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Nadejda Perez Rojas

AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.Sc. (Earth Sciences)

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Biogeochemistry of iron and phosphorus in soils impacted by penguin colonies in Antarctica

TITRE DE LA THÈSE / TITLE OF THESIS

Dr. D. Fortin

DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

Dr. J. Blais

Dr. T. Patterson

Gary W. Slater

Le Doyen de la Faculté des études supérieures et postdoctorales / Dean of the Faculty of Graduate and Postdoctoral Studies

Biogeochemistry of iron and phosphorus in soils impacted by penguin colonies in Antarctica

By
Nadejda Perez Rojas

Thesis submitted to the
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Abstract

Penguin colonies from permanently cold environments have a strong impact on their surrounding ecosystem because their excrements provide ample nutrients to the soils and sediments. The high phosphate content of the penguin guano directly affects primary productivity. However, phosphate solubility is dependent on the presence of iron and other metals, which can form stable PO_4-rich minerals. Phosphate can also be sorbed onto minerals, including iron oxides. The present study investigated the biogeochemistry of phosphorus in a 42 cm-deep soil profile on Gardiner Island in Antarctica in order to assess the effect of penguin excrements on P partitioning in the solid and aqueous phases. The results indicate that the porewaters were slightly acidic (pH 5-6) and contained extremely high levels of dissolved organic carbon (DOC; 120 mM), PO_4 (120 mM), SO_4 (27 mM), NO_3 (18 mM), Cl (320 mM), F (2 mM), Sr (0.10 mM), Ca (18 mM) and Mg (150 mM) at the top of the soil profile. Dissolved iron concentrations were generally low (< 0.04 mM) and increased at a depth of 15-20 cm and at the bottom of the profile. Chemical extraction revealed the presence of two zones of reactive phosphorus (P-ascorbate extractable fraction) in the soil profile, i.e., at the surface and between 16 and 20 cm. Enriched reactive and crystalline iron fractions were also present at a depth of 16-20 cm, but fluctuated throughout the profile. The Fe(II)/Fe(III) molar ratio of the soil was greater than 1 at the surface of the profile and declined with depth. X-ray diffraction analysis showed that the soil likely contained berlinite, strengite and vivianite, along with silicates and quartz. Saturation index calculations also indicated that Ca and Mg-rich phosphate minerals were likely present in the soil. Based on the above results, the presence of penguin colonies on Gardiner Island strongly impacted the geochemical and mineralogical composition of the soil, as observed in other studies on bird guano impacted (ornithogenic) soils. In addition, the presence of both Fe(II) and Fe(III) points to the fact that the soil undergos redox changes, likely as a result of seasonal water table fluctuations. Microcosm experiments with selected samples from the soil profile and an iron-reducing bacterium indeed showed that iron and phosphorus were released into solution as a result of microbial iron reduction. However, abiotic systems also showed a release of phosphorus indicating that non Fe-rich phosphate minerals are soluble under the conditions prevailing in the growth medium.

Résumé

Les colonies de penguins vivant dans les environnements froids ont un impact important sur les écosystèmes environnants car leurs excréments riches en phosphore fertilisent les sols et les sédiments. La solubilité et la biodisponibilité du phosphore est toutefois contrôlée par la précipitation de minéraux phosphatés riches en fer ou autres métaux. Le phosphate peut aussi être sorbé à la surface d'oxydes de fer, limitant ainsi sa disponibilité en tant que nutriment. La présente étude s'est intéressée à la distribution du phosphore (en milieu aqueux et en phase solide) dans un profile de sol (42 cm de profondeur) sur l'île Gardiner en Antarctique, où des colonies de penguins y vivent. Les résultats indiquent que les eaux interstitielles sont légèrement acides (pH 5-6) et contiennent des concentrations très élevées de carbone organique dissout (COD; 120 mM), PO_4 (120 mM), SO_4 (27 mM), NO_3 (18 mM), Cl (320 mM), F (2 mM), Sr (0.10 mM), Ca (18 mM) et Mg (150 mM) à la surface du profile. Les concentrations de fer dissout sont généralement basses (< 0.04 mM) mais elles augmentent à une profondeur de 15 a 20 cm et à la base du profile. Les extractions chimiques ont révélé qu'il y avait deux zones de phosphore réactif (fraction P-ascorbate) dans le sol, soit à la surface et entre 16 et 20 cm. Les sol était aussi enrichi en fer réactif et en fer cristallin entre 16 et 20 cm, mais les concentrations varient énormément en fonction de la profondeur. Le rapport molaire Fe(II)/Fe(III) du sol excède 1 à la surface du profile et diminue ensuite avec la profondeur. Les analyses de diffraction-X ont indiqué que les sol contenait fort probablement de la berlinite de la strengite et de la vivianite, ainsi que des silicates et du quartz. Les calculs d'indices de saturation ont aussi révélé que des minéraux phosphatés riches en Ca et en Mg étaient aussi probablement présents dans le sol. D'après les résultats obtenus, il est clair que les colonies de penguins affectent la composition géochimique et minéralogique du sol où ils résident. Ces résultats sont en accord avec d'autres études de sols ornithogéniques affectés par le guano. De plus, la présence de Fe(II) et de Fe(III) démontre que le sol subit des changements redox suite aux fluctuations saisonnières de la nappe phréatique. Des expériences avec des microcosmes contenant des sédiments du profile de sol et une bactérie réductrice du fer ont en fait révélé que le fer et le phosphate étaient relargués en solution suite à la réduction microbienne du fer. Cependant, le phosphore a aussi été remis en solution dans des microcosmes abiotiques, indiquant que des minéraux non riches en fer, mais contenant du phosphate, sont solubles sous les conditions présentes dans le milieu de culture utilisé.

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I dedicate this work to the very special people in my life, my parents, who always believed in me and supported me in the way parents do.

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1. Introduction

1.1 Characteristics of cold environments

There are many cold environments on planet Earth. According to Morgan-Kiss et al. (2006), more than 70% of the biosphere has temperatures below the freezing point of water or close to it. These environments include polar regions, deep oceans and lakes, and high mountains having different chemical and physical properties (Morgan-Kiss et al., 2006). Soils and waters from permanently cold environments are usually oligotrophic and very low in nitrogen, organic carbon and phosphorus (Gounot, 1999). High alpine and high Arctic ecosystems are characterized by extremely high light levels and variable dark periods. In addition, vegetation periods are very short due to low summer temperatures and vascular plants generally include small shrubs and some perennial plants (Gounot, 1999).

Sea ice cold environments, which cover 13% of the Earth's surface (Lizotte, 2001) have low light, high brine salinity and seasonal darkness. The light level is very low because sea ice and snow cover reflect up to 99% of the surface irradiance (Kudoh et al. 1997; Maykut and Grenfell, 1975; Raven et al. 2000). During the winter, the Southern ocean is covered by thick sea ice, but the recession and break up of the ice in the summer cause rapid fluctuations in the physical environment in the ecosystem (Thomas and Deickmann, 2002). Transitory pond ecosystems represent another type of cold environment. During the summer, ice melts and produces shallow ponds of various sizes. They are characterized by high UV, high irradiance, prolonged desiccation, freeze-thaw cycles and seasonal darkness (Morgan-Kiss et al., 2006).

1.1.1 Antarctica

The Antarctic deserts are the most extreme deserts on the planet. They are characterized by extreme dryness (less than 10cm of precipitation per year) and dramatic temperature fluctuations (i.e., -55 to +10 ^{0}C (Clow et al. 1988; Doran et al. 2002) with an annual average of -20 ^{0}C). The McMurdo Dry Valleys in Antarctica (77-78.5S; 160-164.5E) represent the biggest ice-free area on the continent (Fountain et al. 1999). It consists of dry soils, mountains and year-around frozen lakes. The liquid water below the ice cover is host to abundant microbial life. Phytoplankton, which plays an important role in these ecosystems, experiences low irradiance, narrow spectral light range and phosphorus and nitrogen deficiencies. In addition, all photosynthetic microorganisms have to survive through 5 months of complete darkness during the Antarctic winters (Palmisano et al., 1985; Priscu et al., 1999). There is no evidence that established insects, vertebrates or vascular plants thrive in such environments, and as a result, the McMurdo Dry Valleys are considered Mars analogue sites (Doran et al., 2003; Mckay et al., 2005; Priscu, 1998).

Tundra and maritime Antarctic soils contain moisture and organic matter, but they undergo important freezing-thawing cycles. Tundra vegetation comprises algae, mosses and lichens. Some coastal zones of the Maritime Antarctic are however colonized by penguin colonies (Croxal and Price, 1979). Their presence creates unique tundra ecosystems (Vidal et al., 2003), because penguin guano directly affects the physical and chemical soil properties, and indirectly as a result of enhanced microbial activity creating the ornithogenic soil (Tatur et al., 1997, Sun et al., 2004). Sun et al. (2000; 2001) found that penguin droppings enrich the sediments with nine bio-elements, including S, P, Ca,

Cu, Zn, Se, Sr, Ba and F. The concentration of these elements in nearby lake sediments and soils impacted by the bird's guano far exceeds the levels found in non-impacted sediments. According to Sun et al. (2000, 2001), the accumulation of these elements in ancient sediments provides a very specific marker for the presence of past penguin colonies and their associated guano.

The influence of penguin guano has been shown to impact microbial activity in soils, which in return can affect the cycling of several important soil components, including iron and phosphate.

1.2. Iron Cycling

1.2.1. Distribution of Iron in the Earth's crust

Iron is the fourth most abundant element in the Earth's crust and is present in soils, rocks and sediments. It is either a major or a minor component of igneous minerals (pyroxene, amphibole, olivine and mica), secondary minerals (montmorillonite, illite) and sedimentary minerals (siderite, goethite, limonite, hematite, magnetite, pyrite and others). In addition, weathering of primary minerals containing iron results in the formation of secondary Fe-bearing minerals (Ehrlich, 2002).

Iron is a redox sensitive element which possesses three oxidation states: 0, +2 and +3. Zero-valent iron is however less abundant that ferrous (Fe(II)) and ferric (Fe(III)) iron in the environment. Minerals, such as vivianite, pyrite, marcasite and siderite contain ferrous iron Fe(II) (Cooper et al., 2000; Zachara et al. 2002; Frederickson et al., 1998; Hansel et al., 2003) and form under reducing conditions, whereas under oxic conditions, Fe-(III)-rich minerals dominate, such as iron oxides. The oxidation rate of iron is

however dependent on pH. At pH greater than 5 and under oxic conditions, Fe(II) can be chemically and microbial oxidized to Fe(III), which leads to its precipitation as oxides (Fe_2O_3 and $Fe_2O_3*H_2O$), hydroxides ($Fe(OH)_3$) or oxyhydroxides (FeO(OH)) (Schwertmann and Fitzpatrick, 1992; Cornell and Schwertmann, 1996). Iron oxides are divided into two main groups: crystalline iron oxides (hematite, magnetite, goethite, lepidocrocite) and amorphous or poorly ordered oxyhydroxides (ferrihydrite, green rust) (Fortin and Chatellier, 2003; Fortin and Langley, 2005). Iron oxides can be found in all types of environments (marine and freshwater ecosystems, wetlands, soils and sediments) and they often play an important role in trace element cycling (Fortin et al., 1993; Fortin and Langley 2005; Ferris 2005).

1.2.2. Biological Importance of Iron

Iron represents an important nutrient for different microorganisms. In fact, all prokaryotes and eukaryotes require iron as a nutrient. Some organisms use it in enzymatic reactions that involve electron transfer (aerobic and anaerobic respiration), whereas phototrophs and nitrogen-fixing prokaryotes utilize iron in iron-sulfur proteins – ferrodoxin formation (Emmenegger et al., 2001; Ehrlich, 2002). The solubility of iron can also limit microbial growth, especially in marine ecosystems where soluble iron is limiting (Gelder, 1999; Ehrlich, 2002). In order to increase iron uptake, some microorganisms produce specific chelators (siderophores) to help keep Fe(III) in solution at neutral pH. These include enterobactin from *Salmonella typhimurium* (Pollack and Neilands, 1970), aerobactin from *Enterobacter aerogenes* (Gibson and Magrath, 1969), alterobactin derived from *Alteromonas luteoviolacea* (Reid et al., 1993) and rhodotorulic

acid from yeast *Rhodotorula* (Hersman et al., 2000). Once Fe(III) enters the cell, it gets enzymatically reduced and ferrous iron can be assimilated right away for the formation of heme or non-heme proteins (Ehrlich, 2002).

1.2.3. Microbial iron cycling in freshwater ecosystems

Iron cycling in freshwater ecosystems is depicted in Figure 1.1. Chemical and microbial oxidation and reduction reactions often occur simultaneously in natural environments. In aerobic environments with a pH greater than 4, soluble Fe(II) can be chemically and microbially oxidized and subsequently precipitated as iron oxides (ferrihydrite and other crystalline iron oxides). Under more acidic conditions, the oxidation of Fe(II) is however entirely driven by microbes (Ehrlich, 2002).

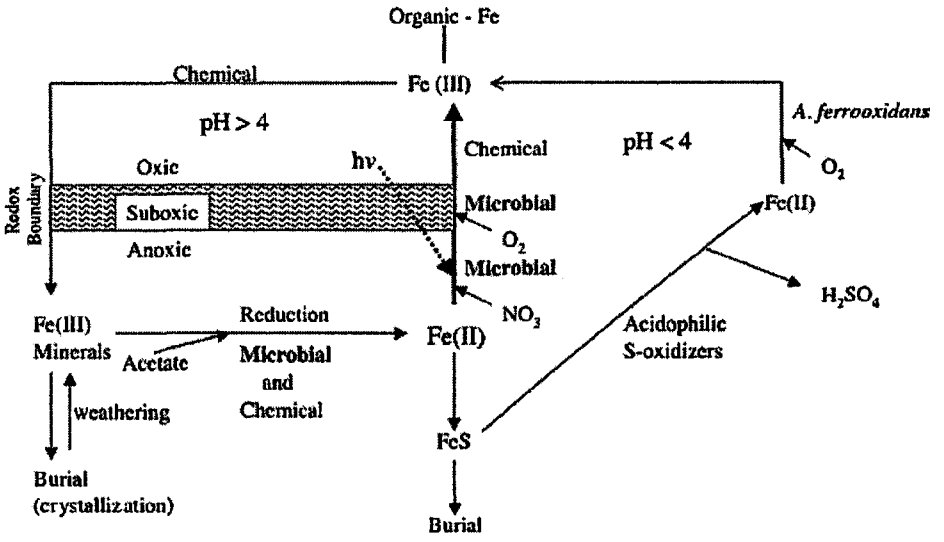


Figure1.1: Microbial iron cycling in freshwater environments, (Emerson and Weiss, 2004).

At low pH conditions (pH <4), Fe(II) can be oxidized by acidophilic iron-oxidizing bacteria, such as *Acidothiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* (Emerson and Weiss, 2004; Harrison, 1984; Blake and Johnson, 2000). The microbial oxidation rate of Fe(II) at low pH is at least a 1000 times faster than the chemical rate (Kasama and Murakami, 2001; Ferris, 2005). Other oxidation pathways include the oxidation of Fe(II) by phototrophic purple bacteria under anaerobic conditions, where Fe(II) is the electron donor for anoxygenic photosynthesis ((Widdel et al., 1993; Emerson and Weiss, 2004). Recently, some nitrate-reducing anaerobic bacteria (*Phodomicrobium vannielii* and *Rhodomicrobium palustris*) and archaea (*Ferroflobus placidus)* have been shown to oxidize Fe(II) and gain energy for their growth (Hafenbrandl et al., 1996; Ehrlich, 2002).

Under more neutral pH conditions, Fe(II) is quickly oxidized to Fe(III) by oxygen. However, some neutrophilic iron oxidizing bacteria can oxidize Fe(II) under low dissolved oxygen concentrations and gain energy from the process. Neutrophilic lithotrophs, such as *Gallionella ferruginea* and *Leptothrix ochraceae,* are known to oxidize ferrous iron under neutral pH conditions (Emerson and Weiss, 2004; Fortin and Langley, 2005). Both bacteria produce copious quantities of poorly ordered iron oxides (ferrihydrite) (Ferris, 2005).

Under reducing conditions, iron oxides can either be reduced by sulfur species or by bacteria (Schwertmann and Cornell, 2000). The following equation shows the abiotic reduction of iron oxides by hydrogen sulfide under microbial sulfate reduction conditions:

$$2\,FeOOH + H_2S = 2\,Fe^{2+} + S + 4\,OH^- \quad (1)$$

Microbial iron reduction is however recognized as a very important pathway or organic carbon degradation in anaerobic sediments (Nealson and Little, 1997; Lovley, 2000, Megonigal et al., 2004).

1.2.4. Microbial reduction of ferric iron

Many studies have reported that microorganisms can reduce amorphous and crystalline iron oxides using iron as a dominant terminal electron acceptor (enzymatic respiration) (Straub et al., 2001; Lovley et al., 2004) or as a supplementary terminal electron acceptor (fermentation reactions) (Ehrlich, 2002). Iron respiration is now being recognized as a significant process in organic carbon mineralization in sediments where nitrate and sulfate respiration do not take place due to insufficient amounts of nitrate or sulfate (Ehrlich, 2002). Equation 2 represents microbial iron reduction:

$$CH_2O + 4\ FeOOH + H_2O = HCO_3 + 4Fe^{2+} + 7OH^- \quad (2)$$

Iron reduction is an important process in diverse anaerobic environments where abundant amounts of organic carbon and iron oxides are present (marine and freshwater sediments, submerged soils, pristine aquifers and petroleum-contaminated environments) (Thamdrup, 2000). Jensen et al. (2003) have shown that in marine sediments enriched with poorly ordered iron oxides, 75% of anaerobic carbon oxidation resulted from microbial iron reduction. The same authors also reported that microbial iron reduction is controlled by several factors, including bioturbation, availability of reactive organic material and concentration and reactivity of iron oxides in sediments. Bioturbation has indeed been shown to significantly increase iron reduction in salt marsh sediments

(Koretsky et al., 2003). Weiss et al. (2004) also linked highly active iron cycling with microbial activity in the rhizosphere of wetland plants where the iron plaque (on the roots' surface) represents a large pool of Fe(III) available for reduction. Microbial iron reduction is also wide spread in acidic environments because Fe(III) is more soluble at low pH conditions. Acidophilic iron-reducing bacteria are facultative aerobes while neutrophillic iron reducers are either facultative anaerobic or strictly anaerobic microorganisms (Johnson and Bridge, 2002).

Microbial iron reduction is dependent on the type of electron donors and electron acceptors. Fredrickson et al. (1998) reported that poorly ordered iron oxides are the main source of iron for anaerobic microbial reduction because they have a higher surface area and they are more bioavailable. However, according to Roden and Urrutia (2002) and Hensel et al. (2003), crystalline iron oxides may undergo microbial reduction as well and because they are more abundant, they can significantly contribute to Fe(II) formation. Amorphous iron oxides have however been shown to be completely reduced, whereas more crystalline oxide species are reduced at lower reduction rate (Roden and Urrutia, 2002; Lovley and Phillips, 1987). Green rust, vivianite, magnetite and siderite are secondary products of microbial iron reduction (Zachara et al., 2002; Fredrickson et al., 1998). The main source of electron donors for iron-reducing microorganisms is the complex organic matter in the sediments, including simple organic acids (acetate), amino acids, sugars, mono aromatic compounds and long chain fatty acids (Lovley et al., 2004).

Microorganisms capable of Fe(III) reduction can either do it minor as a side reaction and not conserve energy for growth (such as sulfate-reducers and methanogenic microorganisms), whereas a vast majority of bacteria and archaea do conserve energy

from iron reduction (Lovley et al., 2004). The best known iron reducers are *Geobacter* sp. (*Geobacter metallireducens, G. sulfurreducens*), *Desulfuromonas* sp. (*D. palmitatis*), *Shewanella* sp. (which are facultative anaerobic bacteria) and some hyperthermophillic microorganisms (Lovley et al., 2004; Ehrlich 2002). According to Lovley et al. (2004), *Geobacter* sp. are the dominating iron reducers in the environment.

Finally, iron-reducing microorganisms have different mechanisms to get iron out of the iron oxides. Some bacteria require direct contact with the iron oxides while others solubilize iron oxides by producing chelators and electron shuttles (e.g. humic acids, quinines) (Hansel et al., 2003; Lovley et al., 2004).

1.3. Phosphorus cycling

Phosphorus is an essential element to life because it is a structural and functional component of all organisms, including phospholipids and phosphoproteins, nucleotides and nucleic acids (Ehrlich, 2002). Phosphorus is also a limiting factor for plant growth. According to Paige et al. (1996), phosphorus cycling is very rapid in fresh waters and large pools of available soluble phosphorus can lead to the eutrophication of lakes. In soils, the process of phosphorus cycling is however slower. Figure 1.2 shows how phosphorus is cycled in the environment.

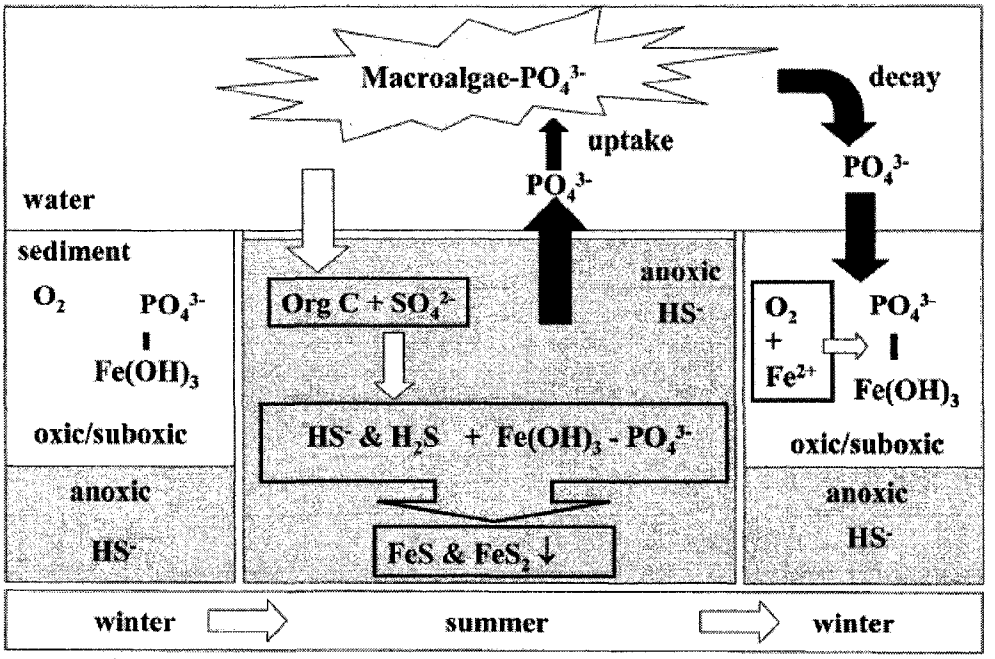


Figure 1.2: Schematic of phosphate cycling (indicated with the filled darker arrows) as redox state changes in sediments. (Rozan et al., 2002)

Phosphorus exists in organic and inorganic forms, which can be soluble or insoluble. The most common soluble inorganic phosphorus species are orthophosphate and metaphosphate, which represent a small portion of the total pool of phosphorus in freshwater systems (Cross and Schlesinger 1995; Chacon et al., 2006). On the other hand, the amount of inorganic P in soils fluctuates between 40% and 80% of the total P (Chacon et al., 2006). Inorganic P easily reacts with Fe, Ca and Al to form insoluble minerals (Rozan et al., 2002; Chacon et al., 2005; 2006; Hyacinthe and Cappellen, 2004; Makris et al., 2005; Kleeberg and Grueberg 2005; Khare et al., 2005). Phosphate-rich minerals include apatite, vivianite and aluminum salt-variscite (Paige et al., 1996). In aquatic environments, the immobilization of phosphorus is however not dramatic because

of lower pools of Fe, Ca and Al in freshwater and marine ecosystems. Phosphate can also bind to iron oxides in freshwater sediments and soils and be immobilized. However, under reducing conditions, phosphate can be released back into solution following the reduction of iron oxides and be taken up by microorganisms and algae (Rozan et al., 2002). Fe(III)-phosphate minerals (such as strengite) can also undergo reductive dissolution in the presence of hydrogen sulfide, as shown in equation 3 (Chacon et al., 2006).

$$2FePO_4 + 3H_2S = 2FeS + 2H_3PO_4 + S \qquad (3)$$

Under reducing conditions, phosphate can react with Fe(II) and precipitate as vivianite and become permanently immobilized (Kleeberg and Gruneberg, 2005). According to Huerta-Diaz et al. (2005), P-bound to Fe-oxyhydroxides in marine sediments can be more mobile than phosphorus associated to other authigenic minerals (carbonate, fluorapatite). In contrast, Al-bound phosphorus is permanently immobile. Alexander (1977) noted that the highest microbial activity on phosphorus solubilization was observed in the rhizosphere, probably because microorganisms use root secretions for acid or chelator generation.

Phosphorus can also occur as a gaseous species, namely phosphine (PH_3). It has been reported to occur in natural environments impacted by bird colonies (Zhu et al., 2006). There are two forms of phosphine, free gaseous phosphine, which is found in waste waters, animal slurries and marshes, and matrix bound phosphine (MBP), found in sediments, soils, manure and animal and human feces (Zhu et al., 2006). In sediments impacted by penguin colonies in Antarctica, guano leachates can interact with weathered

volcanic rocks and produce secondary phosphates, such as struvites ($Mg(NH_4)PO_4.6H_2O$), fluorapatites ($Ca_5(PO_4)_3F$) and brushites ($CaHPO_4.2H_2O$) (Zhu et al., 2006).

1.4. Microbial activity in permanently cold environments

The ability of microorganisms to live and sustain metabolic activity at low temperatures has been known for more than 100 years (Pomeroy and Wiebe, 2001), but Morita et al. (1977) were among the first researchers to measure microbial activity in polar marine environments. Psychrophilic microorganisms have been found in various types of permanently cold environments, from deep oceans to alpine and polar regions where the average annual temperature is below 4 ^{0}C (D'Amico et al., 2006; Pomeroy and Wiebe, 2001). Temperature is a limiting factor because it has an impact on all biochemical processes happening in a cell. Some studies have shown that in permanently cold marine environments, most psychrophiles survive and proliferate under temperature far from their optimal temperature, but their metabolic rates in the summer are comparable to those of mesophiles present in warmer ecosystems (Isaksen and Jorgensen, 1996; Rivkin et al., 1989; Pomeroy and Wiebe, 2001). According to Pomeroy and Wiebe (2001), heterotrophic psychrophilic organisms living in the sea ice have low metabolic rates and a lower ability to oxidize organic carbon. The same authors also state that microorganisms living in cold environments enriched with organic substances (e.g. tide waters, sediments) are less dependable on temperature. In the sub Antarctic Marion Island lakes, higher microbial activities have been measured in lakes impacted by seal feces than in pristine lakes (Robarts et al., 1991).

In order to survive under low temperatures, psychrophilic microorganisms have developed different adaptation mechanisms. Some studies have shown that the lipid membranes of psychrophiles have a higher content of unsaturated fatty acids (Chintalapati et al., 2004; Walker et al., 2006). Gilbert et al. (2004) also reported the production of anti-freeze proteins in bacteria from Antarctic lakes. Bacteria can also produce high concentrations of exopolysaccharides which serve as cryoprotectants (Nicols et al., 2005; Krembs et al. 2002). In addition, psychrophiles produce cold adapted enzymes (Feller and Gerday, 2003).

There is a wide biodiversity of aerobic and anaerobic bacteria, archaea, fungi and microalgae living in cold environments. *Pseudomonas* spp., *Vibrio* spp., *Arthrobacter* spp. and *Micrococcus* spp. are the most frequent bacteria and they dominate over Archaea (with the exception of deep sea waters), whereas archaea (such as *Methanogenium* spp. and *Methanococcus* spp.) are the most widely distributed microorganisms in cold systems (D'Amico et al., 2006). According to Pandey et al. (2004), the dominating cyanobacteria in Antarctic are *Nostoc* spp., *Phormidium* spp. and *Oscillatoria* spp.

Rivkina et al. (2000) observed the existence of a significant amount of microorganisms in permafrost Arctic sediments, whereas Karr et al. (2005) made similar observations in the permanently frozen freshwater Lake Fryxell located in McMurdo Dry Valley in Antarctica. In Fryxell Lake, sulfur is being cycled by a diverse group of sulfate-reducing bacteria from the *Proteobacteria* and *Thermodesulfobacterium* subdivisions and sulfate-reducing archaea from the *Euryarchaeota* group. Studies by Isaksen and Jorgensen (1996) and Knoblauch et al. (1999) have also shown that sulfate reduction in

Arctic marine sediments is the principal carbon oxidation process. Psychrophilic sulfate-reducing bacteria generally have a higher metabolic rate than their mesophilic counterparts (Isaksen and Jorgensen, 1996). Bacterial sulfate reduction has also been detected in Arctic mining tailings and in permafrost sediments impacted by mining operations, where sulfate reduction rates were comparable with those from permanently cold Arctic and Antarctic marine sediments (Karnachuk et al., 2005). Similar results were obtained for mining residues in northern Ontario (Fortin et al., 2000). Laboratory experiments have also shown that iron-reducers and sulfate-reducers can be temperature limited but well cold adapted as indicated by the relatively high reduction rates observed in sediment at 4^0 C (Meier et al., 2005). Finally, a recent study by Vandieken et al. (2006) reported the isolation of two psychrophilic iron-reducing bacteria *Desulforomonas svalbardensis* sp. nov. and *Desulfuromusa ferrireducens* sp. nov. from permanently cold Arctic marine sediments.

1.5. Objectives and hypotheses of the present research

Objectives

1- To determine the role of phosphate on the solid-phase geochemistry of iron in the soil from Antarctica impacted by penguin guano.

2- To determine the mineralogy of the iron- and phosphate-rich minerals in the soil from Antarctica impacted by penguin guano (using X-ray diffraction)

3- To assess the release of iron and phosphate from the same soil from Antarctica during microbial iron reduction.

Hypotheses

1- Given the affinity of phosphate for both Fe(II) and Fe(III), it is expected that the ornithogenic soils will show higher Fe-ascorbate and PO_4-ascorbate fractions than sediments from natural freshwater and marine environments. The concentration of soluble phosphate in the porewaters of the ornithogenic soils should also greatly exceed the levels found in natural freshwater and marine systems.

2- Given the high affinity of phosphate for both Fe(II) and Fe(III), it is expected that the ornithogenic soil will contain more crystalline Fe-phosphate minerals, such as strengite and vivianite, compared to sediments and soils not impacted by bird droppings.

3- It is expected that iron reducing bacteria, such as *Shewanella putrefaciens CN32,* will easily reduce poorly ordered Fe-oxides and Fe-phosphate minerals present in the Antarctica soil because they have been shown to be easily reducible. In addition, bacteria need phosphate to grow and they should preferentially reduce Fe-phosphate minerals.

2. Materials and methods

2.1. Site description and sampling sites

The soil core was sampled by professor Sun from the Chinese University of Science and Technology in Hefei, Anhui Province, during the winter 2006. The sampling site, Gardiner Island, is located in coastal eastern part of Antarctica (S68 34'43", E77 52'33") near Prydz Bay and the Chinese research station Zhongshan (S69 22', E76 24') (Figure 2.1). Several large Adelie penguin rookeries reside on the island (Xie and Sun, 2003) (Figure 2.2.A). One frozen sample of penguin guano was also provided by Prof. Sun.

Control samples were collected near the other Chinese research station called the Great Wall (S69 13', W58 56') from sites free of penguin and other birds influence.

On Gardiner Island, soil samples were taken every cm along a 42 cm-deep profile. The water table fluctuates throughout the year and it was located at a depth around 42 cm during sampling (Figure 2.2.B). The soil has a granular texture (particle size around 1-2 mm) and contains feathers and fragments of penguin bones.

All samples were collected using clean techniques and were kept frozen until being shipped to Ottawa. Upon arrival, they were immediately stored at 4° C and sub-samples were used to measure *in situ* Eh and pH. Porewater and solid-phase geochemistry characterization was later performed according to the techniques described below.

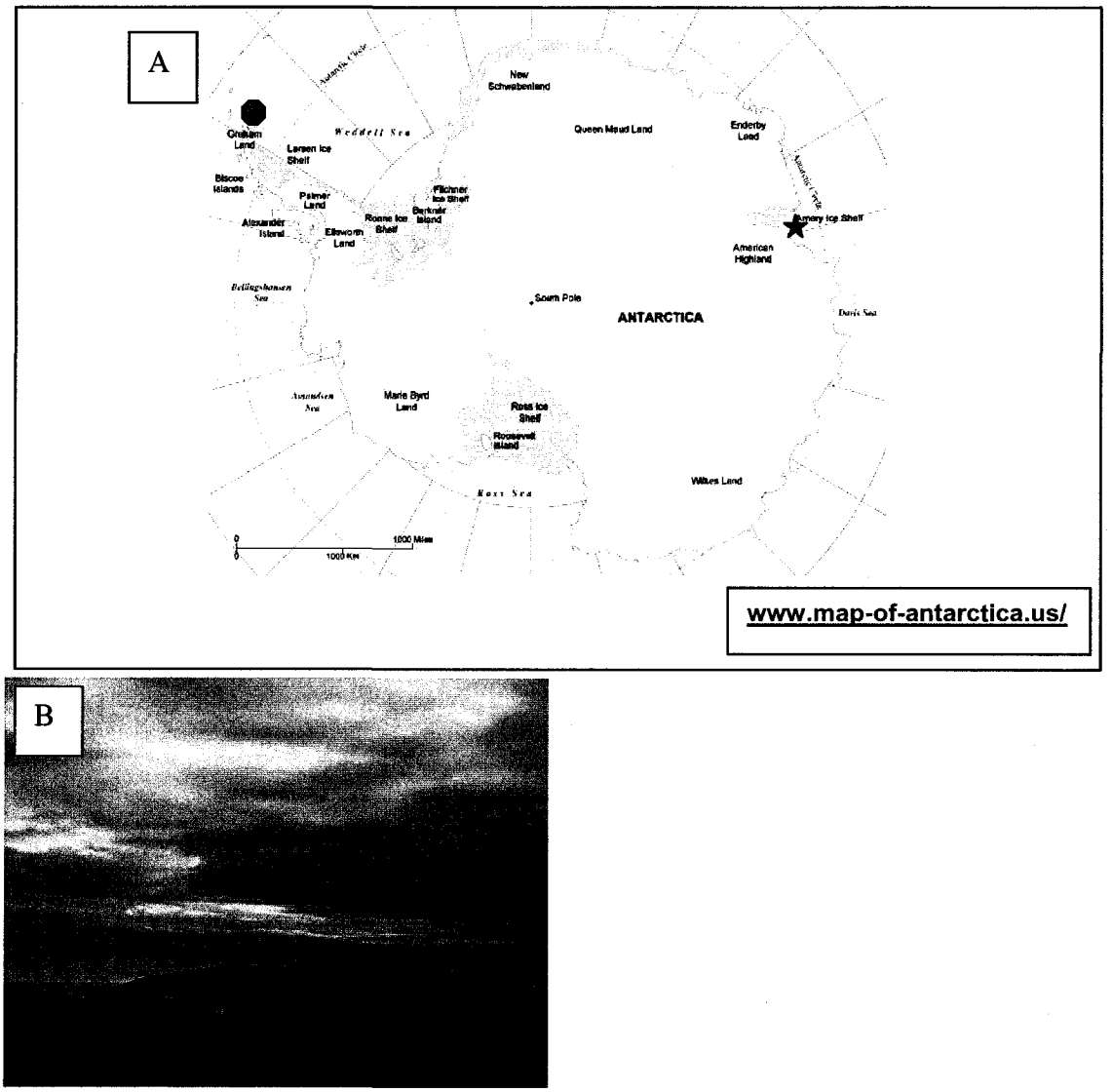


Figure 2.1: (A) Map of the Antarctic continent. The red star shows the location of the sampling site on Gardiner Island, whereas the green octagon indicates the location of the control site. (B) Picture of Gardiner Island.

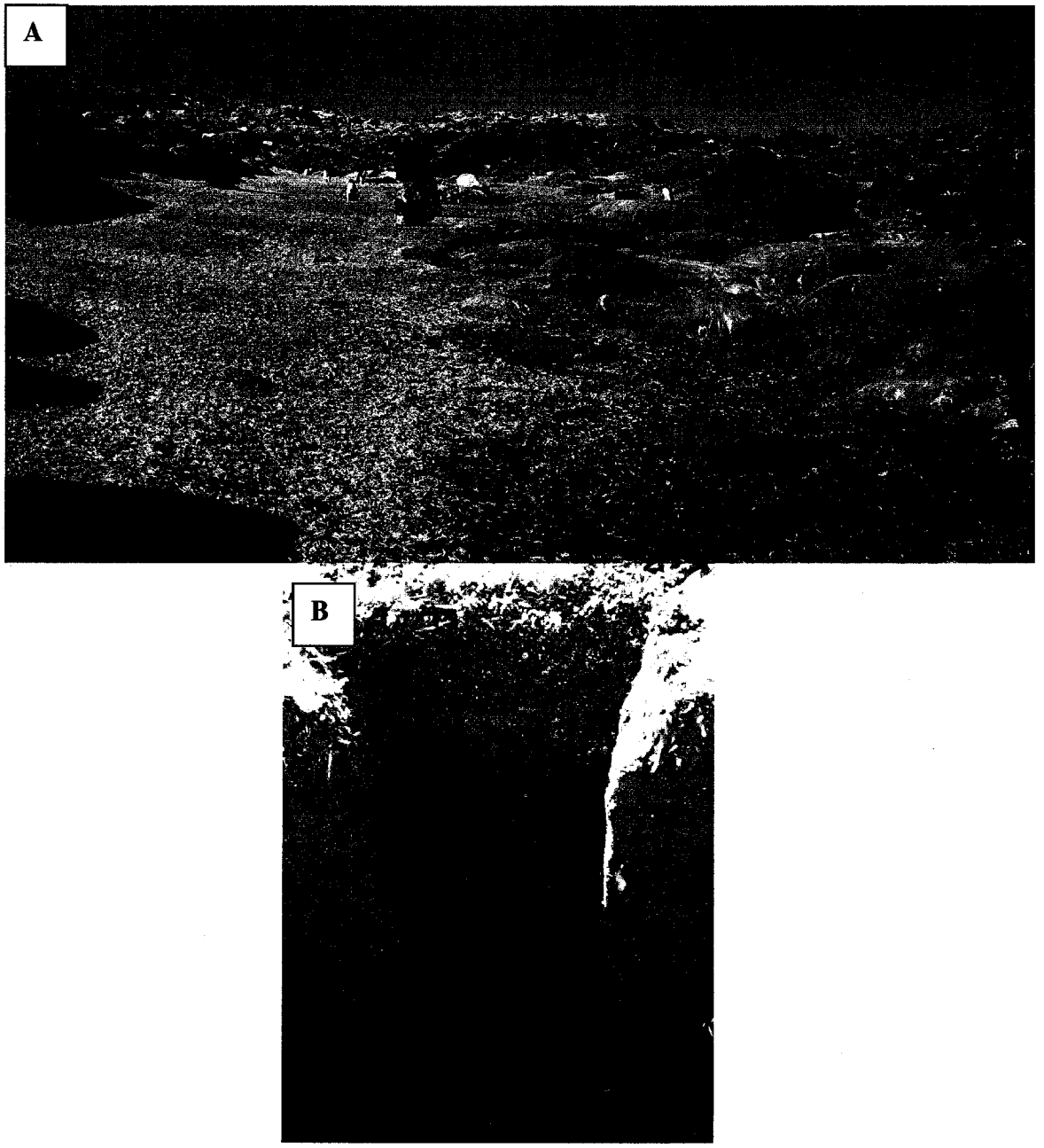


Figure 2.2: (A) Picture of the terrain on Gardiner Island (sampling site). There are no vascular plants present on the site. The ground is covered with penguin feathers and bone fragments. (B) Soil profile (42 cm) where the samples were taken. The water table was located at a depth of 42 cm.

2.2. Eh and pH measurements

Eh and pH measurements were performed in the anaerobic chamber using a VWR pH meter and pH and Eh electrodes (Corning). The pH electrode was calibrated with pH standards at pH 4 and 7, whereas the redox electrode was calibrated with the ZoBell's solution (Nordstrom, 1977). A correction value of + 199 mV (with respect to the hydrogen reference electrode) was applied to all Eh results obtained in the study. The same electrodes were used throughout the study in order to avoid variability between instruments.

2.3. Water content and organic carbon

Water content was determined by placing 1g of sample (in triplicates) into the oven at 105° C for 24 hours. The organic carbon content of the soil used for the water content analysis was determined by loss on ignition (LOI). The samples were heated to 550° C for 4 hours and the loss of weight was recorded (Heiri et al., 2001).

2.4. Pore water analysis

Due to low amount of pore water in the soil, a dilution approach was used to obtain enough volume to perform the analyses. The samples were weighted and mixed with a known volume of ultra-pure water, centrifuged and the supernatant was filtered through a 0.2 nm Millipore filter. Aliquots of the filtered diluted pore water samples were then separated for the analysis of dissolved major cations and anions. The concentration of each element was estimated using the weight and water content of the soil and the dilution factor.

2.4.1. Major cations and anions analysis

Aliquot samples for ICP-AES analysis were acidified with concentrated nitric acid (trace grade) to a final concentration of 1%. Concentrations of total dissolved iron, sulfur, fluoride, potassium, calcium, magnesium and sodium were measured by ICP-AES (Varian Vista-Pro) using Fisher standards acidified with 1% nitric acid.

Main anions, such as nitrate, chloride, sulfate and phosphate were determined by ICP-MS in non-acidified samples.

2.4.2. Sulfate (SO_4^{2-}) analysis

Concentrations of dissolved sulfate were also determined with the turbidity method with stabilized $BaCl_2$ solution (Rodier, 1975). Due to the high sulfate concentrations in the samples, which exceeded the linear range of the method, a dilution was made with ultra-pure water. The absorbance (650nm) was read with a Beckman spectrophotometer.

2.4.3. Phosphate (PO_4^{3-}) analysis

Concentrations of dissolved phosphate were determined with the molybdate-blue complexation method (Koroleff, 1983). Reagent A was made by dissolving 12 g of ammonium molybdate and 0.2908 g of potassium antimony tartrate in 1L of 5N H_2SO_4. The volume of the solution was then brought 2L by adding ultra pure water. Reagent B contained 1.056 g of ascorbic acid dissolved in 200 ml of Reagent A. A KH_2PO_4 solution (1.3mM) was used to make the standards. Phosphate concentration was determined by mixing 1ml of sample with 41 ml of ultra pure water and 8 ml of Reagent B. The

absorbance was read after 10 minutes. The initial phosphate concentrations in all samples were out of linear concentration range of the method. Therefore, a dilution was made with ultra-pure water. The absorbance (840nm) was read with a Beckman spectrophotometer.

2.5. Dissolved organic carbon (DOC)

Dissolved organic carbon concentrations were estimated with a TIC-TOC analyzer (model 1010) calibrated with mono potassium salt standards. Due to limited volume of pore water in each sample, all samples were diluted with ultra-pure water. All samples were analyzed in the G.G. Hatch Isotope Laboratory at the University of Ottawa.

2.6. Solid Phase Geochemistry

Soil samples were sub-sampled for each depth interval (1-5 cm) to provide three replicates for the separate determination of:

1) Amorphous iron (Asc-Fe)
2) Reactive phosphate (Asc-P)
3) Total Amorphous and Crystalline iron (Fe(III) oxides)
4) Total iron in solid phase (complete digestion)
5) Acid Volatile Sulfur and Chromium Reducible Sulfur

2.6.1. Amorphous iron (Asc-Fe) and Reactive phosphate (Asc-P)

Ascorbic acid extracts iron from amorphous Fe (III) oxyhydroxides (such as ferrihydrite), which often comprise 50% of the iron in surface sediments (Kostka and

Luther, 1994). For this purpose, 0.4 g of wet soils were added to 10 ml of ascorbic acid solution, which consisted of 10 g of Na-citrate, 10 g of Na-bicarbonate, 4 g of ascorbic acid in 200 ml of ultra pure deoxygenized water (adjusted to pH 8). The samples were left on a shaker in the anaerobic chamber for 24 hours (Rozan et al., 2002). After 24 hours, the supernatant was filtered with 0.2 um nylon filters (Fisher) and the concentration of iron was determined with the ferrozine method (Stookey, 1970). Phosphate concentrations in the same fraction were determined with the molybdate-blue complexation method (Koroleff, 1983).

2.6.2. Total amorphous and crystalline iron from Fe (III) oxides

According to Kostka and Luther (1994), the dithionite extraction is used to estimate the entire pool of reactive iron in sediments, i.e., iron from ferrihydrite, magnetite and goethite, along with some iron from hematite. 0.25 g of wet soils (in triplicates) was added to 10 ml of dithionite solution, which was made daily by dissolving 20 g of dithionite into 200 ml of deoxygenized 0.2 M Na-citrate and 0.35 M Na-acetate solution (Rozan et al., 2002). The samples were digested for 2 days on a shaker in the anaerobic chamber. The samples were then filtered with a 0.2 um nylon filter (Fisher) and analyzed for total Fe with the same ferrozine method as described earlier.

2.6.3. HCl-extractible Fe (II) and Fe (III)

HCl is effective in extracting iron from amorphous iron (III) oxides and Fe(II) in acid volatile sulfides (AVS), carbonates and phosphate minerals. Triplicate wet samples (0.5g) were mixed with 10 ml of 0.5N HCl for 1 hour on a shaker in the anaerobic

chamber. A 0.1 ml sub-sample was then taken and 5 ml of ferrozine solution (0.1% ferrozine solution with 12 g of HEPES per liter at pH 7) was added for the determination of Fe (II) determination. For Fe total, the sub-sample (0.1 ml) was mixed with 5 ml of hydroxylamine hydrochloride solution (20 ml of 10% NH_2OH added to 12 g of HEPES per liter at pH 7). After 20 minutes, 0.1ml of 0.5% ferrozine solution was added to both extracts and left to react for at least 30 minutes. The extracts were then filtered with a 0.2um nylon filter (Fisher) and the absorbance was read with a spectrophotometer (absorbance 562nm). Fe (III) was estimated by difference between total iron concentration and the concentration of Fe (II) (Kostka and Luther, 1994).

2.6.4. Complete iron digestion

The total iron concentration of the soils was performed by Accutest Laboratories in Ottawa. The wet soils were sequentially digested with aqua regia, hydrofluoric acid (HF) and H_2SO_4 (Canfield, 1989), and the iron concentration was determined with the ferrozine method (Stookey, 1970).

2.7. Sulfate Reduction Rates

Sulfate reduction rates (SRR) are used to estimate the bacterial metabolic activity of sulfate reducing bacteria in marine and freshwater sediments and soils. The ^{35}S radioisotope injection method is the most common method to determine SRR (Kostka et al, 2002; Praharaj and Fortin, 2004). Due to the low amount of pore water in the soils, the samples (3 g) were mixed with 6ml of ultra pure water. The slurry was then injected with ^{35}S and incubated at $+4^0C$ (representing the *in-situ* temperature during the sampling) for 4

hours. The reaction was then stopped with a 20% Zn acetate solution. The samples were centrifuged and the supernatant was analyzed for ^{35}S radioactivity (Beckman LS 1560 scintillation counter). The soil residue was washed two times with ultra pure water and ^{35}S was extracted from the acid volatile sulfur (AVS) and chromium reducible sulfur (CRS) fractions as described in the following section. SRR values were calculated using such parameters as soil density, porosity and SO_4 concentrations in the pore water.

2.8. Solid phase sulfide analysis

The AVS fraction refers to FeS and H_2S, whereas the CRS fraction represents S^0 and FeS_2 (pyrite). These fractions were determined with a two step distillation technique (Fossing and Jorgensen, 1989; Meier et al. 2000). 5 ml of 50 % ethanol was first added to the flasks containing the residues. 7.5 ml of 5N HCl was then added to the soil ethanol mixture and distilled for 25 minutes under nitrogen. AVS was trapped in 10 ml of 5% Zn acetate solution. For CRS analysis 16 ml of 1M Cr^{2+} in 0.5N HCl was added to the residues and the distillation was run for 40 minutes under gentle boiling conditions. The CRS was trapped in 10ml of 5% Zn acetate. Concentrations of sulfide in the traps were determined with the Cline's method (Cline, 1969), using a spectrophotometer. (Absorbance was read at 670 nm).

2.9. Microbial Iron Reduction

Microcosm experiments were set up with soils from selected depths in the profile. The soil samples were placed in a chemically defined growth medium inoculated with *Shewanella putrefaciens* CN-32 (a common iron reducing bacterium in freshwater and

marine environments). The release of ferrous iron, total iron and phosphate was monitored over time, along with pH and Eh. Abiotic and biotic systems containing sterile and non sterile soils with no *S. putrefaciens* cells were also run in parallel to assess the importance of biotic reduction over abiotic reduction.

Shewanella putrefaciens is a gram-negative, facultative anaerobe (Lovley, 1997). It is able to reduce crystalline iron oxides, such as hematite, magnetite and goethite, and poorly ordered hydrous ferric hydroxides (Zachara et al., 1998; Frederickson et al., 1998).

2.9.1. *Shewanella putrefaciens* CN-32 inoculum preparation

S. putrefaciens used in this study was provided by Dr. Danielle Fortin, University of Ottawa. The cultures were maintained aerobically at room temperature on tryptone soy agar (TSA) plates prior their use in the microcosm experiment.

Sub-cultures were grown in the chemically defined medium (CDM) (Appendix A), which consists of 20 mM sodium lactate, 20 mM NH_4Cl_2, 20 mM potassium chloride, 20 mM piperazine-1,4-bis (2-ethanesulfonic acid) (PIPES buffer), 3.9 mM $NaH_2PO_4x2H_2O$ and 2mM trace elements. The first sub-culture was grown in a 50:50 volume ratio of tryptone soy broth (TSB) and CDM, inoculated with one colony of *S. putrefaciens*. The subsequent sub-cultures were grown in a medium with 5:95 and 1:99 of TSB: CDM volume ratios and 100% CDM, as a final step, on a rotary shaker (125 rpm) at room temperature for 24 hours approximately for each step, and for 36 hours for the final step. After the last step, cells were centrifuged at 3000 rpm for 15 minutes and washed in the CDM medium without phosphate added.

Cell concentration was evaluated by using the total protein content determined with the BioRad Protein Assay method (Appendix B). Aerobic cultures of the concentrated cells were prepared via serial dilutions and counted using the Most Probable Number (MPN) technique after 18 hours to verify the results of the BioRad Protein Assay.

2.9.2. Iron Reduction Experiments

The reduction medium is a nutritionally limited medium, without any iron or phosphate added, and used for promotion of iron phosphate reduction and release of phosphate. The reduction medium contained 20 mM sodium lactate, 20 mM NH_4Cl_2, 20 mM potassium chloride, 20 mM piperazine-1,4-bis (2-ethanesulfonic acid) (PIPES buffer) and 2mM trace elements.

In the anaerobic chamber, five grams of soil samples (from 5-10 cm and 15-20 cm intervals in the profile) were added to the bottles containing 200 ml of phosphate-free CDM, along with approximately 10^7 CFU/ml of *S. putrefaciens* cells. These systems are referred to as biotic systems. An abiotic control system contained no cells, but the same amount of sterile gamma-radiated soils. Another control system was setup with non sterile soils in the absence of *S. putrefaciens*. All systems remained under strict anaerobic conditions during the whole length of the experiment. All systems were set up in triplicates.

2.9.3. Sampling

Samples were taken for soluble phosphate, total iron, total ferrous iron, total dissolved iron and ferrous dissolved iron measurements. Before each sampling, the bottles were vigorously shaken and sub-samples were taken for the above measurements and also for pH and Eh analyses. The sub- samples were taken at time 0 and then at day 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

For dissolved Fe(II) and phosphate measurements, samples were filtered through a 0.2 um nylon filter and analyzed with the ferrozine and molybdate-blue complexation methods described earlier. For total Fe(II) analysis, unfiltered samples were digested with 0.5 M HCl and a sub-sample was taken and mixed with ferrozine. For total iron, hydroxylamine was added to the sub-sample, as described in section 2.6.3 and the concentration was determined by ICP-AES. For total phosphate concentration, 6 ml of non-filtered sample were digested with 4 ml of 30% hydrogen peroxide (H_2O_2) and 2 ml of concentrated trace metal grade nitric acid (HNO_3). Vials were placed in the 70^0C oven overnight for complete digestion. On the next day samples were diluted with ultra pure water (1:10) and analyzed by ICP-AES using fresh standard solutions.

On the first and the last days of the experiment, viable plate counts were performed in order to determine if *S. putrefaciens* was alive over the course of the sampling period. For this purpose, 0.5 ml of culture suspension was taken from each experimental bottle and put into a micro centrifuge tube. A dilution series of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} was then performed with sterile CDM. Aliquots of 0.1 ml from the 10^{-4}, 10^{-5} and 10^{-6} dilutions were aseptically added to sterile TSA plates and streaked onto the agar plates. For the abiotic systems, the undiluted cell suspension was used for

cell count. After 18 hours of incubation, plates having between 30 and 300 colonies were counted and the counts were converted into CFU/ml (colony forming units per ml).

2.10 Mineralogy (XRD analysis)

In order to ascertain the mineralogy of the soil samples, X-ray diffraction analysis was used. Soils from selected depths were freeze-dried, finely grounded in the acid washed mortar and analyzed with a Phillips x'pert diffractometer equipped with a Kevex (Si-Li) detector, using a Cu source, a voltage of 45 kV and a current of 40mA. A step scan mode of 0.02^0 with a step time of 2.0 seconds was used between 2^o and 70^o.

3. Results

3.1. Aqueous geochemistry

3.1.1. Physico-chemical description of the soil

Samples were taken from a 42-cm deep soil profile at a 1 cm interval (Figure 2.2B). The soil samples had a sandy granular texture with a particle size of 1-2 mm and they contained bird feathers and bone pieces. The water table was located around 42 cm at the time of sampling (Figure 2.2B), but likely fluctuated between snow melt and dryer conditions.

3.1.2. Eh and pH conditions

The redox (Eh) values of the porewaters ranged between 88 and 174 mV (Figure 3.1), whereas the pH oscillated between 6 and 7 (Figure 3.2).

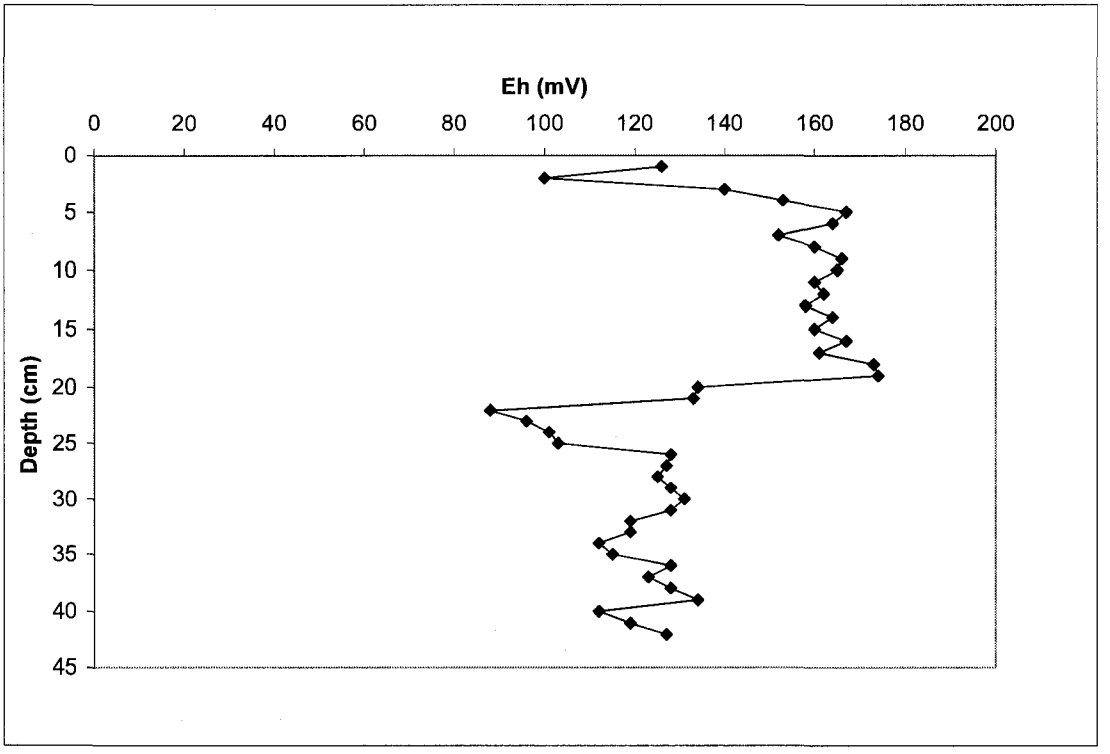


Figure 3.1: Redox conditions measured in the soil samples after their arrival in Ottawa.

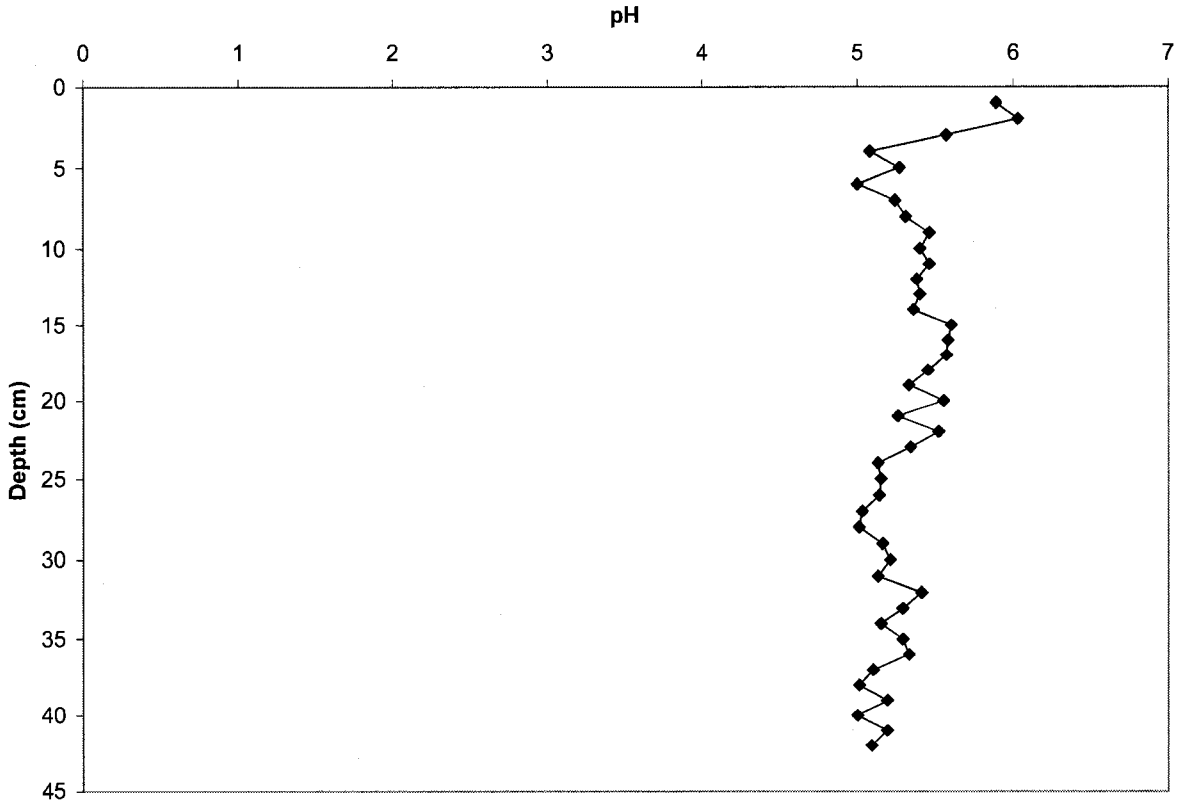
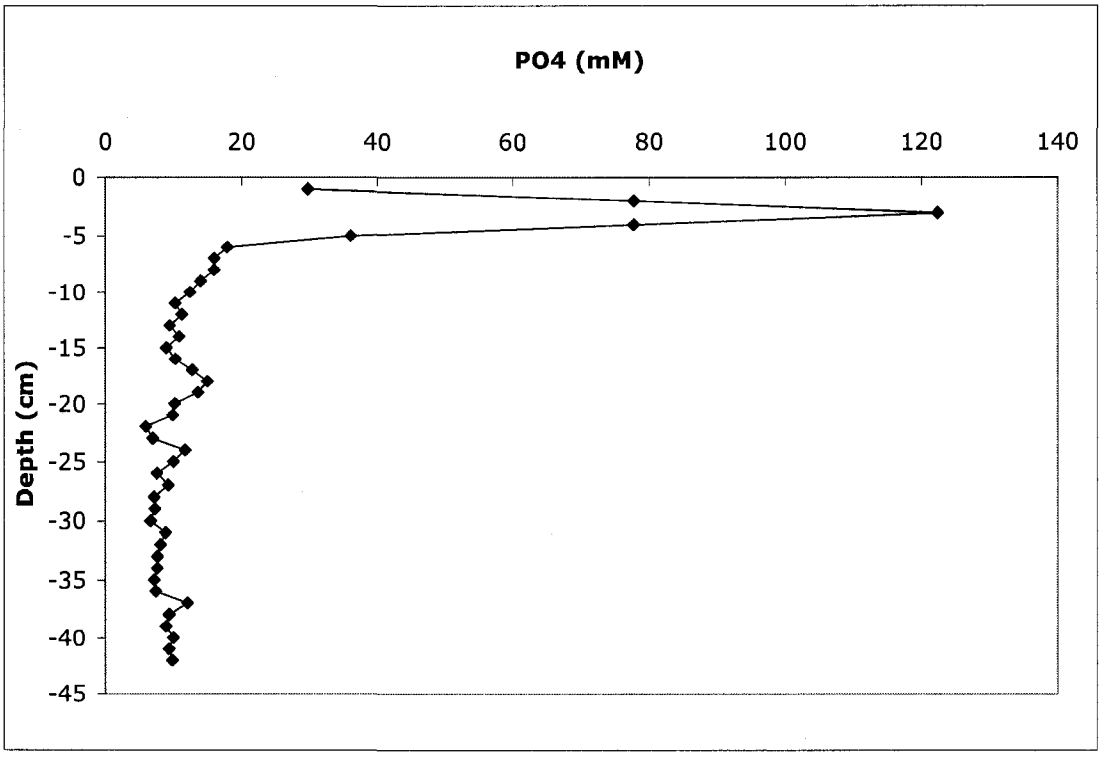


Figure 3.2: pH conditions of the soil samples after their arrival in Ottawa

3.1.3. Major and minor ions

Porewaters extracted from the soil profile were analyzed for major anions (PO_4, SO_4, Cl, F, NO_3) and cations (Fe, Na, K, Mg, Ca, Si, Mn and Sr). Reduced soluble iron and sulfur species were not determined because the prolonged shipment from Antarctica to Ottawa likely resulted in some oxidation of the soil. Dissolved phosphate levels in the

soil ranged from 10 to 125 mM and displayed a sharp increase near the top of the profile, along with a smaller one at a depth of 17 cm (Figure 3.3). Sulfate concentrations were also very high near the top of the profile (~ 28 mM) and declined with depth, oscillating between 10 and 15 mM (Figure 3.3).



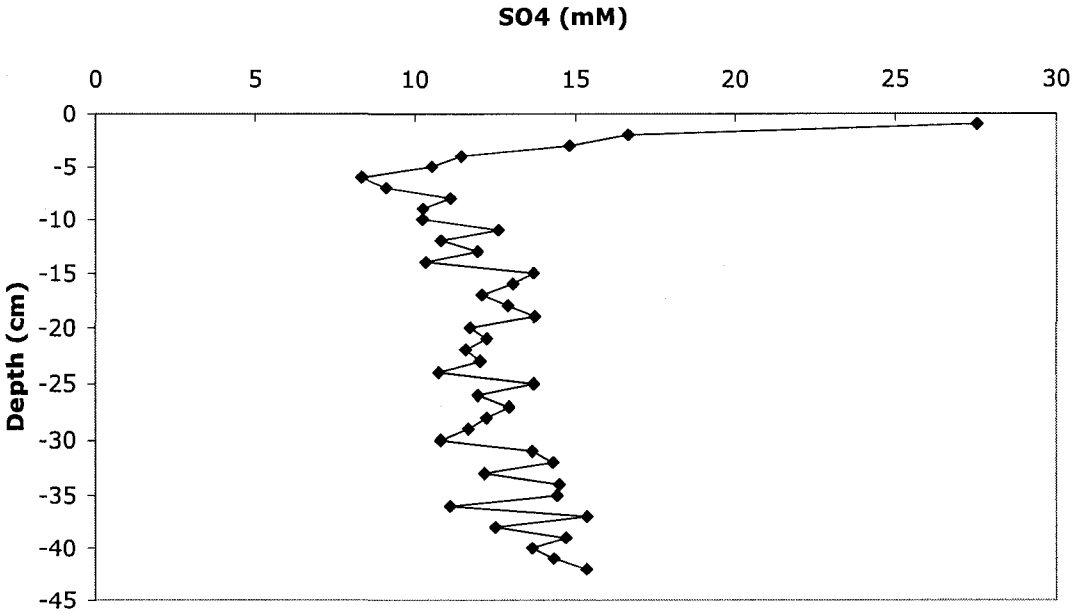


Figure 3.3: Phosphate and sulfate concentrations in the soil porewaters.

Similar concentration trends were also observed for nitrate, fluoride and chloride concentrations, i.e., all anion concentrations increased near the top of the profile (Figure 3.4). For nitrate, concentrations ranged between 2 and 17 mM, whereas they varied between 0.8 and 1.9 mM for fluoride. Chloride levels in the porewaters were extremely high and reached 325 mM at the top of the profile, suggesting seawater input (Figure 3.4).

Major cations analyzed in this project included Fe, Mn, Sr, Si, Na, Mg, K and Ca. As observed for the major anions, several metals showed a sharp concentration increase near the top of the profile, especially Mn, Si Sr, Ca and Mg (Figure 3.5). Fe and Mn concentrations were generally low and ranged between 0.01 and 0.04 mM and 0.01 and 0.09 mM, respectively. Increased Mn concentrations in the porewaters generally occurred above the release of Fe, as shown in Figure 3.5. This is in agreement with the preferential reduction of Mn-oxides over Fe-oxides (Stumm and Morgan, 1996). Si and Sr levels peaked near the top of the profile and ranged between 1.2 and 3.2 mM and 0.02 and 0.10 mM, respectively (Figure 3.5). Na and K concentrations oscillated with depth and ranged between 70 and 125 mM and 9 to 15 mM, respectively (Figure 3.5). Finally, both Ca and Mg increased near the top of the profile and the concentrations varied between 2 and 18 mM and 10 and 150 mM, respectively (Figure 3.5). Based on the concentration profiles of most anions and cations present in the porewaters, it is clear that penguin droppings (guano) and seawater had an impact on the porewater chemistry since most elements were enriched near the top of the profile.

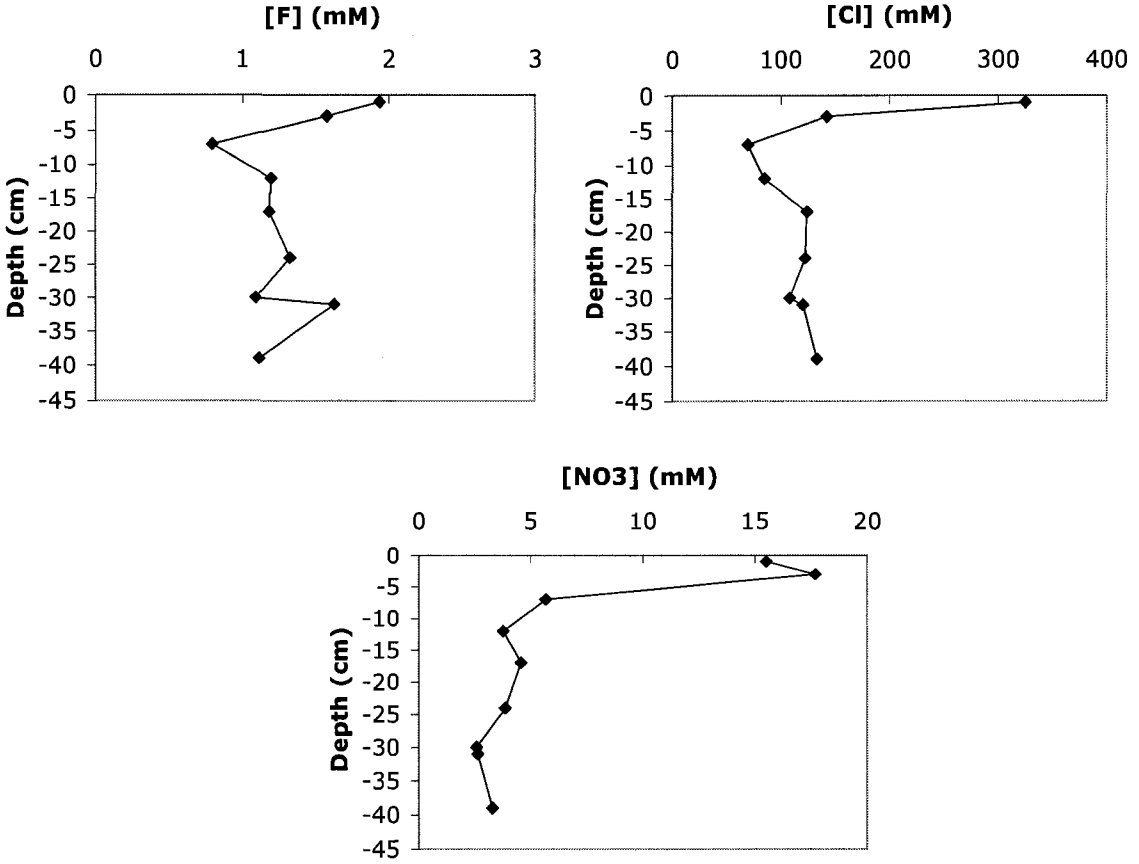


Figure 3.4: Fluoride, chloride and nitrate concentrations in the soil porewaters

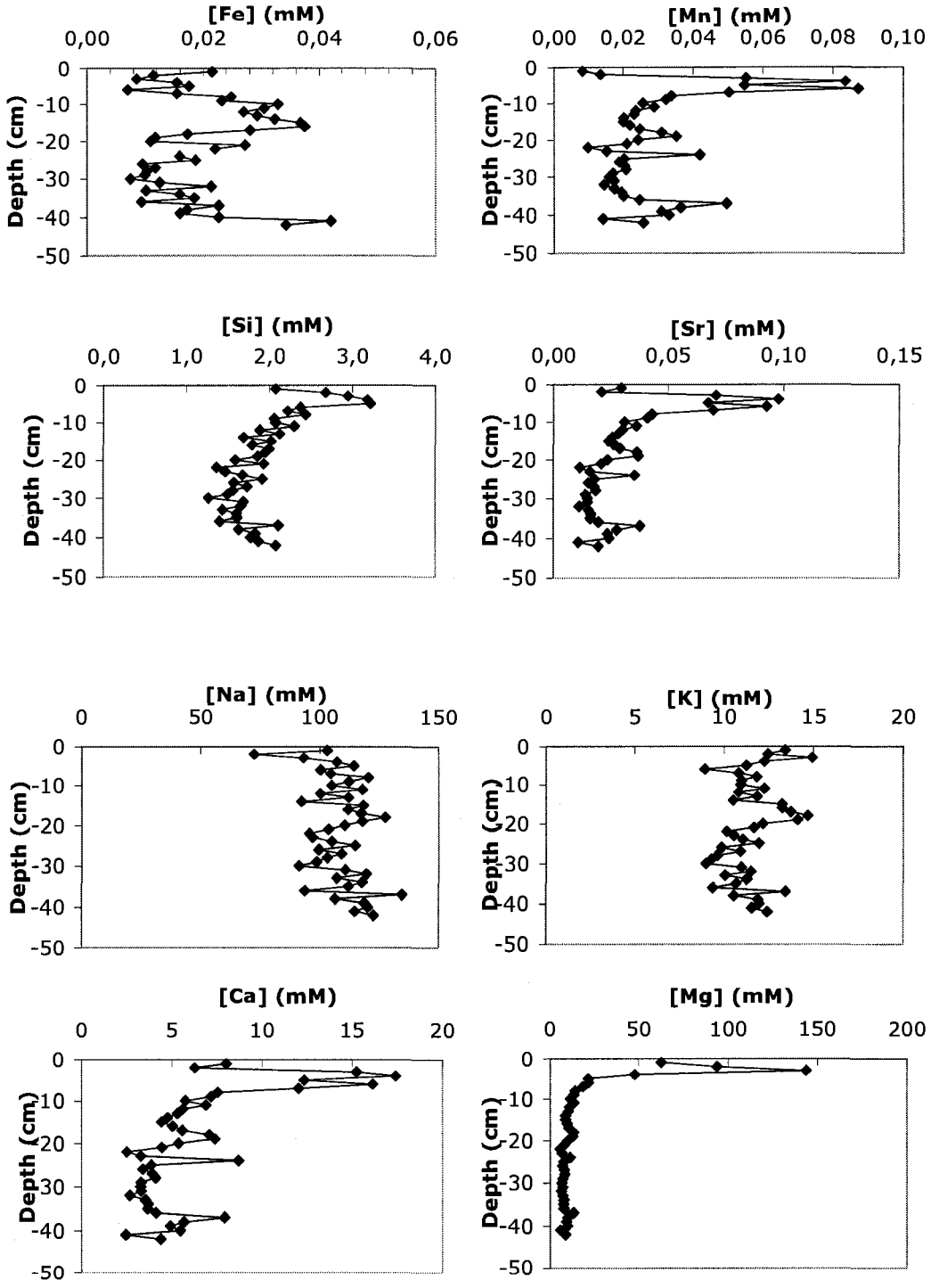


Figure 3.5: Concentration profiles of the major cations in the porewaters (Fe, Mn, Si, Sr, Ca, Mg, Na and K)

3.1.4. Dissolved organic carbon

Dissolved organic carbon (DOC) levels were very high in the soil porewaters (Figure 3.6). Concentrations varied between 39 and 130 mM with an increase at the top of the profile, between 15-20 cm and below 40 cm (Figure 3.6). As mentioned before, the top soil layers were clearly impacted by penguin guano.

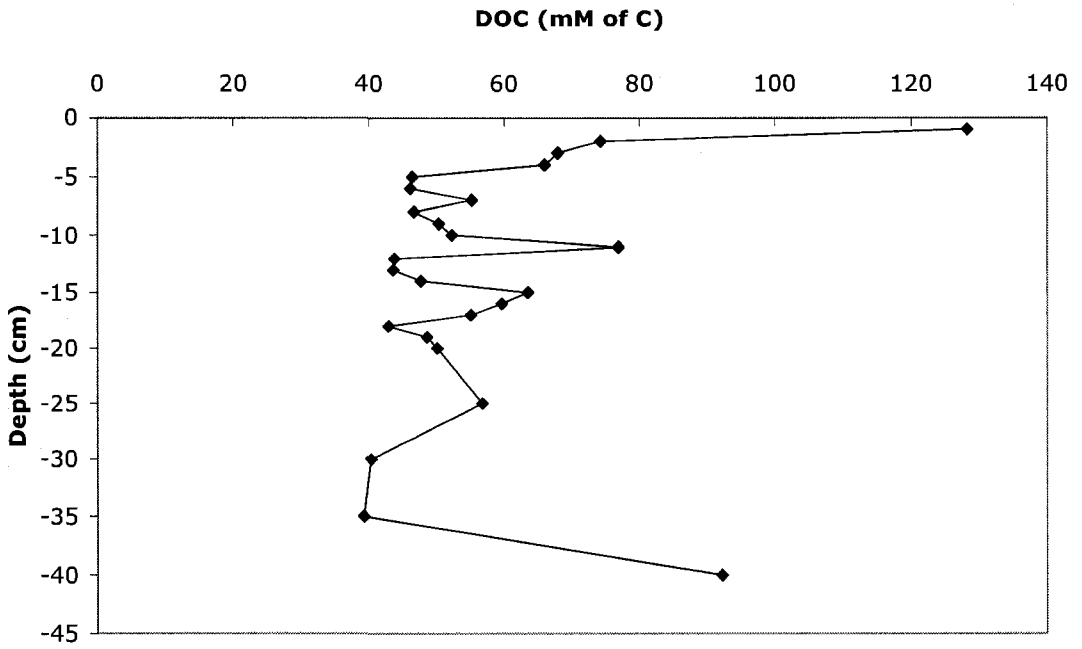


Figure 3.6: Dissolved organic carbon (DOC) concentrations in the porewaters of the soil profile.

3.1.5 Phosphate speciation

Phosphate speciation in the porewaters was carried out with the software Mineql+. The results indicate that under the conditions prevailing in the soil, phosphate existed as either aqueous species of $CaHPO_4$ and $MgHPO_4$ or as $H_2PO_4^-$ and HPO_4^{-2}

(Table 3.1). The porewaters at the top of the profile (1-3 cm) were clearly influenced by Mg-phosphate soluble complexes whereas the porewaters below 3 cm were essentially composed of the soluble $H_2PO_4^-$ complex. The Ca-rich phosphate complex was not present below 20 cm of depth.

Table 3.1: Phosphate speciation in the porewaters of the soil profile

Depth (cm)	**Soluble phosphate species (%)**			
	$CaHPO_4$ aq	$MgHPO_4$ aq	$H_2PO_4^-$	HPO_4^{-2}
1	2.70	28.30	59.20	9.80
2	2.10	39.20	47.60	11.00
3	2.40	28.90	63.00	5.60
4	1.70	6.20	89.80	2.30
5	2.00	4.70	90.00	3.10
6	1.20	2.20	94.10	2.30
7	1.90	4.00	91.10	3.00
8	1.40	3.60	91.50	3.40
9	1.80	4.70	88.80	4.60
10	1.30	3.60	91.00	4.00
15	1.50	4.00	88.20	6.20
20	1.60	4.20	88.50	5.50
25	0.00	1.40	95.60	2.40
30	0.00	1.50	95.30	2.50
35	0.00	1.90	94.20	3.20
40	0.00	1.30	96.40	1.70

3.1.6 Saturation calculations (Mineql+)

Porewater data were also used to calculate (with Mineql+) the saturation indices of various phosphate-rich minerals (Table 3.2). The calculations indicated that the surface porewaters (1 to 3 cm) were saturated with respect to the following minerals: $Ca_4(OH)_2(PO_4)_2$, Ca_2OHPO_4, $Ca_3(PO_4)_2$, $Ca_5OH(PO_4)_3$, strengite, $CaHPO_4$, $Ca_4H(PO_4)_3$, $FePO_4$ and $Mg_3(PO_4)_2$ (Table 3.2). All calculations used ferric iron (Fe(III)) as the dominant soluble Fe species because ferrous iron was not determined. As a result,

ferrous-containing minerals rich in phosphate are not included. Porewaters below 3 cm of depth were under-saturated with respect to several minerals, including $Ca_3(PO_4)_2$, $CaHPO_4$, $Ca_4H(PO_4)_3$, and $Mg_3(PO_4)_2$ (Table 3.2). Near the bottom of the profile (i.e., <30 cm), the porewaters were also under-saturated with respect to $Ca_5OH(PO_4)_3$ (hydroxylapatite).

3.2. Solid phase geochemistry

3.2.1. Soil water content

The water content of the soil in the profile varied between 8 and 18% and increased near the top of the profile (Figure 3.7).

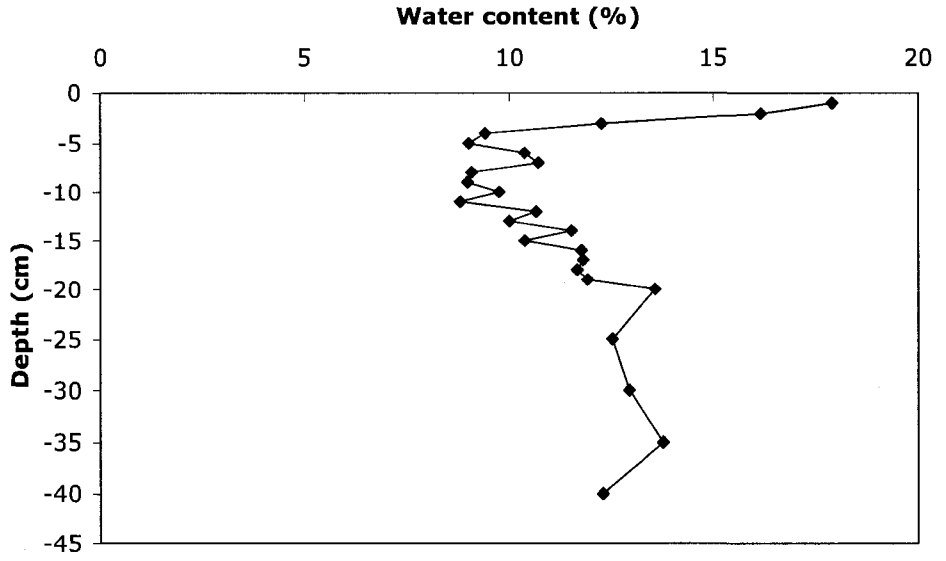


Figure 3.7: Water content (in percentage) of the soil in the profile

Table 3.2: Saturation indices* of various phosphate-rich minerals in the soil profile

Depth (cm)	Minerals								
	$Ca_4(OH)_2(PO_4)_2$	Ca_2OHPO_4	$Ca_3(PO_4)_2$	**$Ca_5OH(PO_4)_3$	strengite	$CaHPO_4$	$Ca_4H(PO_4)_3$	$FePO_4$	$Mg_3(PO_4)_2$
1	24.88	16.46	1.85	8.23	5.79	1.07	1.65	5.19	3.98
2	25.74	16.89	2.58	9.40	6.11	1.38	2.69	5.51	5.48
3	25.26	16.64	2.59	9.17	6.10	1.63	2.95	5.50	4.91
4	22.22	15.13	0.39	5.44	6.04	0.94	0.06	5.44	1.18
5	22.25	15.14	0.17	5.24	5.77	0.71	-0.38	5.17	0.38
6	20.83	14.43	-0.80	3.56	5.37	0.45	-1.62	4.77	-0.93
7	22.00	15.01	-0.02	4.92	5.73	0.65	-0.65	5.13	0.03
8	21.47	14.75	-0.48	4.20	5.68	0.45	-1.30	5.08	-0.17
9	22.16	15.10	-0.06	4.97	5.62	0.52	-0.81	5.02	0.24
10	21.30	14.67	-0.72	3.88	5.73	0.30	-1.68	5.13	-0.34
15	21.89	14.96	-0.38	4.52	5.84	0.34	-1.30	5.24	0.01
20	21.98	15.00	-0.28	4.65	5.24	0.39	-1.16	4.64	0.01
25	19.01	13.52	-2.30	1.15	5.56	-0.14	-3.70	4.96	-1.89
30	18.87	13.35	-2.52	0.86	5.09	-0.29	-4.07	4.49	-2.07
35	19.47	13.75	-2.10	1.58	5.41	-0.17	-3.54	4.81	-1.63
40	18.75	13.39	-2.42	0.90	5.55	-0.13	-3.83	4.95	-2.14

*: S.I. values <1 indicate that the porewaters are under-saturated and the minerals, if present, will undergo dissolution. S.I. values >1 indicate that the solution is super-saturated and the minerals will precipitate.

**: known as hydroxylapatite

3.2.2. Organic matter content (LOI)

The organic matter content of the soil was estimated by loss on ignition (LOI). The levels were generally low and varied from 1.5 to 8% with a net increase near the surface of the soil and around 5-10 cm and 15-20 cm (Figure 3.8).

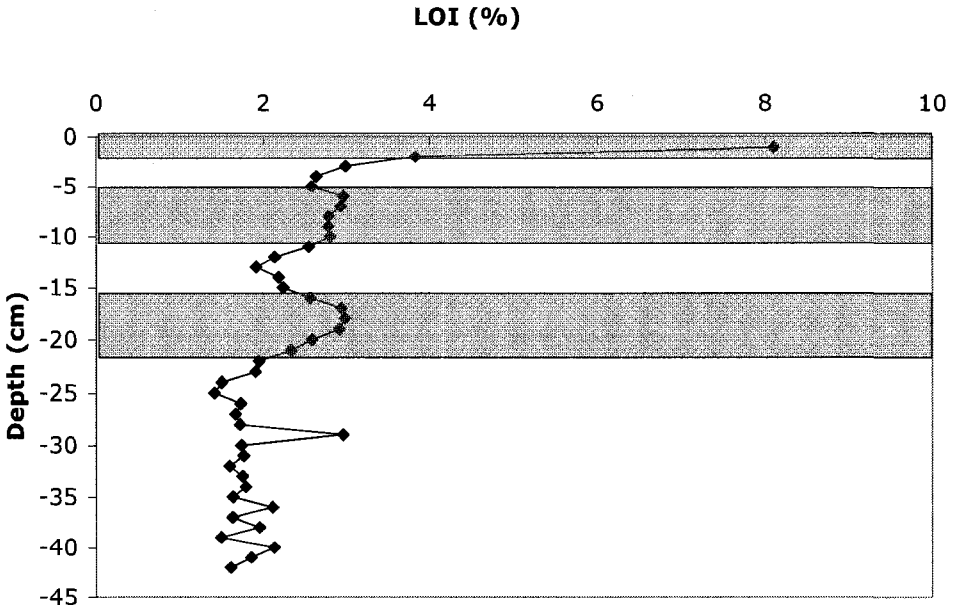


Figure 3.8: Organic matter content (as measured by loss on ignition (LOI)) of the soil. Shaded areas indicate the presence or organic-rich layers in the soil.

3.2.3 Total iron content

The concentration of total Fe (corresponding to iron present in oxides, phosphate, sulfide and silicate minerals) in the soil varied between 0.32 and 0.55 mM/g dry wt. and was fairly constant throughout the profile with the exception of a slight increase near 30-35 cm (Figure 3.9).

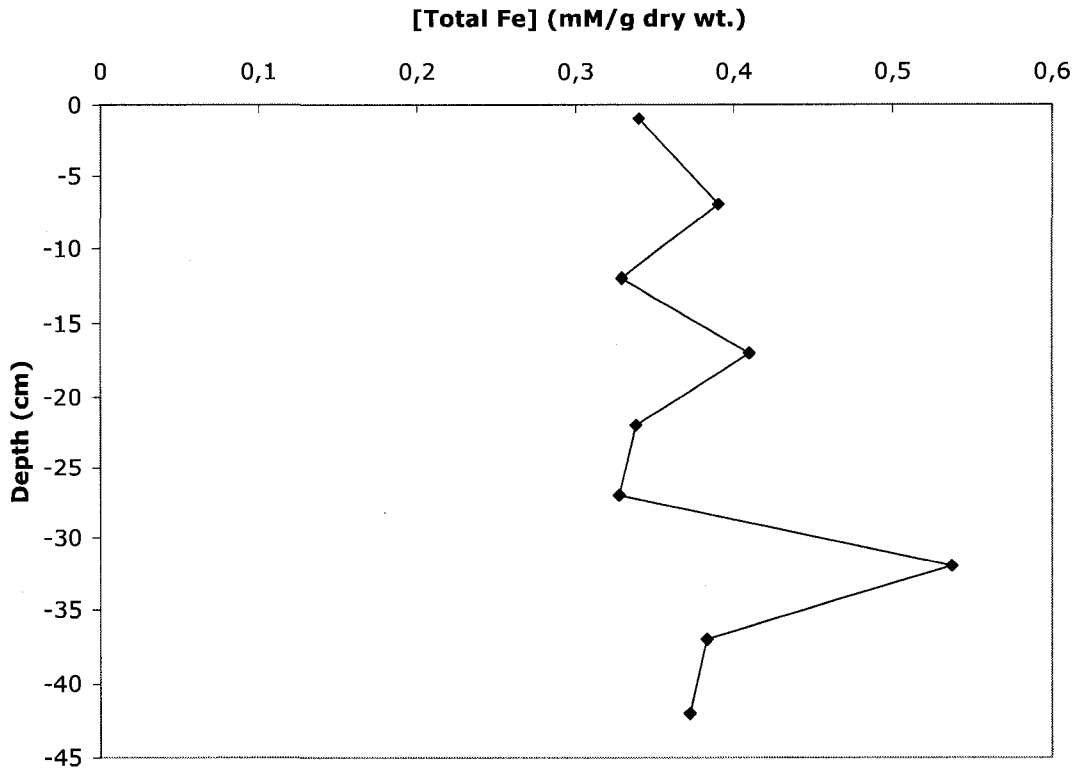
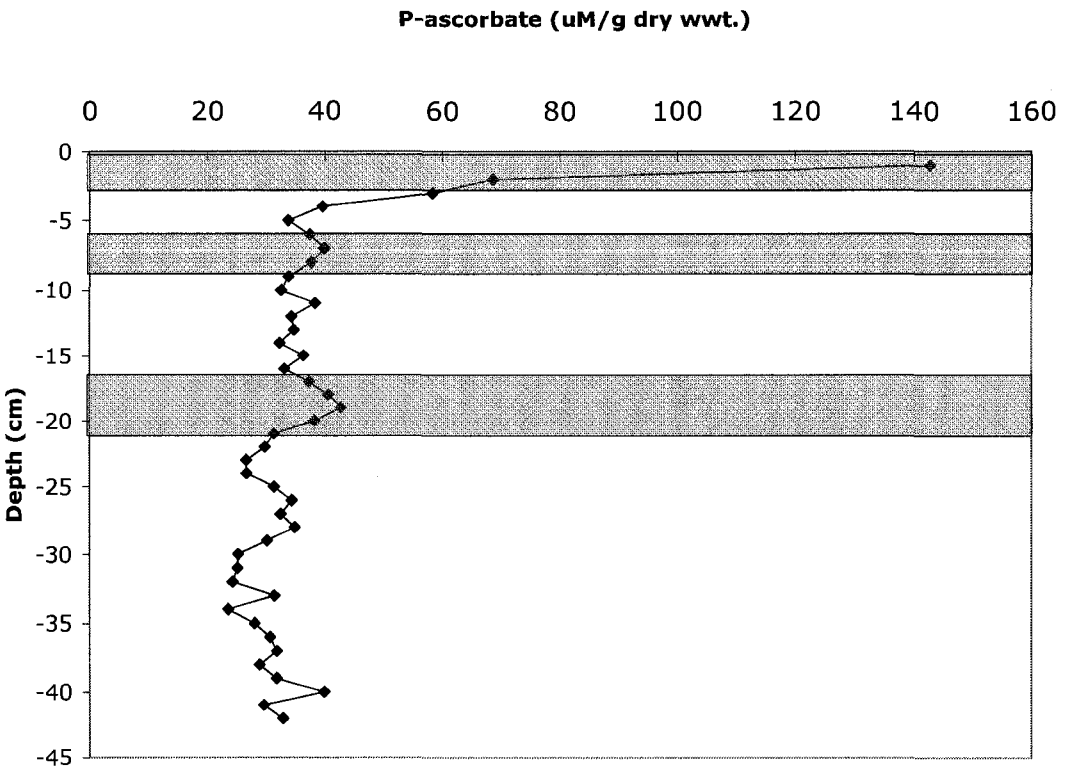


Figure 3.9: Total iron concentration of the soil as a function of depth

3.2.4. Ascorbate extractable iron and phosphorus

Based on the extraction scheme used here (Rozan et al., 2002), ascorbate extractable P corresponds to reactive phosphate, whereas ascorbate extractable Fe is ascribed to amorphous iron (such as iron oxides), but it may contain some Fe from sulfides. Reactive phosphorus was fairly constant with depth but there was a net enrichment near the top of the profile (Figure 3.10). Two other layers were also slightly

enriched, i.e., 6-9 cm and 14-21 cm. The high reactive phosphate content at the top of the profile reflects the accumulation of guano on the soil. Penguin droppings were



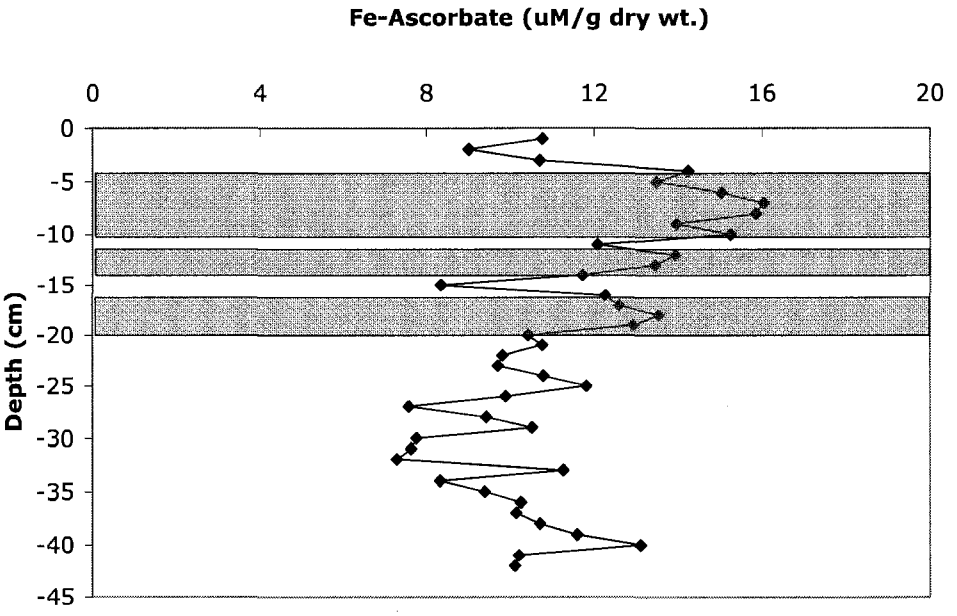


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Figure 3.10: Ascorbate extractable phosphorus and iron fractions as a function of depth. Shaded areas indicate enrichment layers in the soil.

found to contain about 167 uM/g dry wt. of ascorbate extractable phosphorus, which is slightly higher than the content measured in the top part of the soil. Amorphous Fe levels were lower than those measured for reactive phosphate and ranged between 7 and 16 uM/g dry wt. (Figure 3.10). Three enrichment zones were present in the profile, i.e., between 4 and 10 cm, 12 and 14 cm and 16 and 20 cm (Figure 3.10). The ascorbate extractable Fe in guano (around 4 uM/g dry wt.) was however lower than the levels measured in the soil.

3.2.5 Crystalline iron

The crystalline iron fraction was obtained from subtracting the Fe-ascorbate fraction (i.e., amorphous iron) from the Fe-dithionite fraction. The fraction refers to Fe from poorly ordered and crystalline iron oxides and some trace amounts from silicates (Rozan et al., 2002). The soil samples in the profile contained between 39 and 82 uM/g dry wt. of crystalline Fe (Figure 3.11). One net accumulation zone was present between 11 and 17 cm.

3.2.6. Molar Fe(II) / Fe(III) ratio

The molar Fe(II)/Fe(III) ratio (based on the Kostka et al. Method (1994) is shown in Figure 3.12. Three different Fe(II) enrichment zones were present in the soil, i.e., at the surface of the profile, between 5 and 10 cm and 15 an 23 cm.

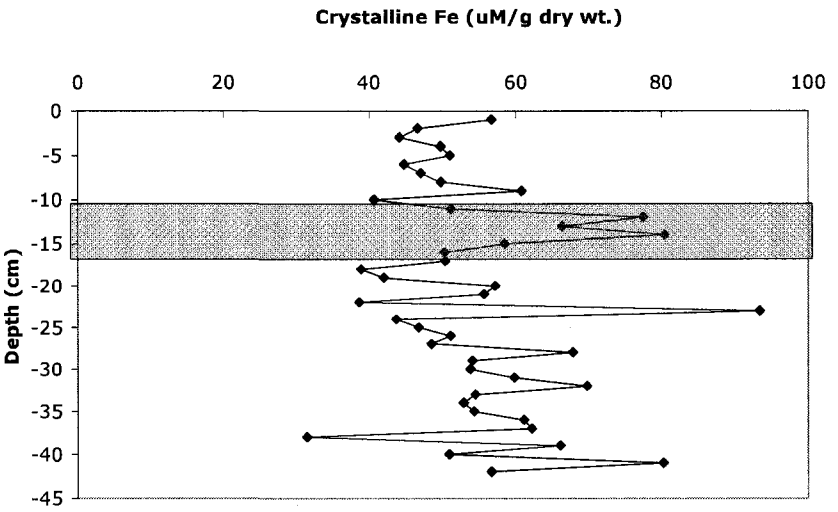

Figure 3.11: Crystalline iron fraction as a function of depth. The shaded area corresponds to a net crystalline Fe-enriched layer in the soil.

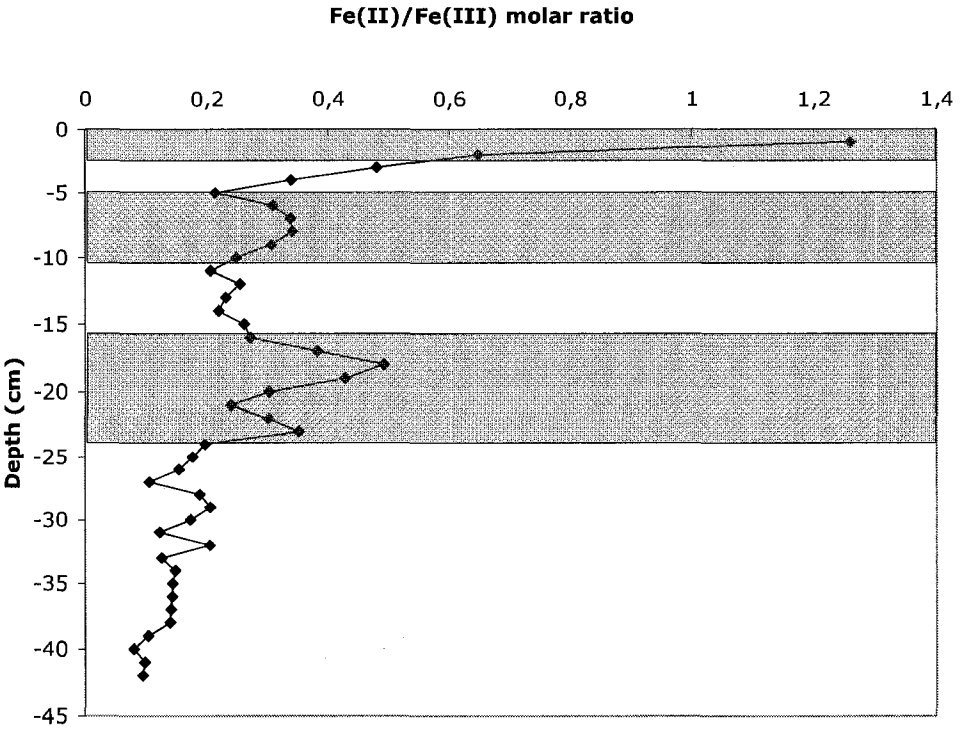

Figure 3.12: Molar Fe(II)/ Fe(III) ratio in the soil. Shaded areas represent soil layers where Fe(II) was enriched.

3.2.7. Acid Volatile Sulfur (AVS) and Chromium Reducible Sulfur (CRS)

The soil samples contained very low levels of AVS (0.11-1.13 uM of S/g dry wt.) and CRS (0.29-11.98 uM of S/g dry wt.) (Figure3.13). AVS and CRS concentrations were depleted between 7 and 15 cm and around 30 cm. S-35 injection did not show any activity of sulfate-reducing bacteria (sulfate reduction rates were equal to zero).

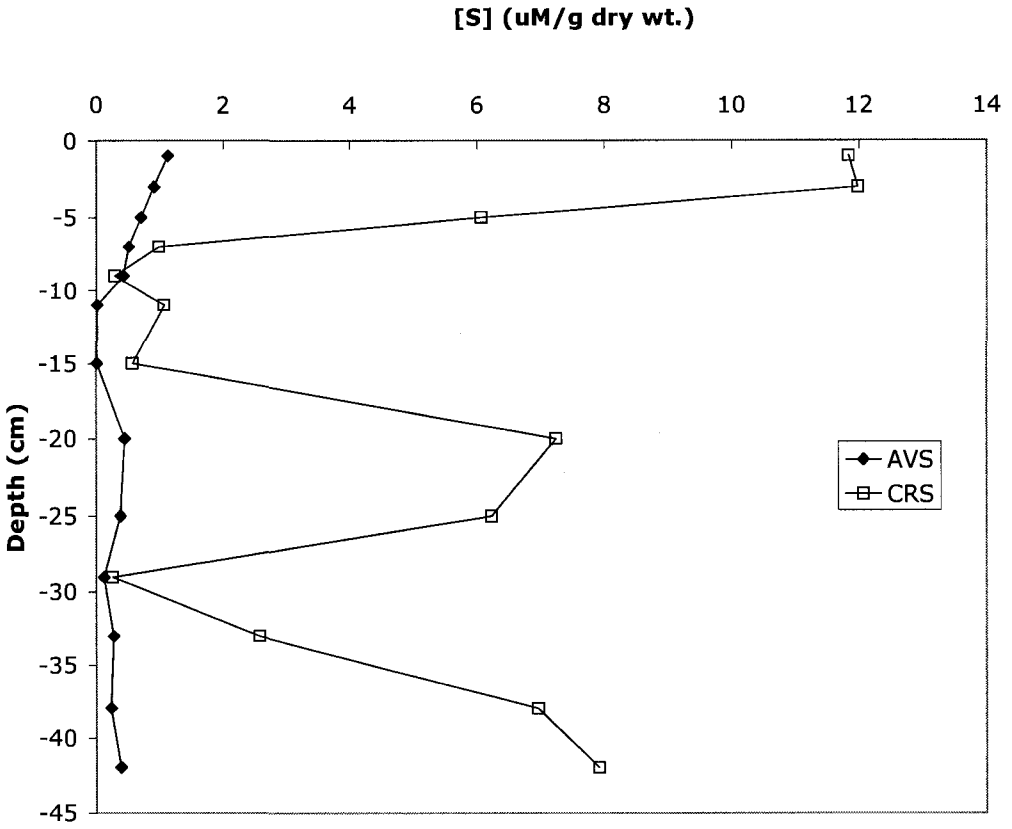

Figure 3.13: Abundance of acid volatile sulfur (AVS) and chromium reducible sulfur (CRS) as a function of depth in the soil profile.

3.2.8 Control soil.

Soil samples from the Chinese Great Wall research station in Antarctica were used as control soil in this study. All 3 samples were taken in an area not impacted by birds and penguin colonies. The soil samples were analyzed for their ascorbate extractable P and Fe contents, crystalline Fe content and Fe(II)/Fe(III) molar ratio (Table 3.3). Based on the results, the control soil contained very low levels of P- and Fe-

ascorbate when compared to the soil in the profile (Figure 3.11). The same trend is observed for the Fe-dithionite fraction, which is lower than that found in the soil in the profile (Figure 3.10). However, the Fe(II)/Fe(III) molar ratio was very similar to those found in the upper soil layers of Gardner Island (Figure 3.12).

Table 3.3 Geochemical composition of the control samples from the Great Wall research station in Antarctica.

Sample*	Fe-ascorbate (uM/g dry wt.)	P-ascorbate (uM/g dry wt.)	Fe-crystalline (uM/g dry wt.)	Fe(II)/Fe(III) (molar ratio)
1	0.41	0.31	0.85	0.89
2	0.44	0.47	0.90	0.46
3	0.44	0.56	0.91	1.06

*: n = 3 for each type of analysis and sample

3.3. Mineralogy

X-ray diffraction patterns from selected soil samples from the profile were almost identical (Figure 3.14). The soil contained quartz and various silicates. Only one phosphate-rich mineral could be partially identified, i.e., berlinite. strengite and vivianite, the most common iron-phosphate minerals found in natural soils and sediments, could not be identified. The X-ray pattern of the penguin guano obviously differed from the ornithogenic soil, as shown in Figure 3.14. Berlinite and frondelite were partially identified based on their most intense reflections. The penguin guano also appeared to contain some mica residues and silicates. Several reflections could not be positively ascribed to known minerals.

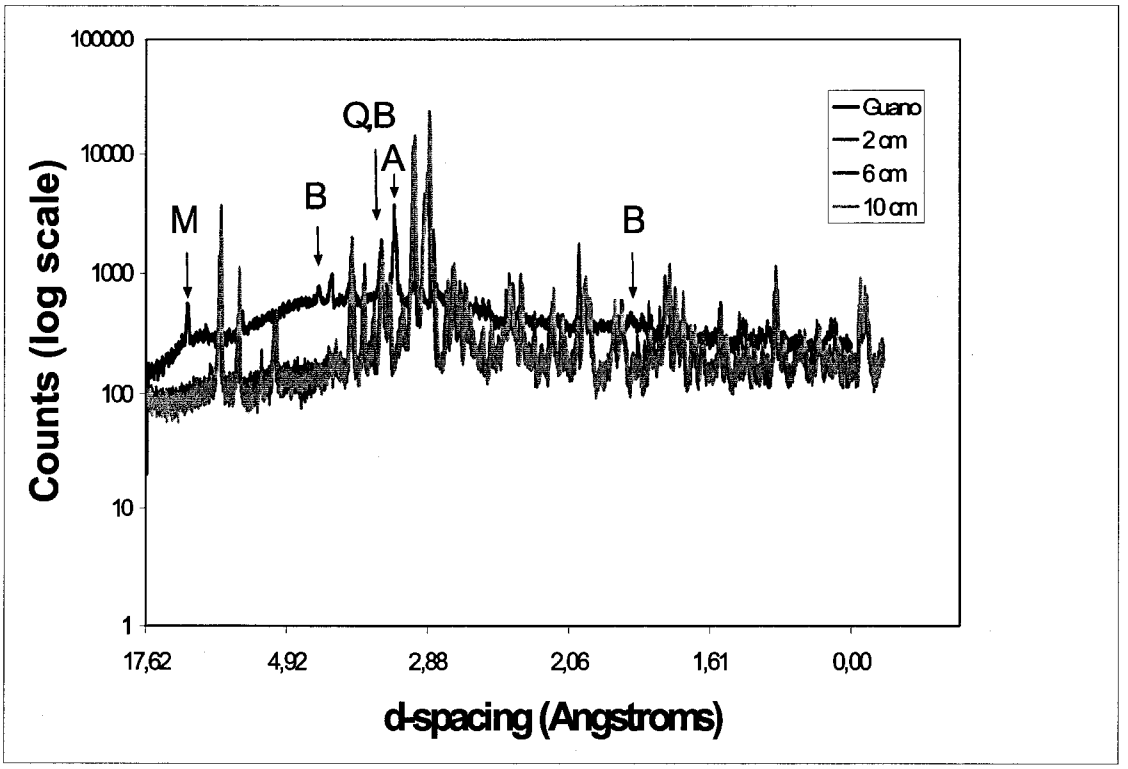

Figure 3.14: X-ray diffraction patterns of the penguin guano and soil within the profile (Q (quartz), B (berlinite), A (albite), M (muscovite), F (frondelite)).

3.4. Microbial iron reduction experiment

3.4.1. Physico-chemical conditions of the abiotic and biotic systems

Soil samples from selected depths (5-10 and 15-20 cm) in the profile were used in microcosm experiments to assess the abiotic and biotic reduction of Fe(III)-rich minerals in the ornithogenic soil. The bacterium *Shewanella putrefaciens* CN-32 was used as the iron reducer. The biotic system contained sterile soil and the iron-reducing bacterium whereas the abiotic systems were composed of sterile and non-sterile soil samples in order to determine if any *in situ* bacteria within the soil were active and capable of

reducing iron. All systems (in triplicate) were monitored over 9 days. pH and Eh conditions for all systems are shown in Appendix C. A slight pH increase was observed for all systems, but it was more pronounced in the biotic systems. The redox conditions fluctuated in the first few days, but all systems became slightly more reducing near the end of the experiment. The number of cells at the beginning and at the end of the experiments in the biotic systems remained stable, i.e., ~ 10_7 CFU/ml, which indicates that the cells were viable. In the biotic systems containing soil from the 5-10 cm interval, there was a clear release of soluble Fe when compared to the 2 abiotic systems (Figure 3.15). The abiotic non-sterile system did not experience any release of soluble Fe which indicates that there were no active iron-reducing bacteria in the soil.

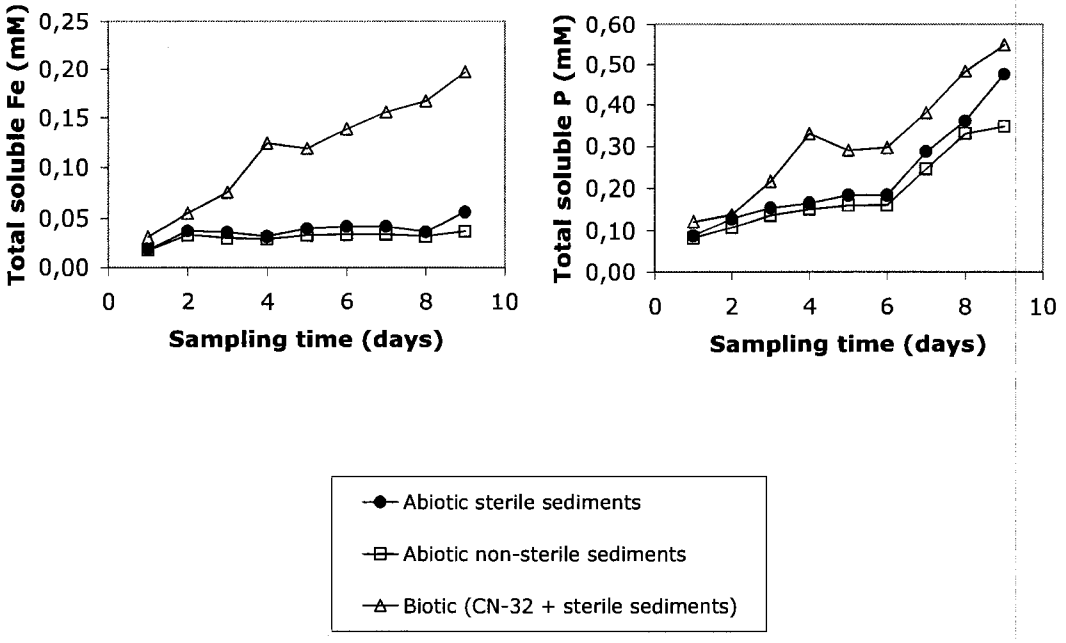

Figure 3.15: Average concentration (n = 3) of total soluble iron and phosphorus released over time in the 2 abiotic systems and the biotic system with *S. putrefaciens* CN-32 containing the soil from the 5-10 cm interval.

Soluble phosphorus increased over time in all systems, but the levels were higher in the biotic system than in the abiotic ones. This suggests that some phosphate-rich minerals were unstable in the chemically-defined medium and underwent dissolution in all systems. In the systems containing the soil from the 15-20 cm interval, very similar trends were observed for both total soluble Fe and P (Figure 3.16). The levels of soluble Fe and P at the end of the experiment were almost identical to those measured in the soil from the 5-10 cm interval.

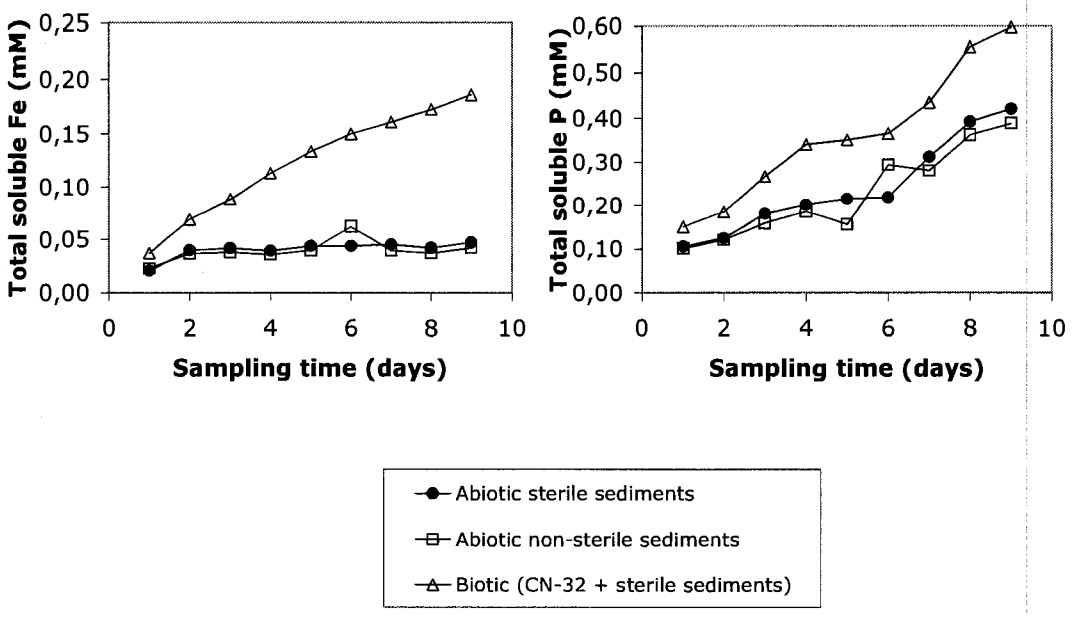

Figure 3.16: Average concentration (n = 3) of total soluble iron and phosphorus released over time in the 2 abiotic systems and the biotic system with *S. putrefaciens* CN-32 containing the soil from the 15-20 cm interval.

4. Discussion

4.1. Impact of penguin guano on the porewater and soil geochemistry and mineralogy

It is well known that penguin colonies play an important role in the transportation of marine-derived phosphorus to terrestrial ecosystems (Sun et al., 2004; Wang et al., 2007). Phosphorus derived from penguin guano is a major nutrient source for those ecosystems because the penguin diet mainly consists of Antarctic krill (up to 99%), which is enriched with phosphorus and fluorine (Sun et al., 2004). The accumulation of penguin droppings therefore directly influences the physical and chemical properties of the soil, and possibly the microbial activity (Zdanowski et al., 2004). The results from this study indicate that the ornithogenic soils from Gardiner Island were slightly acidic and had very high concentrations of dissolved organic carbon (DOC), P, K, Ca, Mg, Mn, Si, Sr, F, Cl and sulfate near the surface of the soil profile and another slight increase at a depth of 15-20 cm (Figures 3.2 to 3.6). Results from this study corroborate the findings of Michel et al. (2006) on ornithogenic cryosols. These authors showed that penguin droppings create an acidic environment and the soils are characterized by their high content of P, K, Ca, Mg and high Al availability. At Gardiner Island, the presence of high levels of Ca and Mg in the surface porewaters clearly influenced the speciation of phosphate (Table 3.1), compared to the deeper soil layers, where phosphate existed as hydroxo-complexes. Our results also indicate that the soluble phosphate concentrations measured at Gardiner Island are extremely high when compared to those generally found in sediments from freshwater and marine environments, i.e., 0.15-0.30 mM and 0.02-0.04 mM, respectively (Rozan et al., 2002). Similarly, sediments from the Dry Valleys of

Antarctica which were not impacted by sea birds communities were alkaline (pH of 7.8-9.8) and contained very low levels of total organic carbon (0-0.1%) (Gilichinsky et al., 2007). From those studies, it is clear that bird colonies strongly impact the porewater geochemistry of the soil where they nest and reside.

The slight increase of phosphate and other metals at a depth of 15-20 cm in the Gardiner Island soil profile is rather intriguing and suggests that the studied site might have been colonized in the past by penguin colonies. Tatur et al. (1997) and Sun et al. (2004) observed that the combination of Sr, P, Ca, Ba, F, S, Cu, Zn and Se is a geochemical biomarker for soils and sediments impacted by penguin colonies in Maritime Antarctica. Sun et al. (2004) also showed that P, Sr, Zn, F, Cu and Se concentrations in ornithogenic soils increased in the sub-surface (around 16 cm) and all elements showed a good correlation with each other. The same authors explained that the sampling site was an active past penguin colony but was later abandoned. It is clear that the site of our study is an active penguin colony at the present time (Figure 2.2), but slightly elevated concentrations of DOC, P, K, Ca, Mg, Mn, Si, Sr at a depth of 15-20 cm indicate that the same site might have been an active penguin colony in the past. It is however difficult to determine the time elapsed between the surface and the sub-surface soil deposition because no geo-chronological analyses were performed on the site. Our results also show that the pool of reactive Fe and P in the soils impacted by penguin colonies at Gardiner Island had increased amounts of ascorbate leachable fractions of Fe and P in comparison to soils from the control site (Chinese Great Wall research station) in Antarctica (25-145 uM/g dry wt versus 0.45 uM/g dry wt for ascorbate-P and 7-16 uM/g dry wt versus 0.4 uM/g dry wt for ascorbate-Fe). The large pool of ascorbate

leachable phosphorus in the surface and sub surface soils can be attributed to the bird colonies, but the iron ascorbate leachable iron fraction is obviously dependent on the mineralogy of the soil the control site, which is related to the local bedrock geology. Unfortunately, no information was provided by Prof. Sun of USTC, regarding the local geology of the control site.

The impact of the bird colonies was also seen in the P/Fe molar ratio (>1) of the soil, which greatly exceeded the ratio generally measured in marine sediments, i.e., 0.01 to 0.20 (Hyacinthe and Van Cappellen, 2004). According to the same authors, the variability of P/Fe ratio depends on the amount of phosphate co-precipitated with Fe(III)-hydroxides, which in return depends on the crystallinity of the Fe solid mineral phases and components of the solution. Their study reported that P/Fe molar ratios decrease with depth due to re-crystallization of Fe (III)-rich solid minerals, which lowers phosphate adsorption. The high and constant P/Fe (i.e., > 1) molar ratio in our study first indicates that precipitation of iron phosphate minerals within the soil dominated over phosphate sorption onto Fe-rich minerals. Second, the constant P/Fe ratio with depth points to the fact that the minerals did not re-crystallize. Vivianite is often reported as being the most common iron phosphate mineral forming in anaerobic sediments (Hyacinthe and Van Cappellen, 2004), but according to our results, it could not be identified by X-ray diffraction (Figure 3.14). The absence of vivianite could be real but one must keep in mind that its abundance in the soils might have been very low and below the detection limit of X-ray diffraction. Saturation index calculations (Table 3.2) did not indicate the presence of vivianite because the concentration of soluble Fe(II) in the porewaters was not measured at the time of sampling in Antarctica (the soil samples

were shipped to Ottawa after being collected, which prevented the analysis of redox-sensitive species). The porewaters were however saturated with respect to strengite and amorphous $FePO_4$, but they could not be identified by X-ray diffraction (Figure 3.14). Our results indicate that the high P/Fe molar ratio of the ornithogenic soils from Gardiner Island reflects the presence of several Fe-phosphate minerals, not only vivianite, as often reported in marine and freshwater sediments (Hyacinthe and Van Cappellen, 2004).

Our results also revealed that iron was present as a reduced and oxidized component of the soil (Figure 3.12) and that the Fe(II)/Fe(III) molar ratio increased at the surface of the profile and at a depth of 15-23 cm. The presence of large quantities of Fe(II) at the surface of the profile is not surprising since the surface soils are essentially composed of fresh penguin droppings. Bird guano enriched in Fe(II) originates from the anaerobic conditions prevailing in their digestive tracks. It is also possible that the large concentrations of DOC and phosphate in the porewaters stabilized the reduced iron and prevented it from being oxidized. Interestingly, the increase of soluble Fe(II) between 10 and 20 cm corresponded to an increase of soluble phosphate in the porewaters (Figure 3.3) around the same depth and to an enrichment of organic matter in the solid phase of soil (Figure 3.8) and in the porewaters (Figure 3.6). It also coincided to increased concentrations of ascorbate extractable phosphorus (Figure 3.10). These results suggest that iron reduction prevailed at that depth, leading to depleted levels of Fe(III) in the soil. Hyacinthe and Van Cappellen (2004) suggested that iron phosphate minerals may be a kinetically efficient terminal electron acceptor for iron reducers. They observed that iron reducing bacteria from *Geobacteraceae* family and *Shewanella putrefaciens* were able to respire on Fe(III) phosphate minerals. However, complete microbial reduction is often

hindered by the sorption of dissolved Fe(II) ions onto the surface of Fe-phosphate minerals (Roden and Zachara, 1996). The microbial diversity of the soils of Gardiner Island is not known, but recent work by Zdanowski et al. (2004) showed that penguin guano contains a wide range of bacterial species, including actinobacteria and firmicutes, which are known to respire on Fe(III)-minerals. Abiotic iron reduction by reduced sulfur species is unlikely here because our result revealed that sulfate-reducing bacteria were not active and that the soils contained little acid-volatile sulfides (Figure 3.13).

Finally, soils impacted by bird guano host various phosphate minerals rarely identified in non-impacted soils (Landis and Craw, 2003). These authors identified potassium bearing phosphate minerals known as taranakite ($K_3Al_5(HPO_4)_6(PO_4)_2*18(H_2O)$) and leucophosphite ($KFe(III)_2(PO_4)_2(OH)*2(H_2O)$) in sediments impacted by sea bird colonies. Zhu et al. (2006) also showed that the interactions between penguin droppings and weathered rocks resulted in the production of secondary phosphates such as struvites ($Mg(NH_4)PO_4*6H_2O$, fluorapatites ($Ca_5(PO_4)_3F$ and brushites ($CaHPO_4*2H_2O$). Aluminum phosphate minerals were also reported by Tatur and Kreck (1990). Apatite or hydroxylapatite ($Ca_5OH(PO_4)_3$) is often a dominant mineral in soils amended by penguin guano because Al-Fe phosphate minerals containing some potassium tend to transform into Ca-phosphates over time (Tatur and Myrcha, 1989). The mineralogical composition of the sediments and penguin guano studied here was difficult to assess because of the complexity of the X-ray diffraction pattern (Figure 3.14). Berlinite ($AlPO_4$) and frondelite ($(Mn(II)Fe(III)_4(PO_4)_3(OH)_5)$) were likely present in the guano, but they could not be identified in the soil samples. Likewise, the porewaters were saturated with respect to $Ca_4(OH)_2(PO_4)_2$, Ca_2OHPO_4,

$Ca_5OH(PO_4)_3$, $CaHPO_4$, $Ca_4H(PO_4)_3$ and $Mg_3(PO_4)_2$, but these minerals were not identified by X-ray diffraction. It is clear that the mineralogical composition of the soil needs to be further investigated and that other analytical techniques, including microprobe analysis and energy dispersive spectroscopy, should be combined to fully assess the complex composition of the soil.

4.2. Stability of Fe-phosphate minerals

Results from the geochemical analysis of the soil and porewaters on Gardiner Island pointed to the fact that iron reduction occurred in the soil, as mentioned in section 4.1. These results prompted us to assess the stability of the iron-phosphate minerals present in the soil under microbial iron reduction conditions. Our results showed that in the presence of *S. putrefaciens* CN32, soluble iron and phosphate concentrations increased over time (Figures 3.15 and 3.16), along with the pH (see appendix C). However, soluble phosphorus was also released over time in the abiotic systems, suggesting that non iron-rich phosphate minerals underwent dissolution in the growth medium used in the study. Based on the ascorbate extraction results (Figure 3.10), there is an excess of phosphorus over iron, which clearly indicates that non iron-rich phosphate minerals are present in the ornithogenic soil. Based on our microcosm results, we can conclude that *S. putrefaciens* CN-32 successfully reduced Fe(III) phosphate minerals and/or Fe(III) hydroxides with sorbed phosphate. However, the similar quantities of both Fe and P released into solution in the biotic system suggest that iron-phosphate minerals were primary reduced, not iron oxides. This is in agreement with Hyacinthe and Van Cappellen (2004), who stated that Fe(III)-phosphate minerals could potentially be used

as terminal electron acceptors during microbial iron reduction. In addition, our extraction results revealed that the pool of amorphous iron (ascrobate Fe) (Figure 3.10) was much smaller than the pool of crystalline Fe (Figure 3.11), which indicates that the iron oxides present in the soil were not easily reducible (Roden and Zachara; 1996). These authors demonstrated that crystalline iron oxides do not undergo microbial iron reduction as easily as amorphous iron oxides.

Zones of potential microbial iron reduction in the soil of Gardiner Island infer that anaerobic conditions must prevail in the soil from time to time. Pictures from the sampling site clearly show that the water table was at the bottom of the profile when the soils were sampled (Figure 3.2), but closer inspection of the soil profile shows that the color of the soil is not uniform, some layers are lighter than others. This change of color suggests that the soils from certain depths might be prone to mineral dissolution, as often observed in the A horizon of soil profiles. According to Chacon et al. (2005), water table fluctuations can affect the composition of soils, microbial activity, availability of nutrients and mineral dissolution. Given the fact that the soil is covered in snow during the colder months on Gardiner Island, it is very likely that the water table fluctuates as a result of snow melt. An elevated water table would then create anoxic conditions affecting the P and Fe cycle in the flooded soil. Reducing conditions would stimulate the activity of iron-reducing bacteria and promote the subsequent reduction of Fe(III)-rich minerals, including iron-phosphate minerals, as shown in our microcosm experiments. According to Chacon et al. (2005), hydrolysis and dissolution of Fe and Al phosphate minerals and the release of clay-associated phosphate minerals could also account for the release of phosphorus under flooded conditions.

5. Conclusion

The present study was conducted to better understand the impact of phosphate-rich penguin guano on the biogeochemistry of iron in permanently cold ornithogenic soil from Gardiner Island, Antarctica. Geochemical analyses revealed that the Gardiner Island soil amended with penguin guano was indeed enriched in phosphate (in both the soluble and solid phases) and hosted a wide range of phosphate-rich minerals. Chemical extraction results also indicated that within the soil profile existed potential zones of microbial iron reduction. Seasonal water table fluctuations were likely responsible for the local development of reducing conditions within the soil profile.

This study however only provides a glimpse of the complex biogeochemical reactions taking place in the soil because we only analyzed one soil profile collected after snow melt. It is clear that further analysis is needed to assess the role of penguin guano on iron and phosphorus cycling in such environments. For instance, more soil profiles should be studied in order to determine if the effect of guano amendment on the mineralogy and geochemistry of the soil is widespread and uniform on the island. In addition, a better control site should be selected, in order to minimize the bias introduced by the local geology. Finally, further studies should include the analysis of reduced soluble Fe and S species on site and the diversity of the microbial populations, especially those involved in iron cycling.

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Appendix A: Preparation of the chemically defined medium (CDM)

Table A-1: Stock solution1, 100x concentrated. Stored at 4° C in the dark.

Compound	MW ($gmol^{-1}$)	Mass added (g)	Concentration (M)
NH_4Cl_2	53.49	11.76	2.2×10^{-3}
KCl	74.56	8.95	1.2×10^{-3}

Table A-2: Stock solution 2, 100x concentrated. Stored at 4° C in the dark.

Compound	MW ($gmol^{-1}$)	Mass added (g)	Concentration (M)
$NaH_2PO_4 \cdot H_2O$	137.99	53.82	3.9×10^{-3}

Table A-3: Stock solution 3, 100x concentrated. Stored at 4° C in the dark.

Compound	MW ($gmol^{-1}$)	Mass added (g)	Concentration (M)
PIPES buffer	335.37	151.0	4.5×10^{-3}

Table A-4: Trace elements stock solution, 1000x concentrated. Stored at 4° C in the dark.

Compound	MW ($gmol^{-1}$)	Mass added (g)	Concentration (M)
Nitrilotriacetic acid	257.10	18.30	7.1×10^{-5}
$MgSO_4 \cdot 7H_2O$	246.48	27.10	1.1×10^{-4}
NaCl	58.44	8.80	1.5×10^{-4}
$MnSO_4 \cdot H_2O$	169.01	4.60	2.7×10^{-5}
$ZnCl_2$	136.80	1.20	8.6×10^{-6}
$FeSO_4 \cdot 7H_2O$	278.02	0.89	3.2×10^{-6}
$CaCl_2 \cdot 2H_2O$	147.02	0.90	6.1×10^{-6}
$CoCl_2 \cdot 6H_2O$	237.93	0.90	3.8×10^{-6}
$Na_2MoO_4 \cdot 2H_2O$	241.95	0.23	9.3×10^{-7}
$NaWO_4 \cdot 2H_2O$	329.90	0.22	6.8×10^{-7}
$NiCl_2 \cdot 6H_2O$	237.70	0.22	9.1×10^{-7}
$CuSO_4 \cdot 5H_2O$	249.70	0.09	3.6×10^{-7}
$AlK(SO_4) \cdot 12H_2O$	474.40	0.09	1.9×10^{-7}
H_3BO_3	61.83	0.09	1.5×10^{-6}

Appendix B: BioRad Protein Assay Procedure

After the last cultivation step of *S. putrefaciens* in 100% CDM, the suspension was diluted through a 10x serial dilution series (10x, 100x, 1000x) using sterile CDM (100ul of inoculum +900ul of CDM). 800ul were then removed from each dilution set and 200ul of BioRad Protein Assay dye reagent was added. The solution was vortexed for 5 minutes after which the absorbance (at 595nm) was read with a spectrophotometer using pure CDM with BioRad dye reagent as a blank solution.

Appendix C: Microbial iron reduction experiments

Table C-1: Average pH in the systems containing the soil from the 5-10 cm interval.

Abiotic sterile		**Abiotic non sterile**		**Biotic**	
Days	pH	Days	pH	Days	pH
1	5.93	1	6.04	1	6.08
2	5.98	2	6.09	2	6.17
3	6.03	3	6.12	3	6.23
4	6.04	4	6.13	4	6.26
5	6.05	5	6.13	5	6.23
6	6.06	6	6.14	6	6.24
7	6.05	7	6.17	7	6.26
8	6.07	8	6.15	8	6.25
9	6.09	9	6.20	9	6.25

Table C-2: Average pH in the systems containing the soil from the 15-20 cm interval.

Abiotic sterile		**Abiotic non sterile**		**Biotic**	
Days	pH	Days	pH	Days	pH
1	5.91	1	6.01	1	6.04
2	5.96	2	6.06	2	6.16
3	5.98	3	6.06	3	6.22
4	5.99	4	6.09	4	6.24
5	5.99	5	6.08	5	6.22
6	5.99	6	6.08	6	6.24
7	6.01	7	6.12	7	6.31
8	6.08	8	6.14	8	6.39
9	6.02	9	6.13	9	6.40

Table C-3: Average Eh in the systems containing the soil from the 5-10 cm interval.

Abiotic sterile		**Abiotic non sterile**		**Biotic**	
Days	Eh (mV)	Days	Eh (mV)	Days	Eh (mV)
1	376	1	370	1	358
2	331	2	321	2	306
3	235	3	248	3	248
4	462	4	438	4	358
5	284	5	288	5	240
6	223	6	239	6	181
7	226	7	239	7	178
8	195	8	226	8	153
9	190	9	219	9	158

Table C-4: Average Eh in the systems containing the soil from the 15-20 cm interval.

Abiotic sterile		**Abiotic non sterile**		**Biotic**	
Days	Eh (mV)	Days	Eh (mV)	Days	Eh (mV)
1	372	1	368	1	347
2	325	2	322	2	279
3	242	3	255	3	234
4	447	4	433	4	327
5	279	5	278	5	204
6	230	6	245	6	177
7	230	7	244	7	178
8	211	8	239	8	145
9	206	9	226	9	149