Equation	R^2
Lydum-Gibbsite #1	control	y = 0.0277x	0.952
	gibbsite 1	y = 0.0456x	0.968
	gibbsite 2	y = 0.0395x	0.968
	gibbsite 3	y = 0.0350x	0.966
	gibbsite 4	y = 0.0416x	0.967
	gibbsite 5	y = 0.0362x	0.968
Lydum-Gibbsite #2	control	y = 0.0101x	0.952
	gibbsite 1	y = 0.0098x	0.957
	gibbsite 2	y = 0.0102x	0.967
	gibbsite 3	y = 0.0109x	0.956
	gibbsite 4	y = 0.0121x	0.942
	gibbsite 5	y = 0.0105x	0.920
Vedersø-Gibbsite #1‡	control	y = 0.0022x	0.862
	gibbsite 1	y = 0.0024x	0.923
	gibbsite 2	y = 0.0025x	0.932
	gibbsite 3	y = 0.0023x	0.909
	gibbsite 4	y = 0.0024x	0.943
	gibbsite 5	y = 0.0024x	0.948

‡ Linear correlation was made for the first four samplings only (3, 25, 50 and 170 hours).

3.3 Phosphorus mobilization and resorption

Figures 3.g, 3.h and 3.i give a graphic representation of ortho-P in solution for the Lydum-Gibbsite #1, Lydum-Gibbsite #2 and Vedersø-Gibbsite #1 experiments respectively. Ortho-P data cannot be taken in account as data showing the amount of P solubilized as a consequence of soil reduction. These data are just showing the amount of P present in solution at the time of the various samplings. Please note that concentrations are most of times too low, moreover low concentrations, such as 0.02 mg/L or less should not be taken in consideration as below the detection limit of the method used (Murphy and Riley 1958; 1962). This is the reason why it was decided to use 5 cm cuvettes in the Lydum-gibbsite #2 experiment, in order to work with higher concentrations of ortho-P in solution.

All of the different gibbsite series in each experiment are supposed to solubilize P at the same extent, as they are supposed to produce the same amount of Fe(II); results from the previous paragraph demonstrate support this statement. Differences in detected ortho-P in solution could then be attributed to the different re-sorbing activities in the different series. The higher gibbsite contents, the bigger sorption activity is believed to be exerted on phosphate in the incubated soils.

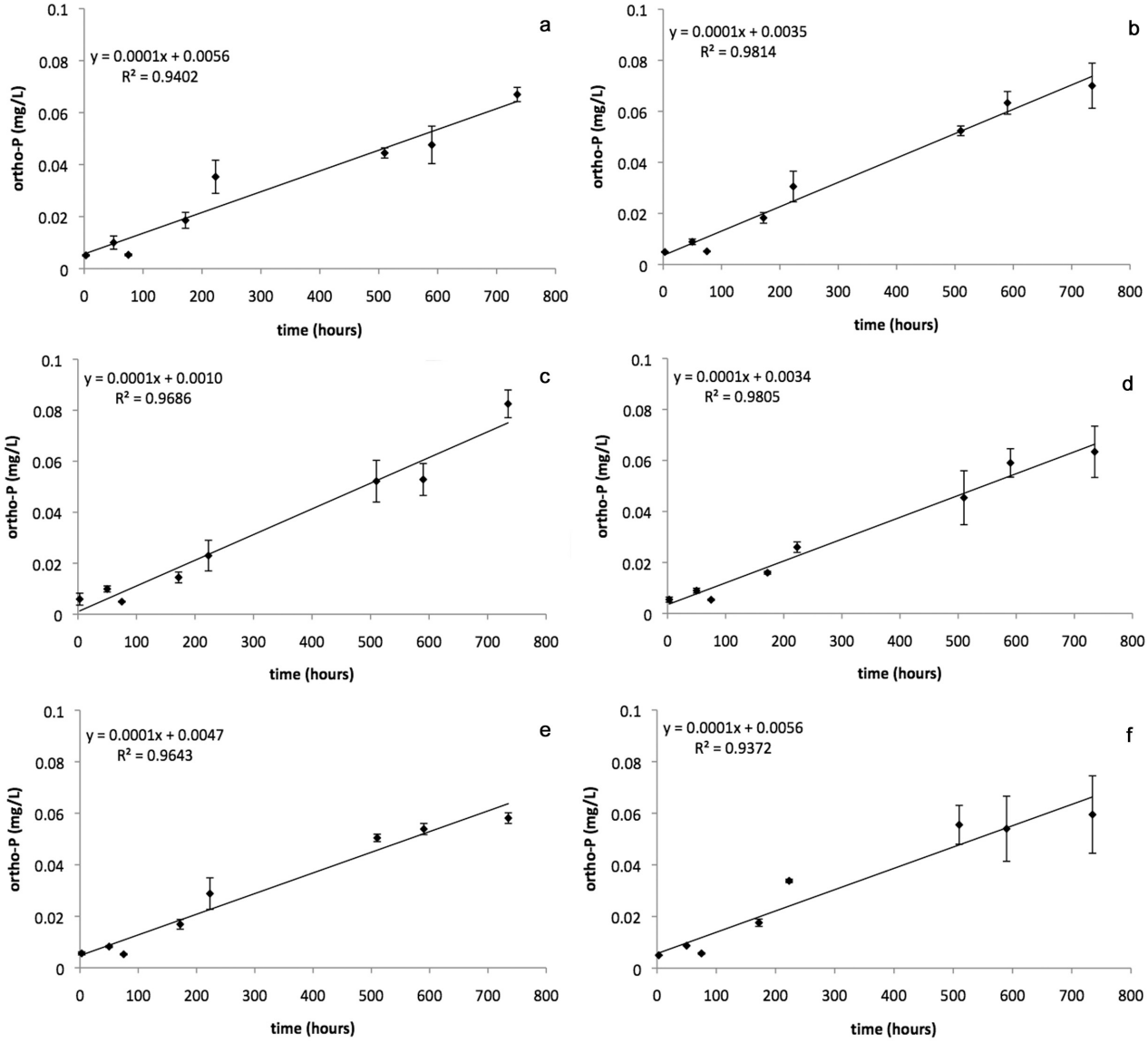


Figure 3.g **Inorganic P release over time for the Lydum-Gibbsite #1 experiment**. The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite 5. Bars refer to standard deviation.

In the first experiment with Lydum soil and gibbsite a positive trend in solubilisation of P could be found. At the end of the experiment (600 hours) the 11.7% of total Olsen-P is found in solution. Every day P concentration in solution rose by 0.00224 ± 0.0001 mg_P/L.

Student's t-test proved that the equations describing the trend lines are equal to each other, indicating that the amount of P found in solution is the same for each gibbsite series, and also that every incubation has been following the same P mobilization dynamic.

For Lydum-Gibbsite #2 trends couldn't be found. It clearly appears that some P is present in solution but it does not seem to be related to the amount of Fe reduced. Moreover, standard deviations are very big in all of the six series.

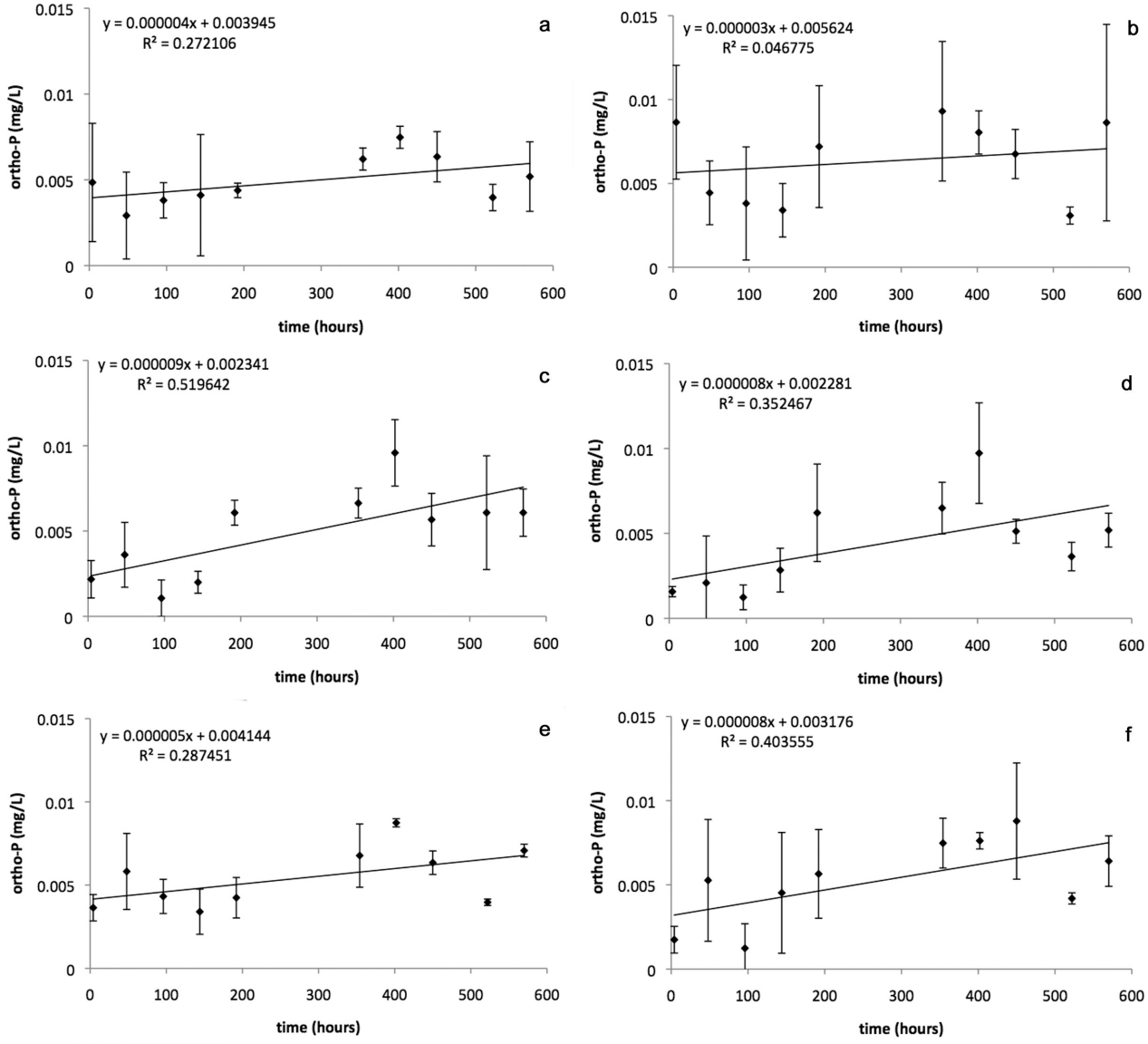


Figure 3.h **Inorganic P release over time for the Lydum-Gibbsite #2 experiment**. The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite. Bars refer to standard deviation.

Figure 3.i shows ortho-P data for the Vedersø-Gibbsite #1 experiment. Analysis for sampling at 170 and 195 hours were not carried out. It is clear, in all the gibbsite series, that the amount of P in solution increases at the beginning and then dropping after 50 hours.

Data from the first three sampling was fitted with an exponential curve, as a straight line could not approximate satisfactory the exponential phase of P release showed that occurred in the beginning of the experiment.

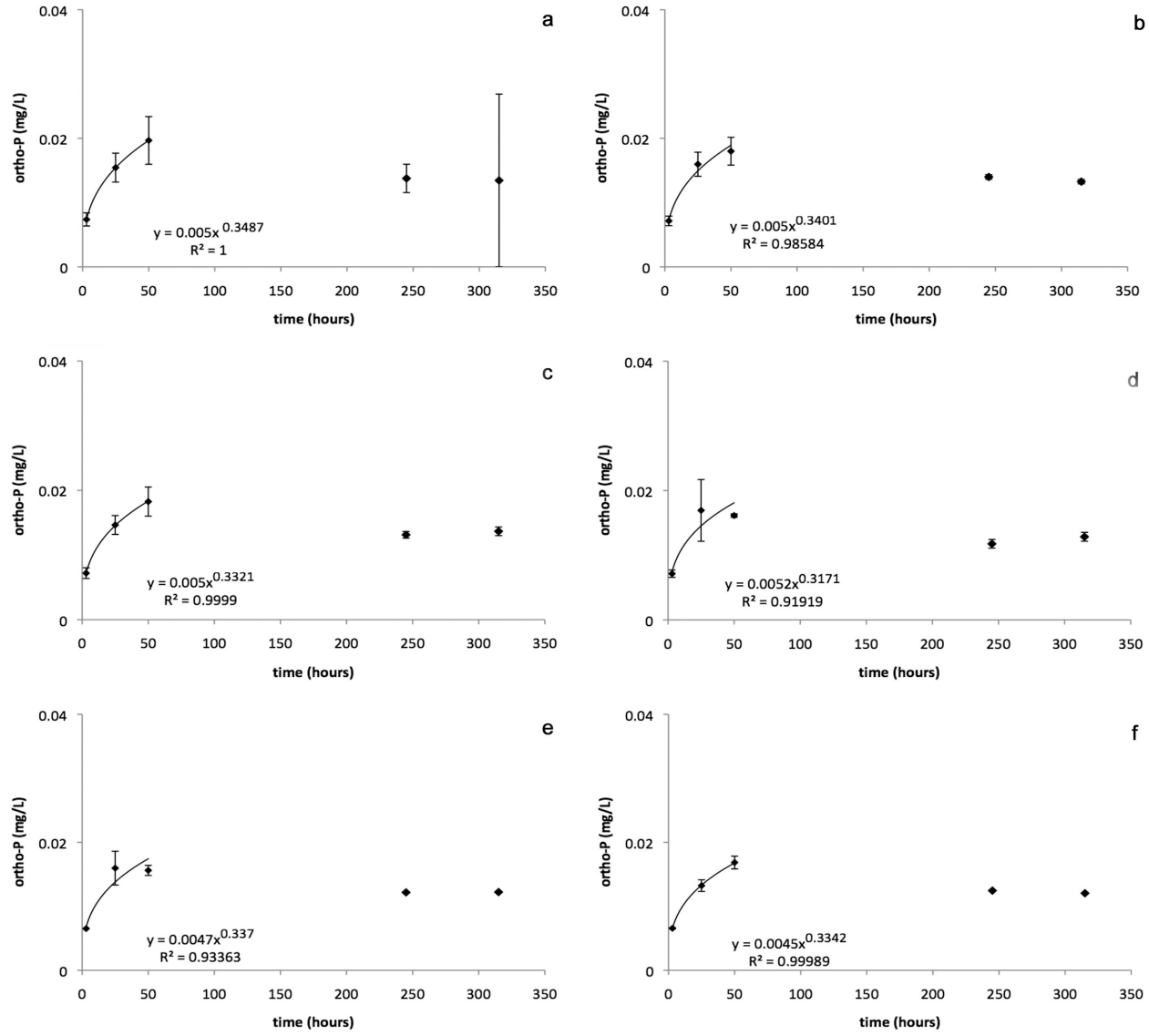


Figure 3.i **Inorganic P release over time for the Vedersø-Gibbsite #1 experiment**. The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite 5. Bars refer to standard deviation.

Table 3.2 shows the equations that describe the linear correlations of ortho-P in solution over time. Note that the equations reported for the Vedersø-Gibbsite #1 experiment refer only to the first three sampling, as other data can not be used due to the drop in Fe(II) occurred between the 4th and the 5th sampling in the experiment.

Table 3.2 **Equations for the linear correlations of ortho-P in solution over time.** Correlations are not reported when R^2 value is lower than 0.750.

Experiment Name	Series Name	Equation	R^2
Lydum-Gibbsite #1	control	$y = 0.0001x + 0.0056$	0.094
	gibbsite 1	$y = 0.0001x + 0.0035$	0.981
	gibbsite 2	$y = 0.0001x + 0.0010$	0.969
	gibbsite 3	$y = 0.0001x + 0.0034$	0.981
	gibbsite 4	$y = 0.0001x + 0.0047$	0.964
	gibbsite 5	$y = 0.0001x + 0.0056$	0.937
Lydum-Gibbsite #2	control	---	0.272
	gibbsite 1	---	0.047
	gibbsite 2	---	0.520
	gibbsite 3	---	0.352
	gibbsite 4	---	0.287
	gibbsite 5	---	0.404
Vedersø-Gibbsite #1‡ ☆	control	$y = 0.005x^{0.3487}$	1
	gibbsite 1	$y = 0.005x^{0.3401}$	0.986
	gibbsite 2	$y = 0.005x^{0.3321}$	1.000
	gibbsite 3	$y = 0.0052x^{0.3171}$	0.919
	gibbsite 4	$y = 0.0047x^{0.337}$	0.934
	gibbsite 5	$y = 0.0045x^{0.3342}$	1.000

‡ correlation was made for the first three samplings only (3, 25 and 50 hours).

☆ exponential correlations were made instead of linear ones.

3.4 Ortho-phosphate ratio on Fe(II)

Fe(II) produced in 1 litre of soil solution and ortho-P in solution were fitted together in order to determine whether a relationship between phosphate in solution and total Fe(II) produced exist. Total Fe(II) was calculated (thanks to equation 26) at each sample.

If present, this relationship could also be compared with the ratio between Olsen-P and CBD extractable iron, which has been previously calculated for each soil.

Table 3.3 show correlations between P mobilization and Fe(II) production. Fe(II) concentration is the independent variable, meaning that P mobilization depends on Fe(II) production.

Clear linear correlations were found only for the Lydum-Gibbsite #1 experiment. Ortho-P concentration increases as Fe(II) concentration in solution increases.

For Lydum-Gibbsite #2 and Vedersø-Gibbsite #2 correlations could not be found. In the first case ortho-P in solution does not seem to be connected to Fe(II) production at all. Ortho-P concentration fluctuates up and down for all the experiment in all six gibbsite series.

In the second case, correlations seem to be present, but nullified by data from the last two samplings, in which a drop of Fe(II) in solution took place, probably due to oxidation of the soil solution in all of the flasks.

Table 3.3 shows a comparison of the calculated Ortho-P/Fe(II) ratio between the three experiments. This ratio was calculated as average of the single Ortho-P/Fe(II) ratios (equation (20), where x=Fe(II) concentration and y=Ortho-P concentration) from all of the gibbsite series in each experiment. Values in Table 3.3 can be used to make a comparison between solution P/Fe ratio and soil P/Fe ratio for the two soils. In general, the ratio in the soil does not correspond to the ratio in the soil solution. The fact that proportions between soil P and soil Fe are not respected once the elements are solubilized (P) and produced (Fe(II)) indicates that desorption is not the only process that takes place, moreover, it suggests that more P should be found in solution.

Table 3.3 **Summary on the ortho-P and Fe(II) ratio for the three experiments**. R^2 and Ortho-P/Fe(II) have been calculated as average of all the R^2 and Ortho-P/Fe(II) single ratios from all of the gibbsite series in each experiment. Ratios are calculated in the mol system.

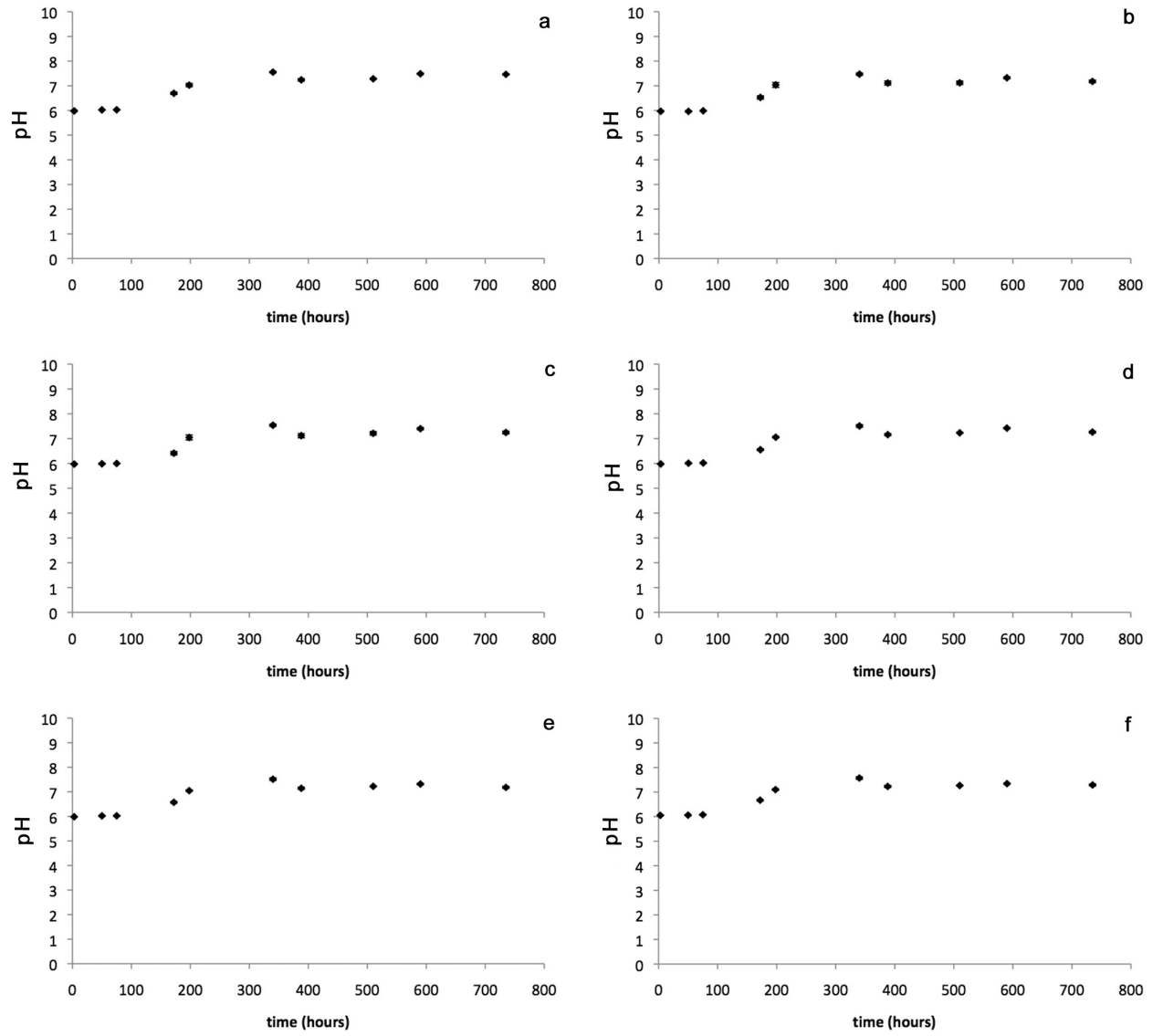
Experiment	Correlation	R^2	Ortho-P/Fe(II)	Olsen-P/CBD-Fe ‡
Lydum-Gibbsite #1	Found. Linear	0.945	$P = 8*10^{-4}$ Fe + 0.0003	$P = 5*10^{-4}$ Fe
Lydum-Gibbsite #2	Not found	0.222	——	$P = 5*10^{-4}$ Fe
Vedersø-Gibbsite #1	Not found	0.102	——	$P = 2.4*10^{-3}$ Fe

‡ Values refer to paragraph 2.2.

3.5 Variation in pH value

Figures 3.j, 3.k and 3.l show how pH has stayed quite stable for the whole duration of the Lydum-Gibbsite #1, Lydum-Gibbsite #2 and Vedersø-Gibbsite #1 experiments respectively.

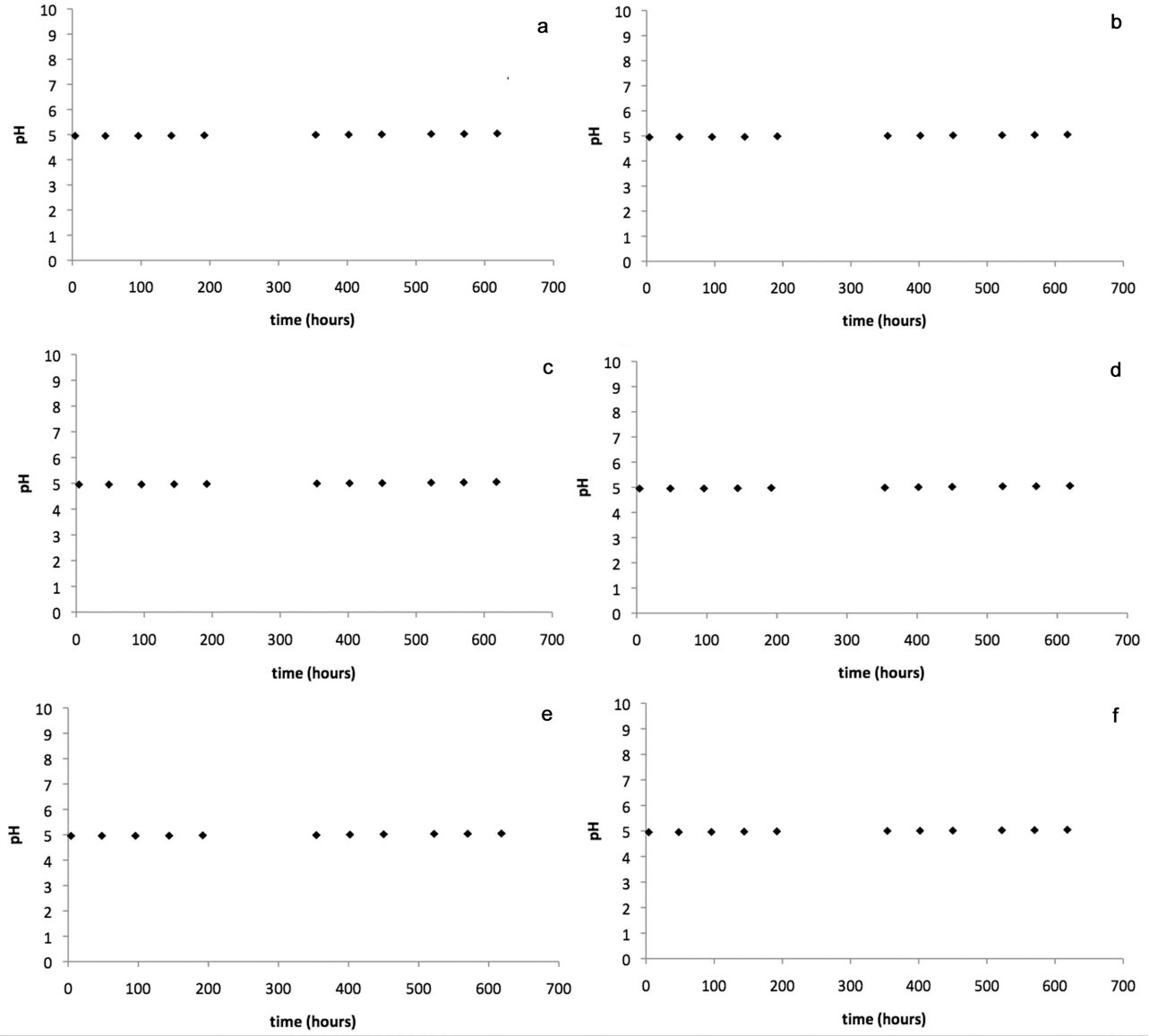
Lydum-Gibbsite #1 has been the only experiment in which pH has been increasing significantly, where else it has been almost constant in the other two experiments. In Figure 3.j a slow and regular growth of pH from 6 to values of approximately 7.5 in the first 600 hours of the experiment. In the last two samplings pH was stable.



time (hours)	3	50	75	172	198	340	388	510	590	735
temperature (°C)	25	26	26	26	27	29	27	27	27	27

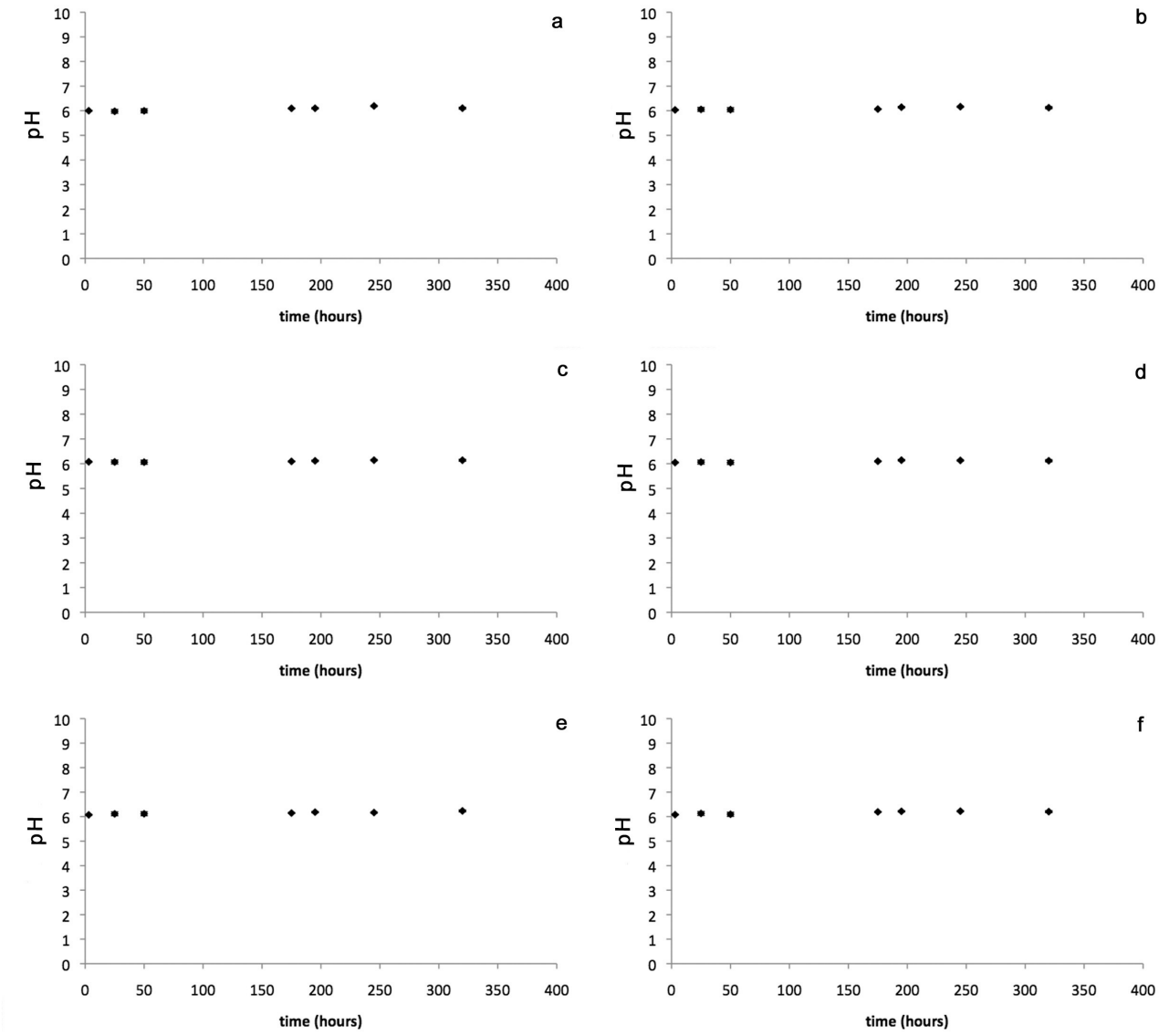
Figure 3.m **pH and temperature values over time for the Lydum-Gibbsite #1 experiment**. The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite 5. Reported temperature was measured while measuring pH. Bars refer to standard deviation.

Figures 3.j, 3.k and 3.m also show how all the different gibbsite series have been following, with very few differences and very small standard deviations (see pH values in appendix B.4), the same trend in all of the three experiments.



time (hours)	4	48	96	144	192	354	402	450	522	570	618
temperature (°C)	25	25	24	25	25	26	25	24	25	25	25

Figure 3.n **pH and temperature values over time for the Lydum-Gibbsite #2 experiment**. The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite 5. Reported temperature was measured while measuring pH. Bars refer to standard deviation.



time (hours)	3	25	50	175	195	245	320
temperature (°C)	25	26	27	26	26	27	25

Figure 3.o **pH and temperature values over time for the Vedersø-Gibbsite #1 experiment**. The letters on the top right of each graph indicate gibbsite concentration: a stands for control; b for gibbsite 1; c for gibbsite 2; d for gibbsite 3; e for gibbsite 4 and f for gibbsite 5. Reported temperature was measured while measuring pH. Bars refer to standard deviation.

4 Discussion

Before starting to discuss the results it is useful to have a look back at the objectives of this thesis. This project was carried out trying to:

(i) confirm that an anoxic incubation of a flooded soil would have lead to reduction of iron(III) oxides and consequent solubilisation of Fe(II);

(ii) confirm that significant loads of phosphorus are released once soil iron(III) oxides are reduced;

(iii) confirm the importance of aluminium-oxides as sorbents for soluble P;

(iv) demonstrate that a positive correlation exists between the amount of P resorbed and the amount of aluminium-oxides added in the soils (of course considering that soils already contain natural aluminium-oxides).

Discussion will start considering whether the objectives have been fulfilled or not; and then continue examining other aspects of interest that will help to fully understand the results this project achieved.

Results shown in paragraph 3.1 clearly confirmed that Fe(II) production occurs under anaerobic conditions, as the iron(III) oxides present in the soil are used as electron acceptors by microorganisms and therefore reduced (Lovley 1991). In each of the three experiments a positive linear correlation was found between Fe(II) production and time of incubation under anoxic conditions. These results also confirm other results obtained in a different study on the same soils (Heiberg et al. 2009). Results show that no significant difference in solubilized Fe(II) production exists between control and the other series where gibbsite was added. Moreover, in all of the three experiments pH values were always the same in all of the different gibbsite series. Therefore, according to equation (13), also total Fe(II) produced is assumed not to be significantly different between the different gibbsite series in each experiment. Soils reduction is then clearly independent from the amount of gibbsite added. Many authors have shown how the presence of Al compounds

in soil can be toxic to bacterial metabolism and activity (Piña and Cervantes 1996). It can be confirmed that microorganisms reducing Fe(III) present in Vedersø (II) and Lydum Å (III) are not affected at all by gibbsite addition up to a concentration of approximately 75 mM and pH values shown in section 3.5.

The concentration of ortho-P in solution has been increasing as soil reduction increased. This seems to confirm the second hypothesis this project has been trying to prove. However, concentration of ortho-P in solution was generally too low to be considered and then discussed. P mobilized in the Lydum-Gibbiste #2 and Vedersø-Gibbsite #1 was found to be under the detection limit for the analysis method for almost the whole duration of the experiments. Also results from samplings taken in the first 200 hours in the Lydum-Gibbsite #1 experiment have to be discarded for the same reason. Moreover, at the end of this experiment only the 11.7% of the total Olsen-P was solubilized that correspond to 2.82 mg_P/kg_{SOIL}. Assuming that an average saturated has soil-water volume ratio that can be approximated to 1 m^3/m^3, and the typical bulk density of a sandy soil has a value of approximately 1600 kg/m^3, the concentration of P released to groundwater would be around 4.5 mg_P/L. It is very difficult to estimate how much of this P would get to any close waterbody. Plant uptake, filtration and resorption in the riparian zones and dilution factors (mix with floodwater, mix with water in the waterbodies and rain) have to be considered. A dilution/dissipation/resorption factor equal to 100 would lead to a P concentration equal to 0.045 mg_P/L. As shown in Table 1.3, lakes can be defined as eutrophic with a P concentration equal or bigger than 0.035 mg_P/L (0.075 mg_P/L for streams).
After considering these aspects, it is more accurate to say that P was mobilized as a result of soil reduction in amounts that are not always significant; as that both of the soils could have potentially been releasing much more P. Only in the first experiment significant loads of P were released.

To see if gibbsite is to be considered as a good sorbent we have to discuss what the sorption isotherms (Figure 2.b; section *a* and *b*) indicate and relate all the considerations with the data of ortho-P solubilization shown in paragraph 3.3. Figure 2.b shows that gibbsite has sorption properties towards P. The amount of P sorbed to gibbsite is anyway much smaller than the amount of P that stays in solution. Sorption power of gibbsite increases only when gibbsite is present in big amounts. A PAC for gibbsite series could not be calculated, as specific surface of gibbsite is not known. Thanks to the sorption isotherms it is possible to know the amount P that could be sorbed by gibbsite at different soluble P concentrations. In series gibbsite 5 (where gibbsite was added at 100 $g_{GIBBSITE}/kg_{SOIL}$), this value is now going to be calculated as reference value with an equation

similar to equations 8 and 10. Sorption isotherm at pH 6 (figure 2.c section a) shows that gibbsite could sorb about 3 $\mu g_P/g_{GIBBSITE}$ with a soluble P concentration of 0.0056 mg_P/L (amount of P solubilized in the Lydum-Gibbsite #1 experiment at 600 hours).

$$100\ g_{GIBBSITE}/kg_{SOIL} * 0.00297\ mg_P/g_{GIBBSITE} = 0.297\ mg_P/\ kg_{SOIL} \quad (27)$$

This value represents the highest sorption power for gibbsite in the whole project as the other gibbsite series contain much less gibbsite.
Comparing this value with the PACs of the two soils (equations 8 and 10) it clearly appears that sorption power of the gibbsite used in this project is totally inadequate and too small to have any significant role in P resorption.
As already discussed, only in the Lydum-Gibbsite #1 experiment, significant loads of P were mobilized. In this experiment any relevant difference between the different gibbsite could not be found, indicating that the addiction of gibbsite has not been making any change on P mobility. This ineffectiveness is due to its poor sorption skills.
More P was though expected to be solubilized, as P/Fe ratio in soil was supposed to be found with no changes in solution as ortho-P/Fe(II). Table 3.3 shows that ortho-P/Fe(II) ratio for Lydum Å (III) is smaller than soil P/Fe ratio, while no correlation between ortho-P and Fe(II) could be found for Vedersø (II). These two facts indicate that a resorption process took place in all the experiments, and it has been very effective, as P was not mobilized in significant amounts in the Lydum-Gibbsite #2 and Vedersø-Gibbsite #1 experiments. Iron(III) oxides are believed to be the leading players of this process. It is believed that soils themselves had been re-sorbing significant loads of the P that was mobilized during soil reduction as both of the soils had a very low degree of saturation of P (equations 9 and 11) and soils were not totally reduced. Consequentially all the non-reduced available iron(III) oxides have been sorbing P in significant loads in all of the experiments.

The last objective of the project is therefore not proved right, as gibbsite did not play any relevant role in P resorption.

Lydum-Gibbsite #1 and Lydum-Gibbsite #2 produced results very dissimilar to each other. The two experiments were supposed to produce similar results, as the same soil was used. The soil was actually expected to be more reduced in the second experiment, as more acetate was added to obtain a faster and more potent Fe(III) reduction. In order to understand why the two experiments

produced different results it is important to point out all the aspects and variables that made the experiments different:

• in the firs experiment sodium acetate was added as 0.5 mM buffer at pH 6 while in the second experiment acetate was added as 1 mM buffer at pH 5;

• pH has been fluctuating from 6 to approximately 7.5 in the first experiment (Figure 3.j) while it has been stable at values very close to 5 in the second experiment (Figure 3.k);

• the first experiment was carried out with temperature, in the glove box, that was always around 26±0.5° C while temperature has been varying much more in the second experiment (26±2° C);

• the first experiment was carried out inside the MBRAUN glove box while the second experiment was carried out in the COY glove box;

As both of the glove boxes are believed to be equally efficient in creating and maintaining an anaerobic environment, moreover the more acetate added the more promotion of bacterial activity is believed to take place; differences in temperature and pH values are pointed at as the causes of the differences in results between the two experiments.

Vedersø (II) degree of saturation in P was around the 20%. Part of the Olsen-P could be sorbed to Al naturally present in the soil. The amount of Al present in the soil is approximately 1.3 mg/kg. Natural aluminium oxides have been proved to have relevant PAC values (Murray and Hesterberg 2006). Thus the real saturation in P of the iron-oxides could even be much smaller than the value expressed in equation 11.

It can be concluded that any significant P mobilization took place in the experiment with Vedersø (II) because:

• phosphorous content was too little in the soil. In this case P would have been too much diluted to be detected with the analysis method that was used in this project;

• a significant amount of P was naturally sorbed by non-soluble Al compounds in the soil. In this case, the amount of P sorbed to iron oxides could have been too little to be detected;

• phosphorus could not be mobilized as precipitation of amorphous iron phosphates took place after the drop on Fe(II) production that occurred during the Vedersø-Gibbsite #1 experiment.

Figure 3.e shows clearly that a drop on Fe(II) production took place during the Vedersø-Gibbsite#1 experiment in all the series. Oxidation of the solution is believed to be the reason for such a drop. As described in chapter 2, in the MBRAUN glove box, presence of oxygen was checked very often with the light bulb method. Though it is possible, that oxygen was introduced in the glove box without being detected by the light bulb. The light bulb had a small hole in the glass, and gas

exchange between the bulb and the glove box could have been too slow to detect oxygen in time. It is likely that oxygen was introduced in an amount, which could quickly be expelled by the glove box filter in a short time, but in a concentration big enough to oxidize part of the Fe(II) in each of the eighteen flasks.

The stop on Fe(II) production between the 4th and the 5^{th} sampling could have also been due to the exhaustion of reducible Fe(III) pools. Total Fe(II) produced after 170 hours was estimated to be about the125% of the total oxalate extractable iron present in the soil. Thus is very likely that, the resting iron oxides could not be reduced by microorganisms as they could be part of mineral iron(III) oxides non-reducible pools.

5 General Conclusions and Future Prospects

As seen through previous chapters, the project failed to prove its main hypothesises right. It clearly appears that the gibbsite chosen for this project cannot sorb P to any significant extent. Several values for specific surface of gibbsite can be found in literature, with a range that goes from 4.5 m^2/g to 100 m^2/g (Rozic et al. 2001; Lützenkirchen 2006). Acros Organic does not provide this information. Therefore, it is believed that gibbsite was not fine enough, thus its specific surface was too little to have a relevant sorption activity. Gibbsite sorption capacity should have been measured for various gibbsites coming from different producers and distributors of chemical matherials, and consequently a finer and more efficient gibbsite than the one provided by Acros Organic should have been used.
If a better sorbent were chosen, there would still be a basic fault in the project anyway: the experiments were carried out with the wrong soils. A good soil for this kind of experimentation would be, according to author's considerations and conclusions found in other studies (Szilas et al. 1998): (*i*) a soil with a much higher P/Fe ratio, thus more saturated with P than Lydum Å (III) and Vedersø (II), in order to limit the resorption of P by non-reduced available iron(III) oxides; (*ii*) a soil not very rich in Fe, in order to have a significant reduction process with the majority of the Fe(III) in soil conversed to Fe(II) during the experiment time frame.

The experiment is not to be considered as a complete failure anyway. The experiment demonstrated that, in agricultural soils with properties similar to Lydum Å (III) and Vedersø (II) (at ranges of pH and temperature measured in this project), anaerobic conditions occurring for a short-medium long time frame (up to 600-700 hours) have little or no relevant effects on P mobilisation and migration from soil to groundwater; as the resorption process by non-reduced available soil iron(III) oxides is very potent in these kind of soils.

Any scientific work, as far as the author and the supervisors know, has not been published yet on aluminium oxides and aluminium silicate minerals ability on P resorption in soil under anoxic

conditions. Therefore this project has a very challenging aim that has to be persecuted until good and relevant results are obtained. New series of experiments are needed.

The same project should be repeated with some changes in its materials and methods. Soils used should have a degree of P saturation (as calculated in equations 9 and 11) of approximately 75%; with a content of oxalate extractable iron of approximately 3 g_{Fe}/Kg_{SOIL} and thus an Olsen-P content of approximately 15 mg_P/kg_{SOIL}. Content of Al in soil should then be as less relevant as in Lydum Å soil in order to be able to ignore, in calculations and speculations, the sorption of P to natural occurring aluminium oxides and aluminium silicate minerals. With such a soil and with an efficient set up (similar to the one in Lydum-Gibbsite #1 experiment) a complete or nearly complete reduction of the soil is supposed to take place. Therefore, the majority of soil Olsen-P would get dissolved in solution and hypothesized aluminium sorbing-activity would appear clear and would be easily quantifiable and qualifiable.

A better sorbent should be chosen, as Acros Organics' gibbsite is inadequate.

It would also be wise to change the used methods to some extent as well, in order to have a more efficient set up. Soil reduction should be promoted with a different acetate buffer, as the 0.5 mM - pH 6 turned out to be very efficient in promoting reduction but not very good on keeping stable the pH; and the 1 mM - pH 5 turned out to be good at keeping the pH stable but did not promote bacterial activity efficiently. A larger amount of soil should be incubated as 10 g gave such a low output in both Fe(II) and ortho-P production, thus part of the results could not be trusted because under analysis detection limits. In order to have a more precise control on soil's reduction, Fe(II) sampling should be taken with an acid extraction method, which allows to detect the total Fe(II) produced during soil reduction. Moreover the soil-water ratio in the soil solution should be similar to natural soil-water ratios of saturated soils, in order to have a more representative model in which variables such as the K_d ratio could be used without any speculation, assumption or difficult calculations. If repeated, with such new changes in its material and methods, and with the experience gained, the project is supposed to provide much more satisfying results.

If project hypothesis were proved right, gibbsite and kaolinite would then be likely tool to use in stopping P flow from agricultural fields to streams and lakes. Buffer zones soils could be enriched with cheap, effective and non-polluting gibbsite or kaolinite in order to immobilize P passing by. As a positive consequence the P input to waterbodies could be reduced to sustainable limits.

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Appendix A

Extracts from Buffalo-P Project presentation

By the
Ministry of Food, Agriculture and Fisheries.
Directorate for Food, Fisheries and Agri Business

Project title:

Best Management of Stream Banks, Buffer zones and Floodplains for reducing Agricultural Phosphorus Losses.

Project manager:

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The project falls within the following research area:

Nutrients in soils and water:

· Availability, transformation, transport and loss of dissolved, colloidal and particulate phosphorus from the soil system through leaching and erosion including methods to quantitative measurements of concentrations and fluxes. Availability of phosphorus in crops and loss to the aquatic environment should be related to amount and type of phosphorus fertilizer and a regional perspective.

· Development and tests of methods to reduce phosphorus loss from agricultural areas.

· Models and mechanisms for transport of nitrogen (and phosphorus) from field to lakes andestuaries, including the establishment and significance of tile drains.

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Project duration:

Four years, from 2006 to 2009.

Main objectives:

1.To quantify bank erosion and bank failure rates and losses of phosphorus forms with bank material in natural and regulated Danish stream types and describe the main factors influencing the bank erosion process for development of a decision support tool supporting end users in applying Best Management Practice (BMP).

2.To determine the phosphorus retention efficiency of differently managed buffer strips receiving runoff from agricultural land and to devise rules for their management and placement in landscapes.

3.To quantify and model the spatial and temporary net deposition of sediment and attached phosphorus forms on natural and restored floodplains and investigate the content of phosphorus in floodplain soils and the risk for phosphorus mobilization along gradients in phosphorus content, duration of inundation and redox conditions in floodplain soils.
4.To risk assess Danish riparian soil types with respect to in situ phosphorus mobilization following drainage termination, identify the controlling factors for phosphorus release or retention, evaluate their ability to retain tile drainage water phosphorus and develop guidelines for Best Management Practice (BMP).

Project summary:

Through a basic, innovative and strategic research strategy this project will develop and test guidelines, methods and tools that can support end users in implementing targeted mitigation measures in streams, buffer zones and floodplains for immediate reductions in the agricultural losses of phosphorus to the aquatic environment. The research strategy chosen is directed at increasing our understanding of the various mechanisms responsible for sorption, storage and loss of different phosphorus forms in the ca. 400,000 ha cultivated Danish low-lying soils and riparian areas along our ca. 65,000 km watercourses, these areas being currently responsible for more than half of the agricultural phosphorus loss to the aquatic environment. Changes in the agricultural use and practice on low-lying soils in riparian areas and the function of tile drains can on relatively small areas give multiple advantages: (i) a reduction in the phosphorus loss from stream bank erosion; (ii) an increase in the phosphorus sorption and storage potential on riparian areas through establishment of uncultivated buffer strips/buffer zones along watercourses, irrigation of riparian areas with tile drainage water from upland farmed fields and allowing temporary inundations of entire floodplains with river water. We hypothesize that such mitigation strategies will ensure a certain and immediate reduction in the losses of dissolved and particulate phosphorus forms from agricultural areas to streams, lakes and coastal waters, thereby helping to obtain a good ecological quality as required in the EU Water Framework Directive.

Through controlled laboratory experiments the project will investigate the biogeochemical processes that control the phosphorus sorption/desorption kinetics under aerobic and anaerobic conditions in the geochemical types of Danish low-lying soils differing in soil phosphorus content, phosphorus sorption capacity and degree of phosphorus saturation. The experiments will enable us to develop a dynamic model for the seasonality phosphorus sorption in Danish low-lying soils. The loss of sediment and phosphorus via bank erosion will be quantified in natural meandering and straightened and channelized Danish stream types within two representative Danish river basins

using the erosion pin method in a replicate research design consisting of 72 stream sites and a study of longer-term bank retreats in natural reaches using aerial photographs in ArcView. The research design will enable us to conduct statistical analyses of the biotic and abiotic factors controlling bank erosion and analyze the sources, forms and potential bioavailability of phosphorus in stream bank sediments. The storage and dynamics of dissolved and particulate phosphorus forms in uncultivated buffer strips and buffer zones along streams will be investigated by *in situ* measurements of phosphorus deposition, controlled field experiments in small runoff plots and by applying an existing GIS-based runoff pattern model to describe the sedimentation of eroded soil material in buffer zones. The ability of floodplains for sorption and storage of phosphorus forms will be investigated at sites being naturally inundated and at sites where the interaction between river and floodplains has been restored, utilizing a combination of *in situ* measurements of the deposition of sediment and phosphorus, and by dating of soil cores from profiles of natural radioactive tracers. Finally, we will validate the developed dynamic models for the phosphorus sorption/desorption kinetics in typical Danish low-lying soils by establishing water and phosphorus-balances for small plots being irrigated with tile drainage water.

The outcome of the project in the form of guidelines, models and tools has great strategic and applied advantages as it supports the end users being responsible for the management of streams and riparian areas in optimizing their buffering potential for phosphorus losses from adjoining or upstream agricultural areas. The scientific quality of the project will be ensured through publication of at least 10 papers in peer reviewed international journals and organization of two project workshops with participation of an Advisory Board of 3 well known international scientists working within the project area. One of the project workshops will be held as 1-2 special sessions in connection with the 5th International Phosphorus Workshop (IPW5) to be held in September 2007 in Silkeborg, Denmark. The project involves a high educational commitment through a PhD-student and a Post Doc financed by the project, connected to two Danish research schools (RECETO and SOAS), development and organization of a PhD course ”Phosphorus Biogeochemistry in Wetlands”, and attachment of several MSc and BSc students to the project.

Project background.

Agriculture is today the main driving force for the phosphorus (P) pressure on the Danish aquatic environment. The state of P in inland and coastal waters is in many cases so high that many rivers, lakes and estuaries are impacted to such an extent that a good ecological quality cannot be obtained. Therefore, the objective of reaching a good ecological quality in water bodies as required by the EU Water Framework Directive (EU 2000) will demand that the P-loss from agricultural areas are

reduced. Consequently, responses in the form of introduction of general and targeted mitigation measures against agricultural P-losses in source areas and along the different P-pathways are needed. This demand that knowledge on the short and longer-term effects of the different mitigation measures adopted by River Basin Managers and farmers to reduce P-losses from agricultural land are developed. One way to reduce agricultural P-losses is to adopt general mitigation measures at source areas as done in the Danish Action Plans for the Aquatic Environment II and III for combating nutrient pollution.

The general mitigation measures implemented in the Action Plan III against P in source areas aims at halving the P-surplus in Danish agriculture before 2015. Danish agricultural soils have been enriched in total P-content during the last century so the effects of the adopted general mitigation measure will develop over longer time periods. Therefore, movement of particulate P and dissolved P along important pathways as soil erosion and surface runoff, stream bank erosion and tile drainage water will continue to supply P-forms to surface waters for decades. The only way to obtain immediate reductions in agricultural P losses are to adopt targeted mitigation measures for reducing P-mobilization in source areas or increase the natural buffer potential for the transport of particulate and dissolved P-forms via different hydrological pathways to surface waters. In Denmark, a major part of the present P loss from agricultural areas to surface waters has been shown to be derived from riparian areas due to losses of P from stream bank erosion and P-loss from tile drained organic soils. However, riparian areas will under natural conditions function as important buffers for phosphorus delivered via groundwater, drainage water or surface water due to various biogeochemical processes as sedimentation, sorption, denitrification and biological uptake.

Targeted mitigation measures are part of the Governmental Action Plan II (1998) and III (2004) that allows formerly cultivated riparian areas along the river continuum to be transformed into wetlands, wet meadows and wide uncultivated buffer zones. Our current knowledge on how, where, with what effect and with what risks Best Management Practices (BMP) can be introduced by end users for utilizing the natural phosphorus buffering potential in riparian areas to obtain immediate reductions in the agricultural P-loading of surface water bodies are, however, strongly limited. BMPs need to be developed for implementation of targeted mitigation measures as: (i) installation of uncultivated buffer zones along streams to capture P enriched sediments and dissolved P transported towards the stream edge with surface runoff; (ii) letting tile drainage water from adjoining arable fields irrigate riparian soils for sedimentation, sorption and uptake of P-forms; (iii) blocking ditches or cutting tile drains in the river corridor; (iv) allowing river water to inundate the floodplains for shorter or longer time periods. Restoration of the natural hydrological regimes in rivers and installation of wide buffer zones will also allow for changes in vegetation cover and

stream maintenance which in turn will reduce bank erosion and bank failures as sources of sediment and phosphorus. Many of the flood plains have, however, been intensively farmed for decades with a high P-surplus and hence the soil P-content may in some places be very high. Re-wetting of such floodplains soils may therefore create a risk for release of former agricultural P for shorter or longer periods as shown from monitoring of some recreated wetlands and lakes under the Danish Action Plan for the Aquatic Environment II. There is a strong need for development of BMPs for riparian areas to take full advantage of the P filtering effect of these areas providing efficient means for reducing agricultural P releases to the aquatic environment. The BMPs will be based on current understanding and quantitative modeling of the physics and biogeochemistry of phosphorus in wetland areas.

State of the Art

Riparian areas constitute an important part of river basins being situated as a border zone (ecotone) between upland areas and surface water bodies. Many biogeochemical processes (sorption/desorption reactions, biological uptake, leaching, erosion and sedimentation) governs the fate of phosphorus forms entering riparian areas via direct agricultural inputs of fertilizer and manure P, eroded material or tile drainage water from bordering agricultural fields and through inundation of floodplains with river water.

Many studies of phosphate sorption to aerobic soils and sediments have demonstrated that the phosphate sorption capacity is linearly correlated with the content of iron(III) and aluminium oxides, usually expressed as the content of oxalate- or dithionite extractable iron and aluminium. Generally, humic substances increases sorption capacity indirectly by stimulating the formation of amorphous iron(III) oxides which have large phosphate sorption capacities. During anoxic conditions iron(III) is reduced to iron(II) which leads to partial or full dissolution of iron(III)oxides with a concurrent release of phosphate to solution. However, the phosphate released may resorb to redox-stable phases such as clay silicates and aluminium oxides, and – depending on pH, phosphate and iron(II) solution concentrations – iron(II) phosphates such as vivianite may precipitate establishing an upper maximum solution P concentration. Several workers have observed higher P release the higher is the phosphate saturation of the sediments and high internal loadings have been forecasted at startup of constructed wetlands. Many studies demonstrate that the P retention of lowland soils is strongly depending on the management of the soils, in particular with respect to pH, redox, the P sorbents and their degree of saturation. However, at the moment there is no general model to predict P sorption/release rates under reducing conditions.

Main project hypotheses

1) The capacity for lowland soils to retain P during surface runoff, irrigation and inundatation depends on the sediments P sorption capacity, the degree of P saturation, the amount of Fe and redox-stable sorbents, the inherent P sorption/release kinetics and the hydraulic load.

2) The P sorption capacity is strongly variable between and within lowland soils.

3) Buffer zone soils and stream banks are enriched with P from agricultural practice and soil and tillage erosion on bordering fields which in turn may become a source of P-loss to surface waters through bank erosion.

4) Stream bank erosion rates and the resulting P-loss is higher along disturbed and regulated streams than along natural, undisturbed streams.

5) Vegetated buffer zones along watercourses are effective in intercepting particulate P transported with surface runoff from agricultural fields.

6) Irrigation of riparian areas with tile drainage water can intercept both particulate P and dissolved P and thereby reduce agricultural P-losses to surface waters.

7) The role of colloid P-mobilization, transport and flocculation in buffer zones and on riparian areas are an important part of the P-budgets.

8) Natural or restored temporary inundated floodplains are significant net sinks for agricultural derived P-forms transported in the river system.

9) Influx of fluvial sediment or sediment from tile drains may increase the P-binding potential of riparian areas.

10) Large scale mass failures happening over periods of decades dominates in naturally meandering streams as opposed to every day weakening and fluid entrainment processes (bank erosion) in regulated streams.

Project design and common methods

The project utilizes a combination of controlled laboratory experiments, field experiments and field measurements to test and validate our hypothesis on the function of floodplains, buffer zones and river banks as sources and sinks for dissolved, colloidal and particulate phosphorus forms. In the project we will involve the most important geochemical types of Danish low-lying soils. The investigations will be performed in 2 river basins representing the major geomorphologic regions in Denmark and we will cover the entire river continuum from spring to the mouth. River Odense Å, representing the most common geomorphologic region in Denmark (moraine landscape). River Odense Å has been a Pilot River Basin for the EU Water Framework Directive Basic Analysis and involved in several EU Research Projects under the 5th and 6th Water Framework Program

(EUROHARP, EUROLIMPACS). The Regional Authority (County of Funen) has completed and planned several larger scale restoration projects in the Odense Å river basin under the Second Action Plan for the Aquatic Environment that can be adopted as research areas. River Skjern Å, representing the second most common geomorphologic region in Denmark namely the old moraines and outwash plains in western and southern Jutland. The lower part of the River Skjern Å and its main tributary Omme Å was restored during 2000-2002 transforming 1800 ha agricultural land to wetlands and wet meadows. The Ringkjøbing county and the regional farming organizations are planning for initiation of Action Plans in the catchment for reducing nitrogen loadings of Ringkjøbing Fjord. The project utilises as far as possible the same field, laboratory and statistical methods across the Work Packages. A list of common methods is shown in Table 1. Thus, all P-fractionation is done at one of the participants (SDU), all batch experiments and oxalate extractions at the Faculty of Life Sciences University of Copenhagen, all intact soil column experiments at DJF and analysis of river water and tile drainage water at NERI. However, a traditional analysis as total P will be done at all laboratories involved and here the project will assure that an inter-comparison of the different methods is conducted during the project period.

Appendix B
Data tables

B.1.Soil characteristics

Soil pH.

Soil Name	pH A	pH B	ST. Dev	Mean	CV %
Lydum 3 (0 -25 cm)	4.84	4.85	0.01	4.85	0.15
Vedersø 3 (0- 25 cm)	4.42	4.41	0.01	4.42	0.16

Oxalate extractions.

Soil Name	g A	g B	dilution	Abs A (Al)	Abs B (Al)	ppm Al in A	ppm Al in B
Lydum 3 (0 -25 cm)	1.001	1.012	10	0.008	0.008	17.75	17.75
Vedersø 2 (0- 25 cm)	1.024	1.01	1	0.22	0.21	54.775	52.275

	g Fe / kg soil in A	g Fe / kg soil in B	Mean	ST.DEV.	CV%
Lydum 3 (0 -25 cm)	13.66	13.35	13.51	0.22	1.62
Vedersø 2 (0- 25 cm)	0.13	0.13	0.13	0.00	0.33

Soil name	Gram A	Gram B	Dilution	Abs A (Fe)	Abs B (Fe)	ppm Fe in A	ppm Fe in B
Lydum 3 (0 -25 cm)	1.001	1.012	100	0.197	0.195	546.95	540.46
Vedersø 3 (0- 25 cm)	1.024	1.01	1	0.19	0.187	5.24	5.15

	g Al / kg soil in A	g Al / kg soil in B	Mean	ST.DEV.	CV%
Lydum 3 (0 -25 cm)	0.443	0.438	0.441	0.003	0.77
Vedersø 2 (0- 25 cm)	1.337	1.294	1.316	0.031	2.33

Standards i ppm Fe	Abs	Abs	Mean	ST.DEV.	CV%
0	0	-0.001	-0.001	0.0007	-141.42
5	0.166	0.167	0.167	0.0007	0.42
10	0.313	0.313	0.313	0.0000	0.00
15	0.439	0.434	0.437	0.0035	0.81
20	0.551	0.542	0.547	0.0064	1.16
30	0.695	0.687	0.691	0.0057	0.82

Standards i ppm Al	Abs
0	0
5	0.021
10	0.042

15	0.062
20	0.082
30	0.121

CBD extractions.

Soil name	Gram A	Gram B	Dilution	Abs A	Abs B	ppm Fe in A	ppm Fe in B
Lydum 3 (0-25)	0.5075	0.5098	10	0.134	0.114	224.01	225.62
Vedersø 3 (0-25)	0.5073	0.5003	1	0.069	0.067	18.56	18.18

	Gram Fe pr kg soil in A	Gram Fe pr kg soil in B	Mean	ST.DEV.	CV%
Lydum 3 (0-25)	88.28	88.51	88.40	0.16	0.19
Vedersø 3 (0-25)	1.83	1.82	1.82	0.01	0.46

Standards i ppm Fe	Abs (første kørsel)	Abs (2 Kørsel)
0	0	0
4	0.137	0.129
10	0.322	0.293
20	0.548	0.486
30	0.69	0.602

Soil name	Gram A	Gram B	Dilution	Abs A	Abs B	ppm Al in A	ppm Al in B
Lydum 3 (0-25)	0.5075	0.5098	1	0.006	0.006	1.36	1.36
Vedersø 3 (0-25)	0.5073	0.5003	1	0.004	0.005	0.81	1.08

	Gram Al pr kg soil in A	Gram Al pr kg soil in B	Mean	ST.DEV.	CV%
Lydum 3 (0-25)	0.536	0.534	0.535	0.00	0.32
Vedersø 3 (0-25)	0.079	0.108	0.094	0.02	21.76

Standards i ppm Al	Abs 1	Abs 2	ST.DEV.	Mean	CV %
0	0	0.001	0.001	0.0005	141.4
4	0.015	0.015	0.000	0.015	0.0
10	0.038	0.037	0.001	0.038	1.9
20	0.074	0.073	0.001	0.074	1.0
30	0.109	0.107	0.001	0.108	1.3
40	0.144	0.143	0.001	0.144	0.5

TOC analysis.

Soil name	1	2	3	mean	ST.DEV.	CV %	korigeret mean
Lydum 3 (0-25)	1.5637	1.5499	1.5151	1.5429	0.0250	1.62	1.583
Vedersø 3 (0-25)	0.5274	0.5478	0.5103	0.5285	0.0188	3.55	0.542

Olsen-P.

Prøve	Gram A	Gram B	Abs A	Abs B	mg P/L in A	mg P/L in B	mg P/kg in A	mg P/kg in B	mean	ST.DEV.	CV %
Lydum 3, 0-25 cm	1.0068	1.0062	0.0898	0.0876	0.1226	0.1197	24.36	23.80	24.08	0.40	1.66
Vedersø 3, 0-25 cm	1.005	1.0035	0.0056	0.0074	0.0109	0.0133	2.17	2.65	2.41	0.34	14.09

	mg P/kg in A	mg P/kg in B	mean	ST.DEV.	CV%
Lydum 3, 0-25 cm	24.36	23.80	24.08	0.40	1.66
Vedersø 3, 0-25 cm	2.17	2.65	2.41	0.34	14.09

Standards i mg P/L	Abs
0	0
0.05	0.0336
0.1	0.0693
0.2	0.1504
0.3	0.2235
0.4	0.2985

Soil texture.

Soil name	Gram soil	4 min	8 min	2 hours	16 hours
Lydum 3 (0-25)	50.06	5.5	5	4	3
Vedersø 3 (0-25)	50.19	1	1	0.5	0.5

	ler +silt g/L	ler	silt	skål	Skålens vægt i gram	Grov sand	finsand
Lydum 3 (0-25)	5.2	3.1	2.1	4	219.86	27.15	17.65
Vedersø 3 (0-25)	1	0.5	0.5	3	154.99	45	4

	% Ler i jorden	% silt i jorden	% grovsand i jorden	%finsand i jorden	summen
Lydum 3 (0-25)	6.2	4.2	54.3	35.3	100
Vedersø 3 (0-25)	1	1	90	8	100

B.2.Sorption isotherms

Gibbsite pH 5.

Prøver	Abs A	Abs B	Abs C
1	0	0	0
2	0.004	0.004	0.003
3	0.03	0.031	0.029
4	0.186	0.186	0.183
5	0.069	0.069	0.068
6	0.24	0.24	0.239
7	0.079	0.078	0.079
8	0.142	0.144	0.14
9	0.202	0.208	0.208

	udtag af filtratet i ml	Dilution	ppm P i A	ppm P i B	ppm P i C	mean mg/L
1	2.5	1.5	0.005	0.005	0.005	0.005
2	2.5	1.5	0.013	0.013	0.011	0.012
3	2.5	1.5	0.066	0.068	0.064	0.066
4	2.5	1.5	0.384	0.384	0.378	0.382
5	0.5	7.5	0.727	0.727	0.717	0.724
6	0.5	7.5	2.468	2.468	2.458	2.465
7	0.1	37.5	4.145	4.094	4.145	4.128
8	0.1	37.5	7.352	7.454	7.251	7.352
9	0.1	37.5	10.407	10.713	10.713	10.611

	uM P i A flasken	uM P i B flasken	uM P i C flasken	Mean
1	0.158	0.158	0.158	0.158
2	0.421	0.421	0.355	0.399
3	2.130	2.196	2.065	2.130
4	12.388	12.388	12.191	12.322
5	23.474	23.474	23.145	23.365
6	79.694	79.694	79.365	79.584
7	133.809	132.165	133.809	133.261
8	237.371	240.659	234.084	237.371
9	336.002	345.865	345.865	342.578

	Start koncentration	Sorption A	Sorption B	Sorption C	Sorption - snit	Stdev
		umol/kg	umol/kg	umol/kg	umol/kg	umol/kg
1	0.00	-15.78	-15.78	-15.78	-15.78	0
2	5.00	460.11	457.92	464.49	460.84	3.348022375
3	10.00	786.96	780.38	793.53	786.96	6.575385413
4	25.00	1267.77	1261.20	1280.92	1269.96	10.04406713
5	40.00	1663.55	1652.59	1685.46	1667.20	16.74011188
6	100.00	2041.59	2030.63	2063.51	2045.24	16.74011188
7	150.00	1673.89	1783.48	1619.09	1692.15	83.70055938
8	250.00	1262.86	934.09	1591.63	1262.86	328.7692706
9	350.00	742.24	413.47	413.47	523.06	189.8150269

standards i ppm	Abs

P	
0	0
0.01	0.005
0.05	0.032
0.1	0.071
0.15	0.108
0.2	0.144
0.4	0.294
0.5	0.365

Gibbsite pH 6.

Prøver	Abs A	Abs B	Abs C	udtag af filtratet i ml	Fort
1	-0.0017	-0.002	0.0006	2.5	1.5
2	0.0114	0.0035	0.0016	2.5	1.5
3	0.0551	0.0557	0.0509	2.5	1.5
4	0.2397	0.2383	0.2385	2.5	1.5
5	0.0802	0.0823	0.0913	0.5	7.5
6	0.2618	0.2501	0.2697	0.5	7.5
7	0.0905	0.082	0.082	0.1	37.5
8	0.0746	0.0933	0.0697	0.05	75
9	0.0994	0.1154	0.1017	0.05	75

	ppm P i A	ppm P i B	ppm P i C	mean mg/L	st dev	uM P i A flasken
	0.006	0.005	0.010	0.007	0.003	0.18
	0.032	0.016	0.012	0.020	0.010	1.03
	0.120	0.121	0.111	0.118	0.005	3.87
	0.491	0.489	0.489	0.490	0.002	15.86
	0.852	0.873	0.964	0.896	0.059	27.51
	2.679	2.561	2.759	2.666	0.099	86.49
	4.779	4.351	4.351	4.494	0.247	154.28
	7.958	9.839	7.465	8.421	1.253	256.92
	10.453	12.062	10.684	11.066	0.870	337.47

	uM P i B flasken	uM P i C flasken	P i oplsøning Snit - uM
1	0.16	0.33	0.25
2	0.52	0.40	0.46
3	3.91	3.60	3.75
4	15.77	15.79	15.78
5	28.19	31.12	29.65
6	82.69	89.06	85.88
7	140.48	140.48	140.48
8	317.65	241.00	279.33
9	389.44	344.94	367.19

	Start koncentration uM	Sorption A umol/kg	Sorption B umol/kg	Sorption C umol/kg	Sorption - snit umol/kg	Stdev umol/kg
1	0.00	-18.19	-16.24	-33.13	-23	9.2
2	5.00	396.71	448.03	460.37	435	33.8
3	10.00	612.84	608.94	640.12	621	17.0

4	25.00	913.68	922.77	921.47	919	4.9
5	40.00	1248.94	1180.73	888.41	1106	191.5
6	100.00	1350.57	1730.59	1093.98	1392	320.3
7	150.00	-428.01	952.39	952.39	492	797.0
8	250.00	-691.70	-6765.46	899.82	-2186	4045.2
9	350.00	1253.26	-3943.55	506.22	-728	2809.7

standard i ppm P	Abs				mg/g	
					-7.2705E-07	2.98323E-07
0	0				1.40454E-05	1.08997E-06
0.010	0.003				2.00374E-05	5.48505E-07
0.052	0.0343				2.96801E-05	1.58802E-07
0.103	0.0723				3.57086E-05	6.18336E-06
0.155	0.1108				4.49319E-05	1.03408E-05
0.207	0.1427				1.58927E-05	2.57306E-05
0.413	0.3007				-7.05687E-05	0.0001306
0.516	0.3851				-2.35046E-05	9.07111E-05

Kaolinite pH 5

Prøver	Abs A	Abs B	Abs C	udtag af filtratet i ml	Dilution
1	0.046	0.036	0.037	2.5	1.5
2	0.06	0.06	0.061	2.5	1.5
3	0.094	0.089	0.089	2.5	1.5
4	0.259	0.257	0.257	2.5	1.5
5	0.07	0.069		0.5	7.5
6	0.103	0.103	0.103	0.5	7.5
7	0.15	0.152	0.154	0.5	7.5
8	0.212	0.215	0.215	0.5	7.5

	ppm P i A	ppm P i B	ppm P i C	mean mg/L
1	0.096	0.076	0.078	0.083
2	0.124	0.124	0.126	0.125
3	0.193	0.183	0.183	0.186
4	0.526	0.522	0.522	0.523
5	0.721	0.711		0.716
6	1.054	1.054	1.054	1.054
7	1.529	1.549	1.569	1.549
8	2.155	2.186	2.186	2.175

	uM P i A flasken	uM P i B flasken	uM P i C flasken	Mean
1	3.09	2.44	2.50	2.68
2	4.00	4.00	4.07	4.03
3	6.22	5.90	5.90	6.00
4	16.98	16.85	16.85	16.89
5	23.28	22.96	0.00	23.12

6	34.04	34.04	34.04	34.04		
7	49.37	50.02	50.67	50.02		
8	69.58	70.56	70.56	70.24		

	Start koncentration	Sorption A	Sorption B	Sorption C	Sorption - snit	Stdev
		umol/kg	umol/kg	umol/kg	umol/kg	umol/kg
1	0	-309.11	-243.90	-250.42	-267.81	35.92
2	5	99.59	99.59	93.07	97.41	3.77
3	10	377.86	410.47	410.47	399.60	18.83
4	30	1301.83	1314.88	1314.88	1310.53	7.53
5	40	1671.87	1704.48		1688.17	23.06
6	55	2095.84	2095.84	2095.84	2095.84	0.00
7	75	2563.32	2498.11	2432.89	2498.11	65.21
8	100	3041.70	2943.87	2943.87	2976.48	56.48

standatd i ppm P	abs				sorption mean mg/g kaol	st dev mg/g
				1	-8.64638E-06	8.98597E-07
0	0			2	3.14505E-06	9.41986E-08
0.01	0.006			3	1.29012E-05	4.70993E-07
0.05	0.034			4	4.2311E-05	1.88397E-07
0.1	0.071			5	5.45033E-05	5.76846E-07
0.15	0.111			6	6.76651E-05	7.63512E-13
0.2	0.149			7	8.06524E-05	1.63157E-06
0.4	0.295			8	9.60969E-05	1.41298E-06

B.3.Main experiments

Lydum-Gibbsite #1.

Summary

LYDUM + GIBBSITE	NaAcetate 0.5micro mol pH6					
flask	0	1	2	3	4	5
GIBBSITE (g)	0	0.025	0.05	0.1	0.5	1
GIBBSITE (g/L)	0	0.05	0.1	0.2	1	2
GIBBSITE (g/Kg ds)	0	2.5	5	10	50	100

soil weighted	0	1	2	3	4	5
A	11.87	11.87	11.87	11.87	11.87	11.87
B	11.87	11.87	11.87	11.87	11.87	11.87
C	11.87	11.87	11.87	11.87	11.87	11.87

Gibbsite added	0	1	2	3	4	5
A	0	0.025	0.05	0.1	0.5	1
B	0	0.025	0.05	0.1	0.5	1
C	0	0.025	0.05	0.1	0.5	1

IRON II (mg/l)

time	0	st dev	1	st dev	2	st dev	3	st dev	4	st dev	5	st dev
3	0.021	0.004	0.020	0.004	0.021	0.001	0.029	0.001	0.032	0.002	0.025	0.007
50	0.016	0.005	0.018	0.006	0.013	0.008	0.023	0.002	0.029	0.009	0.035	0.003
75	0.102	0.007	0.117	0.040	0.070	0.035	0.068	0.049	0.102	0.003	0.128	0.013
172	6.729	0.189	7.905	0.652	6.302	0.792	5.783	0.649	6.009	2.966	7.012	0.956
388	13.192	0.357	20.737	0.533	17.217	1.253	16.859	0.793	19.452	2.600	16.630	1.491
510	15.267	0.724	25.026	0.722	22.007	3.097	19.961	1.443	22.525	1.291	18.804	0.584
570	15.405	0.699	26.884	0.884	23.872	1.562	20.652	1.685	24.426	1.550	19.720	2.225
735	18.433	0.921	30.440	0.476	26.190	2.386	24.481	1.919	28.001	1.329	23.763	1.114

ORTHO P (mg/l)

time	0	st dev	1	st dev	2	st dev	3	st dev	4	st dev	5	st dev
3	0.005	0.000	0.005	0.000	0.006	0.002	0.005	0.001	0.006	0.001	0.005	0.000
50	0.010	0.003	0.009	0.001	0.010	0.001	0.009	0.001	0.008	0.000	0.009	0.000
75	0.005	0.000	0.005	0.000	0.005	0.000	0.005	0.000	0.005	0.000	0.006	0.000
172	0.019	0.003	0.018	0.002	0.014	0.002	0.016	0.001	0.017	0.002	0.018	0.001
223	0.035	0.006	0.031	0.006	0.023	0.006	0.026	0.002	0.029	0.006	0.034	0.001
510	0.044	0.002	0.052	0.002	0.052	0.008	0.045	0.011	0.050	0.001	0.056	0.008
590	0.048	0.007	0.063	0.004	0.053	0.006	0.059	0.006	0.054	0.002	0.054	0.013
735	0.067	0.003	0.070	0.009	0.083	0.005	0.063	0.010	0.058	0.002	0.059	0.015

Fe(II) analysis.

Fe (ii) ppm	3hours	50 hours	75 hours	172 hours	223 hours
0.05	0.019	0.0243	0.0234	0.0255	0.02
0.1	0.0383	0.0492	0.0477	0.0425	0.0409
0.2	0.0968	0.0957	0.0931	0.0952	0.0961
0.5	0.2375	0.2428	0.236	0.2402	0.2432
1	0.4744	0.48866	0.4728	0.4729	0.48
2	0.9507	0.9709	0.9515	0.9436	0.9423
3	1.431	1.4485	1.4282	1.4211	1.424
	340 hours	388 hours	510 hours	590 hours	735 hours
	0.0217	0.0226	0.0243	0.0229	0.0187
	0.0415	0.046	0.0457	0.0446	0.0427
	0.0918	0.0961	0.0945	0.0884	0.0853
	0.2238	0.2335	0.2316	0.22	0.2159
	0.4419	0.4655	0.4666	0.4452	0.4298

	0.8989	0.9294	0.9211	0.9046	0.8831	
	1.3733	1.4032	1.3873	1.3571	1.3278	

3 HOURS							
	0	1	2	3	4	5	
A	0.0021	0.0017	0.0011	0.0009	0.001	0.0009	
B	0.0006	0	0.0009	0.0006	0.0019	0.0038	
C	0.0006	0.001	0.0013	0.0006	0.0009	0.0012	
	Equation	y = 0.4781x - 0.0039		R^2	0.99996	DILUTION	2
50 HOURS							
	0	1	2	3	4	5	
A	0.0048	0.004	0.0023	0.0067	0.0092	0.0099	
B	0.0042	0.0065	0.0052	0.0074	0.0098	0.0103	
C	0.0065	0.0061	0.0057	0.0065	0.0057	0.0089	
average	0.005166667	0.005533333	0.0044	0.006866667	0.008233333	0.0097	
st. dev	0.001193035	0.001342882	0.001835756	0.000472582	0.002214347	0.00072111	
	Equation	y = 0.4834x + 0.0012		R^2	0.999	DILUTION	2
75 HOURS							
	0	1	2	3	4	5	
A	0.0234	0.0189	0.0055	0.00193	0.0229	0.0293	
B	0.024	0.0371	0.0208	0.0247	0.0233	0.0321	
C	0.0208	0.0228	0.0189	0.0172	0.0221	0.0259	
	Equation	y=0.4764x - 0.0015		R^2	0.99999	DILUTION	2
172 HOURS							
	0	1	2	3	4	5	
A	0.7742	0.8499	0.7747	0.6461	0.3186	0.7823	
B	0.7952	0.9999	0.8212	0.7725	0.8191	0.9587	
C	0.8188	0.9559	0.6407	0.6338	0.995	0.7479	
	Equation	y = 0.4733x - 0.0001		R^2	0.99997	DILUTION	4
223 HOURS							
	0	1	2	3	4	5	
A	0.506	0.6594	0.4686	0.5355	0.5765	0.5342	
B	0.5946	0.678	0.6158	0.5852	0.6151	0.6029	
C	0.5403	0.751	0.4561	0.5575	0.6932	0.4908	
	Equation	y = 0.4745x - 0.0005		R^2	0.9999	DILUTION	10
245 HOURS							
	0	1	2	3	4	5	
A	0.5715	0.7885	0.6947	0.6425	0.6687	0.6038	
B	0.546	0.7728	0.7303	0.6675	0.6976	0.7025	
C	0.5453	0.8406	0.6116	0.6536	0.8234	0.6064	
	Equation	y = 0.4564x - 0.0048		R^2	0.99981	DILUTION	13.33333333
315 HOURS							
	0	1	2	3	4	5	
A	0.3174	0.4755	0.4122	0.3722	0.4072	0.3778	
B	0.303	0.478	0.4243	0.4065	0.432	0.4268	
C	0.3029	0.4982	0.3687	0.4014	0.5225	0.3595	
	Equation	y = 0.4669x - 0.0002		R^2	0.99998	DILUTION	20
510 HOURS							

	0	1	2	3	4	5	
A	0.2743	0.428	0.387	0.318	0.3654	0.3372	
B	0.2712	0.4266	0.4333	0.3606	0.4046	0.3262	
C	0.2512	0.4489	0.3264	0.3619	0.4036	0.317	
	Equation	y = 0.4616x + 0.0013		R^2	0.99998	DILUTION	26.66666667
195 HOURS	0	1	2	3	4	5	
A	0.2517	0.4614	0.3916	0.3159	0.3914	0.3418	
B	0.2522	0.4365	0.3834	0.371	0.403	0.3641	
C	0.2725	0.4635	0.4329	0.3569	0.4417	0.2904	
	Equation	y = 0.453x - 0.0029		R^2	0.99996	DILUTION	26.66666667
196 HOURS	0	1	2	3	4	5	
A	0.3009	0.4965	0.4673	0.3674	0.4492	0.3798	
B	0.3173	0.5105	0.4362	0.4092	0.4469	0.4117	
C	0.2867	0.4971	0.3885	0.4301	0.4863	0.3794	
	Equation	y = 0.4436x - 0.005		R^3	0.99993	DILUTION	26.66666667

Ortho-P analysis.

P ppm	3hours	50 hours	75 hours	172 hours	223 hours
0.01	0.005	0.005	0.005	0.0067	0.005
0.05	0.0337	0.0337	0.0321	0.0343	0.0321
0.1	0.0714	0.0714	0.0681	0.0724	0.0681
0.15	0.1104	0.1104	0.1031	0.1128	0.1031
0.2	0.146	0.146	0.1399	0.1488	0.1399
0.4	0.3009	0.3009	0.2864	0.3046	0.2864
	340 hours	388 hours	510 hours	590 hours	735 hours
	0.0028	0.0028	0.0063	0.0063	0.0049
	0.0283	0.0283	0.0341	0.0341	0.0343
	0.0654	0.0654	0.0684	0.0684	0.0731
	0.1022	0.1022	0.105	0.105	0.1122
	0.1349	0.1349			0.1523
	0.2981	0.2981	0.292	0.292	0.3077

3 HOURS	0	1	2	3	4	5	
A	-0.0007	-0.001	0.0012	-0.0011	-0.0007	-0.001	
B	-0.001	-0.0011	-0.0009	-0.001	-0.0009	-0.001	
C	-0.001	-0.001	-0.0015	0	-0.0002	-0.0009	
	Equation	y = 0.7599x - 0.004		R^2	0.99987	DILUTION	1.25
50 HOURS	0	1	2	3	4	5	
A	0.0009	-0.001	-0.0002	-0.0009	-0.0007	-0.0007	
B	-0.0009	-0.0007	0.0002	-0.0007	-0.0009	-0.0007	
C	-0.0006	-0.0002	-0.0007	-0.0002	-0.001	-0.0007	
	Equation	y = 0.7599x - 0.004		R^2	0.99987	DILUTION	1.25
75 HOURS	0	1	2	3	4	5	
A	-0.0006	-0.001	-0.001	-0.0007	-0.0009	-0.0004	
B	-0.0009	-0.0011	-0.0009	-0.001	-0.0007	-0.0009	

C	-0.001	-0.0009				-0.0007	-0.0007	
	Equation	y = 0.7233x - 0.0039		R^2		0.99987	DILUTION	1.25
172 HOURS	0	1	2	3	4	5		
A	0.0068	0.0067	0.0043	0.0063	0.0062	0.0067		
B	0.0077	0.0092	0.0068	0.0068	0.0071	0.0081		
C	0.0104	0.0084	0.0062	0.007	0.0085	0.0083		
	Equation	y = 0.7671x - 0.0031		R^2		0.99981	DILUTION	1.25
223 HOURS	0	1	2	3	4	5		
A	0.0143	0.0098	0.0054	0.0103	0.0098	0.0155		
B	0.0145	0.0162	0.0117	0.0125	0.0118	0.0154		
C	0.0208	0.0153	0.0111	0.0106	0.0167	0.0161		
	Equation	y = 0.7233x - 0.0039		R^2		0.99987	DILUTION	1.25
340 HOURS	0	1	2	3	4	5		
A	0.0568	0.0691	0.0629	0.0734	0.0796	0.0763		
B	0.0544	0.0732	0.0658	0.0795	0.0758	0.0706		
C	0.0702	0.0729	0.0651	0.0763	0.0842	0.0892		
	Equation	y = 0.7354x - 0.0033		R^2		0.99972	DILUTION	1.25
388 HOURS	0	1	2	3	4	5		
A	0.0261	0.0255	0.0259	0.0049	0.0222	0.0046		
B	0.028	0.0406	0.0264	0.0052	0.0048	0.0034		
C	0.0287	0.0399	0.0349	0.0356	0.0065	0.0057		
	Equation	y = 0.7354x - 0.0033		R^2		0.99972	DILUTION	1.25
510 HOURS	0	1	2	3	4	5		
A	0.0242	0.0265	0.024	0.0164	0.0264	0.0276		
B	0.0222	0.0287	0.0308	0.0255	0.0255	0.0261		
C	0.0222	0.0273		0.0283	0.0272	0.0344		
	Equation	y = 0.7354x - 0.0033		R^2		0.99972	DILUTION	1.25
590 HOURS	0	1	2	3	4	5		
A			0.0252	0.0292	0.0273	0.0248		
B	0.0217	0.0358		0.0299	0.0281	0.0236		
C	0.0277	0.0321	0.0304	0.0352	0.0298	0.037		
	Equation	y = 0.7354x - 0.0033		R^2		0.99972	DILUTION	1.25
591 HOURS	0	1	2	3	4	5		
A	0.0217	0.024	0.0289	0.0328	0.0322	0.0345		
B	0.0247	0.0293	0.0258	0.0286	0.03	0.0217		
C	0.0222	0.0189	0.0322	0.0403	0.0302	0.0386		
	Equation	y = 0.7354x - 0.0034		R^3		0.99995	DILUTION	1.25

pH values. (With temperature)

time (hours)	3	50	75	172	198	340	388	510	590	735

temperature (°C)	25	26	26	26	27	29	27	27	27	27
0A	5.95	6.03	6.02	6.66	6.95	7.56	7.3	7.3	7.49	7.46
0B	5.99	6.02	6.02	6.68	7.05	7.52	7.21	7.26	7.51	7.44
0C	6	6.02	6.04	6.75	7.08	7.58	7.21	7.29	7.46	7.49
1A	5.95	5.94	5.98	6.58	7.15	7.51	7.19	7.2	7.36	7.24
1B	5.98	5.98	5.99	6.55	7	7.49	7.12	7.1	7.33	7.16
1C	5.97	5.97	5.99	6.45	6.96	7.42	7.02	7.06	7.28	7.13
2A	6	5.95	5.96	6.23	6.98	7.52	7.09	7.2	7.36	7.22
2B	5.95	5.99	6	6.52	7.09	7.54	7.1	7.22	7.42	7.25
2C	5.97	6.02	6.04	6.49	7.07	7.56	7.17	7.22	7.42	7.27
3A	5.97	6	6.03	6.55	7.03	7.55	7.21	7.25	7.44	7.3
3B	5.98	6	6	6.59	7.09	7.53	7.14	7.22	7.44	7.27
3C	5.99	6.03	6.03	6.52	7.05	7.45	7.13	7.23	7.39	7.22
4A	5.99	6.04	6.01	6.55	7.05	7.57	7.2	7.22	7.38	7.24
4B	5.99	6.01	6.02	6.57	7.07	7.51	7.17	7.24	7.28	7.17
4C	5.97	6.01	6.04	6.6	7.02	7.46	7.06	7.21	7.29	7.13
5A	6.04	6.05	6.1	6.67	7.09	7.55	7.22	7.26	7.3	7.32
5B	6.09	6.05	6.05	6.68	7.1	7.58	7.22	7.26	7.37	7.32
5C	6.03	6.08	6.08	6.66	7.11	7.59	7.25	7.28	7.36	7.23

Vedersø-Gibbsite #1.

Summary

VERDERSO III + GIBBSITE			with NaAcetate 0.5mM at pH6			
flask	0	1	2	3	4	5
GIBBSITE (g)	0	0.025	0.05	0.1	0.5	1
GIBBSITE (g/L)	0	0.05	0.1	0.2	1	2
GIBBSITE (g/Kg ds)	0	2.5	5	10	50	100
soil weighted	0	1	2	3	4	5
A	11.88	11.87	11.87	11.87	11.87	11.87
B	11.89	11.87	11.87	11.87	11.87	11.88
C	11.87	11.87	11.87	11.87	11.87	11.87
Gibbsite added	0	1	2	3	4	5
A	0	0.025	0.05	0.1	0.5	1
B	0	0.0251	0.0501	0.1	0.5	1
C	0	0.0251	0.05	0.1	0.5	1

IRON II (mg/l)												
time	0	st dev	1	st dev	2	st dev	3	st dev	4	st dev	5	st dev
3	0.018	0.003	0.015	0.131	0.015	0.003	0.022	0.015	0.011	0.002	0.010	0.001
25	0.130	0.007	0.126	0.003	0.130	0.007	0.125	0.004	0.124	0.003	0.121	0.006
50	0.148	0.007	0.148	0.009	0.155	0.015	0.142	0.004	0.137	0.000	0.140	0.001
245	0.266	0.043	0.301	0.082	0.303	0.046	0.268	0.016	0.277	0.021	0.304	0.044

315	0.288	0.047	0.318	0.075	0.304	0.011	0.257	0.026	0.293	0.031	0.285	0.026

ORTHO P
(mg/l)

time	0	st dev	1	st dev	2	st dev	3	st dev	4	st dev	5	st dev
3	0.007	0.001	0.007	0.001	0.007	0.001	0.007	0.001	0.007	0.000	0.007	0.000
25	0.015	0.002	0.016	0.002	0.015	0.001	0.017	0.005	0.016	0.003	0.013	0.001
50	0.020	0.004	0.018	0.002	0.018	0.002	0.016	0.000	0.016	0.001	0.017	0.001
245	0.014	0.002	0.014	0.000	0.013	0.001	0.012	0.001	0.012	0.000	0.012	0.000
315	0.013	0.013	0.013	0.000	0.014	0.001	0.013	0.001	0.012	0.000	0.012	0.000

Iron(II) analysis.

Fe (ii) ppm	3hours	25 hours	50 hours	170 hours	195 hours	245 hours	315 hours
0.05	0.0222	0.0112	0.0112	0.015	0.0237	0.0221	0.0117
0.1	0.0427	0.0276	0.0276	0.0322	0.0486	0.0446	0.0459
0.2	0.0945	0.077	0.077	0.0682	0.0895	0.0835	0.0933
0.5	0.2323	0.2006	0.2006	0.172	0.2385	0.2389	0.2402
1	0.4653	0.4154	0.4154	0.4202	0.4855	0.4633	0.4524
2	0.9399	0.8811	0.8811	0.8344	0.9622	0.9341	0.9386
3	1.4005	1.3588	1.3588	1.3123	1.4197	1.434	1.4087

3 HOURS	0	1	2	3	4	5	
A	0.0032	0.0024	0.0032		0.0018	0.0017	
B	0.0027	0.0027	0.0021	0.0067		0.0013	
C	0.0039	0.0027	0.002	0.0017	0.0012	0.0013	
	Equation	y = 0.468x - 0.001		R^2	1	DILUTION	2
25 HOURS	0	1	2	3	4	5	
A	0.0092	0.0088	0.011	0.0074	0.0076	0.0077	
B	0.0076	0.0074	0.0088	0.0076	0.0073	0.0083	
C	0.011	0.0088	0.0077	0.0089	0.0088	0.0057	
	Equation	y = 0.465x - 0.021		R^2	0.999	DILUTION	2
50 HOURS	0	1	2	3	4	5	
A	0.0145	0.0112	0.0189	0.0129	0.0107	0.0112	
B	0.0114	0.0133	0.0135	0.0118	0.0107	0.0117	
C	0.0143	0.0155	0.0126	0.011	0.0109	0.0115	
	Equation	y = 0.465x - 0.021		R^2	0.999	DILUTION	2
170 HOURS	0	1	2	3	4	5	
A	0.0626	0.0602	0.0823	0.0604	0.0599	0.0754	
B	0.0471	0.0558	0.0652	0.0569	0.0697	0.0688	
C	0.057	0.076	0.0597	0.0634	0.0681	0.0552	
	Equation	y = 0.439x - 0.021		R^2	0.998	DILUTION	2
195 HOURS	0	1	2	3	4	5	
A	0.0581	0.0649	0.069	0.0475	0.0446	0.0647	

B	0.0403	0.0433	0.0525	0.0448	0.0577	0.0604	
C	0.0507	0.0674	0.0477	0.0544	0.0421	0.0518	
	Equation	y = 0.475x + 0.001		R^2	0.999	DILUTION	2
245 HOURS							
	0	1	2	3	4	5	
A	0.0629	0.0582	0.0775	0.0585	0.0537	0.0741	
B	0.045	0.0502	0.0636	0.053	0.0631	0.0695	
C	0.0623	0.0873	0.0558	0.0601	0.0618	0.0542	
	Equation	y = 0.4769x - 0.0066		R^2	0.99975	DILUTION	2
315 HOURS							
	0	1	2	3	4	5	
A	0.0675	0.0613	0.0674	0.0609	0.0581	0.0671	
B	0.0503	0.0586	0.0691	0.0491	0.0721	0.0643	
C	0.071	0.0905	0.0638	0.0571	0.0621	0.0554	
	Equation	y = 0.4707x - 0.0048		R^2	0.99979	DILUTION	2

Ortho-P analysis.

Ortho-P ppm	3hours	25 hours	50 hours	245 hours	320 hours
0.05	0.0311	0.0331	0.0331	0.0319	0.0319
0.1	0.068	0.0697	0.0697	0.0619	0.0619
0.2	0.1451	0.1449	0.1449	0.1409	0.1409
0.4	0.2891	0.2974	0.2974	0.287	0.287

3 HOURS							
	0	1	2	3	4	5	
A	0.0013	-0.0001	0.001	0.0006	-0.0002	-0.0001	
B	0	0.0009	-0.0001	-0.0002	-0.0002	-0.0002	
C	0	0	0	0.0004	-0.0002	-0.0002	
	Equation	y = 0.737x - 0.005		R^2	1	DILUTION	1
25 HOURS							
	0	1	2	3	4	5	
A	0.0018	0.0004	0.0011	0.0009	0.0011	-0.0002	
B	0.0002	0.0018	0	-0.0001	0	0.0004	
C	0.0005	0.0009	0.0005	0.0034	0.002	-0.0002	
	Equation	y = 0.756x - 0.005		R^2	0.999	DILUTION	1
50 HOURS							
	0	1	2	3	4	5	
A	0.004	0.0011	0.0027	0.0012	0.0006	0.0018	
B	0.0013	0.0027	0.001	0.0011	0.0012	0.0012	
C	0.002	0.0016	0.002	0.001	0.0009	0.0011	
	Equation	y = 0.756x - 0.005		R^2	0.999	DILUTION	1
245 HOURS							
	0	1	2	3	4	5	
A	0.0013	0.0027	0.0024	0.0004	0.0013	0.0018	
B	0.0043	0.0023	0.0017	0.0012	0.0012	0.0015	
C	0.0017	0.0028	0.0018	0.0013	0.0013	0.0011	
	Equation	y = 0.7366x - 0.0077		R^2	0.99934	DILUTION	1
320 HOURS							

	0	1	2	3	4	5	
A	0.0031	0.0023	0.0018	0.0017	0.0013	0.0013	
B	0.0018	0.0021	0.0026	0.0013	0.0013	0.0011	
C	0.0017	0.0018	0.0027	0.0023	0.0013	0.0011	
average	0.0022	0.002066667	0.002366667	0.001766667	0.0013	0.001166667	
st. dev	0.000781025	0.000251661	0.000493288	0.000503322	0	0.00011547	
	Equation	y = 0.7366x - 0.0077		R^2	0.99934	DILUTION	1

pH values. (With temperature).

Time (hours)	3	25	50	175	195	245	320
temperature (°C)	25	26	27	26	26	27	25
0A	5.98	5.89	6.07	6.08	6.12	6.2	6.14
0B	6.01	6	6.01	6.1	6.11	6.21	6.11
0C	6.01	6.04	5.91	6.11	6.07	6.17	6.06
1A	6	6.03	6.05	6.03	6.14	6.13	6.11
1B	6.03	6.06	6.04	6.1	6.14	6.17	6.12
1C	6.07	6.07	6.04	6.07	6.15	6.19	6.14
2A	6.04	6.05	6.03	6.07	6.09	6.1	6.12
2B	6.09	6.03	6.06	6.06	6.08	6.13	6.09
2C	6.1	6.13	6.1	6.15	6.18	6.2	6.21
3A	6.04	6.07	6.05	6.13	6.13	6.13	6.14
3B	6.02	6.07	6.05	6.04	6.17	6.11	6.12
3C	6.08	6.06	6.05	6.13	6.13	6.16	6.1
4A	6.09	6.11	6.13	6.14	6.18	6.18	6.23
4B	6.07	6.09	6.11	6.14	6.16	6.19	6.25
4C	6.06	6.14	6.11	6.16	6.21	6.13	6.22
5A	6.06	6.12	6.1	6.18	6.17	6.22	6.19
5B	6.1	6.15	6.09	6.2	6.25	6.21	6.23
5C	6.08	6.12	6.1	6.2	6.23	6.24	6.19

Lydum-Gibbsite #2.

Summary Soil.

LYDUM + GIBBSITE		with NaAcetate 1mM at pH 5				
flask	0	1	2	3	4	5
GIBBSITE (g)	0	0.025	0.05	0.1	0.5	1
GIBBSITE (g/L)	0	0.05	0.1	0.2	1	2
GIBBSITE (g/Kg ds)	0	2.5	5	10	50	100
soil weighted	0	1	2	3	4	5
A	12.81	12.81	12.81	12.81	12.81	12.81
B	12.81	12.81	12.81	12.81	12.81	12.81
C	12.81	12.81	12.81	12.81	12.81	12.81
Gibbsite	0	1	2	3	4	5

added						
A	0	0.025	0.05	0.1	0.5	1
B	0	0.025	0.05	0.1	0.5	1
C	0	0.025	0.05	0.1	0.5	1

IRON II (mg/l)

time	0	st dev	1	st dev	2	st dev	3	st dev	4	st dev	5	st dev
4	0.043	0.002	0.047	0.131	0.042	0.002	0.063	0.002	0.066	0.003	0.046	0.008
48	0.094	0.005	0.092	0.003	0.101	0.001	0.112	0.007	0.104	0.019	0.091	0.002
96	0.214	0.025	0.229	0.107	0.227	0.017	0.304	0.064	0.366	0.022	0.267	0.006
144	1.059	0.081	0.956	0.118	0.958	0.122	1.004	0.073	1.198	0.117	0.927	0.166
192	2.000	0.102	1.876	0.081	1.677	1.291	2.167	0.099	1.946	0.131	1.558	0.103
354	3.243	0.095	3.006	0.411	3.073	0.223	3.273	0.167	3.651	0.087	2.544	
402	3.502	0.064	3.472	0.264	3.569	0.382	3.780	0.289	4.328	0.171	3.205	0.203
450	4.166	0.366	5.261	0.173	4.698	0.247	4.276	0.278	4.161	0.291	4.330	0.160
522	6.032	0.642	5.542	0.340	5.876	0.607	6.409	0.223	6.764	1.421	6.749	0.876
570	6.589	1.082	5.899	0.342	6.200	0.679	6.943	0.168	7.914	1.062	6.679	2.432
618	5.783	2.219	5.644	0.307	6.512	0.077	6.967	0.162	8.308	1.897	6.741	0.444

ORTHO P (mg/l)

time	0	st dev	1	st dev	2	st dev	3	st dev	4	st dev	5	st dev
4	0.005	0.003	0.009	0.003	0.002	0.001	0.002	0.000	0.004	0.001	0.002	0.001
48	0.003	0.003	0.004	0.002	0.004	0.002	0.002	0.003	0.006	0.002	0.005	0.004
96	0.004	0.001	0.004	0.003	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.001
144	0.004	0.004	0.003	0.002	0.002	0.001	0.003	0.001	0.003	0.001	0.005	0.004
192	0.004	0.000	0.007	0.004	0.006	0.001	0.006	0.003	0.004	0.001	0.006	0.003
354	0.006	0.001	0.009	0.004	0.007	0.001	0.006	0.002	0.007	0.002	0.007	0.001
402	0.007	0.001	0.008	0.001	0.010	0.002	0.010	0.003	0.009	0.000	0.008	0.000
450	0.006	0.001	0.007	0.001	0.006	0.002	0.005	0.001	0.006	0.001	0.009	0.003
522	0.004	0.001	0.003	0.001	0.006	0.003	0.004	0.001	0.004	0.000	0.004	0.000
570	0.005	0.002	0.009	0.006	0.006	0.001	0.005	0.001	0.007	0.000	0.006	0.002

Iron(II) analysis.

Fe (ii) ppm	4hours	48 hours	96 hours	144 hours	192 hours	354 hours
0.05	0.0262	0.0262	0.0211	0.0193	0.0193	0.0193
0.1	0.0464	0.0464	0.0452	0.0472	0.0472	0.0472
0.2	0.084	0.084	0.0984	0.092	0.092	0.092
0.5	0.2019	0.2019	0.2422	0.2209	0.2209	0.2209
1	0.3833	0.3833	0.4785	0.4407	0.4407	0.4407
2	0.8066	0.8066	0.9579	0.9063	0.9063	0.9063
3	1.2836	1.2836	1.4354	1.39	1.39	1.39

402 hours	450 hours	522 hours	570 hours	610 hours
0.0193	0.0193	0.022	0.025	0.021
0.0472	0.0472	0.043	0.047	0.043
0.092	0.092	0.086	0.092	0.088
0.2209	0.2209	0.226	0.239	0.228
0.4407	0.4407	0.449	0.478	0.452
0.9063	0.9063	0.905	0.965	0.92
1.39	1.39	1.421	1.426	1.38

4 HOURS						

	0	1	2	3	4	5	
A	-0.0006	0.0005	-0.0001	0	-0.0006	-0.0007	
B	0	0.0001	-0.0007	-0.0006	-0.0001	0.0018	
C	-0.0002	0.0004	-0.0004	-0.0006	0.0004	-0.0004	
	Equation	y = 0.4211x - 0.0075		R^2	0.9979	DILUTION	2.5
48 HOURS							
	0	1	2	3	4	5	
A	0.0009	0.0004	0.0009	0.0013	0.0031	0.0001	
B	0.0002	0	0.001	0.0021	0.0001	0	
C	0.0001	0.0004	0.0011	0.0024	0.0005	0.0003	
	Equation	y = 0.4211x - 0.0075		R^2	0.9979	DILUTION	5
96 HOURS							
	0	1	2	3	4	5	
A	0.0193	0.0155	0.0211	0.0328	0.0355	0.0239	
B	0.0219	0.0327	0.0221	0.0212	0.0316	0.0247	
C	0.0172	0.0146	0.0189	0.0302	0.035	0.025	
	Equation	y = 0.4788x - 0.0001		R^2	0.99998	DILUTION	5
144 HOURS							
	0	1	2	3	4	5	
A	0.0826	0.073	0.0919	0.0892	0.0996	0.0704	
B	0.0947	0.0939	0.0839	0.0784	0.0961	0.0967	
C	0.0963	0.0782	0.0697	0.0907	0.1163	0.0698	
	Equation	y = 0.4617x - 0.0066		R^2	0.99965	DILUTION	5
192 HOURS							
	0	1	2	3	4	5	
A	0.1689	0.162	0.1764	0.1915	0.1866	0.1475	
B	0.1777	0.1753	0.01753	0.1855	0.1696	0.1355	
C	0.1877	0.1626	0.2509	0.2035	0.1632	0.1288	
	Equation	y = 0.4617x - 0.0066		R^2	0.99965	DILUTION	5
354 HOURS							
	0	1	2	3	4	5	
A	0.2848	0.2683	0.2893	0.2999	0.3326	xxx	
B	0.3022	0.3101	0.2887	0.2786	0.3217	xxx	
C	0.2915	0.2344	0.2534	0.3085	0.3373	0.2407	
	Equation	y = 0.4617x - 0.0066		R^2	0.99965	DILUTION	5
	st dev	0.095043251	0.410641553	0.222610557	0.166698763	0.086665669	
402 HOURS							
	0	1	2	3	4	5	
A	0.1525	0.1478	0.1611	0.1627	0.2021	0.136	
B	0.1544	0.1677	0.1742	0.158	0.187	0.1522	
C	0.1583	0.1456	0.1393	0.1831	0.1906	0.1359	
	Equation	y = 0.4617x - 0.0066		R^2	0.99965	DILUTION	10
450 HOURS							
	0	1	2	3	4	5	
A	0.1746	0.2321	0.2224	0.176	0.1754	0.2008	
B	0.2052	0.2313	0.1998	0.1986	0.2008	0.1931	
C	0.1774	0.2455	0.2087	0.1979	0.1804	0.186	
	Equation	y = 0.4617x - 0.0066		R^2	0.99965	DILUTION	10
522 HOURS							

	0	1	2	3	4	5	
A	0.251	0.248	0.274	0.286	0.368	0.208	
B	0.266	0.27	0.293	0.288	0.324	0.338	
C	0.309	0.239	0.237	0.305	0.237	0.26	
	Equation	y = 0.4692x - 0.0077		R^2	0.99926	DILUTION	10
570 HOURS	0	1	2	3	4	5	
A	0.262	0.282	0.3	0.324	0.435	0.327	
B	0.288	0.278	0.314	0.331	0.361	0.431	
C	0.358	0.253	0.252	0.34	0.338	0.04	
	Equation	y = 0.4774x + 0.0002		R^2	0.99979	DILUTION	10
618 HOURS	0	1	2	3	4	5	
A	0.189	0.197	0.235	0.247	0.381	0.257	
B	0.069	0.213	0.239	0.256	0.283		
C	0.307			0.264	0.251	0.239	
	Equation	y = 0.4607x - 0.003		R^2	0.99997	DILUTION	12.5

Ortho-P analysis.

P ppm	4hours	48 hours	96 hours	144 hours	192 hours
0	0.146	0.146	0.146	0.146	0.146
0.002	0.153	0.153	0.153	0.153	0.153
0.01	0.183	0.183	0.181	0.181	0.181
0.02	0.213	0.213	0.223	0.223	0.223
0.03	0.248	0.248	0.251	0.251	0.251
0.04	0.283	0.283	0.283	0.283	0.283
0.08	0.425	0.425	0.428	0.428	0.428
0.1	0.495	0.495	0.498	0.498	0.498
	354 hours	402 hours	450 hours	522 hours	560 hours
	0.146	0.146	0.145	0.145	0.145
	0.153	0.153	0.151	0.151	0.151
	0.181	0.181	0.176	0.176	0.176
	0.223	0.223	0.215	0.215	0.215
	0.251	0.251	0.251	0.251	0.251
	0.283	0.283	0.282	0.282	0.282
	0.428	0.428	0.416	0.416	0.416
	0.498	0.498	0.492	0.492	0.492

4 HOURS	0	1	2	3	4	5	
A	0.147	0.155	0.148	0.149	0.151	0.149	
B	0.159	0.163	0.151	0.148	0.152	0.15	
C	0.158	0.168		0.148	0.154	0.147	
	Equation	y=3.4881x+0.1453		R^2	0.99981	DILUTION	1.805555556
48 HOURS	0	1	2	3	4	5	
A	0.159	0.161	0.153	0.158	0.159	0.148	
B	0.147	0.155	0.15	0.147	0.154	0.162	
C	0.151	0.152	0.159	0.146	0.165	0.164	
	Equation	y=3.4881x+0.1453		R^2	0.99981	DILUTION	1.45
96 HOURS							

	0	1	2	3	4	5	
A	0.152	0.16	0.151	0.15	0.153		
B	0.156	0.155	0.147		0.155	0.147	
C	0.154	0.147	0.148	0.148	0.157	0.151	
	Equation	y= 3.5104x + 0.1466		R^2	0.99951	DILUTION	1.805555556
144 HOURS							
	0	1	2	3	4	5	
A	0.151	0.159	0.15	0.15	0.151	0.151	
B	0.152	0.153	0.153	0.154	0.156	0.167	
C	0.166	0.152	0.151	0.156	0.157	0.154	
	Equation	y= 3.5104x + 0.1466		R^2	0.99951	DILUTION	1.48
192 HOURS							
	0	1	2	3	4	5	
A	0.157	0.156	0.16	0.169	0.155	0.155	
B	0.156	0.162	0.163	0.156	0.155	0.158	
C	0.158	0.173	0.16	0.159	0.16	0.167	
	Equation	y= 3.5104x + 0.1466		R^2	0.99951	DILUTION	1.48
354 HOURS							
	0	1	2	3	4	5	
A	0.161	0.162	0.163	0.163	0.158	0.164	
B	0.163	0.18	0.16	0.158	0.167	0.168	
C	0.16	0.164	0.164	0.165	0.163	0.161	
	Equation	y= 3.5104x + 0.1466		R^2	0.99951	DILUTION	1.48
402 HOURS							
	0	1	2	3	4	5	
A	0.166	0.169	0.172	0.163	0.167	0.166	
B	0.163	0.163	0.172	0.177	0.167	0.164	
C	0.164	0.165	0.164	0.169	0.168	0.164	
	Equation	y= 3.5104x + 0.1466		R^2	0.99951	DILUTION	1.48
450 HOURS							
	0	1	2	3	4	5	
A	0.164	0.16	0.16	0.156	0.159	0.172	
B	0.157	0.158	0.161	0.156	0.162		
C	0.159	0.165	0.154	0.159	0.159	0.16	
	Equation	y=4.449x + 0.1444		R^2	0.99958	DILUTION	1.81
522 HOURS							
	0	1	2	3	4	5	
A	0.155	0.152	0.155	0.153	0.156	0.156	
B	0.155	0.155	0.174	0.155	0.156	0.158	
C	0.159	0.154	0.159	0.158	0.157	0.157	
	Equation	y=4.449x + 0.1444		R^2	0.99958	DILUTION	1.48
560 HOURS							
	0	1	2	3	4	5	
A	0.156	0.156	0.164	0.157	0.165	0.159	
B	0.157	0.165	0.166	0.16	0.167	0.168	
C	0.167	0.19	0.158	0.163	0.165	0.164	
	Equation	y=4.449x + 0.1444		R^2	0.99958	DILUTION	1.48

pH values with temperature

time (hours)	4	48	96	144	192	354	402	450	522	570	618

temperature (°C)	25	25	24	25	25	26	25	24	25	25	25
0A	4.95	4.95	4.95	4.96	4.97	4.99	5.00	5.01	5.03	5.04	5.06
0B	4.96	4.95	4.96	4.96	4.97	5.00	5.00	5.00	5.02	5.02	5.03
0C	4.95	4.95	4.95	4.96	4.98	4.99	5.00	5.02	5.03	5.03	5.05
1A	4.95	4.96	4.96	4.96	4.98	5.00	5.01	5.02	5.03	5.03	5.04
1B	4.95	4.96	4.96	4.97	4.99	5.01	5.01	5.03	5.03	5.05	5.06
1C	4.95	4.97	4.96	4.96	4.99	5.00	5.02	5.02	5.03	5.04	5.06
2A	4.95	4.95	4.95	4.97	4.98	4.98	5.00	5.01	5.03	5.03	5.05
2B	4.95	4.95	4.96	4.97	4.97	4.99	5.00	5.01	5.03	5.04	5.05
2C	4.96	4.96	4.96	4.96	4.98	5.01	5.01	5.01	5.02	5.04	5.06
3A	4.95	4.95	4.95	4.96	4.97	4.98	5.00	5.02	5.04	5.04	5.06
3B	4.95	4.96	4.96	4.97	4.99	5.00	5.01	5.02	5.04	5.04	5.05
3C	4.95	4.95	4.95	4.96	4.97	4.98	5.01	5.02	5.04	5.05	5.07
4A	4.97	4.97	4.97	4.97	4.98	4.98	5.00	5.02	5.03	5.05	5.06
4B	4.95	4.95	4.95	4.95	4.97	4.99	5.00	5.02	5.04	5.04	5.05
4C	4.95	4.96	4.96	4.97	4.98	4.99	5.02	5.02	5.04	5.03	5.04
5A	4.95	4.96	4.96	4.97	4.97	5.00	5.01	5.01	5.02	5.02	5.04
5B	4.95	4.95	4.97	4.97	4.99	5.00	5.00	5.01	5.03	5.04	5.04
5C	4.95	4.95	4.95	4.98	4.99	5.00	5.01	5.02	5.03	5.04	5.06