THE EFFECTS OF GEOCHEMICAL CONDITIONS ON THE ESTABLISHMENT AND GROWTH OF MANGROVE SEEDLINGS IN ACID SULFATE SOIL ENVIRONMENTS

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ABSTRACT

Extensive excavation of mangrove soils for aquaqulture ponds without correct knowledge and technique leads to the disturbance of acid sulfate soils (ASS) due to exposure of iron sulfide to air. This condition has been recorded in many places, particularly in southeast Asia, where the disturbance of ASS generates numerous environmental problems, such as: poor soil and water quality, reduction of aquaculture production, death of vegetation and aquatic life, and more.

ASS is a stress factor that is responsible for the failure of some mangrove restoration projects. However, there is evidence that natural revegetation of mangroves has occurred in some abandoned ponds. Available published papers on the geochemical factors that affect the success or failure of rehabilitation in ASS areas are very few, and this makes it difficult to achieve successful rehabilitation. Geochemical studies of mangrove rehabilitation in ASS environments are essential, since different areas may have different geochemical conditions. Additionally, the interactions among geochemical factors in ASS environments are complex and can affect the response of mangrove seedlings.

The establishment of mangrove seedlings in ASS environments would deal with several potential problems, particularly acid conditions and high concentration of metals. This research focuses on the concentration of two major elements released in ASS environments aluminium (Al) and iron (Fe), and two mobile metals under ASS conditions nickel (Ni) and copper (Cu).

The main objective of this study is to evaluate various geochemical factors involved in ASS environments, which in turn influence the response of mangrove seedlings to ASS. This study also seeks to determine the accumulation and translocation of metals within mangrove seedling tissues in relation to the concentration of metals in the soils of various environments, and their relationship to the mangrove seedlings' establishment and growth.

To achieve these objectives, a laboratory study was conducted at the Aquaculture Laboratory at QUT. For comparison, a field study was conducted in abandoned aquaculture ponds situated in the Mare District, adjacent to the Gulf of Bone, South Sulawesi, Indonesia. The study species in the laboratorory trials was *Rhizophora stylosa*, and the species examined in the field study included mainly *R. stylosa* and *R. mucronata*.

The study used three replications in subsurface soils near roots, and in the surface layer for some variables. The variables examined include pH, pH_{fox}, redox potential, Titratable Actual Acidity (TAA), Titratable Potential Acidity (TPA), Titratable Sulfidic Acidity (TSA), water-soluble sulfate, HCl extractable sulfur (S_{HCl}) or KCl extractable sulfur (S_{KCl}), Peroxide Sulfur (S_P), Peroxide oxidisable sulfur (S_{POS}), pyrite, organic content, grain size, total metal and metal fractionation. The density, establishment and the growth of Rhizophoraceae were also determined.

Both the experimental and field study demonstrated that the general geochemical condition required by mangrove seedlings are: higher pH and pH_{fox}, and a reducing environment. Compared to the existing acidity (TAA) and other associated properties that count for the existing acidity level, such as water-soluble sulfate, extractable sulfur, exchangeable Al and Fe, the amount of potential acid (TPA and TSA) and pyrite on surface soils strongly correlated with the acidity, density, establishment and growth of the seedlings in the field study area. Higher amounts of potential acidity, including pyrite in surface soils, provided higher opportunities to oxidise in oxidative environments, which then release water-soluble sulfate, extractable sulfur, and exchangeable Al into subsurface soils, decrease pH and $pH_{fox,}$ and affect the density, establishment and growth of mangrove seedlings in the field study area.

The experimental study showed that the number of seedlings survived in non-ASS environment was higher compared to that in ASS environments. Lower sulfate and total extractable sulfur provided a good environment for mangrove seedlings to live under the non-ASS experimental environments. However, measured sulfur species as a single factor did not directly affect the density, establishment and growth of the seedlings in the field area. Sulfide correlates negatively to the establishment and growth of the seedlings. The type of environments (non-ASS and ASS) did not significantly affect the values of either the seedlings' total fresh length or their root length in the experiment at work. Mangrove seedlings can still grow and survive in high acidity but with lower values of density, establishment, and relative growth rate.

The concentration of metals in the environment influenced the concentration of metals in root tissues of *Rhizophora stylosa* seedlings. However, increasing concentration of metals (Fe, Al, Ni and Cu due to ASS disturbance in both experiment and field studies as well as addition of Ni and Cu in the experimental study did not increase BCF values. The selective mechanisms

are clearly shown in this study, where the seedlings tended to accumulate metals to certain amount based on their function and limited adsorption of non-essential metals. In regards to high levels of metals, mangrove seedlings regulated, retained metals mainly in their roots and employed an exclusion strategy, distributed them to aerial parts with low mobility and excreted them through leaf tissues.

The amount of potential acid (TPA and TSA) and pyrite in the surface soils strongly correlated with the acidity, density, establishment and growth of the seedlings. The presence of pyrite in surface soils allowed oxidation process to occur, which then enhanced the release of water-soluble sulfate, extractable sulfur, and exchangeable Al to subsurface soils, thus influencing the density and growth of mangrove seedlings. In contrast, the existing acidity (TAA) of both surface and subsurface soils, and associated existing acidity (water-soluble sulfate, extractable sulfur, exchangeable Al and Fe) in subsurface soils did not directly control the density, establishment and growth of the mangrove seedlings in the field study area. Exchangeable Al had a negative correlation with the establishment of the seedlings.

The free inundation of seawater produced an improvement in the soil quality of the study area, including higher pH (field and oxidisable), and low organic content. Free tidal inundation also generated low existing acidity, potential acidity and pyrite percentage on surface soils and reducing environments, therefore reducing the opportunity for pyrite to oxidise. Accordingly, the amounts of water-soluble sulfate, extractable sulfur and exchangeable Fe and Al on subsurface soils were low. Low organic material in these sites caused a low amount of S_P and S_{POS}. Furthermore, physical and geochemical factors, such as: pH, redox potential, grain size, sulfur species affected metal concentrations in both soils and roots. All these processes highlight the importance of tidal inundation in improving soil quality and providing a good environment, which results in higher density, establishment and relative growth of mangrove seedlings in mangrove restoration projects. Good water circulation also allows propagule supply, therefore enabling mangroves to establish naturally.

This study provides a better understanding of the response of mangrove seedlings under conditions of various ASS, high metal concentrations, and non-ASS environments, as well as a recommended best strategy for achieving successful restoration in similar conditions.

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ABBREVIATIONS

AASS	……..	Actual Acid Sulfate Soils
AAS	……..	Atomic Absorption Spectroscopy
Al	……..	Aluminium
AHD	……..	Australian Height Datum
ANCOVA	……..	Analysis of Covariance
ANOVA	……..	Analysis of Variance
ANZECC	……..	Australian and New Zealand Environment and Conservation New Zealand
APHA	……..	American Public Health Association
ARMCANZ	……..	Agriculture and Resource Management Council of Australia and New Zealand
ASS	……..	Acid Sulfate Soils
BCF	……..	Bioconcentration Factor
BCR	……..	Commision of the European Communities Bureau of Reference Council
Cu	……..	Copper
Eh	……..	Redox potential
Fe	……..	Iron
ICP-OES	……..	Induced Coupled Plasma – Optical Emission Spectroscopy
LC	……..	Lethal Concentration
LOI	……..	Loss on Ignition
Ni	……..	Nickel
PASS	……..	Potential Acid Sulfate Soils
PCA	……..	Principle Component Analysis
pH_{fox}	……..	Field Oxidisable pH
pH_{ox}	……..	Laboratory Oxidisable pH
POCAS	……..	Peroxide Oxidisable Combined Acidity and Sulfur
PVC	……..	Polyvinylcarbon
RGR	……..	Relative Growth Rate
S_{HCl}	……..	Hydrogen Chloride extractable Sulfur
S_{KCl}	……..	Kalium Chloride extractable Sulfur
SOB	……..	Sulfate Oxidizing Bacteria
S_P	……..	Peroxide Sulfur

S_{POS}	……..	Peroxide Oxidisable Sulfur
SRB	……..	Sulfate Reduction Bacteria
TAA	……..	Titratable Actual Acidity
TF	……..	Translocation Factor
TPA	……..	Titratable Potential Acidity
TSA	……..	Titratable Sulfidic Acidity

GLOSSARY

Acid Sulfate Soils	Soils or sediments that contain accumulated iron sulfides, mainly in the form of pyrite (FeS_2), in the upper layers of soils
Actual Acid Sulfate Soils	s that contain high sulfuric acid generated from oxidation of pyritic layer through drainage or disturbance
Adsorb	Taking up and holding a gas, liquid, or dissolved element in a thin layer of molecules on the surface of a solid substance
Apoplastic pathway	The route followed by water moving through plant cell walls and intercellular spaces (the apoplast)
Australian Height Datum	Mean sea level that is based on official tide gauges around the coastline
Bioaccumulation	A process by which elements are taken up by a plant from exposure to a contaminated environment (soil, sediment, water)
Bioavailable	A form of an element ready to be taken up by a plant
Bioconcentration Factor	The bioaccumulation of an element/substance by a plant from all possible routes, measured by the ratio of steady state concentration of a toxic substance in a plant relative to its environment
Cortex	The cells located between the epidermis and the vascular cylinder of a root tissue
Endodermis	A thin layer of parenchyma of root, located outside the vascular cylinder. It regulates the flow of water
Epidermis	The outermost layer covering the root of a plant
Iron-plaque	Deposits of iron (ferric) compounds which coat the surface of a root as a result of ferrous oxidation activity in the plant root and associated microorganisms
Jarosite	Yellow or brown hydrous iron sulfate mineral ($KFe_3(SO_4)_2(OH)_6$)
Leachate	Soluble constituent that is washed out from a mixture of soil solids
Lethal Concentration$_{50}$ (LC_{50})	A concentration of a compound that causes death in 50% or more of exposed seedlings

Parenchyma	Thin-walled cells of a root located between the epidermis and pericycle in a root constituting the cortex, and tissue specialised for food storage
Pneumatophora	Breathing roots that grow out vertically of the soil and maintain air routes during high tide
Porewater	The water that exists within soil grains
Potential Acid Sulfate Soils	Soils that contain pyrite that have not been oxidised or exposed to air
Propagule	Partially germinated seeds that grow out from the seed coat and the fruit prior to detaching from the plant
Pyrite	Pale-bronze or brass-yellow, isometric mineral: FeS_2; the major form of the sulfide minerals that spread widely
Rehabilitation	A program to recover ecosystem functions in a degraded ecosystem or habitat
Relative Growth Rate	Rate of plant growth that is measured based on the increase in plant height in a certain period
Remediation	A clean-up program or method used to remove hazardous materials from an area
Restoration	A program to return a former mangrove forest area to its original community structure, including the species and natural functions
Rhizosphere	A narrow zone surrounding the plant root where the biology and chemistry of the area is influenced by the exudation of compounds from root and microbial activity nearby
Salt gland	Organs that consist of several cells designed to excrete salt. They are located near the epidermis and covered by cuticle
Seedling	A very young plant grown from a propagule
Stele	The central part of the root consisting of the xylem and phloem together with supporting tissues
Translocation Factor	A measurement of the ratio of shoot to root concentration to assess the mobility of an element/substance

Turgor	The rigidity or fullness of a cell due to high water content as a result of differing solute concentrations between a semipermeable membrane

STATEMENT OF ORIGINAL AUTHORSHIP

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference has been made.

Signature:

QUT Verified Signature

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CHAPTER 1: INTRODUCTION

1. Background

The mangrove ecosystem has numerous important functions, such as providing physical barriers against waves (Tomasick et al., 1997, Alongi, 2002), supplying energy and organic matter to adjacent systems (Tomasick et al., 1997, Kathiresan and Bingham, 2001, Alongi, 2002), and providing habitats for many species of aquatic organisms (Tomasick et al., 1997, Alongi, 2002), as well as having economic value, such as fuelwood, construction, traditional medicine (Walters et al., 2008, Alongi, 2002). However, over-exploitation of this ecosystem for economic reasons have a severe negative impact on the environment.

In many countries, including Indonesia, Thailand, Vietnam, the Philippines, and Ecuador, the replacement of mangroves by aquaculture ponds is a major reason for mangrove loss (Stevenson, 1997, Saenger, 2002). The massive excavation of mangrove soils leads to the disturbance and air exposure of acid sulfate soils (ASS), which are soils or sediments that contain accumulated pyrite (FeS_2) (Fitzpatrick et al., 1998, Fitzpatrick, 2003, Fanning et al., 2010, Johnston et al., 2010a). Pyritic layer commonly occurs and stable particularly in low coastal areas, including in mangrove ecosystem, where iron, sulfate, and organic material are abundant (Fitzpatrick, 2003). Using these components, iron monosulfide (FeS) is generated and transformed into iron sulfide through a series of reactions that involve microorganisms (Berner, 1970, Benning et al., 2000, Burton et al., 2006, Kraal et al., 2013).

Disturbance of ASS generates sulfuric acid (Powell and Ahern, 2000, Fitzpatrick, 2003, Fanning et al., 2010), releases a huge amount of iron and aluminium, and elevates other metal levels (Fitzpatrick et al., 1998, Cook et al., 2000, Macdonald et al., 2004). This process removes dissolved oxygen from the water (Cook et al., 2000). Under low pH (< 4) and anoxic condition, ferric iron becomes an effective oxidant agent in oxidizing pyrite and the presence of *Thiobacillus ferooxidant* increases pyrite oxidation rate (Schippers and Jørgensen, 2002, Cook et al., 2004).

In addition, excessive concentration of iron in water resulting from acid sulfate soils can enhance blooms of toxic cyanobacteria such as *Lyngbya majuscula* (Muller, 2006). Acidity also damages infrastructure such as roads, concrete and steel pipes, drains, etc. that are constructed in ASS areas (Ahern and McElnea, 2000, Hicks et al., 2002). Drainage of peaty

acid sulfate soils can also produce a significant amount of greenhouse gases, such as carbon dioxide (CO_2) and nitrous oxide (N_2O) (Hicks et al., 2002). When acidic waters that contain high levels of soluble metals and deoxygenated waters flow into water systems, it may cause fish disease and reduction of fish and aquaculture production, as well as the death of vegetation and aquatic life (Dent, 1986, McDonald, 2007).

Disused aquaculture ponds resulting from ASS disturbance are reported in many places, particularly in southeast Asia, such as Indonesia, Vietnam and Cambodia (Stevenson, 1997). In Indonesia, around 70% of aquaculture ponds were affected by ASS from a total of 450,000 ha of aquaculture ponds (DJPB, 2011). These numbers illustrate the extent of the environmental issues faced by many countries.

Several physical and chemical methods have been identified and implemented to remediate and manage ASS impacts in these coastal environments. These include chemical neutralisation, forced oxidation and leaching, and seawater flushing (Sammut et al., 1999). However, since ASS remediation is time consuming and costly (Stevenson, 1997), these strategies are problematic, especially for developing countries. For instance, up to 90 tonnes of lime is required to neutralise one hectare of severe ASS (Tan, 1983).

The restoration of coastal environments affected by ASS disturbance is a significant challenge, made even more important by the negative impact that such disturbance has on the natural mangrove ecosystem. When the benefit of restoring degraded aquaculture ponds to natural function is greater than the benefit of the remediation to regain their production, restoration of mangrove sites should be considered (Sammut et al., 1999, Stevenson, 1997), and perhaps become a priority program.

Direct planting of mangrove seedlings has been carried out in numerous mangrove rehabilitation sites in a cost effective strategy. The success of direct planting in this rehabilitation method has been claimed in some areas; nonetheless, other such projects have had failures (Stevenson, 1997, Lewis et al., 2006). The factors behind the success or failure of mangrove rehabilitation using the direct planting method has been scrutinised intensively (Lewis and Marshall, 1997, Field, 1998, Lewis, 2005, Lewis et al., 2006). Many experts suggest that ASS is one stress factor that results in the failure of some mangrove restoration

projects. Therefore, suitable remediation techniques to remove stress factors such as acid and toxic leachates need to be addressed before attempting mangrove restoration (Stevenson, 1997, Wolanski, 2006).

However, there is also some evidence that natural revegetation of mangrove has occurred in some abandoned ponds (e.g. in Haad Sao Kai, Ranong Province, Thailand, and in Tiwoho, North Sulawesi, Indonesia). In these ponds, free seawater inundates and circulates round the ponds through the broken dikes, and in some case, drainage work has improved the hydrology of these sites and restored mangrove growth, which highlights the role of suitable hydrology in mangrove restoration (Stevenson, 1997, Djamaluddin, 2006, Lewis et al., 2006). Tidal buffer allows acid neutralization in acid sulfate soils area due to the presence of bicarbonate (HCO_3-) and carbonate (CO_3^{2-}) in seawater (Indraratna et al., 2002).

Despite intensive investigation into the role of hydrology in mangrove restoration (McKee, 1993, McKee, 1995a, McKee, 1995b, Field, 1998, Lewis, 2005), there has been very little research conducted to understand the geochemical factors that affect the success or failure of rehabilitation in ASS areas; this has contributed to difficulties in achieving successful rehabilitation. This knowledge gap includes the tolerance of mangrove seedlings to acid sulfate soil conditions, geochemical conditions, including neutralizing capacity of the soils, and the effects of seawater tidal inundation on the geochemistry of soil that affect the survival and growth of mangrove seedlings. Hence, a detailed understanding of the tolerance of juvenile mangroves to ASS conditions is critical to effective and successful rehabilitation of a mangrove ecosystem.

Mangroves provide a biogeochemical buffer for several pollutants, including heavy metals, as mangroves can retain metals (Saenger et al., 1990, Jones et al., 2000, MacFarlane and Burchett, 2000, MacFarlane et al., 2003, Silva et al., 2006, Zhang et al., 2007a). The responses of mangrove seedlings to metals have been widely investigated. Mangrove seedlings show tolerance to high concentrations of exposed metals, but a negative effect on growth was observed in the seedlings when they are exposed to certain metals. For instance, the growth of *Avicennia marina* (Forsk.) Vierh. seedlings was affected significantly when there was a high concentration of Zn (MacFarlane and Burchett, 2002). Exposure of *Rhizophora mangle* L. to high concentrations of Cd, Pb or Hg did not affect the growth of the

seedlings, while a high concentration of Hg did. Some visual changes were also noted in cases of high metal exposure (Walsh et al., 1979).

In acid sulfate soil environments, the main potential problems for mangrove seedlings are high acidity and high concentrations of mobile Al and Fe, since they are released in significant amounts compared with other heavy metal (Lockhart, 1996, Cook et al., 2000, Preda and Cox, 2002). In various ASS-contaminated sites, the concentration of other metals such as Ni, Cu, Zn, Cd, Pb, and Cr were high when compared to background levels determined from pre-industrial settings using drill holes and shallow cores (Preda and Cox, 2002). The levels of these metals are usually low in most coastal systems (e.g. Pumicestone Passage, Queensland) (Preda and Cox, 2002) and such low concentrations of metals may not affect mangrove seedlings. However, under certain conditions such as prolonged drought (Clark et al., 1997), ASS can release high concentration of these metals. A pulse of this type may be highly detrimental to the health of mangrove seedlings.

Previous research in disturbed ASS area shows that the concentrations of Al and Fe in parts of mature mangroves are high relative to other metals (Preda and Cox, 2002, Silva et al., 2006). Mature mangroves may be categorised as Al and Fe accumulator plants. However, no research has confirmed whether mangrove seedlings, when grown in unpolluted soils and replanted in an ASS conditions where there were acidic conditions and high concentration of metals, behave the same as existing mature mangroves. This evaluation is important, since the adaptation capability of seedlings has not been established yet.

Broad information about adaptation of other terrestrial plants to ASS environments is available and can be used as a basis for research. For instance, *Eucalyptus camaldulensis* has been widely replanted for afforestation in Vietnam, including on ASS in the Mekong River delta (Nguyen et al., 2005). The selection of this species was based on its fast growing characteristic without considering the condition of sites; this gap led to failure on a large scale (Jong et al., 2006). Therefore, a detailed understanding of factors including the tolerance of local plants to ASS conditions is critical in order to achieve successful and effective rehabilitation.

The tolerance of some terrestrial plant species to acid conditions, or to high concentrations of Al or Fe, has been examined (Kidd and Proctor, 2000). However, these studies carried out research in Al or Fe solutions and only provide evaluation on the visual impact on seedlings, and do not consider the geochemical aspects. Geochemistry studies in ASS conditions are very important, since the interaction among the factors in such environments is complex and can influence the tolerance of mangrove seedlings.

1. The aims, hypothesis, and scope of the study

Based on the argument of some experts and the available evidence on mangrove restoration in disturbed ASS environments, the following question arises: "Is remediation of abandoned ponds in disturbed ASS environments necessary to promote the development of healthy restored mangrove ecosystems?".

The establishment of mangrove seedlings under ASS environments would deal with several potential problems, particularly acidic conditions and high concentration of metals. Therefore, some other detailed questions that arise are: "Which geochemical conditions are required for mangrove seedlings to establish and grow?"; and "what is the tolerance of mangrove seedlings to acid conditions and high levels of metals?".

From these research questions, the hypothesis that this study tests is: "mangrove seedlings can survive replantation under ASS conditions, including elevated metal concentrations, but with some disruptions to growth responses."

This hypothesis is primarily based on results from previous research which indicates that mangrove seedlings are able to grow in very high concentration of metals, although with negative effects on the rate of growth (Walsh et al., 1979, MacFarlane and Burchett, 2002). It is hypothesised that similar effects may occur in ASS conditions. Mangrove seedlings may respond negatively to even higher metal concentrations in ASS due to the acidic conditions. It is also important to evaluate the response of mangrove seedlings to severe acid sulfate soil conditions with high concentration of metals.

To test the hypothesis, a laboratory experiment was carried out. The laboratory study was accomplished by propagating *Rhizophora stylosa* (Vierh) propagules from a 'clean' site for seven months and subjecting them to a range of conditions. Rhizophoraceae has a broad

distribution around the world (Duke, 2006), and includes one of the mangroves most tolerant to unhealthy environments (Vanucci, 2002). Rhizophoraceae is a salt-excluding species (i.e. species that exclude salt at the roots) (Lawton et al., 1981, Hogarth, 2007).

A detailed understanding of the mechanisms by which mangrove seedlings retain metals requires the evaluation of various geochemical factors (pH, redox potential, organic content, grain sizes). Further examination of various acidity variables as well as metal forms was conducted in the field study to determine the factors that influence the tolerance of mangrove seedlings in natural ASS conditions.

To achieve a better understanding of the natural response of mangrove seedlings to various geochemical factors, a field study was also carried out in abandoned ponds that are affected by ASS in Mare, District of Bone, Province of South Sulawesi, Indonesia.

The results from the experimental and field study were compared to achieve the following objectives:

Objective #1: to determine the accumulation and translocation of metals within mangrove seedlings with respect to the concentration of metals in the soils of various environments. The relationship between metal concentrations and establishment and growth of mangrove seedlings is also to be determined.

This research focuses on the concentration of two major elements released in ASS environments: Al and Fe (Dent, 1986, Cook et al., 2000, Fitzpatrick et al., 1998). Two metals which are mobile under ASS conditions, Ni and Cu (Preda and Cox, 2001, Nordmyr et al., 2008), are also examined in this research. The different functions of the elements investigated (i.e. Fe and Cu are essential elements, and Al and Cu are non-essential elements) enables comparison of their accumulation pattern in the mangrove parts.

Objective # 2: to evaluate various geochemical factors involved in ASS environments, to determine the response of mangrove seedlings to ASS.

Assessment of the mechanisms by which mangrove seedlings retain metals requires the evaluation of various physical and geochemical factors (pH, redox potential, organic content and grain sizes). Some sulfur species were also evaluated to understand the process involved

in acidic environments (Ahern et al., 2004). The effects of tidal inundation on the geochemical properties were also examined.

Together, these determinations answer the main aim of this research, which is to investigate the effect of geochemical conditions on the response (survival and growth) of Rhizophoraceae seedlings, and to examine the interactions of geochemical factors involved in ASS condition.

The research provides a better understanding of the geochemistry of ASS disturbance and the ability of mangrove seedlings to cope with those conditions. Together, these results provide suitable recommendations to environmental managers involved in mangrove ecosystem restoration affected by ASS disturbance. The uniqueness of this study is that it combined biological measurements with various geochemical and ASS approaches, therefore it is suitable for ASS environments.

It is critical to examine the problem through a geochemistry study of mangrove rehabilitation in ASS environments, since different areas may have different geochemical/mineralogical conditions. In addition, the interactions among geochemical factors in ASS environments are complex, and can affect the response of mangrove seedlings. The results from this biogeochemical study can assist in determining the geochemical conditions mangrove seedlings can tolerate, and whether remediation that promotes the development of healthy restored mangrove ecosystems is required.

2. Structure of the thesis

To cover the objectives of the research, this thesis comprises nine chapters. The contents of each chapter is summarised below.

Chapter 1 briefly introduces the background of the issues, the current research and the gap in knowledge that indicates the need for this research. This chapter then outlines the research questions and hypothesis, as well as the aim, objectives and scope of the research.

Chapter 2 presents the fundamental theory related to ASS to provide a basic understanding of the study area, and identifies the major potential problems in establishing mangrove

growth in such conditions. This chapter also reviews the gaps in knowledge in publications on the responses of mangrove seedlings in ASS environments, and the geochemical factors that influence the establishment of seedlings in such conditions.

Chapter 3 describes in detail the nursery design, propagation, experimental design, and the type of analysis applied in both the experimental and field study. It also describes the field study sites.

Chapter 4 examines how different environments (ASS and non-ASS) affect the survival of *R. stylosa* seedlings. Assessment of the growth of the seedlings (i.e. total length and root length) within different environments was carried out. The chapter also presents an evaluation of the interaction of various geochemical key factors (pH, redox potential, sulfide, sulfate, total sulfur, organic content and grain size) in the assessed environments, as well as the relationship of the geochemical factors that influence the survival of mangrove seedling in those experimental environments.

Chapter 5 discusses the survival response of *R. stylosa* seedlings to ASS environments. Bioconcentration factors and translocation factors of metals within the parts of the mangrove seedlings (stem, leaf, and root tissues) are analysed. Furthermore, the relationship between metal concentrations in both root tissue and soil under different environments is examined. The geochemical factors that influence the distribution and accumulation of metals within root tissue and soil are also discussed.

Chapter 6 examines the general geochemical conditions in which mangrove seedlings are established naturally, and/or are replanted in abandoned aquaculture ponds. This chapter also evaluates the interactions between the measured physical and geochemical variables of subsurface soils near root areas. The impact of tidal inundation on the improvement of soil quality is discussed.

Chapter 7 evaluates the role of acidity on seedling establishment and development by examining some acidity properties, including total existing acidity and total potential acidity in soils. Acid leachate is identified from the surface soils, thus determining their correlations and interactions with pH and sulfur species on subsurface soil layers, and their relationships to the establishment and growth of seedlings. This chapter also evaluates the effect of tidal

inundation on some acidity properties of surface and subsurface layers to gain a better understanding of acidity roles in the establishment and growth rate of mangrove seedlings.

Chapter 8 provides a general discussion on the geochemical conditions required by seedlings to be established and grow, based on the results from the various non-ASS and ASS environments studied in the experimental and field research. Through this comparison, a better understanding, particularly of the response of mangrove seedlings to acid conditions, as well as to high concentrations of metals, can be achieved. The study also contributes to knowledge of the role of tidal inundation in the improvement of soil quality that influenced the mangrove seedlings' establishment and growth. The significance of the study is discussed, and strategies are recommended to assist environmental management to achieve effective and successful mangrove restoration in similar conditions.

The other outcomes of this research are an oral presentation and publication in the conference proceedings for the Asian Conference on Sustainability, Energy, and Environment in Osaka, Japan (3 – 6 May, 2012). The material of the paper is mainly drawn from the study results of Chapter 6. The abstract for the conference proceeding is presented in Appendix F, pp. 197.

CHAPTER 2: LITERATURE REVIEW

The geochemical factors behind the success or failure of mangrove rehabilitation in ASS environments are poorly understood. This chapter reviews the potential problems and the gaps in knowledge about the responses of mangrove seedlings in ASS environments, as well as the geochemical factors that influence the establishment of mangrove seedlings under such conditions. To provide a better understanding on this topic, an overview of the background of the study areas is presented.

1. The pyrite formation processes

Pyrite (FeS2) is formed through transformation from iron monosulfide (FeS) in a series of reactions that involves microorganisms. In reducing environment of marine surface sediments, FeS is abundant as result of precipitation of high level of dissolved ferrous iron and H_2S produced by sulfate reduction bacteria. This iron monosulfide is immediately transformed to the stable FeS_2 as FeS reacts with dissolved H_2S in strictly anoxic environment, or polysulfides (S_n^{2-}) in suboxic environment (Berner, 1970, Benning et al., 2000, Burton et al., 2006, Kraal et al., 2013).

Several factors can inhibit the formation of stable FeS_2. Availability of organic matter and reactive iron are primary factors in pyrite formation. Inadequate organic matter required by bacteria limits sulfate reduction processes and pyrite formation (Berner, 1970, Lin et al., 2000, Jasińska et al., 2012). However, high level of organic compounds in soil forms Fe^{2+} complex and minimises pyrite formation (Morse and Wang, 1997, Morse, 1999, Kraal et al., 2013). Although the presence of high sulfide concentration generated by microorganism due to decomposition of high organic matter, the level of pyrite will not reach high concentration without the presence of high level and reactivity of iron (Berner, 1970, Jasińska et al., 2012). In the area of carbonate muds, the combination of high organic matter and sulfide and poor iron minerals forms low content of pyrite (Berner, 1970).

Diffusion of sulfate into soil from overlaying water causes a production of sulfide, thus its availability became a limiting factor of pyrite formation (Berner, 1970). Rapid formation of FeS that occurs in Fe rich environment reduces H_2S production, which lowers pyrite formation (Berner, 1970, Burton et al., 2006, Kraal et al., 2013). Slow reaction between FeS

and H_2S under severe anoxic conditions also minimizes pyrite formation (Benning et al., 2000, Kraal et al., 2013). Low conversion of polysulfide pathway and conversion of H_2S to HS^- at high pH also limit pyrite formation (Morse and Wang, 1997, Morse, 1999, Burton et al., 2006, Kraal et al., 2013).

2. Basic concepts and the occurrence of acid sulfate soils

Acid sulfate soils (ASS) are saline soils or sediments that contain accumulated iron sulfides (Fitzpatrick et al., 1998, Fitzpatrick, 2003, Fanning et al., 2010, Johnston et al., 2010a), mostly in the form of pyrite (Powell and Ahern, 2000), in the upper layers of soils under waterlogged or highly reducing environments such as sulfidic conditions (Fitzpatrick et al., 1998).

There are two basic types of acid sulfate soils: potential acid sulfate soils (PASS) and actual acid sulfate soils (AASS). Soils that contain pyrite that has not been oxidised are categorised as Potential Acid Sulfate Soils (PASS) (Fitzpatrick et al., 1998). The iron sulfide layer is stable and maintained by permanent groundwater under anaerobic reducing conditions (Powell and Ahern, 2000). The pH of the PASS soils or sediments is usually near neutral (approximately 7.00) (Fitzpatrick et al., 1998), or may be weakly acid to weakly alkaline (Powell and Ahern, 2000).

When soils containing pyrite are exposed to the air through drainage or disturbance, sulfuric acid is formed. The production of sulfuric acid leads to a decrease in pH, usually to less than 4 (Powell and Ahern, 2000, Fitzpatrick, 2003). Such soils or sediments are known as actual acid sulfate soils (AASS) (Fitzpatrick et al., 1998). The AASS materials are recognised by the present of yellow or debris-coloured jarosite [$KFe_3(SO_4)_2(OH)_6$] (Fitzpatrick et al., 1998, Fitzpatrick, 2003), often in combination with dark reddish marks (Fitzpatrick et al., 1998).

The availability of dissolved sulfate, iron and organic matter and the presence of anaerobic conditions (Fitzpatrick, 2003) are several conditions that favour ASS development. A stable (Powell and Ahern, 2000) and low coastal environment (Australian Height Datum < 5 m), such as barrier estuaries and coastal lakes enhance the accumulation, and stimulate the development of ASS (Fitzpatrick, 2003).

The oxidation of pyrite under acid sulfate soil conditions has several stages, which involve both chemical and microbiological processes, and are influenced by the pH, as shown below (Dent, 1986). The initial stage is the slow oxidation of pyrite that produces ferrous iron, and elemental sulfur or sulfate (Dent, 1986, White and Melville, 1993):

$$FeS_2 + ½\ O_2 + 2H^+ \rightarrow Fe^{2+} + 2S + H_2O \quad \text{..... (1)}$$

The next stage is oxidation of elemental sulfur, which generates sulfate and acid (sulfuric acid). This process also reacts very slowly, however catalysation of autothropic bacteria may enhance the process in neutral pH conditions:

$$S + {}^3/_2O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \quad \text{..... (2)}$$

Besides iron sulfides, oxidation of iron monosulfide can also generate acidification. However, this infrequently occurs, because only very small amounts of FeS exist:

$$2FeS + {}^9/_2O_2 + (n+2)H_2O \rightarrow Fe_2O_3.nH_2O + 2SO_4^{2-} + 4H^+ \quad \text{..... (3)}$$

Overall, pyrite oxidation generates precipitation of ferric hydroxide and sulfuric acid, which is represented by the following equation (Dent, 1986) :

$$FeS_{2(s)} + {}^{15}/_4O_{2(g,aq)} + {}^7/_2\ H_2O \rightarrow Fe(OH)_{3(s)} + 2SO_4^{2-}{}_{(aq)} + 4H^+{}_{(aq)} \quad \text{.....(4)}$$

Pyrite oxidation can also produce ferrous iron. The ferrous iron generated can be further oxised to ferric iron (White et al., 1993, Cook et al., 2004):

$$FeS_2 + {}^7/_2O_{2(aq)} + H_2O \rightarrow Fe^{2+}{}_{(aq)} + 2SO_4^{2-}{}_{(aq)} + 2H^+{}_{(aq)} \quad \text{.....(5)}$$

$$Fe^{2+}{}_{(aq)} + H^+{}_{(aq)} + {}^1/_4O_{2(g,aq)} \rightarrow Fe^{3+}{}_{(aq)} + {}^1/_2H_2O \quad \text{.....(6)}$$

Under anoxic and low pH (< 4), Fe^{3+} becomes soluble and reacts as an oxidant agent in pyrite oxidation (Schippers and Jørgensen, 2002, Cook et al., 2004, Carey and Taillefert, 2005). Under such condition, *Thiobacillus ferrooxidans* can boost the rate of pyrite oxidation (Cook et al., 2004):

$$FeS_2 + 14Fe^{3+}{}_{(aq)} + 8H_2O \rightarrow 15\ Fe^{2+}{}_{(aq)} + 16H^+{}_{(aq)} \quad \text{.....(7)}$$

The sulfuric acid that is produced under pyrite oxidation releases soluble and colloidal iron (Hicks et al., 2002), attacks the insoluble aluminium in clay and releases soluble Al^{3+} in huge amounts (Fitzpatrick et al., 1998, Macdonald et al., 2007), and elevates other trace metals

(Cook et al., 2000, Sundstörm, 2002). The processes described show that the primary potential risks to mangrove seedlings' life in ASS environments are iron, sulfur, aluminium and acid.

Complex environments in ASS areas lead to the formation of different states of sulfur (Armstrong and Armstrong, 2005, Johnston et al., 2009b, Johnston et al., 2010b). The form of sulfur depends on various factors; for instance, in a very reducing environment caused by tidal inundation, the reduction of sulfate occurs. In this condition, sulfate-reducing bacteria (SRB), for instance *Desulfovibrio desulfuricans*, oxidise organic matter and yield readily soluble hydrogen sulfide (H_2S) (Armstrong and Armstrong, 2005).

The overall reaction of sulfur reduction is:

$2H^+ + SO_4^{2-} + CH_2O \rightarrow H_2S + 2CO_2 + 2H_2O$

The sulfide equilibriums are:

$H_2S \rightarrow HS^- + H^+ \rightarrow S^{2-} + 2H^+$

(Armstrong and Armstrong, 2005).

Neutral, or near neutral pH and alkaline mediums (pH between 5.5 and 9) promote the reduction of sulfate to sulfide (Starkey, 1946, Postgate, 1959, Willow and Cohen, 2003, Labrenz and Banfield, 2004). However, microbial sulfate reduction can occur in acid environments (pH <5), with some possible negative impacts on the reduction process, or on the bacteria (Willow and Cohen, 2003, Church et al., 2007, Koschorreck, 2008).

The initiation of sulfate reduction requires reducing environments (Postgate, 1959, Zagury et al., 2006), therefore low or negligible sulfide concentration are observed at high oxidative-acidic environments (Connell and Patrick, 1968, Willow and Cohen, 2003) due to low adaptive capability of SRB to such environments (Dolla et al., 2006). Loss of sulfide (particularly H_2S) is due to several reasons, including the escape of gas from the soil. The loss of sulfide may also be due to precipitation with iron or other metals (Connell and Patrick, 1968, Quicksall, 2009, Johnston et al., 2009b, Johnston et al., 2010b), or it may be oxidised back to sulfate by sulfate-oxidising bacteria (SOB) (e.g. *Thiobacillus denitrificans*), that are able to use nitrate as an oxidant under anoxic conditions (Lyimo and Mushi, 2005).

3. Major potential problems faced by mangrove seedlings in acid sulfate soil areas

In ASS environments, mangrove seedlings are exposed to several stresses, which include high concentration of metals and sulfur species. Most of ASS research have focused on the effects of major elements Fe and Al on plants, while little is known regarding on the effects of trace metals Cu and Ni in ASS conditions. Therefore, references from non-ASS study are included in this literature review to provide general information on the effects that mangrove seedlings may encounter under ASS conditions. In ASS environments, other geochemical factors, such as pH, redox potential, organic content, grain size, and the type of mineralogy interact with each other and may negatively influence the establishment of the seedlings.

2.1. Metals

Iron and aluminium are the main metals that are released in ASS environments in large amounts (Dent, 1986, Fitzpatrick et al., 1998, Cook et al., 2000, Macdonald et al., 2007) Elevated concentrations of other metals also occur in ASS environments, including nickel (Ni) and copper (Cu). Nickel has high mobility under ASS conditions (Preda and Cox, 2001, Nordmyr et al., 2008). In ASS-contaminated sites in the Logan estuary, southeast Queensland, Ni had concentrations exceed ANZECC standard compared to other heavy metals (Lockhart, 1996).

Iron is an essential element for plants, particularly as an electron carrier for photosynthesis (Wilkins and Wilkins, 1997, Bertrand et al., 2002). Nevertheless, in certain concentrations, in lowland areas, iron toxicity occurs and causes lower photosynthetic rate (Audebert and Sahrawat, 2000). Visual symptoms of iron toxicity in rice is bronze color of leaves (Sahrawat, 2004, Becker and Asch, 2005), growth inhibition (Dent, 1986, Audebert and Sahrawat, 2000) and significant reduction of yield (Audebert and Sahrawat, 2000, Sahrawat, 2004, Becker and Asch, 2005, Fageria et al., 2008). Yield reduction is an indirect effect of iron toxicity caused by inhibition of the uptake of other essential nutrients by plants (Fageria et al., 2008).

The increase of uptake and toxicity of Fe is strongly influenced by several plant and environmental factors, such as plant genotypes, low soil pH, high level of soluble iron

released from parent material, reducing environment, microbial activities, increase in ionic concentration, low soil fertility, soil organic content and interaction with other nutrient (Fageria et al., 2008)

Copper is an essential element for plants, particularly as an electron carrier (Wilkins and Wilkins, 1997, Bertrand et al., 2002), and for structure and catalysis purposes (Bertrand et al., 2002). In certain concentrations, Cu can replace chlorophyll Mg of plants and cause disruption to the process of photosynthesis (Bertrand et al., 2002, Mishra and Dubey, 2005) and cell death (Bertrand et al., 2002). Visible toxicity of *A. marina* is observed at a soil concentration of less than 400 μg Cu/g (MacFarlane and Burchett, 2001).

Aluminium is a non-essential metal (Mishra and Dubey, 2005) and is a major risk in plant growth (Dent, 1986), including in acid soil environments (Samac and Tesfaye, 2003, Kochian et al., 2004). The most common reported effects of high concentrations of Al on plants are production of a shallow root system and inhibition of cell division (Kidd and Proctor, 2000, Samac and Tesfaye, 2003, Kochian et al., 2004), which influence their water and nutrient uptake (Samac and Tesfaye, 2003, Kochian et al., 2004).

Nickel has no essential role in plant metabolism (Bertrand et al., 2002, Kabata-Pendias and Pendias, 2001) and is known to have a potential negative impact on photosynthesis at high concentrations (Bertrand et al., 2002, Mishra and Dubey, 2005). Nickel significantly delays germination and inhibits dry matter production (Nedhi et al., 1990). Limited growth of plants (Nedhi et al., 1990, Kabata-Pendias and Pendias, 2001) and injuries due to excessive concentration of Ni have been widely observed (Kabata-Pendias and Pendias, 2001). Strong inhibition of nutrient absorption, root development, and metabolism are also evident in plants under Ni stress (Kabata-Pendias and Pendias, 2001).

2.2.1.1. Mangrove responses to metal exposure

Mangrove seedlings from different species show different levels of tolerance to high concentration of metals under laboratory conditions. The survival and growth of *R. mangle* seedlings was not affected by exposure to the metals Cd, Pb, and Hg at concentrations of more than 100 μg/g soils (Walsh et al., 1979, MacFarlane and Burchett, 2001). However, seedlings showed various symptoms, and 65% of seedlings died at extremely high levels of

500 μg Hg/g soils (Walsh et al., 1979). This study did not evaluate further the factors that affect survival in these conditions, and suggested that the tolerance of *R. mangle* seedlings to high concentrations of these metal exposures is due to the development of non-toxic sulfides in the root, detoxification in seedling tissues, and the exclusion mechanism, or a combination of these factors (Walsh et al., 1979).

Avicennia marina seedlings also tolerate high metal concentrations, including Cu. Several negative impacts such as visual toxicity started to occur when the concentration of Cu was 400μg /g (MacFarlane and Burchett, 2001). It was found that LC_{50}—a concentration of a compound that causes death in 50% in exposed seedlings—was 566μg Cu /g soil. Total inhibition of emergence occurred at an extremely high Cu concentration of 800μg Cu /g soil (MacFarlane and Burchett, 2002).

Based on the various potential problems described above that affect mangrove seedlings, it is critical to examine the response of seedlings to high concentration of metals in ASS environments.

2.2.1.2. Factors affecting the distribution and accumulation of metals in mangrove parts

In response to metal exposure, plants commonly either exclude or accumulate metals. These types of strategy affect the distribution and accumulation of metals. In the exclusion mechanism, the uptake and/or translocation of metals from root to shoot is restricted (Baker, 1981, Kochian et al., 2004) to prevent excessive metal uptake into plant parts (Levitt, 1980). Metal exclusion is the most common mechanism in metal tolerant species (Baker, 1981). In the accumulation mechanism, metals are accumulated in some part of the plant body (Verkleij and Schat, 1990, Kochian et al., 2004) and detoxified in the shoots (Baker, 1981). Metal accumulation can occur in some plant species that grow mainly on soils that contain a high concentration of metals (Baker, 1981, MacFarlane et al., 2003, MacFarlane et al., 2007).

Most studies of mangroves reveal that there is a common pattern in the way that the plants exclude metals, with higher metal concentration in the roots and lower concentration in the

aerial parts (leaves and stems). This type of distribution is affected by factors such as the ion exclusion mechanism and excretion of metals from the leaves.

These mechanisms are similar to the physiological adaptation to high levels of salt, where mangroves excrete and exclude salt through their leaves or roots. Based on their response to salts, mangroves are categorised into two types: salt-excreting species that excrete salt from the leaves, and salt-excluding species that exclude salt at the roots (Lawton et al., 1981).

Rhizophora stylosa and *Ceriops australis* are categorised as salt-excreting species (Bell and Duke, 2005). The ion exclusion mechanism in the root is considered to be involved in the low accumulation of Zn and negligible Pb in the aerial parts of *R. mucronata* seedlings (Thomas and Eong, 1984). *Avicennia marina* excretes the excess metals through salt glands on the leaves (MacFarlane and Burchett, 2000). Preliminary research on herbicides suggested that mangroves take up herbicides in a similar manner to salt through their exclusion and excretion mechanism (Bell and Duke, 2005).

The different patterns of metal distribution and accumulation in mangrove seedlings may also explain their responses to essential and non-essential metals. Essential metals such as Cu and Zn are distributed to all seedling parts, while non-essential metals like Pb are not distributed evenly to other parts by the *R. mucronata* seedlings (Thomas and Eong, 1984). A similar pattern of uptake of Pb is also observed in *R. mangle* (Walsh et al., 1979).

However, certain metals (e.g. Cd, Fe, Cu, Co, Mo, Pb, Sn, Ti, Ag, Cr, Zr, V, and Ga) are generally accumulated more in roots than in shoots (Siedlecka, 1995). Several elements (e.g. Ni) are uniformly distributed between roots and shoots (Siedlecka, 1995).

The distribution and accumulation of metals in the tissues of mangrove are also controlled by the mobility of metal (MacFarlane, 2002) in the plant. The mobility of an element is often identified by the translocation factor. The translocation factor (TF) is measured as the ratio of shoot to root concentration (Regvar and Vogel-Mikus, 2008).

The bioconcentration factor (BCF) is used to describe the bioaccumulation of a substance, or the uptake from the adjacent component, for instance, through speciation from water in aquatic systems (Jørgensen et al., 1998). The BCF values provide the steady state

concentration of a toxic substance in an organism in all possible routes relative to its environment (Jørgensen et al., 1998).

In general, the concentration of metals in the roots and leaves of mangrove seedlings increases as the concentration of metal in the soil increases. At low concentrations of Cu in soils under laboratory conditions, limited Cu was taken up and accumulated in the leaves (MacFarlane and Burchett, 2001). This was confirmed in field conditions (MacFarlane and Burchett, 2002).

The concentration of metals in mangrove parts is several times higher than the concentration in soils. For instance, the concentration of Cu in roots is 2.3 to 10 times compared to its concentration in soils. The concentration of Cu in leaves is 0.1 to 0.9 times compared to its concentration in soils (MacFarlane and Burchett, 2002). Cu concentration in leaves were found to be higher as salinity increases and soil pH decreases (MacFarlane, 2002).

2.2. Sulfur

Sulfur is one of the important macronutrients for plant development and for uptake by the plant in the form of sulfate (Buchner, 2008). Excess sulfur can decrease yield, which is accompanied by increased uptake of Cu, Mn and Fe by plant. This in turn causes severe and harmful changes to the metabolism of the plant cell (Rennenberg, 1984). Damage to roots by dissolved sulfide (H_2S) or any other factor destroys the oxidising capability of roots, and in consequence exaggerates Fe toxicity (Kabata-Pendias and Pendias, 2001).

2.2.2.1. Mangroves' responses to high levels of sulfide

High concentrations of sulfide, which is accompanied by the change of redox potential, may influence the ability of mangrove seedlings to survive. Although a field study in Mtoni and Mbeni, Dar es Salaam, Tanzania, revealed that *Avicennia marina* and *Rhizophora mucronata* occur in coastal areas that have high concentrations of sulfide (0.0025-0.96 mM in Mtoni and 1.5–24.5 mM in Mbeni), a glasshouse experiment demonstrated that the seedlings of *A. marina* and *R. mucronata* did not grow in reducing soil (-27 to -198 mV) that contains a high concentration of sulfide of 0.5–6 mM (Lyimo and Mushi, 2005).

Complete inhibition of photosynthesis occured in some mangrove species (A*egiceras corniculatum* (L.) Blanco, *Avicennia marina* (Forsk.) Vierh., *Bruguiera gymnorrhiza* (L.)

Lamk., and *Rhizophora stylosa* Griff.) in the presence of sulfide (Youssef and Saenger, 1998).

4. The assessment of plants' tolerance to acid sulfate soil conditions

Although it is well known that mangrove seedlings tolerate high concentrations of many metals, the tolerance of *R. stylosa* seedlings to ASS conditions, and the ability of those seedling species to distribute and accumulate metals under ASS conditions, are poorly understood. Previous research has shown that the levels of Fe in parts of mature mangroves are relatively high compared with other metals. However, there has been little research to observe the concentrations of Al in mangrove parts.

The concentration of Fe in root tissues of *Kandelia candel* in the Mai Po area, Hong Kong, which is protected under the Ramsar Convention, is 4225.8 µg/g, and the concentration of Zn is 122.9 µg/g (Ong Che, 1999). The concentration of Fe in the roots and leaves of *R. mangle* in a polluted area of Rio de Janeiro, Brazil is 1011 and 37.2 µg/g respectively, compared to 4856 µg/g of soil concentration. Here, the concentration of Cu in roots and leaves is 5.1 and 0.1 µg/g, compared to 2.8 µg/g concentration in soil (Silva et al., 1990). In a mangrove area that is dominated by *R. mangle* in Surui, Guanabara Bay, Rio de Janeiro, Brazil the concentration of Al in soils is 9021 µg/g (Farias et al., 2007). There has been little research into Al in mangrove parts.

In ASS contaminated areas in the Pumicestone region of southeast Queensland, Australia, Fe concentrations in the pneumatophores of mature *A. marina* are found in relatively high amounts of up to 4687 mg/kg compared to other metals such as Zn, which occurs in concentrations up to 72 mg/kg (Preda and Cox, 2002). However, there is little information about the tolerance of mangrove seedlings to high concentrations of metals in acid sulfate soil environments.

Understanding of the tolerance of mangrove seedlings to high levels of sulfur species in acid sulfate soil environments is also poor. Most of the research on sulfur has been conducted to assess the impact of sulfide on mangroves (Youssef and Saenger, 1998, Lyimo and Mushi, 2005). Poor understanding of the effect of geochemical conditions on mangrove seedlings

under ASS environments leads to a high failure rate in mangrove restoration projects in such environments (Stevenson, 1997, Lewis et al., 2006).

5. Interaction of geochemical factors in acid sulfate soil environments

The interaction of geochemical factors in ASS environments is complex and can affect the initial geochemical conditions. The formation of sulfuric acid in ASS environment enhances the mobility of some metals, especially Ni, Cu, Zn, Cr, Co, which results in elevated concentrations of those metals (Preda and Cox, 2001). Acidic conditions do not influence less mobile elements, such as Mo and Pb (Preda and Cox, 2001).

The concentration and distribution of metals in ASS environments are influenced by geochemical factors. In the ASS affected study area in the Pimpama catchments, Southeast Queensland (Preda and Cox, 2001), complexes with iron, manganese and organic compounds, and the grain size of soil were geochemical factors that controlled the distribution of released metals. Fine-grained soils, such as clay minerals have a high specific surface area and a strong adsorptive capability (Salomons and Forstner, 1984). Therefore, higher concentrations of metal were found in fine-grained soils than in sandy soils in mangrove areas (Tam and Wong, 2000). In addition to those variables, redox potential (Eh) influences accumulation of metals and their mobilisation in mangrove soils (Harbison, 1986).

Several metal ions are adsorbed and co-precipitated with hydrous oxides of Fe, Mn and Al in either sediments or soils. For example; Fe oxides co-precipitates V, Mn, Ni, Cu, Zn, Mo; and Mn oxides co-precipitate Fe, Co, Ni, Zn, Pb (Alloway and Ayres, 1997). Similar results were found in an ASS-affected study area in the Pimpama catchments, Queensland. Most metals associate with Fe oxides and clays, while Co and Ni associate mostly with Mn oxides. Zn is between these two oxides, since it has affinity for both Fe and Mn oxides (Preda and Cox, 2001).

An experiment using a draining and leaching technique showed that strong acidic conditions slightly alter the texture of the soil to more clay, but there is no significant change in the mineral composition of the soil (Golez, 1995). But field studies show that acidic conditions can enhance the weathering of the parent rocks of pyrite, and liberate major and minor metals

from the structures as well as trace metals from mineral phases that have adsorbed them (Preda and Cox, 2001). In acid conditions, several changes are stimulated in certain minerals, including decomposition of pyrite to jarosite as result of oxidation (Preda and Cox, 2004).

The presence of high organic matter or peat (McElnea et al., 2004), redox, and inundation conditions (Burton et al., 2008, Johnston et al., 2009b) are some factors responsible for the high variation in ASS areas and lead to a complex environment. Such complex environments create a unique form of iron mineral (Burton et al., 2008, Johnston et al., 2009b) and cause the formation of different states of sulfur matter (Armstrong and Armstrong, 2005, Johnston et al., 2009b, Johnston et al., 2010b). Thus, the geochemical conditions formed will affect the seedlings' establishment and growth, as discussed in previous sub-sections.

6. The neutralising capacity of soil

The ability of an environment to naturally recover from acid condition is mainly influenced by the neutralising capacity of the soil, which is governed by various factors. Complexation of major cations by organic matter (Indraratna et al., 2002, Hazelton and Murphy, 2007, Nelson and Su, 2010, Löfgren et al., 2011), oxides, and hydroxides (Nelson and Su, 2010, Löfgren et al., 2011) raises the neutralising capacity of soil.

High cation exchange capacity also influences the neutralizing capacity of soil (Hazelton and Murphy, 2007, Nelson and Su, 2010, Glover et al., 2011, Löfgren et al., 2011). Clayey soil has lower exchange capacity compared to sandy soil, therefore it is acidified more slowly (Hazelton and Murphy, 2007).

Dissolution of minerals, including clay minerals, significantly influences the neutralizing capacity of soil (Nelson and Su, 2010, Glover et al., 2011) at long period (Nelson and Su, 2010). High soil buffering capacity also occurs in the presence of high carbonates content, which decreases acidification (Indraratna et al., 2002, Nelson and Su, 2010, Glover et al., 2011). Carbonate/bicarbonate consisted in seawater tidal acts as buffering agent in acid sulfate area. Besides neutralizing acid, tidal action also reduces pyrite oxidation as it increases drain water (Indraratna et al., 2002).

7. Summary of literature review

The main potential problem that mangrove seedlings may encounter in ASS environments is a high concentration of metals, particularly Fe and Al, which are the major acid elements released in ASS environments (Dent, 1986, Fitzpatrick et al., 1998, Cook et al., 2000). Elevation of Ni and Cu, which are mobile in acid conditions, may also become a problem in such environments. These metals are known to be toxic to plants in very high concentration.

The effects of sulfur species is another potential problem for mangrove seedlings in ASS environments, as they are known to be very toxic to plants (Rennenberg, 1984, Kabata-Pendias and Pendias, 2001). Other geochemical factors, such as pH, redox potential, organic content, grain size, and pyrite interact with each other and may affect the establishment of mangrove seedlings in ASS environments.

Mangrove seedlings from various species have shown their tolerance to a high concentration of metals (MacFarlane and Burchett, 2001). Of two common responses that occur in plants (exclusion/avoiding, and accumulating), exclusion of metals appears to be a common pattern in mangrove seedlings, with higher metal concentration in roots and lower in aerial parts (leaves and stems). This type of distribution is affected by several factors, such as the ion exclusion mechanism (Thomas and Eong, 1984) and excretion of metals in leaves (MacFarlane and Burchett, 2000).

The distribution and accumulation of metals in the tissues of mangroves are also controlled by the mobility of metal and the concentration of metal in the soil (MacFarlane, 2002). The concentration of metals in roots and leaves of mangrove seedlings increases several times as the concentration of metal in the soil increases (MacFarlane and Burchett, 2002).

The tolerance of *R. stylosa* seedlings for acid sulfate soil (ASS) conditions, and the ability of that species to distribute and accumulate metals under ASS conditions, are poorly understood. Previous ASS studies only provide evaluation of the visual impact on seedlings and do not consider the geochemical aspects. Geochemical study of ASS conditions is very important, since the interaction among the factors in such environments is complex and can influence the tolerance of mangrove seedlings. Geochemical factors also influence the neutralising

capacity of soil, which together with seawater tidal buffer, are essential aspects on natural recovery of the area that affected by acid sulfate soils.

CHAPTER 3: METHODS

This chapter describes the general methods used in this study. The research methods are organised under the headings of Experimental Study, Field Study and Analysis.

1. Experimental study

1.1. Nursery setting

The experimental study was conducted in the indoor nursery at the Aquaculture Laboratory, Q Block, Queensland University of Technology, Brisbane, Australia. Artificial seawater was used for propagation and treatments (Ye et al., 2005) by dissolving commercial sea salts to 17 ‰. This level is considered suitable for mangrove developments that are grown in a nursery environment (Clarke and Johns, 2002).

A flow-through system was applied in this research (Figure 3.1). To imitate tidal works, artificial seawater from a large reservoir was pumped up through pipes once a day to fill each of the larger containers that contained a propagule/seedlings pot. To imitate daylight, the lights were set for a 12:12 photoperiod (Walsh et al., 1979) using 36-watt growth lights.

Pots without holes at the bottom were used for propagation (Figure 3.2). The size of each pot was 140 mm in diameter and 115 mm high. Eight holes (each with a diameter of 5 mm) were made around the upper rim of the pot to minimise disturbance of the soils from the water flow. The pot was then placed into a larger container (200 mm x 190 mm). The larger container had two holes, each of 5 mm diameter. The upper hole acted to maintain the level of water used to submerge the propagule pot. The lower hole worked to discharge the water after about seven hours. After the process, the water was drained via a discharge pipe located at the bottom of the larger container.

1.2. Propagule and soil collection

Mangrove propagules and soils were collected in Myora Springs, Stradbroke Island, south-east Queensland. The selection of the site was based on the consideration that Stradbroke Island has an environment that is better in terms of water quality, metal levels and non-Acid sulfate soil compared to western parts of Moreton Bay. The soils collected were for propagation, control and metal treatment mediums.

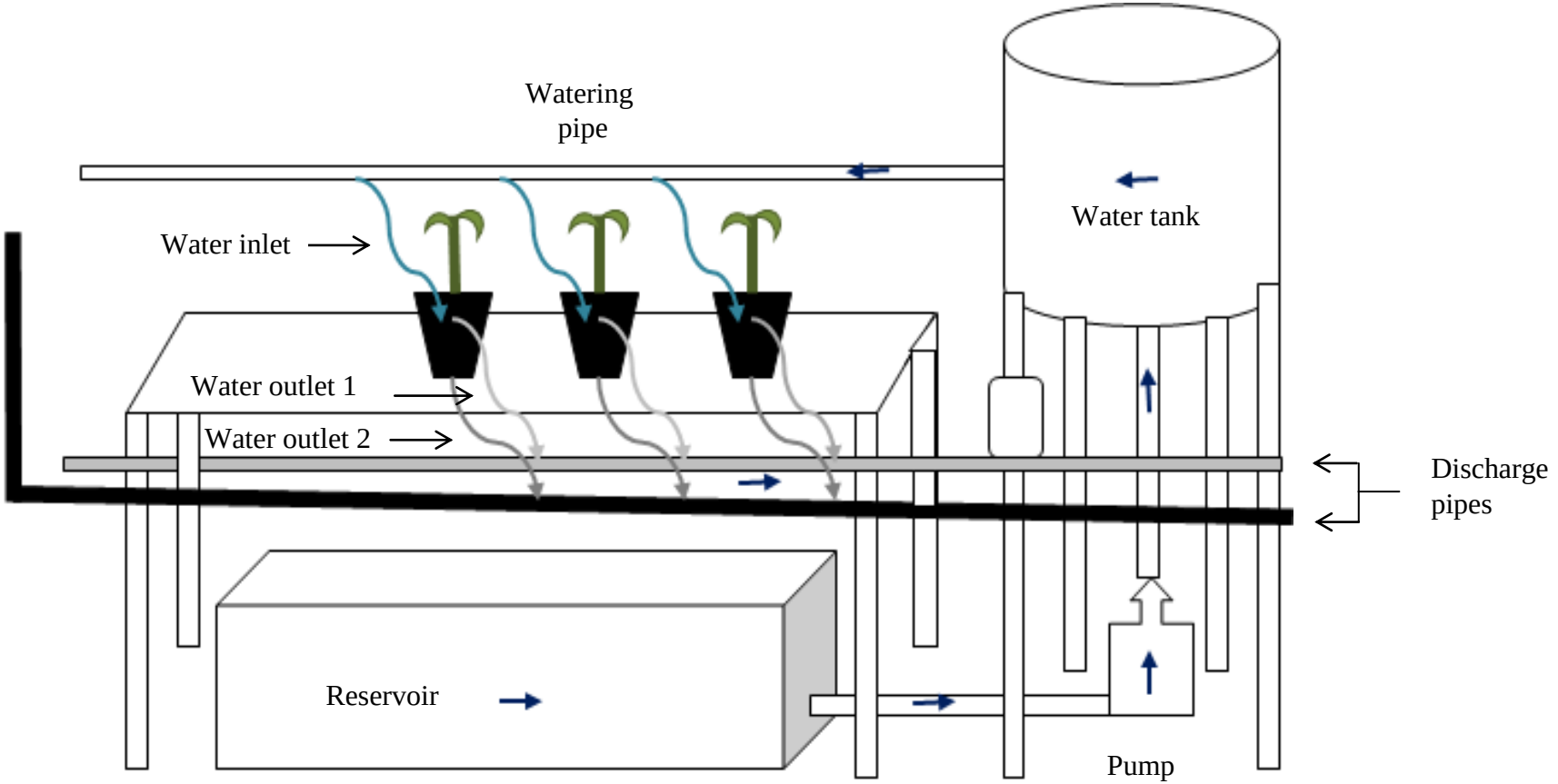


Figure 3.1. The flow through water system applied in the mangrove nursery and experiment. One high tide a day was applied to each pot. Water outlet 1 was designed to maintain water level and water outlet 2 was to discharge water

Figure 3.2. Prototype of propagation pots used in the mangrove nursery. The smaller medium pot was placed into a larger container pot designed to maintain water level then discharge it to imitate tidal conditions. Ø represents the diameter

The soils for the ASS treatments were collected from the mangrove area around Brighton Park at the mouth of North Pine River, Bramble Bay, southeast Queensland. This location is categorised as Potential Acid Sulfate Soil (PASS), where ASS occurs within five metres of the upper layer and has a proportion of oxidisable sulfur above the recommended 'action level' in at least one soil layer (Ahern and McElnea, 2000).

The upper soils were collected and mixed in a large tank before being distributed to the pots to minimise the variability of soil characteristics.

The collection of the dropped propagules was carried out under Self-Assessable Code MP05. The collection of soils in Brighton was carried out under a marine parks permit QS2009/MAN24. The maps of collection sites for the laboratory study are presented in Appendix G.1, pp. 199.

1.3. Propagation (Germination)

Propagules that had similar weight and length were chosen for propagation (MacFarlane and Burchett, 2002). The basal part of *Rhizophora stylosa* propagule was submerged in 25% seawater for two days to stimulate root development before planting (Clough, 1984). The propagation of *R. stylosa* was conducted in a flow-through system for seven months (Figure 3.2). Fertiliser (i.e. Fish Emulsion), with a nutrient composition of N 9%: P 2%: K 6%, was added into the seawater in each pot at the beginning of the propagation and at the beginning of treatments.

1.4. Pollutant treatments & growth measurements

Seven-month-old seedlings were replanted in larger pots and used for control, metal and ASS treatments, with three replications of each treatment. The experimental medium pot had a diameter about 20 mm larger than the propagation pot. Pollutant treatments (heavy metal and ASS exposures) were conducted for up to eleven weeks, or depending on the mortality of the seedlings.

1.4.1. Acid sulfate soils treatment methods

In this experimental study, the soils from Brighton were acidified using sulfuric acid to a pH around 3.5. Three levels of ASS treatments were carried out. Acid Sulfate Soils with a low level of metal formed the ASS control. The other two treatments each had different metals added, based on recommended soil quality guidelines of ANZECC standard, i.e. above trigger level and above high level (ANZECC and ARMCANZ., 2000) (Table 3.1).

Table 3.1. Experimental design with three replications applied in the research

Soil type	**Metals**				
	None	Cu_{70*}	Cu_{280**}	Ni_{25*}	Ni_{55**}
Clean (Myora)					
ASS (Brighton)					

Notes: * concentration above trigger level of recommended soil quality guidelines of ANZECC and ARMCANZ.
** concentration above high level of recommended soil quality guidelines of ANZECC and ARMCANZ.

1.4.2. Heavy metal treatment methods

This treatment mainly followed the procedure described by (MacFarlane and Burchett, 2001, MacFarlane and Burchett, 2002):

Two concentrations of Ni and Cu in the form of metal salt solution ($NiCl_2$, $CuCl_2.2H_2O$) were added to each pot in each treatment (Table 3.1). The selection of chloride metals was based on the consideration that this form can be tolerated by mangroves, therefore can reduce the effect of toxicants from the Cl (Burchett et al., 1984).

1.4.3. Growth measurements

Several measurements were carried out in order to assess the response of mangrove seedlings under metals and ASS contamination. The measurements include recording of apparent health conditions, survival of seedlings, and measurement of height of whole seedlings, as well as average root length. Average root length was determined by recording the image of the root and analysing it using the software ImageJ.

Relative growth rates were determined using the following calculation:

$$RGR = (\ln m_2 - m_1) / (t_2 - t_1)$$

Where: m_2 and m_1 = plant height at the end and beginning of the experimental period
$t_2 - t_1$ = the time gap (Poorter and Garnier, 2007).

2. Field study

2.1. Study site description

The field study was carried out from July to December 2011 (rainy season) in six different environments in abandoned pond areas in Mare ($04^o51'S$, $120^o18'E$), district of Bone, province of South Sulawesi Selatan, Indonesia (Figure 3.3 and Figure 3.4). The ponds with $\pm$ 70 ha were previously a mangrove forest and were cleared for extensive shrimp ponds in 1993. The ponds had successful production in the first years, but failed and have been abandoned since then. Although lime and fertiliser were applied in huge amounts, failed or poor rates of production had been experienced by local the community. Such conditions are a typical pattern in abandoned ponds in different places; therefore this is a suitable location for studying the research problem.

The sites used are described below:

Site 1 is located at the bank of a blocked small creek that has no mangroves. In this location, two out of six *Rhizophora mucronata* seedlings that were replanted at the beginning of the study survived over a three-month period.

Site 2 is a reservoir pond that can be visually categorised as AASS, by the oily red scum and yellowish jarosite accumulated on top of soils. No Rhizoporaceae seedlings were found in this site. There was one mangrove fern (*Acrostichum sp)* seedling. This species is often found in a cleared or disturbed area.

Site 3 is a drainage area with yellowish jarosite on the surface soils. Mangrove fern (*Acrostichum sp)* seedlings occur (Figure 3.5).

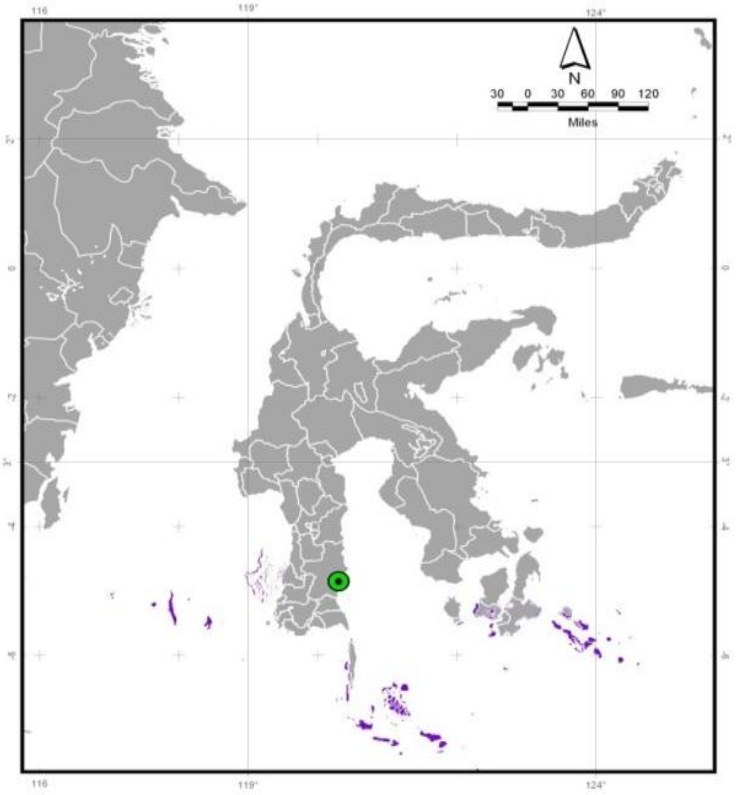


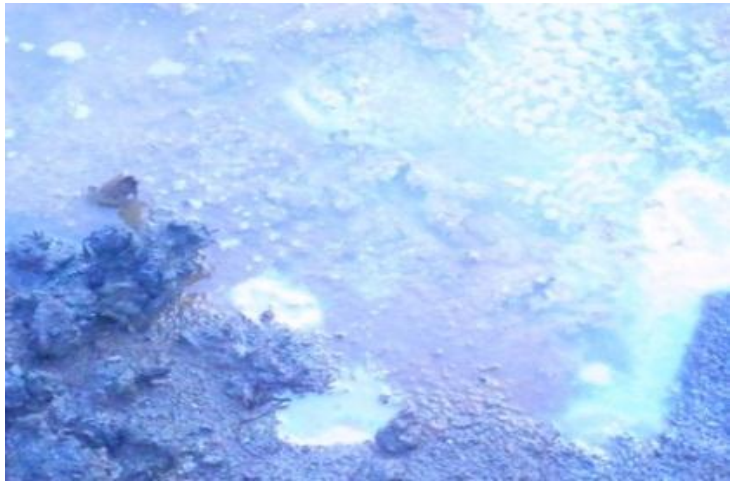
Figure 3.3. Location of field study in Mare, Distric of Bone, Province of South Sulawesi, Indonesia

Figure 3.4. Aerial photograph of the field study location in Mare, Distric of Bone, Province of South Sulawesi, Indonesia (Microsoft, 2012)

a. Site 1

b. Site 2

c. Site 2



d.Site

Figure 3.5. Location of Study Sites 1-3 in Mare, South Sulawesi, Indonesia

e. Site 4.

f. Site 5.

g. Site 6.

h. Site 7.

Figure 3.6. Location of Study Sites 4-7 in Mare, South Sulawesi, Indonesia

Site 4 was at the bank of a creek, it had both naturally occurring *R. stylosa and R. mucronata*, and replanted *R. mucronata* seedlings. At a different area within the same creek, Site 5 was located. In this area, about five species of natural growth mature mangroves existed. The species were *R. stylosa, R. mucronata, Avicennia marina,* and *Sonneratia* sp. Naturally and replanted *R. mucronata* seedlings also existed. Site 6 was an abandoned pond that had free seawater circulation from broken dikes. Natural *R. stylosa and R. mucronata* seedlings had grown in this site.

Site 7 was a control site, which is located at the beach outside an abandoned area. In this site, natural and replanted *R. stylosa* and *R. mucronata* seedlings had grown (Figure 3.6).

Over the sites, there were more naturally occurring *R. stylosa* seedlings than there were *R. mucronata* seedlings. The number of replanted *R. mucronata* seedlings was greater than of the naturally occurring ones. Therefore, naturally occurring pre-existing *R. stylosa* seedlings were used as research samples in the study, except in Site 1, where replanted *R. mucronata* seedlings were used as research samples.

2.2. Sample collection

Six replicate samples of porewater were collected using peeping bottles built from a 250 ml bottle connected to a PVC pipe at the bottom. This pipe served to hold the bottle firmly upright inside the soil. The upper side of the bottle had holes around it and was covered with a soft net to minimise the amount of the soil getting inside (Figure. 3.7). The bottles were put inside the holes in the soil and buried at low tide, and kept there overnight to ensure the bottles were filled with porewater. After removal, the porewater was transferred into sample bottles and preserved with zinc acetate for sulfide analysis.

Six replicates of 15 cm soil cores were collected around mangrove seedlings at each site. A seedling was defined as being no more than one metre high, and without branches.

Figure 3.7. Peeping bottle designed to collect porewater sulfide. The upper side of the bottle had holes around it, covered with a soft net, and was connected to a PVC pipe.

The density and survival rates of plants were estimated by counting, marking and measuring all individuals in six 1m x 1m plots randomly placed at each site. Plots were revisited at the end of the trial, and the same plants were examined. The plant height measured was the above ground height. The relative growth rate (RGR) of the seedlings was determined for the three-month period using Poorter and Garnier's (2007) calculation, as described in the experimental method section. The roots of mangroves were rinsed with deionised water for total metal analysis.

2.3. Analysis

Mangrove seedlings and soils from both experimental and field studies were analysed for several parameters.

2.3.1. Analysis of metals

The analysis involved measurement of metals in water, soils and mangrove tissues.

2.3.1.1. Water analysis

Analysis of total metals in water were carried out once a week or fortnightly in the first month of the experiment to identify any significant metals released into the discharge water due to the application of the flow-through water system. The water samples were not filtered (Preda and Cox, 2001). Since the samples were analysed shortly after collection, no preservation was performed. The metal analyses in water were determined using ICP-OES.

2.3.1.2. Soil and mangrove analysis

The total metal analysis procedure was similar to the method used by previous researchers (Khrisnamurty et al., 1976, MacFarlane and Burchett, 2001, Defew et al., 2005, Silva et al., 2006). The soils were oven dried at 85^{o} C for 48 hours to prevent oxidation (Ahern et al., 2004). Mangrove tissues (stem, leaf and root) were oven dried at 60^{o}C for 24 hours (Defew et al., 2005, MacFarlane and Burchett, 2001). The results from the experiment show that root tissues accumulated metals in significant amounts compared to other tissues under the examined soil concentrations. The bioconcentration factors (BCF) of each metal in leaf and stem tissues are less than one, which is smaller compared to the BCF of greater than one in the root tissues. See Appendix A1, pp. 151–153). Therefore, only root tissues were analysed in the field study.

Soils and mangrove tissues were digested with concentrated nitric acid and hydrogen peroxide. The digestates were filtered and added to a 50 ml flask for soil and roots, and 25 ml for mangrove propagules and leaves (Khrisnamurty et al., 1976). Total metals analysis of samples of the experimental study was performed using ICP-OES. The analysis of total Al and Fe of samples of the field study was also conducted using ICP-OES, while the analysis of total Ni and Cu of the samples of field study was conducted using AAS.

The bioconcentration factor of metals was determined using the ratio of the concentration of a toxic substance (i.e. metal) in the root tissue of mangrove seedlings relative to its environment (i.e. total metal in sub-soils) (Jørgensen et al., 1998).

The metal fractionation analysis followed the protocols for the Commission of the European Community Bureau of Reference (BCR) (Davidson et al., 1994). The procedure consists of three steps of sequential extraction to determine metal fractionation. The first step is an acetic acid

extraction to determine exchangeable, water and acid soluble forms. The second step uses hydroxylamine hydrochloride to determine reducible forms (iron and manganese oxide bounds). The third step is hydrogen peroxide oxidation coupled with ammonium acetate extraction to determine the oxidisable form (organic matter/sulfide bound) (Davidson et al., 1994). The metal concentrations from sequential extraction were determined by AAS Hitachi Z-2000.

2.3.2. Analysis of grain size and pyrite

The soil analysis of the samples from the experimental and field studies involves the measurement of grain size percentage and colour of soils near roots (10-15 cm depth). Determination of grain size was conducted using classical wet sieve analysis by employing a stainless steel 63 μm sieving net (Percival and Lindsay, 1997), involving percentages of silt, clay and very fine sand (< 63 μm) and sand (> 63 μm).

The percentage of pyrite on the surfce and sub-layer soils was measured through Titratable Sulfidic Acidity (TSA) analysis (Konsten and Sarwani, 1990). The estimation of pyrite was based on the calculation:

Pyrite = (TSA: 22,4) x 0,1

Determination of pyrite in the study area enabled assessment of the role of pyrite on geochemical conditions that affect mangrove seedlings' establishment and growth.

2.3.3. Sulfur analysis

Total sulfur, water-soluble sulfate and sulfide were analysed to evaluate the nature of the geochemical condition and their interaction with other geochemical parameters. Through this evaluation, a better understanding of the geochemical aspects that are involved in the establishment of mangrove seedlings in ASS environments can be obtained.

Water-soluble sulfate levels in both the experiment and the field study were extracted using deionised water (Page and Steinbock, 2009) and determined using the Turbidimetry method (APHA, 1999). The determination of sulfide in porewater was conducted using the blue methylene method, and determined by spectrophotometer (APHA, 1999).

Total sulfur soil from the experiment were analysed in the form of HCl-extractable sulfur (Tot S_{HCl}). The advantage of this method of analysing ASS samples is that it recovers most forms of sulfate, including soluble and exchangeable sulfate, sulfate from gypsum and other insoluble sulfate compounds, such as jarosite, natrojarosite, as well as sulfur from organic matter (Ahern et al., 2004). The measurement of the total sulfur in samples from the experiment used the Turbidimetry method (APHA, 1999).

The Peroxide Oxidisable Combined Acidity and Sulfur (POCAS) method was used to analyse samples from the field study, since the samples contain high organic contents. This method allows tracking of existing, potential, and net amounts of acidity and sulfur in soils. The samples were sent to the Soil Laboratory, Research Institute of Coastal and Aquaculture, Maros, Indonesia.

The POCAS method involves several steps, which consist of determining levels of KCl extractable Sulfur (S_{KCl}), titratable actual acidity (TAA), peroxide sulfur (S_P), titratable peroxide acidity (TPA), peroxide oxidisable sulfur (S_{POS}), and titratable sulfidic acidity (TSA) (White and Melville, 1993). S_{KCl} determines the adsorbed and soluble sulfate (Ahern et al., 2004). The S_P determines the sulfate contained in soils through oxidising the soils to generate maximum acidity from reduced sulfidic material (Ahern et al., 2004). The S_{POS} estimates the net potential acid risk of the soil from the unoxidised sulfur compounds by determining the difference between S_{POS} and S_{KCl} (Ahern et al., 2004). The TSA value was determined by calculating the difference between the value of TPA and the TAA (White and Melville, 1993).

2.3.5. Organic content

To enable assessment of the role of organic matter in geochemical conditions as well as its relationship to heavy metal concentration in soil and in the mangrove seedlings, an estimate of organic content in soil samples was conducted. The concentration of organic content were determined using the Loss on Ignition (LOI) method, following the method described by (Heiri et al., 2001). Sample soils were oven dried for 24 hours at 105^{o} C before heating them at 550^{o} C.

2.3.6. The chemical-physical measurements

These measurements are of pH and redox potential (Eh) using a water quality meter. These measurements will assist in determining the effect of measured parameters on the solubility, mobilisation and precipitation/deposition of metals.

The detailed procedures of the analysis used in the study are presented in Appendix E, pp. 181 - 195.

2.4. Statistical analysis

This research applied several statistical analyses, using Excel and SPSS 17 to assess the distribution of different metals, geochemical parameters and their correlations. Normality tests and transformation were employed for non-normal variables. The transformation types depended on the type of skewness. The Kruskal Wallis analysis was used to examine whether the variables did not fulfill the normality.

Chi-square analysis was used to examine the association between the two main types of environments and the survival of the mangrove seedlings. ANOVA was used to compare the differences in value from different parameters under four different treatments. A post-hoc pairwise comparison of sample means with Tukey's significant difference test was used when the ANOVA results showed a significant difference ($p < 0.05$). The General Linear Model (ANCOVA) was used to examine whether survival days affect the final root length of the *R. stylosa* seedlings. Linear regression was used to determine the relationships between metal concentrations in the root tissues or in the soils and both the RGR and the final root length of the seedlings.

Principle Component Analysis (PCA) was employed to identify geochemical trends. Standardised regression was used to examine the relationship between Al and Ni concentrations in the root tissues and the soils and the RGR and final root length of the seedlings, and between the density, establishment, growth and other geochemical variables. The Pearson correlation was employed to identify the correlation and interaction within the geochemical variables (Chapters 4, 6, 7), and to test the relationship between metal concentrations in soils and mangrove tissues (Chapters 5).

CHAPTER 4: THE EFFECTS OF ACID SULFATE SOIL CONDITIONS ON THE ESTABLISHMENT AND GROWTH OF *RHIZOPHORA STYLOSA* SEEDLINGS, AND GEOCHEMICAL FACTORS INVOLVED: AN EXPERIMENTAL STUDY

1. Introduction

The establishment of mangrove seedlings in an area is controlled by many factors, including the availability of suitable environmental conditions. Mangrove restorations that do not consider the ecological requirements or assess stress factors often result in major failures or financial loss. This problem has been reported in West Bengal, India, and the Philippines (Lewis, 2005). In North Sulawesi, Indonesia mangrove restoration has been conducted unsuccessfully five times in eight years in the same abandoned fish ponds because prior ecological assessments have not been made (Lewis et al., 2006).

Highly degraded conditions in acid sulfate soil (ASS) environments affect the survivability and growth of mangrove seedlings. Potential problems that should be taken into account before replanting seedlings in such environments are high levels of released metals, sulfate and acid. Seedling mangroves have been reported to tolerate high concentration of trace metals, such as: Cd, Cu, Hg, Pb (Walsh et al., 1979, MacFarlane and Burchett, 2001). However, little is known regarding the effects of Fe, Al, and Ni on mangrove seedlings, particularly under acidic environments.

Iron and aluminium are the elements that are released in huge amount during iron sulfide oxidation in ASS disturbed environments (Dent, 1986, Fitzpatrick et al., 1998, Cook et al., 2000, Macdonald et al., 2007). Although Fe acts as an essential nutrient for plants, in exceeding amount under low pH it has been reported to cause "bronzing" of rice leaves as a toxic effect (Sahrawat, 2004, Becker and Asch, 2005), negatively affect plants growth (Dent, 1986, Audebert and Sahrawat, 2000), and decrease yield (Audebert and Sahrawat, 2000, Sahrawat, 2004, Becker and Asch, 2005, Fageria et al., 2008). High Al concentration is widely known to inhibit root elongation of some terrestrial plant seedlings grown under acidic conditions (Marschner, 1991, Barceló and Poschenrieder, 2002, Bertrand et al., 2002, Kochian et al., 2004).

Copper and nickel are a few of trace metals whose concentrations elevate during pyrite oxidation (Lockhart, 1996). Under very high Cu soil concentration, toxic symptoms and total inhibition of emergence appear in *Avicennia marina* seedlings (MacFarlane and Burchett, 2001, MacFarlane and Burchett, 2002). Excessive concentration of Ni in soil reduces growth of plants (Nedhi et al., 1990, Kabata-Pendias and Pendias, 2001) and injuries (Kabata-Pendias and Pendias, 2001). High Ni concentration environment also diminishes root development (Kabata-Pendias and Pendias, 2001).

Sulfate is rarely reported to have a severe impact on plants because it is a nutrient sulfur form required by plants (Rennenberg, 1984). In disturbed ASS areas, increased level of sulfate or sulfide can occur due to its dynamic environments (Armstrong and Armstrong, 2005, Johnston et al., 2009b, Johnston et al., 2010b). Although increased level of sulfide has only a small impact on their aboveground biomass, such condition raises the ratio of root and aboveground biomass of seedlings of *Rhizophora mangle* (McKee, 1993). In high concentration, sulfide can damage mangrove seedlings, causing stomatal closure, decreased gas exchange, inhibit growth, and low survival (Youssef and Saenger, 1998, Kathiresan and Bingham, 2001). The growth of mangrove seedlings is also influenced by acidity (Kathiresan and Thangam, 1990).

Understanding the geochemical characteristics formed in an environment is important for determining which type of environment will provide suitable conditions for seedlings to live and vice versa. To achieve this objective, this chapter firstly examines the effects of experimental environments on the establishment of the *Rhizophora stylosa* seedlings and their relative growth rates, root length and other visual health conditions. Then, this chapter identifies the geochemical characteristics formed in non-ASS and ASS environments, and determines the relationship between geochemical factors and the growth variables. To achieve a better understanding in the geochemical processes occurred in the experiment that influence the establishment of the seedlings, interactions within these variables are also examined. This knowledge is critical to achieving a successful rehabilitation program.

2. Methods

2.1. Collection, propagation and treatment

Mature *R. stylosa* propagules and soils for growth medium as well as non-ASS treatment were collected from Myora Springs, North Stradbroke Island, while the soils for ASS treatment were collected from Brighton, southeast Queensland, Australia. The propagules were grown allowing seven months to establish themselves under standard laboratory conditions before being transferred into each of the larger experimental conditions. The soils for the medium of control and metal treatments were added with Ni in the form of $NiCl_2$ in concentrations of 25 and 55 μg/g, and Cu was added in the form of $CuCl_2$ at concentrations of 70 and 280 μg/g. The duration of the treatment was up to 80 days.

2.2. Measurement

Before propagation, several geochemical analysis of mediums were conducted to ensure the suitability of the soil for propagation. The measurement included pH, redox potential, organic content, total concentration of Fe, Al, Ni and Cu. During seven weeks of the treatment period, the pH of the mediums were measured to determine the influence of the artificial seawater supplied.

After experiment period, the geochemical analysis of the experimental soils surrounding the roots ($\pm$ 10 cm depth) measured sulfide, pH, redox potential, organic content, grain size, water-soluble sulfate, S_{HCl}, total metal, and porewater sulfide.

The measurement of pH and redox potential (Eh) was performed using a water quality meter. The organic content was determined using LOI method (Heiri et al., 2001). The grain size was determined by wet sieving analysis (Percival and Lindsay, 1997), through which the percentages of silt, clay and very fine sand (< 63 μm) and sand (> 63 μm) were attained. The water soluble sulfate was established by extracting water from soil samples using deionized water. The samples were shaken for 30 minutes, centrifuged and filtered using 0.45 μm membrane (Page and Steinbock, 2009). The HCl-extractable sulfur (Tot S_{HCl}) were established by extracting soil with 4M HCl (1 : 40) (Ahern et al., 2004). Both extracted samples for water soluble sulfate and Tot S_{HCl} were further analysed for sulfate using the Turbidimetry method (APHA, 1999). The

porewater sulfide was analysed using the blue methylene method, and determined by spectrophotometer (APHA, 1999).

Growth measurements included apparent health conditions, survival of seedlings, and measurement of the total height of seedlings, and their average root length. The average root length was determined by recording the image of the root and analysing it using the software ImageJ. Relative growth rates were determined using the following calculation:

$$RGR = (\ln m_2 - m_1) / (t_2 - t_1)$$

Where: m_2 and m_1 = plant height at the end and beginning of the experimental period
$t_2 - t_1$ = the time gap (Poorter and Garnier, 2007).

2.3. Statistical analysis

Data are presented as mean +/- standard deviation. Chi-square analysis was used to examine test the association between the two main types of environments and the survival of the mangrove seedlings. The General Linear Model (ANCOVA) was used to examine the difference in either the relative growth rate (RGR) or root length of the *R. stylosa* seedlings within the different environments. The ANCOVA was also used to examine whether survival days affect the RGR and the final root length of the *R. stylosa* seedlings. Linear regression was used to determine the relationship between Al and Ni concentrations in the soils and the RGR and final root length of the seedlings.

Principal Component Analysis (PCA) was used to identify the characteristics of geochemical conditions of the experimental environments. To examine the correlation between geochemical factors and survival days, two-tail Pearson correlation analysis was employed.

3. Results

3.1. Propagation and experimental mediums

The average pH of soils that were used for propagation was 7.29 $\pm$ 0.16STD. This value is in the normal range for mangrove areas (Ramanathan et al., 1999). The average redox potential measured was -53 $\pm$ 4, which is in the range of other Eh values of mangrove soils (+100 to -250 mV) (Saenger, 2002). The range of LOI was between 2.48 $\pm$ 0.57 %, above the organic matter content in mangrove soils used as control in other research (1.98%) (Tam and Wong, 2000), but

was far below the value in the Ho Chung mangrove swamp that is close to the discharge sewer point in HKSAR, China, where it was 34 ± 10% (Yu et al., 2005).

The average concentrations of total Fe and Al in the soils at the beginning of propagation were 943 ± 108μg/g and 1095.8 ± 105.4 μg/g, respectively. No limit value is prescribed in the ANZECC standard, however these concentrations are below those found in other clean mangrove sites or in ASS-contaminated areas. The concentration of Fe found in the soils in a clean mangrove site in Brazil was 2464 ppm per dry weight (Harris and Santos, 2000), while the concentration of Fe found in an ASS-contaminated site in Logan River estuary, Australia was up to 50,000 mg/kg (Lockhart, 1996). The Al concentration in the soils in an ASS-contaminated site in the Pumiceston region, Australia was up to 14,694 mg/kg (Preda and Cox, 2002).

The concentrations of total Ni and Cu in propagation soils were 4.30 ± 0.9 μg/g and 1.4 ± 0.4 μg/g, respectively. These concentrations are also far below the trigger value of the ANZECC and ARMCANZ standard (2000), which are 21 for Ni and 65 ppm Cu. See Table 4.1 for the detailed measurement of metal concentrations in propagation soils.

Table 4.1. The concentration of metals in propagation soils (n = 5). Data are presented as mean +/- standard deviation.

Sample	Concentration (μg/g)			
	Al	Fe	Ni	Cu
1	1202	983	4.50	1.5
2	1159.5	1083	2.00	2
3	855.5	733	5.50	0.5
4	1072.5	883	4.50	1.5
5	1189.5	1033	5.00	1.5
Average	1095.8 ± 105.4	943 ± 108	4.30 ± 0.9	1.4 ± 0.4

During the experimental period, the pH of the mediums had changed according to the pH of the artificial seawater supplied. The soil pH of non-ASS environment mediums decreased slightly up to 0.6 point, while the pH of ASS environment mediums increased nearly one point (Figure 4.1).

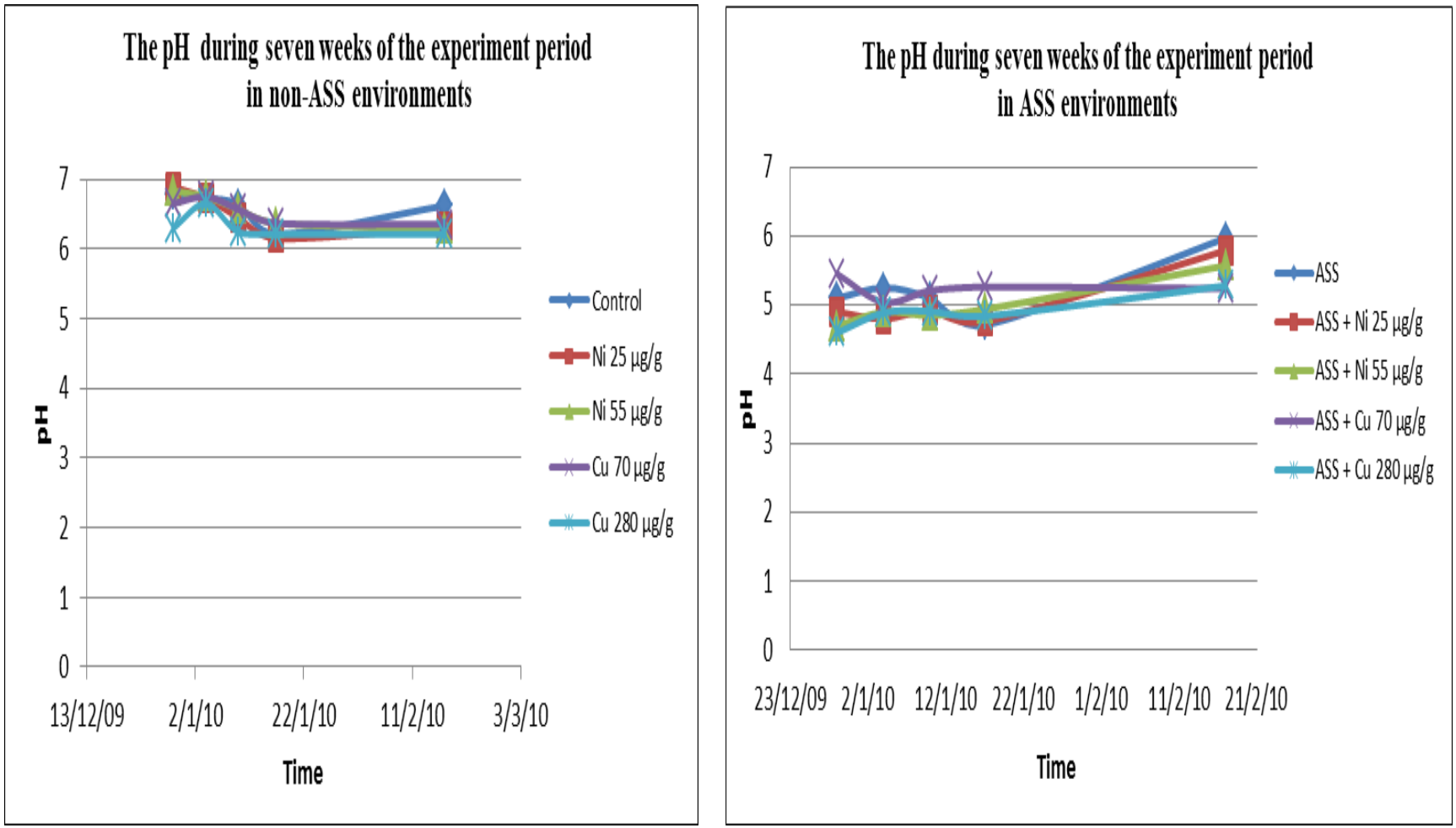


Figure 4.1. The pH of soils during seven weeks of the experiment period in non-ASS and ASS environments

3.2. Seedlings survival and growth rates

There was very strong evidence to conclude that the establishment of mangrove seedlings depended on the type of experimental environment. Figure 4.2 shows that the proportion of seedlings that survived in the non-ASS environments was higher (80%) than those in the ASS environments (27%) ($x^2 = 6.6$, df = 1, p = 0.010). See Appendix A.1, Table A.1.2, pp. 146 for the detail data. However, root treatment during measurement before seedlings replanting may also affect seedlings condition and reduce their ability to establish.

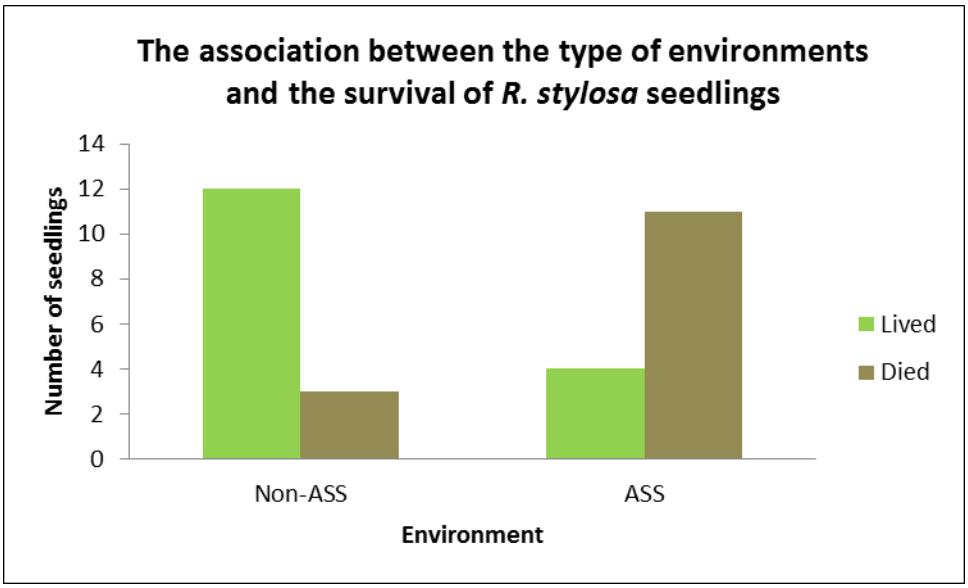


Figure 4.2. The number of *R. stylosa* seedlings survived in non-ASS and ASS environments (n=30)

Loss of turgor was the primary visual effect on the dying seedlings in both non-ASS and ASS environments. Loss of leaves from both surviving and dying seedlings was also commonly recorded in all environments. The time taken for the new leaves to unfurl was delayed for the surviving seedlings in the ASS environments.

In general there was a pattern which showed that the averages of total fresh length and the relative growth rate (RGR) of the seedlings were lower in ASS environments (Table 4.2). However, ANCOVA results show that there was no statistical difference in either the RGR or

root length difference of the *R. stylosa* seedlings within the environments. There was also no effect of survival days on either the RGR or length root difference (Table 4.3).

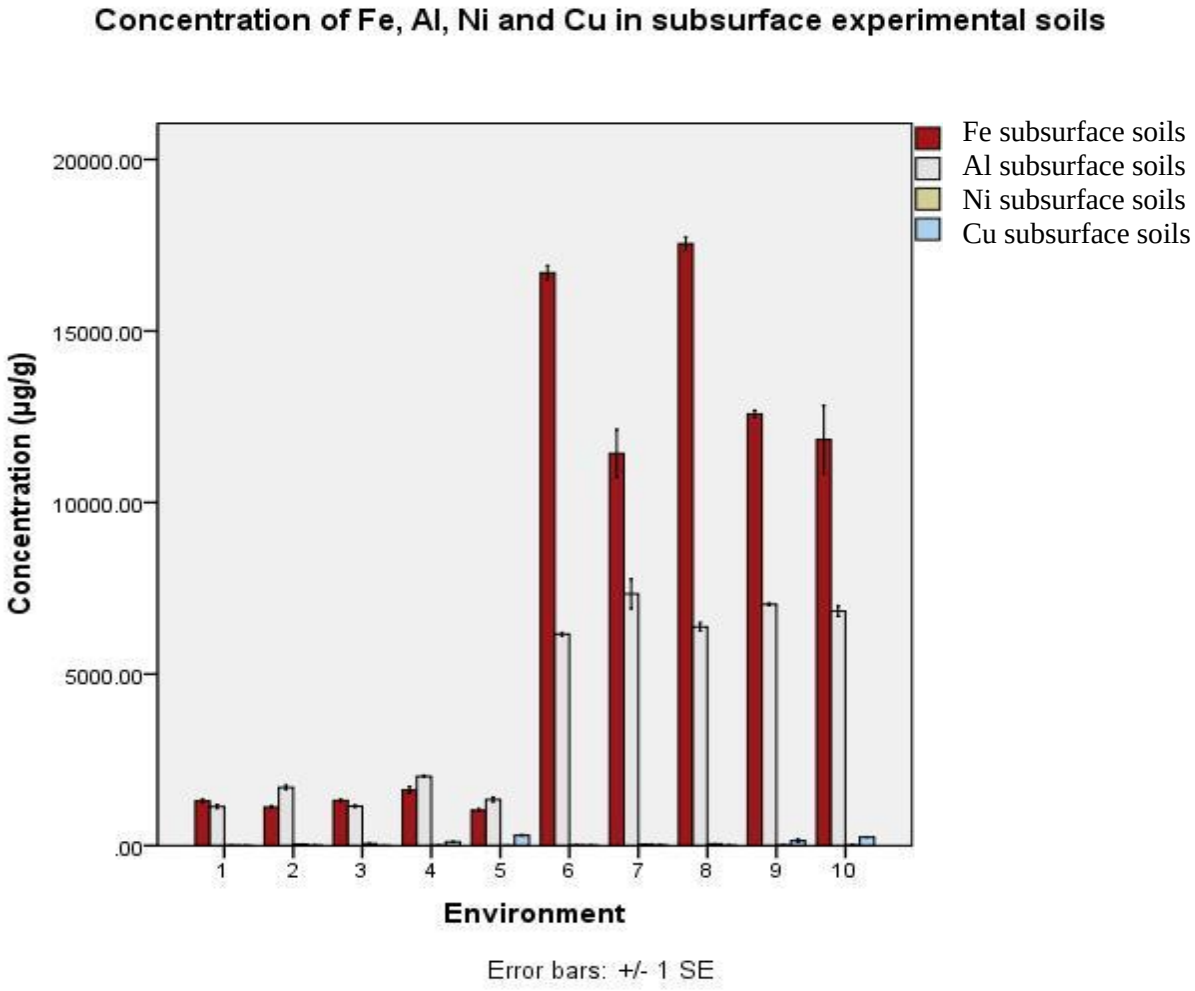


Figure 4.3. The average concentrations of metals in subsurface experimental soils (n=30). Environments: 1. Control; 2. Ni 25µg/g; 3. Ni 55µg/g; 4. Cu 70 µg/g; 5. Cu 280 µg/g; 6. ASS; 7. ASS+Ni 25µg/g; 8. ASS+Ni 55 µg/g; 9. ASS+Cu 70 µg/g; 10. ASS+Cu 280 µg/g. Values are mean $\pm$ SE

The ranges of average concentrations of Fe, Al, Ni, and Cu in the ASS subsurface soils were 11429.68–17545.27 µg/g, 6161.67–7336.40 µg/g, 9.17–47 µg/g, and 5.83-248.33µg/g, respectively (Figure 4.3). The concentration of the metals in subsurface soils can be seen in the Appendix A1. Table A.1.1, pp. 145.

Table 4.2. Total height and root length of *Rhizophora stylosa* seedlings before and after treatments, and relative growth rate (n = 30). Data are presented as mean +/- standard deviation.

Environment	Total height (cm)			RGR	Length (cm)		
	Before	After	Difference	(cm / d)	Before	After	Difference
Control	46.7 ± 2.5	48.7 ± 1.5	2.0 ± 1.7	0.0005 ± 0.0004	8.2 ± 4.2	8.4 ± 4.1	0.3 ± 0.2
Ni 25 µg/g	54.0 ± 7.0	55.1 ± 5.4	1.1 ± 1.7	0.0003 ± 0.0004	10.5 ± 2.0	10.9 ± 1.9	0.4 ± 0.2
Ni 55 µg/g	47.5 ± 5.7	48.8 ± 6.7	1.3 ± 1.1	0.0003 ± 0.0002	8.2 ± 0.3	8.7 ± 0.9	0.5 ± 0.6
Cu 70 µg/g	50.4 ± 9.2	51.0 ± 8.8	0.6 ± 0.7	0.0002 ± 0.0002	9.1 ± 3.2	9.6 ± 3.4	0.5± 0.2
Cu 280 µg/g	48.0 ± 3.1	48.8 ± 3.3	0.8 ± 1.0	0.0002 ± 0.0003	10.9 ± 0.6	11.0 ± 0.6	0.1 ± 0.0
ASS	46.8 ± 5.7	47.1 ± 5.2	0.3 ± 0.6	0.0001 ± 0.0002	8.2 ± 0.3	8.3 ± 0.5	0.1 + 0.5
ASS + Ni 25 µg/g	47.5 + 5.9	48.5 ± 5.8	1.0 ± 1.7	0.0003 ± 0.0004	9.2 ± 1.8	9.2 ± 1.4	0.0 ± 0.4
ASS + Ni 55 µg/g	48.8 ± 1.0	49.0 ± 1.0	0.2 ± 0.3	0.0001 ± 0.0001	9.1 ± 0.7	9.1 ± 0.7	0.1 ± 0.0
ASS + Cu 70 µg/g	46.0 ± 3.6	46.2 ± 3.4	0.2 ± 0.3	0.0002 ± 0.0002	10.4 ± 2.0	10.5 ± 1.9	0.2 ± 0.2
ASS + Cu 280 µg/g	45.8 ± 2.5	46.2 ± 2.5	0.3 ± 0.6	0.0001 ± 0.0001	8.5 ± 0.8	8.6 ± 0.9	0.1 ± 0.1

Table 4.3. ANCOVA results for relative growth rate, environments, and survival days (n = 30).

Variables	df	F	p
Relative growth rate:			
Environment	6	0.071	0.998
Survival days	1	4.204	0.061
Environment*Survival days	6	0.137	0.989
Root length addition:			
Environment	6	0.071	0.968
Survival days	1	4.204	0.235
Environment*Survival days	6	0.137	0.908

df: degrees of freedom, F: value to test of null hypothesis, p : significance values, * : interaction between the variables

Regression analysis (Table 4.4) shows that RGR did not correlate with metal concentration in subsurface soils ($p > 0.05$). There was no relationship between the values of metal concentration in subsurface soils on root length difference of the seedlings ($p > 0.05$), except on the Fe concentration in subsurface soils ($r = -0.358$, $r^2 = 0.128$, $n = 30$, $p = 0.052$). There was an indication of the negative influence of Al in subsurface soils on the addition of root length, although it was not strong ($r = -318$, $r^2 = 0.101$, $n = 30$, $r = 0.052$).

Table 4.4. Result of regression analysis between relative growth rate, root length difference, and metal concentrations in subsurface soils (n = 30). Bold values indicate that the variables have p value that is closer to 0.05

Variable:	p	r
RGR:		
Fe subsurface soil concentration	0.081	-0.324
Al subsurface soil concentration	0.136	-0.279
Ni subsurface soil concentration	0.915	0.020
Cu subsurface soil concentration	0.161	-0.263
Root length addition:		
Fe subsurface soil concentration	**0.052**	**-0.358**
Al subsurface soil concentration	0.087	-0.318
Ni subsurface soil concentration	0.929	0.017
Cu subsurface soil concentration	0.073	-0.332

p : significance values, r : correlation values

Principal Component Analysis shows that there were three groups with certain geochemical aspects involved (Figure 4.4). The non-ASS environment cluster (Group A) was characterised by higher pH, lower Eh, lower sulfur species, and higher sand content. This group had a higher number of survival days. Another sub-group of non-ASS environment (Group B) had higher organic content and sulfide concentrations. Acid sulfate soils environments (Group C) are characterised by higher Eh, sulfate and sulfur concentrations, higher silt/clay percentages, and lower pH. Acid sulfate soil environments had a lower survival time.

This characterisation was supported by Pearson correlation analysis, which shows that the number of survival days increases with higher pH, lower redox potential, higher sand percentage, lower sulfate and S_{HCl}, and higher sulfide concentrations (Table 4.6). However, the correlation

analysis does not support the sub-group in ASS (Group B) and shows that there was no correlation between organic contents and other variables, including sulfide.

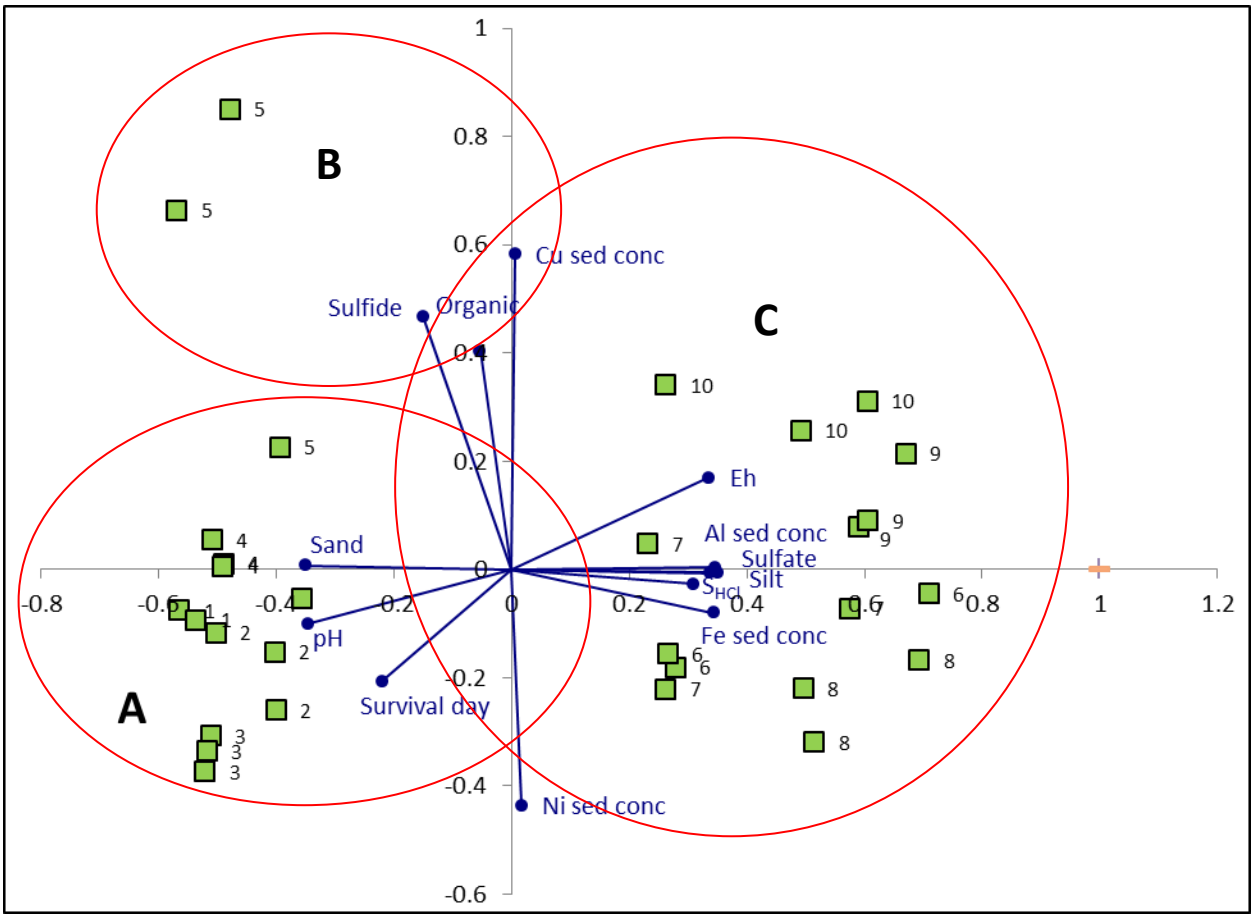


Figure 4.4. Principal Component Analysis of the geochemical factors affecting the establishment of *R. stylosa* seedlings. Environments: 1. Control; 2. Ni 25µg/g; 3. Ni 55µg/g; 4. Cu 70 µg/g; 5. Cu 280 µg/g; 6. ASS; 7. ASS+Ni 25µg/g; 8. ASS+Ni 55 µg/g; 9. ASS+Cu 70 µg/g; 10. ASS+Cu 280 µg/g. Notes: the boxes represent the type of environments. The concentration of metals in subsurface soils are written by the element name followed by "sed conc"

Table 4.5. Geochemical data on the experimental environments (n=30). Data are presented as mean +/- standard deviation.

Environment	pH	Eh	Sand (%)	Silt/clay (%)	Organic (%)	Sulfide (mg/l)	Water-soluble sulfate (mg/l)	S_{HCl} (%)
Control	6.62 ± 0.23	-250 ± 55	91.30 ± 2.10	6.90 ± 2.10	3.30 ± 0.13	1.24 ± 0.90	0.86 ± 1.01	0.14 ± 0.02
Ni 25 µg/g	6.65 ± 0.06	-288 ± 7	90.50 ± 1.42	9.47 ± 1.42	4.47 ± 0.54	0.50 ± 0.14	0.48 ± 0.25	0.24 ± 0.09
Ni 55 µg/g	6.64 ± 0.05	-285 ± 12	94.33 ± 1.08	5.67 ± 1.08	3.50 ± 0.14	0.30 ± 0.03	0.29 ± 0.21	0.16 ± 0.03
Cu 70 µg/g	6.74 ± 0.05	-280 ± 22	91.17 ± 2.45	8.83 ± 2.45	3.25 ± 0.07	2.74 ± 0.19	0.63 ± 0.04	0.14 ± 0.02
Cu 280 µg/g	6.42 ± 0.19	-213 ± 51	90.80 ± 0.96	9.20 ± 0.96	4.93 ± 1.62	8.60 ± 7.51	0.29 ± 0.10	0.15 ± 0.01
ASS	5.54 ± 0.79	-37 ± 119	66.63 ± 5.39	33.37 ± 5.39	3.27 ± 0.28	0.16 ± 0.01	5.70 ± 3.66	0.35 ± 0.03
ASS + Ni 25 µg/g	5.54 ± 0.55	-18 ± 89	71.87 ± 3.27	28.13 ± 3.27	4.09 ± 1.31	0.09 ± 0.04	9.41 ± 4.59	0.21 ± 0.07
ASS + Ni 55 µg/g	5.36 ± 0.44	-33 ± 110	67.60 ± 0.96	32.40 ± 0.96	3.12 ± 0.41	0.08 ± 0.04	11.42 ± 2.09	0.34 ± 0.03
ASS + Cu 70 µg/g	4.78 ± 0.42	178 ± 25	70.03 ± 1.77	29.97 ± 1.77	3.30 ± 0.37	0.06 ± 0.05	7.45 ± 1.33	0.40 ± 0.03
ASS + Cu 280 µg/g	5.31 ± 0.42	143 ± 136	72.63 ± 0.67	27.37 ± 0.67	5.06 ± 1.31	0.09 ± 0.05	9.75 ± 2.10	0.33 ± 0.03

Table 4.6. Correlation between survival days and geochemical factors (n=30)

	Survival days	pH	Eh	Sand	Silt	Organic	Sulfide	Sulfate	S_{HCl}
Survival days	1	.695**	-.664**	.464**	-.464**	0.097	.448**	-.602**	-.410*
pH		1	-.956**	.859**	-.859**	0.083	.755**	-.901**	-.796**
Eh			1	-.822**	.822**	-0.016	-.729**	.875**	.780**
Sand				1	-1.000**	0.095	.722**	-.915**	-.792**
Silt					1	-0.095	-.722**	.915**	.792**
Organic						1	0.261	-0.116	-0.152
Sulfide							1	-.802**	-.684**
Sulfate								1	.734**
S_{HCl}									1

** Correlation is significant at the 0.01 level (1-tailed). * Correlation is significant at the 0.05 level (1-tailed).

4. Discussion

The type of the environment, ASS versus non-ASS, significantly affected the establishment of *R. stylosa* seedlings in experimental conditions. Higher pH, lower Eh, lower sulfate concentrations and HCl extractable sulfur contents in non-ASS environments provided a good environment for mangrove seedlings to live. Similar pH and redox conditions have frequently been recorded in other mangrove research, underlining the significant impact that geochemical variables have on the establishment of mangroves. Other research has concluded that mangroves usually occur in neutral or near neutral pH environments (Kusmana, 1990, Ramanathan et al., 1999, Kitaya et al., 2002, Bosire et al., 2003) and reducing environments (McKee, 1993, Matthijs et al., 1999, Gleason et al., 2003).

In contrast, ASS environments that had opposite geochemical conditions to non-ASS environments that inhibited mangrove seedlings from establishing. The addition of sulfuric acid into the experimental medium that formed an ASS environment created high concentrations of water-soluble sulfate, and increased the percentages of S_{HCl} in the soils. In such acid conditions, exposure of soils to air increased the redox potential (Oxmann et al., 2010), and released high levels of metals and sulfate through oxidation of iron sulfide (Dent, 1986, Fitzpatrick et al., 1998, Cook et al., 2000, Macdonald et al., 2007), which in turn negatively affected the establishment of mangrove seedlings under the ASS mediums. This process was supported by the results of the Pearson correlation (Table 4.6). This correlation analysis shows that there were high associations between low survival days and high acidity (low pH), greater redox potential and high of both water-soluble sulfate and HCl-extractable sulfur. The Pearson analysis also confirms that low survival days was not caused by high water-soluble sulfate and extractable sulfur but high acidity of the ASS environment. This argument is represented by strong correlation amongst low pH, high water soluble sulfate and HCl extractable sulfur.

The concentrations of sulfide in all environments were categorised as low compared to the values around *R. mangle* stand areas in Hummingbird Cay, Exuma, Bahamas, which varied between 0–133 mg/l (Nickerson and Thibodeau, 1985), and to the sulfide values (2 mM, or 64 mg/l) where mangrove seedlings encountered stress symptoms, damaged root cell membrane, causing stomatal closure and decreased photosynthetic gas exchange (Youssef and Saenger, 1998). Strong correlations amongst sulfide, sulfate, pH, Eh and survival days in

the Pearson correlation analysis explain that under acidic conditions the oxidative ASS environments were dominated by high concentrations of sulfate compared to sulfide, in which the acid condition led to low survival days.

Strong correlations between grain size (sand, and silt/clay plus very fine sand) and establishment of seedlings (survival days) were due to the influence of the experimental mediums rather than to natural geochemical factors. Non-ASS mediums contained sandy soils from Myora, while ASS medium contained higher silt/clay and very fine sand from Brighton.

Despite its effect on the seedlings' establishment, the type of environment did not significantly affect either the RGR or the final values of root length of the seedlings. The number of survival days did not affect either the RGR or final root length of the seedlings in various environments.

High metal concentrations in soils in the experimental environments did not affect either the RGR or increase in root length, with the exception of the effect of Fe on decreased root length. One reason for the decrease in root length may be that the effect of the total concentration of Fe in soils on root length was greater than the Al and Ni concentration, due to the excessive concentration of Fe compared to the concentrations of Al and Ni in the ASS subsurface soils.

The presence of Fe subsurface soil concentration enhances Al accumulation in the seedlings. The accumulation of Al in tobacco (*Nicotiana tabacum* L.), leading to lethal conditions, only occurred when ferrous existed in the same nutrient medium. The existence of ferrous enhances the production of peroxidation lipids and causes a loss of membrane integrity. The disintegration of membranes causes Al to bind to oxygen donors on the surface of plasma membranes and poisons the plant (Ono et al., 1995, Chang et al., 1999). Similar conditions inhibit the growth, and cause leaf necrosis of *Phragmatis australis* (Nguyen et al., 2005). In this experimental study of mangrove growth there was an indication of the influence of Al on the decrease in root length, although it was not strong (see Table 4.4); this supports the role of Fe soil concentration in the Al effect.

Another factor that can explain the decrease in root length is the effects of acid associated with the presence of high Fe concentration in mid soils. The acid may come from the leachate

of Fe minerals (e.g. jarosite) due to watering (artificial tidal), or from the addition of acid (sulfuric acid) during the acidification process that releases Fe from the soils' minerals. This is supported by high levels of sulfur species and Fe in the soils of ASS environments (see Table 4.5 and Appendix A.1, Table A.1.1. pp. 145).

The amount and the period of exposure, as well as exclusion mechanisms, might be responsible for the absence of adverse impacts on the final total height and root length caused by the experimental conditions. High Al concentrations (up to 29 mg/l) in acid mineral soils at Sheriffmuir, Scotland (pH 4.3 $\pm$ 0.11) did not have a severe effect on the root development of Birch seedlings (*Betuala pendula* Roth). In contrast, exposure to low Al concentrations (2–5 mg/l) caused inhibition of root elongation in that type of soil (Kidd and Proctor, 2000). In this study, exposure of mangrove seedlings to high metal concentrations did not have a significant effect on seedlings. This suggests that mangroves are able to adapt high concentrations of Al.

Low or no toxic effects on root development has been found in some Al-resistant varieties of maize (*Zea mays* L.) after long exposure to Al concentration (Gunsé et al., 2000, Kidd et al., 2001, Barceló and Poschenrieder, 2002). These plants showed inhibition of root elongation immediately after they were exposed in 50 µM Al, but the root development recovered after few hours and gained comparable conditions to the control plants after 24 hours (Gunsé et al., 2000, Kidd et al., 2001, Barceló and Poschenrieder, 2002). Such recovery from root growth inhibition is interpreted as a shock response (Parker, 1995) and is revealed in both Al-resistant and sensitive varieties of wheat (Barceló and Poschenrieder, 2002). Similar processes might also have affected the seedlings in the current study, where they faced loss of leaves earlier in the treatment, but then most of the seedlings in non-ASS environments recovered.

Exclusion of metals from roots is responsible for the lack of effect on either final length or root length of the seedlings. This argument is supported by the evidences that the examined metals were accumulated in root tissues with limited distribution to aerial parts, particularly non-essential metal Al. The discussion regarding on the concentration of metals in mangrove seedlings tissues is discussed in the Chapter 5. Exclusion of Al from root tip or apex is one type of plant tolerance mechanism (Delhaize and Ryan, 1995, Delhaize et al., 1993, Barceló

and Poschenrieder, 2002, Kochian et al., 2004) and is also responsible for root development, as it prevents the accumulation of Al in the plant (Barceló and Poschenrieder, 2002).

5. Conclusions

The type of the environment significantly affected the establishment of *R. stylosa* seedlings in experimental conditions, where the number of seedlings survived in non-ASS environments is greater than in ASS environments. Higher pH, lower Eh, lower sulfate and HCl extractable sulfur in non-ASS environments provided a good environment for mangrove seedlings to live in. In contrast, ASS environments that have opposite geochemical conditions inhibit mangrove seedlings from becoming established.

The type of environment did not affect either the values of seedlings' total fresh length or their root length. However, excessive concentrations of Fe compared to Al and Ni in subsurface soils negatively affected the increase in root length of the seedlings. The presence of Fe soil concentration enhances Al accumulation on the seedlings, which is shown by indications of the influence of Al on the decrease in root length growth, although it was not strong.

CHAPTER 5: THE BIOACCUMULATION AND TRANSLOCATION OF METALS IN *RHIZOPHORA STYLOSA* (GRIFF.) SEEDLING PARTS IN ACID SULFATE SOILS AND METAL ENVIRONMENTS: A LABORATORY EXPERIMENT

1. Introduction

The rehabilitation of mangroves in an Acid Sulfate Soil (ASS) environment potentially poses numerous problems, including high concentration of heavy metals. In this environment, aluminium and iron are released in significant amounts compared with other heavy metals (Lockhart, 1996, Cook et al., 2000, Preda and Cox, 2002, Macdonald et al., 2004). Other mobile metals such as nickel and copper are frequently found in elevated concentrations above the baseline in soil. Nickel concentrations, for instance, exceed the ANZECC standard compared to other trace metals observed in ASS-contaminated sites in the Logan estuary (Lockhart, 1996).

Laboratory-based studies (Walsh et al., 1979, Thomas and Eong, 1984, MacFarlane and Burchett, 2002) as well as field studies (Saenger et al., 1990, Jones et al., 2000, MacFarlane and Burchett, 2000, MacFarlane et al., 2003, Zhang et al., 2007b) revealed that various species of mangrove seedlings show tolerance to high concentrations of metal exposure. Through the Bioconcentration Factor (BCF) analysis, seedlings appeared to accumulate higher levels of metals in root tissues compared to those in soils, and uptake a limited amount of metals in aerial parts, i.e. stem and leaf tissues (Walsh et al., 1979, Thomas and Eong, 1984, Silva et al., 1990, Zheng, 1997, MacFarlane and Burchett, 2002, Alongi et al., 2003, Zhou et al., 2011). The BCF is a measurement of the ratio of a toxic substance in an organism relative to its environment (Jørgensen et al., 1998). The bioconcentration of various metals in different species of mangrove seedlings have been extensively assessed (see Table 5.1 and Table 5.2); however, the response of mangrove seedlings to acid sulfate soil conditions, particularly to Fe, Al, and Ni is poorly understood.

Higher concentrations of Fe and Al were found in the pneumatophora of mature *Avicennia marina* in the ASS area in the Pumicestone region, Queensland, Australia (Preda and Cox, 2002). However, nursery-grown mangrove seedlings that are transplanted into an ASS environment may behave differently from mature naturally grown mangroves that are well

established before the onset of ASS conditions. Therefore, a detailed understanding of the tolerance of mangrove seedlings to ASS conditions is important to achieve effective and successful rehabilitation.

The mobility of metal also controls the distribution and accumulation of metals in mangrove tissues (MacFarlane, 2002). To assess the mobility of a metal, a translocation factor is usually measured. The Translocation Factor (TF) is determined by measuring the ratio between shoot (i.e.leaf) and root concentration (MacFarlane et al., 2007, Regvar and Vogel-Mikus, 2008).

Zinc, the most mobile of all metals tested (Cu and Pb), accumulates to the highest concentration in leaf tissue of the mature *A. marina*, while other elements are accumulated to a limited degree (MacFarlane, 2002). Non-accumulation of Pb occurs in all parts of *R. mucronata* and very limited accumulation of Pb occurs in the parts of *A. alba* seedlings, with the exception of the roots for both species (Thomas and Eong, 1984). This indicates the low mobility of Pb. The TF of several metals can be seen in Table 5.1 and Table 5.2.

This study examines the response of *Rhizophora sytlosa* seedlings to exposure to high concentrations of Fe, Al, Ni, and Cu, and is based on a laboratory study that mimics ASS conditions and environments with high metal concentration. Under these environments, the bioconcentration and translocation factors of those metals in stem, leaf, and root tissues were determined. The correlations between the concentration of metals in the root and subsurface soils were examined. Additionally, the geochemical factors that influence the concentration of metals in root tissues and subsurface soils as well as the geochemical process involved were evaluated.

2. Methods

2.1. Propagule and soil collection and treatment

Mangrove propagules and soils were collected in Myora Springs, Stradbroke Island, south-east Queensland based on the consideration that the site has a better environment in terms of water quality, metal levels and non-acid sulfate soils. The soils collected were for propagation, control and metal treatment mediums.

Table 5.1. The concentration of essential and non-essential metals in soils and Rhizophoraceae tissues, based on published works.

Species	Location	Soils (µg/g)	Stem conc (µg/g)	Stem BCF	Leaf conc (µg/g)	Leaf BCF	Root conc (µg/g)	Root BCF	TF
Essential									
Fe:									
R. apiculata	Tamil Nadu, India[1]	29,100			103.8	0.004			
R. mangle	Rio de Janeiro, Brazil[6]	4856			37.2	0.008	1011	0.21	0.04
R. mucronata	Tamil Nadu, India[1]	29,101			140.2	0.005			
Mn:									
R. apiculata	Tamil Nadu, India[1]	385							
R. mangle	Rio de Janeiro, Brazil[6]	52			101	1.94	15.3	0.29	6.60
R. mucronata	Tamil Nadu, India[1]	385			391	1.02			
R. stylosa	Yingluo Bay, China[9]	46.6			48	1.03	10	0.21	4.8
Zn:									
R. mucronata	Laboratory based[7]	10	ND	ND	16.1	1.61	321.5	32.15	0.05
	Laboratory based[7]	250	42.4	0.17	56.7	0.23	792.3	3.17	0.07
	Tamil Nadu, India[1]	50			40.3	0.81			
R. apiculata	Tamil Nadu, India[1]	50			16.8	0.34			
R. mangle	Rio de Janeiro, Brazil[6]	18			7.2	0.4	19.9	1.11	0.36
R. stylosa	Western Australia[2]	29			6.6	0.23	15	0.53	0.43
	North Australia[4]	53			26	0.48			
	Yingluo Bay, China[9]	47			5.9	0.13	6.2	0.13	0.95
	Hainan Island, China[3]				5.7	0.2			

Table 5.2. The concentration of essential and non-essential metals in soils and Rhizophoraceae tissues, based on published works (cont.)

Species	Location	Soils (µg/g)	Stem conc (µg/g)	Stem BCF	Leaf conc (µg/g)	Leaf BCF	Root conc (µg/g)	Root BCF	TF
Cu:									
R. apiculata	Tamil Nadu, India[1]	24			10.25	0.43			
R. mangle	Rio de Janeiro, Brazil[6]	2.8			0.1	0.04	5.1	1.82	0.02
R. mucronata	Tamil Nadu, India[1]	24			19.9	0.83			
	E. India[5]	2.8			1.8	0.64			
R. stylosa	Western Australia[2]	14			3.7	0.27	6.5	0.47	0.57
	North Australia[4]	39			16	0.42			
	Yingluo Bay, China[9]	19			0.6	0.03	1.1	0.06	0.55
	Hainan Island, China[3]				2.4	0.18			
Non essential:									
Ni:									
R. stylosa	Yingluo Bay, China[9]	14.6			0.75	0.05	0.8	0.05	0.94
Pb:									
R. mangle	Laboratory based[8]	250	ND	ND	ND	ND	758.1	3.03	ND
	Rio de Janeiro, Brazil[6]	9.9			0.01	0	0.2	0.02	0.07
R. mucronata	Laboratory based[7]	50	ND	ND	0.9	0.02	9.6	0.19	0.09
	Laboratory based[7]	250	5.1	0.02	1	0.004	209.9	0.84	0.005
	Tamil Nadu, India[1]	8[4]			12.61	1.58			
R. apiculata	Tamil Nadu, India[1]	8[4]			12.23	1.53			
R. stylosa	North Australia[4]	16			2.2	0.14			
	Yingluo Bay, China[9]	10			0.8	0.08	0.9	0.09	0.83
	Hainan Island, China[3]				20	0.7			
Cd:									
R. mangle	Laboratory based[8]	500	27.6	0.06	20.9	0.04	517.7	1.04	0.04
R. stylosa	Yingluo Bay, China[9]	0.077			0.15	1.95	0.25	3.25	0.6

1. (Agoramoorthy et al., 2008), 2. (Alongi et al., 2003), 3. (Lian et al., 1999), 4. (Saenger et al., 1990), 5. (Sarangi et al., 2002);[6] (Silva et al., 1990), 7. (Thomas and Eong, 1984), 8. (Walsh et al., 1979), 9. (Zheng, 1997).

Soils for ASS treatments were collected from the mangrove area around Brighton Park, Bramble Bay, southeast Queensland. This location is categorised as Potential Acid Sulfate Soil (PASS), where ASS occurs within five metres of the upper layer and has a proportion of oxidisable sulfur above the recommended 'action level' in at least one soil layer (Ahern and McElnea, 2000).
Seven-month-old seedlings that were harvested from a nursery were replanted into each of the larger experimental mediums. Nickel in the form of $NiCl_2$ (by as much as 25 and 55 μg/g) and Cu in the form of $CuCl_2$ (by as much as 70 and 280 μg/g) were applied to soils used for the mediums of control, metal treatments and ASS plus metal treatments. The concentrations were based on the trigger level and above high level of the recommended soil quality guidelines of ANZECC standard (ANZECC and ARMCANZ., 2000). Three replications were applied in this experiment.

2.2. Measurement

Weekly analysis of water discharged during the first month was conducted to examine the total metals leachate from the experimental mediums. No preservation used since water samples were measured shortly after collection. Water samples were not filtered (Preda and Cox, 2001). Soil samples were oven dried at 85 ^{o}C for 48 hours (Ahern et al., 2004) and ground with ceramics mortar and pestle. Mangrove tissues were oven dried at 60^{o}C for 24 hours (Defew et al., 2005, MacFarlane and Burchett, 2001). One g soil samples and plant tissues (leaf, stem and root) were digested with concentrated HNO_3 and H_2O_2 (Khrisnamurty et al., 1976). Total metal of water, soils, and mangrove tissues samples were determined using ICP-OES.

The subsurface soils were tested for pH and redox potential) using a water quality meter. Organic content was determined using LOI method (Heiri et al., 2001). Water soluble sulfate was determined by extracting water from soil samples with deionized water. The samples were shaked for 30 minutes, centrifuged and filtered using 0.45 μm membrane (Page and Steinbock, 2009). HCl-extractable sulfur (Tot S_{HCl}) were determined by extracting soil with 4M HCl (1 : 40) (Ahern et al., 2004). Both extracted samples were further analysed for sulfate using the Turbidimetry method (APHA, 1999). Porewater sulfide was analysed using the blue methylene

method, and determined using spectrophotometer (APHA, 1999). All measurements used three replications.

2.3. Statistical analysis

The General Linear Model (ANCOVA) was used to examine whether survival days affect the values of bioconcentration of metals in mangrove tissues in different environments. ANOVA was used to determine the differences between the values of Bioconcentration Factors (BCF) of metals in each part of the mangrove seedlings (root, leaf, and stem) within the environments. If there was a significant difference within the environments, a post hoc pairwise analysis was applied to compare sample means between the environments.

One-tailed correlation analysis using the Pearson correlation was employed to test the relationship between metal concentrations in soils and roots. A Two-tailed Pearson correlation analysis was used to examine the correlation between metal concentration in the subsurface soils and the geochemical factors.

3. Results

3.1. Experimental conditions

Weekly analysis of discharged water during the first month showed that very little, or undetectable amounts, of metals were washed out in ASS treatments, except for Fe. As a comparison, the concentration of artificial seawater used to supply the propagation is shown in Table 5.3.

Table 5.3. The average concentration of metals in discharged water (n = 18). Data are presented as mean +/- standard deviation.

Environment	Concentration (mg/l)			
	Al	Fe	Ni	Cu
ASS	0.006 ± 0.009	0.170 ± 0.020	ND	ND
ASS + Ni 25 µg/g	0.006 ± 0.009	0.240 ± 0.020	0.010 ± 0.010	ND
ASS + Ni 55 µg/g	0.004 ± 0.005	0.210 ± 0.010	0.040 ± 0.010	ND
ASS + Cu 70 µg/g	0.002 ± 0.04	0.310 ± 0.010	ND	0.090 ± 0.017
ASS + Cu 280 µg/g	0.002 ± 0.04	0.298 ± 0.190	ND	0.040 ± 0.010
Artificial seawater	ND	ND	0.008 ± 0.013	0.038 ± 0.015

ND = not detected

Artificial ASS generated elevated levels of metals, particularly Fe and Al, which were released through the acidification process in the soils. The Fe and Al concentrations in the artificial ASS soils increased around 22% and 37%, respectively, from the initial concentrations of Brighton soils that used in ASS mediums. The concentrations of Fe and Al in ASS soils were around 10 times higher than in the Control soils that used soils from Myora (Table 5.4).

a. b.

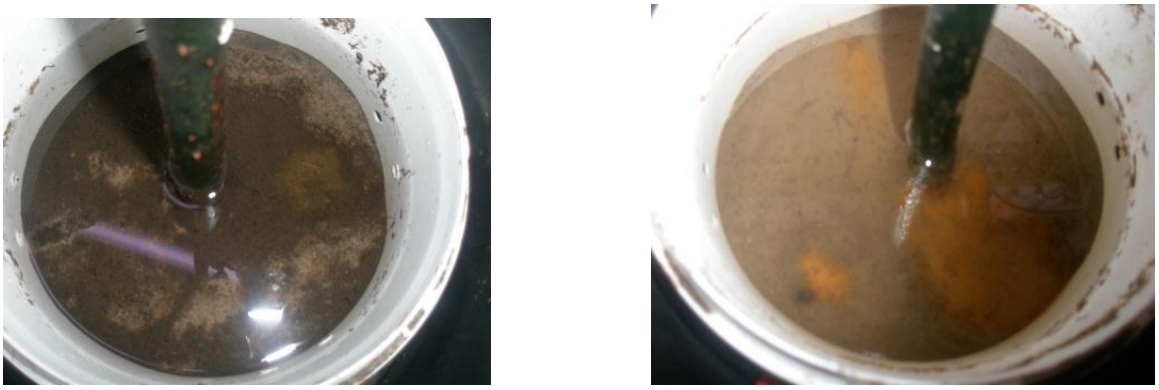


Figure 5.1. Soil colour at the beginning of the experiment. a). Typical non-ASS soils. b). Yellow jarosite existed at the surface of all artificial ASS soils.

The acidification process elevated Ni and Cu about 17% and 29%, respectively from the initial un-acidified Brighton soils. The acidified soils contained Ni and Cu, which were 35% and 77% higher than in the Control soils (Table 5.4).

Table 5.4. Comparison of metal concentrations obtained from Myora, Brighton, and acidified Brighton soils (n = 9). Data are presented as mean +/- standard deviation.

Soil	Concentration (µg/g)			
	Fe	Al	Ni	Cu
Myora	1227.54 ± 180.37	982.75 ± 10.96	7.75 ± 0.35	1.75 ± 0.35
Brighton	13963.55 ± 698.18	3681.5 ± 217	10.00 ± 2.47	5.50 ± 0.24
ASS	17905.6 ± 2251.53	5850 ± 120.2	12.00 ± 3.89	7.75 ± 0.35

During the experiment, the pH value of the ASS mediums increased close to neutral as a result of regular seawater supply. This is similar to natural conditions, where the pH of soils is influenced by the pH of water during high tide.

3.2. Bioconcentration and translocation factors of metals in seedling parts

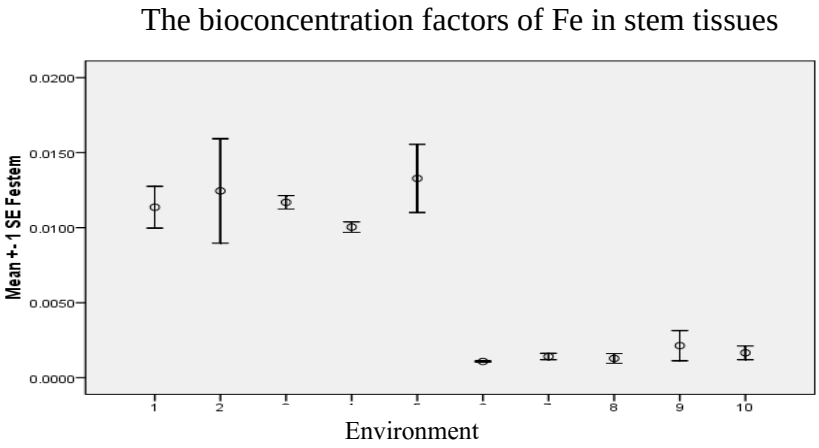
ANCOVA results showed that the values of bioconcentration factors of metals in mangrove seedling parts were not affected by the survival days ($p > 0.05$; Appendix B, pp. 156-163). The bioconcentration factors of metals in mangrove parts are presented in Figure 5.2-5.5. See Appendix A.2, pp. 151–153 for their detail concentrations. The translocation factors of metals in examined environments are presented in Figure 5.6 and Appendix A.3, pp. 154).

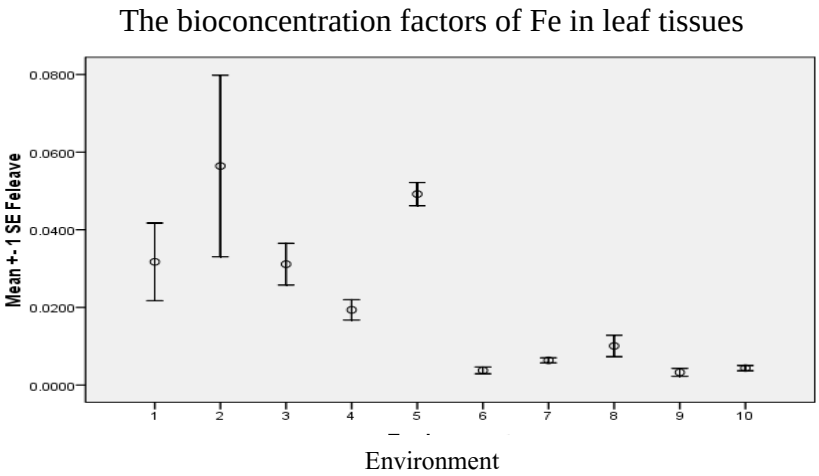
It becomes an important issue if the BCF or TF value is higher than 1, for this suggests that the seedlings accumulate more than the soils do. In this study, the BCF values were often less than 1 even in a very high concentration of metals. This was because beyond a certain high concentration of metals, the level accumulated remained stable.

3.3.1. Iron

In general, the BCF values of all non-ASS environments were strongly different from all ASS environments (Figure 5.2). A similar conclusion was generated by ANOVA that showed that the BCF values of Fe concentration in stem, leaf and root tissues were significantly different within the examined environments ($p < 0.05$). ANOVA analysis showed more detailed results for stem, leaf and root tissues (Appendix C, pp. 165).

There were three groups of BCF values of Fe in leaf tissues within the environments. The first group was for all environments, except Ni 25 μg/g and Cu 280 μg/g; the second group was consisted of ASS+Ni 55 μg/g and all non-ASS environments, except Ni 25μg/g. The third group was for all non-ASS environments. The BCF of Fe in root tissues was consisted of four groups, where two groups were dominated by ASS environments, while other two groups were consisted of non ASS (Figure 5.2). The first group was consisted of all non-ASS environments, except Cu 70 μg/g that were in a group of ASS. The second group was consisted of non ASS environment, except Ni 25 μg/g and Cu 70 μg/g. The third group was consisted of all ASS environments with addition of two non-ASS environments (ASS Cu 70 μg/g and Cu 280 μg/g). The fourth group





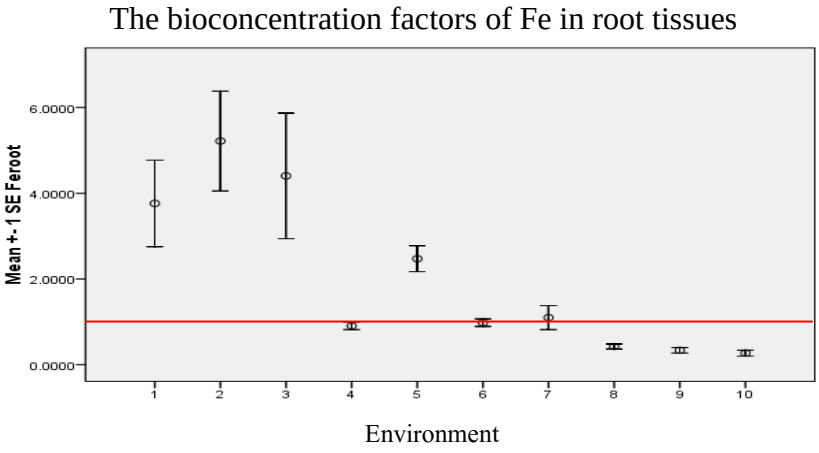


Figure 5.2. The error bars of bioconcentration factors of Fe in stem, leaf, and root tissues under different environments. Environments:1. Control; 2. Ni 25μg/g; 3. Ni 55μg/g; 4. Cu 70 μg/g; 5. Cu 280 μg/g; 6. ASS; 7. ASS+25 μg/g; 8. ASS+Ni 55 μg/g; 9. ASS+Cu 70 μg/g; 10. ASS+Cu 280 μg/g. Mean levels were compared using ANOVA, with all F values having 9, 20 df.

was consisted of all ASS (except ASS with addition of Cu 280 μg/g), with addition of two non-ASS environments (Cu 280 μg/g, ASS, and ASS plus Ni 25 μg/g).

The BCF values in stem and leaf were less than 1, while in root the values were higher than 1 in non-ASS, and less than 1 in ASS environments.

3.3.2. Aluminium

Although Figure 5.3 shows a wide range for Al BCFs in both stem and leaf tissues, ANOVA results show that the BCFs of Al found in stem and leaf tissue within all environments were very low (< 1) and similar ($p > 0.05$). The BCF values of Al in root tissues were significantly different within the environments ($p < 0.05$; Appendix C.5, pp. 171). In general, there were two groups of the BCFs of Al in root tissues within the examined environments. The first group was consisted of, all environments, except Cu 280 μg/g. The second group was consisted of non-ASS, except Cu 70 μg/g (Appendix C.6, pp. 172).

3.3.3. Nickel

The ANOVA results show that bioconcentration factors of Ni in stem, leaf and root tissues within environments were similar ($p > 0.05$; Appendix C.7, pp. 173), although Figure 5.4 shows ranges of concentration of Ni in the Control site in both stem and leaf tissues. The BCF values of Ni in stem and leaf were generally less than 1, while the BCF values in root tissues were generally higher than 1.

3.3.4. Copper

ANOVA results show that there was a significant difference in BCFs of Cu found in stem and leaf tissues within the environments ($p < 0.05$; Appendix C.8, pp. 174). In those tissues, Control had significantly different BCF values to other environments (Figure 5.5). The BCF values of Cu in stem and leaf tissues were less than 1, except in the Control environment.

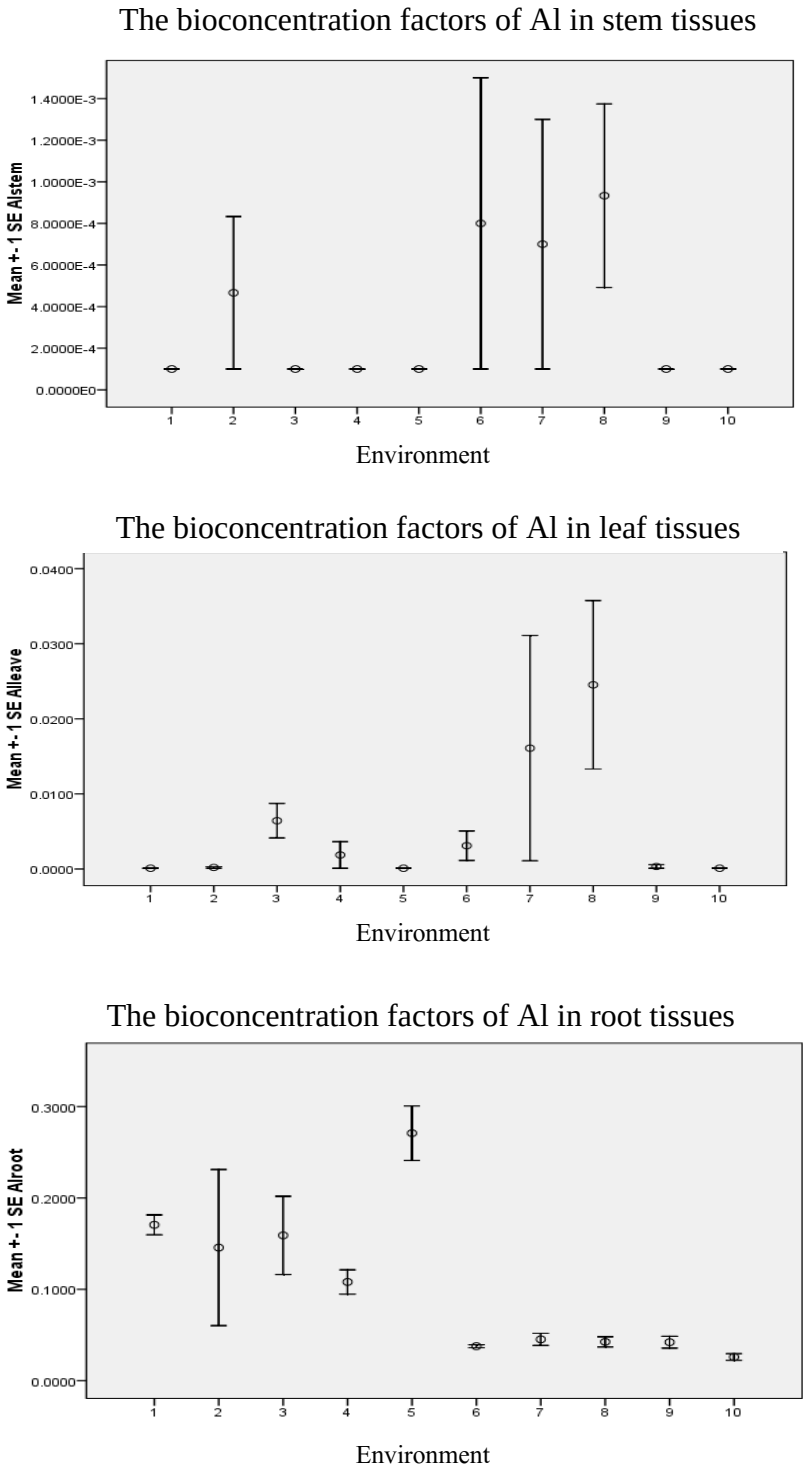


Figure 5.3. The error bars of bioconcentration factors of Al in stem, leaf, and root tissues under different environments. Environments:1. Control; 2. Ni 25μg/g; 3. Ni 55μg/g; 4. Cu 70 μg/g; 5. Cu 280 μg/g; 6. ASS; 7. ASS+25 μg/g; 8. ASS+Ni 55 μg/g; 9. ASS+Cu 70 μg/g; 10. ASS+Cu 280 μg/g. Mean levels were compared using ANOVA, with all F values having 9, 20 df.

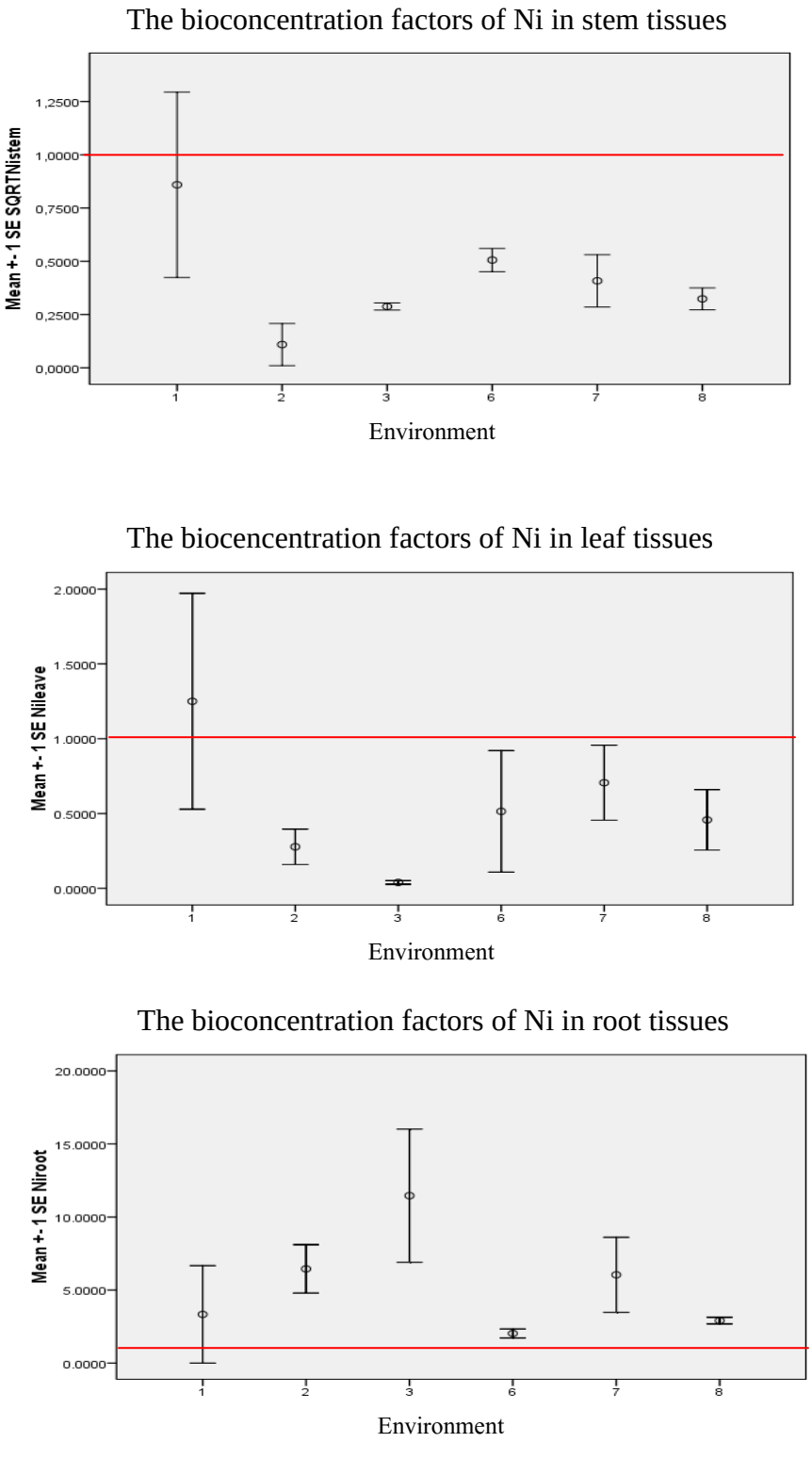


Figure 5.4. The error bars of bioconcentration factors of Ni in stem, leaf, and root tissues under different environments. Environments:1. Control; 2. Ni 25µg/g; 3. Ni 55µg/g; 6. ASS; 7. ASS+25 µg/g; 8. ASS+Ni 55 µg/g. Mean levels were compared using ANOVA, with all F values having 5, 12 df.

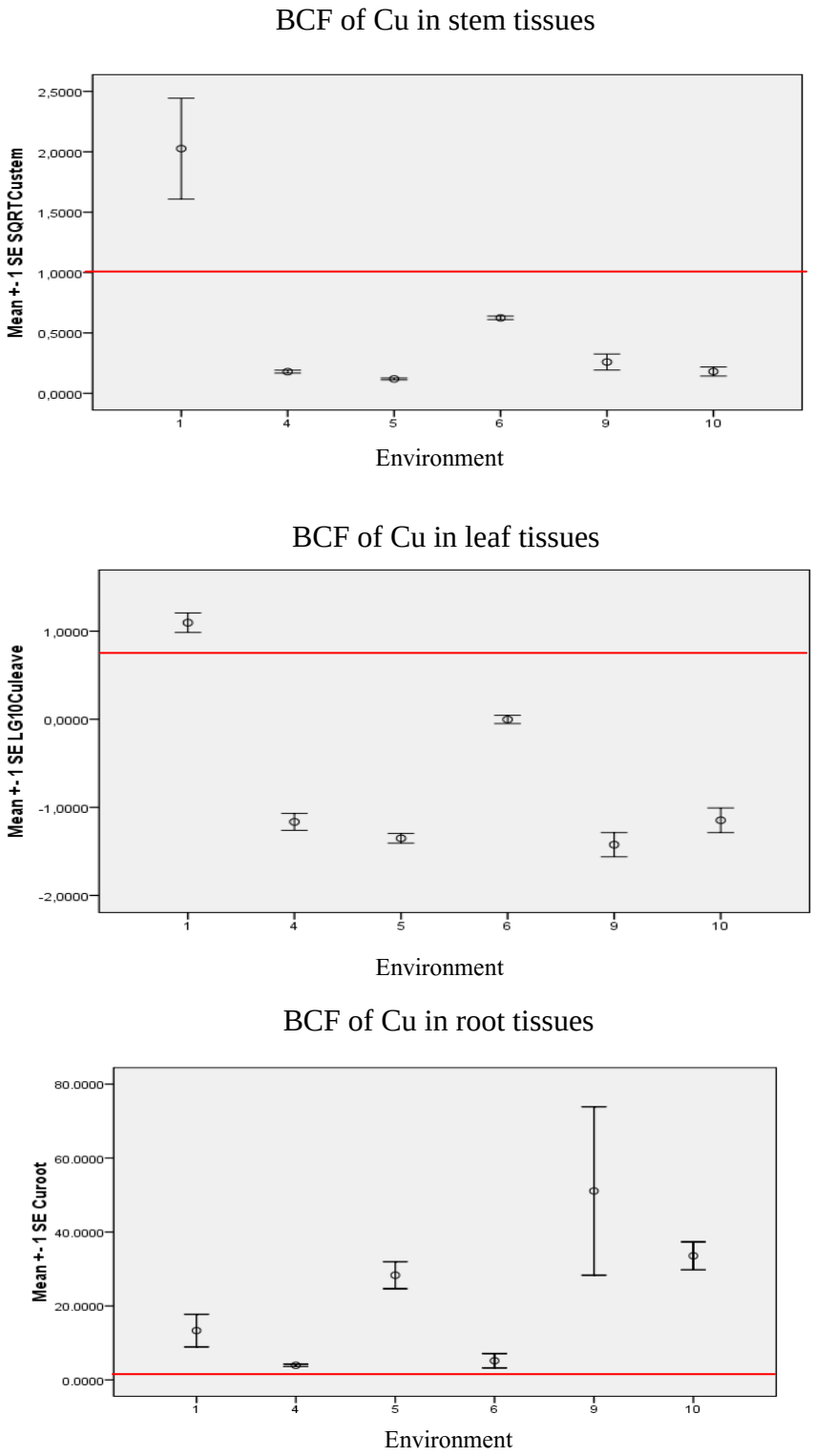


Figure 5.5. The error bars of bioconcentration factors of Cu in stem, leaf, and root tissues under different environments. Environments:1. Control; 4. Cu 70 μg/g; 5. Cu 280 μg/g; 6. ASS; 9. ASS+Cu 70 μg/g; 10. ASS+Cu 280 μg/g. Mean levels were compared using ANOVA, with all F values having 9, 20 df.

ANOVA results also show that the BCF values of Cu in root tissues were different in the different environments ($p < 0.05$; Appendix C.8, pp. 174). There were two groups: The first group consisted of all environments, except ASS+Cu 280 μg/g, and the other group consisted of all environments, except Cu 70 μg/g (Figure 5.5). The BCF values of Cu in root tissue were higher than 1.

Table 5.5. Summary of average bioconcentration factors of metals in mangrove tissues within the examined environments

Metal	Bioconcentration Factor				
	Non-ASS Environments		ASS Environments		Value
	Control	Metal Addition	ASS	Metal Addition	Comparison*
Stem:					
Fe	< 1	< 1	< 1	< 1	ASS lower
Al	< 1	-	< 1	-	Similar - ASS higher
Ni	> 1	< 1	< 1	< 1	Similar
Cu	> 1	< 1	< 1	< 1	Similar
Leaf:					
Fe	< 1	< 1	< 1	< 1	ASS lower
Al	< 1	< 1	< 1	< 1	Similar - ASS higher
Ni	> 1	< 1	< 1	< 1	Similar
Cu	> 1	< 1	< 1	< 1	Similar
Root:					
Fe	**> 1**	> 1	**> 1**	< 1	ASS lower
Al	< 1	-	< 1	< 1	ASS lower
Ni	> 1	**> 1**	> 1	**> 1**	Similar - ASS lower
Cu	> 1	**> 1**	> 1	**> 1**	Similar - ASS higher

- Value comparison represents the comparison between non-ASS and ASS mediums

The translocation factors of Fe, Al, Ni and Cu in all environments low (< 1) (Figure 5.6, Figure 5.7, and Appendix A.3, pp. 154).

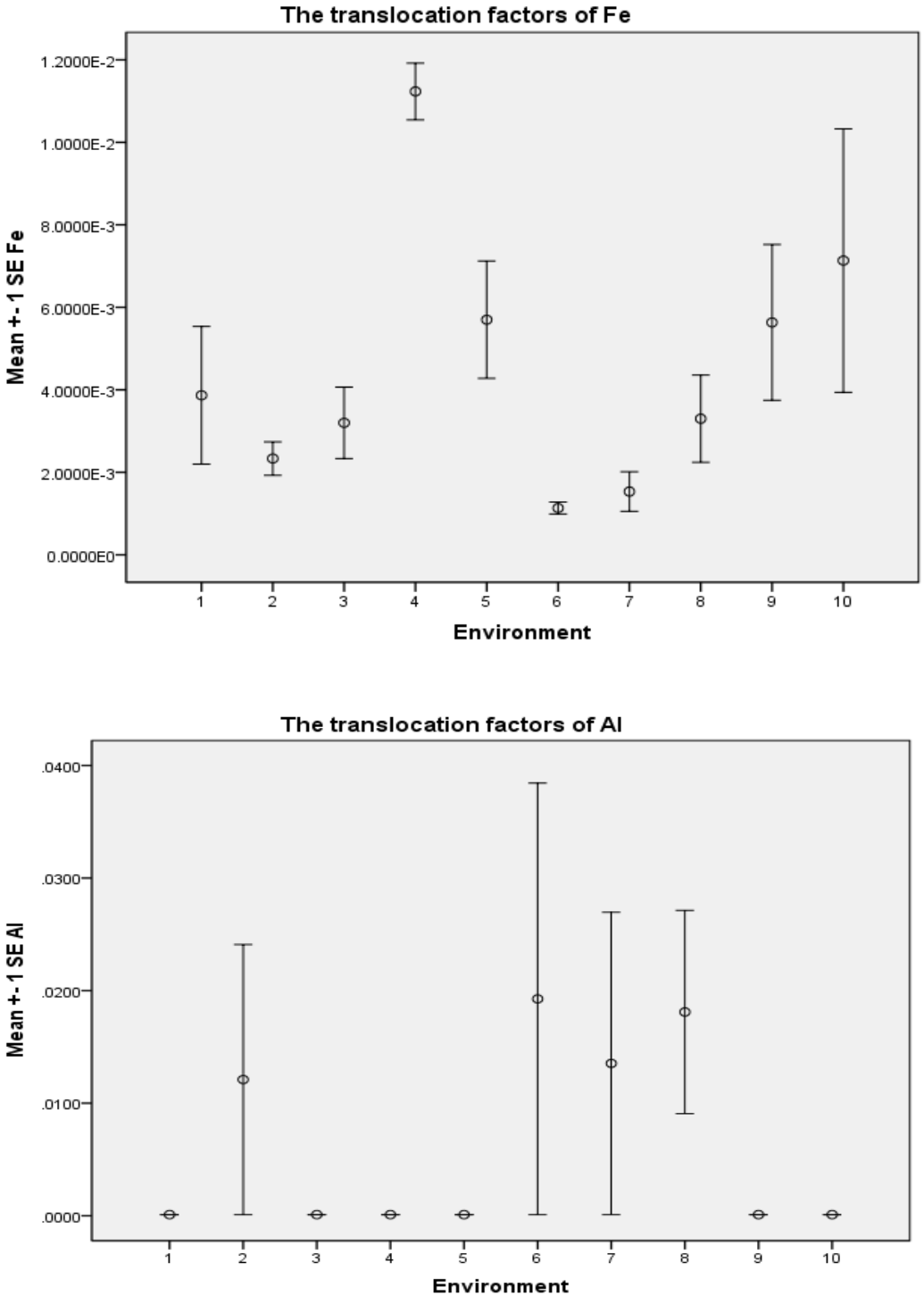
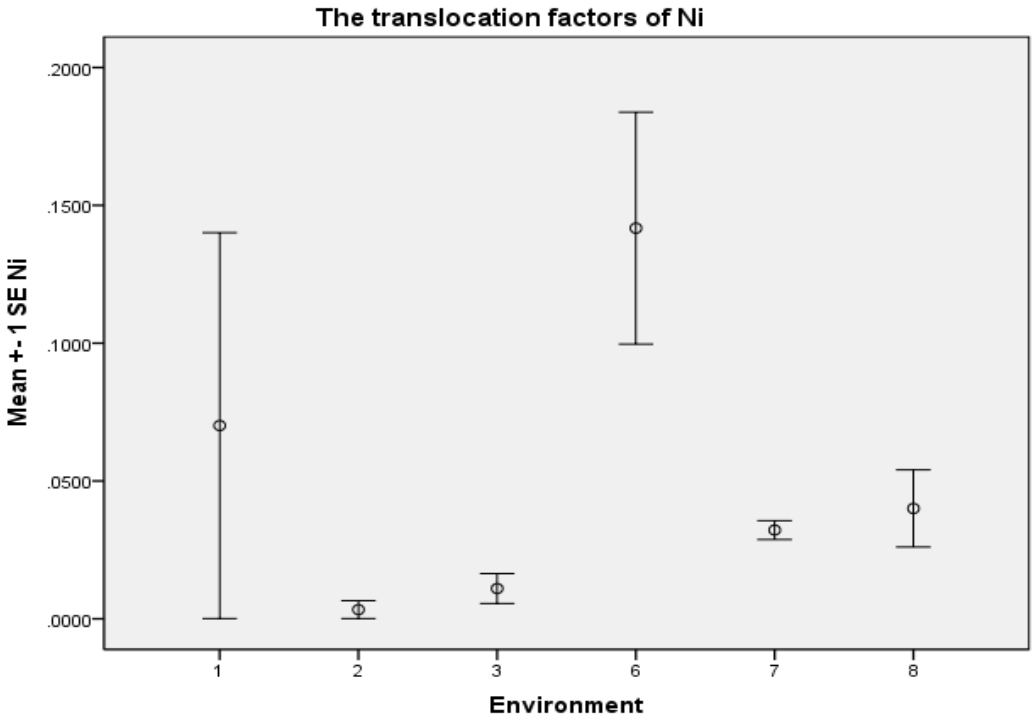


Figure 5.6. The error bars of translocation factors of Fe and Al under different environments. Environments:1. Control; 2. Ni 25μg/g; 3. Ni 55μg/g; 4. Cu 70 μg/g; 5. Cu 280 μg/g; 6. ASS; 7. ASS+25 μg/g; 8. ASS+Ni 55 μg/g; 9. ASS+Cu 70 μg/g; 10. ASS+Cu 280 μg/g.



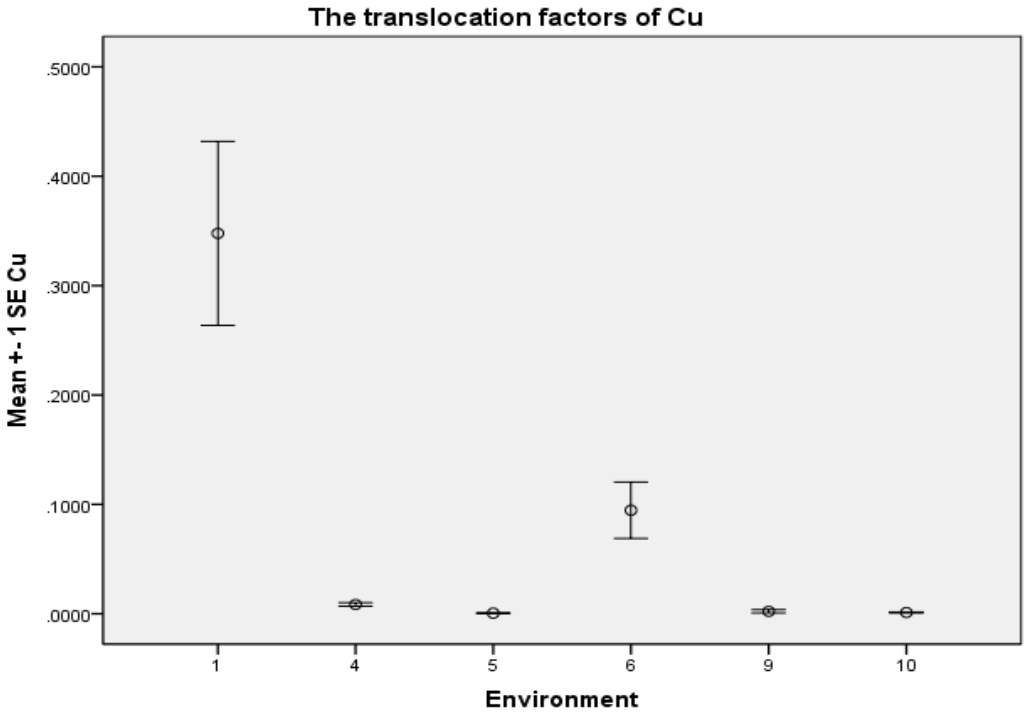


Figure 5.7. The error bars of translocation factors of Ni and Cu under different environments. Environments:1. Control; 2. Ni 25μg/g; 3. Ni 55μg/g; 4. Cu 70 μg/g; 5. Cu 280 μg/g; 6. ASS; 7. ASS+25 μg/g; 8. ASS+Ni 55 μg/g; 9. ASS+Cu 70 μg/g; 10. ASS+Cu 280 μg/g.

3.3. Factors influencing metal concentrations in roots and soils

The concentrations of Fe in roots correlated positively with the concentration of Fe and Al in the soils ($p < 0.05$). There was an indication of the influence of Al soils on the concentration of Al in root tissues, although the correlation was not strong. Concentrations of Al in the root did not correlate with the soil concentration ($p < 0.05$). Both Ni and Cu concentrations in the root had strong correlations with their concentrations in the soils ($p < 0.01$) (Table 5.6).

Table 5.6. Pearson matrix correlation between metal concentrations in root tissues and subsurface soils (n = 30)

Roots	Subsurface soils			
	Fe	Al	Ni	Cu
Fe	.533**	.450*	0.207	-0.323
Al	0.077	0.128	-0.159	0.194
Ni	-0.137	-0.193	.861**	-.711**
Cu	0.121	0.302	-.452*	.875**

**. Correlation is significant at the 0.01 level (1-tailed).
*. Correlation is significant at the 0.05 level (1-tailed).

Table 5.7. Pearson matrix correlation between metal concentrations in root tissues and subsurface soils, and geochemical factors (n = 30)

Metal concentrations		pH	Eh	LOI	Sulfide	Sulfate	S_{HCl}
Fe	Roots	-0.217	0.148	-0.16	-0.297	.320*	0.247
	subsurface soils	-.807**	.767**	-0.211	-.391*	.891**	.790**
Al	Roots	-0.153	0.087	0.29	.345*	0.047	-0.174
	subsurface soils	-.840**	.852**	-0.059	-.396*	.924**	.743**
Ni	Roots	0.208	-.334*	-0.056	-0.271	-0.156	-0.078
	subsurface soils	0.036	-0.144	-0.094	-.361*	0.098	0.088
Cu	Roots	-.364*	.488**	.375*	.352*	0.220	0.173
	subsurface soils	-.365*	.463*	0.268	0.345	0.309	0.214

**. Correlation is significant at the 0.01 level (1-tailed). *. Correlation is significant at the 0.05 level (1-tailed).

The pH, Eh, and sulfur species strongly influenced the concentration of major cations (Fe and Al) in soils in the experimental environments ($p < 0.01$, $p < 0.05$). Similar relationships between the concentrations of Fe and Al in root tissues occurred, although they were not strong.

The pH and Eh also strongly influenced the concentration of Cu in both root tissues and subsurface soils ($p < 0.05$, $p < 0.001$). The concentrations of Cu in root tissues were strongly influenced by the organic content and sulfide ($p < 0.05$). There was also an indication of the

effects of these variables on the Cu subsurface concentrations. Influences of water-soluble sulfate and extractable sulfur on the concentrations of Cu in subsurface soils and root tissues were also indicated, but the relationships were not strong. The concentrations of Ni in root tissues were significantly influenced only by redox potential, although there was an indication of the influence of pH, and sulfide on the concentration of Ni in root tissues. Sulfide also negatively influenced the concentration of Ni in subsurface soils (Table 5.7).

4. Discussion

4.1. Bioconcentration factors and translocation factors of metals in seedling parts

The results highlight the finding that the mangrove seedlings accumulated Fe, Al, Ni, and Cu mainly in roots, but these elements were distributed to a limited extent in stem and leaf tissues in response to high concentrations of metals, particularly in ASS conditions. Besides accumulation of metals in roots, large amount of metals bound to the cell wall during metal transportation was responsible for the higher concentration of metal in roots compared to shoots (Greger, 2004). Washing seedling roots using deionized water only (without using chemicals) might leave strongly bound mineral fraction, which counts to higher amount of total metal in roots.

The ratio of metal levels in roots compared soils were high (BCF were about 1 or greater than 1) in both non-ASS and ASS mediums, with the exception of the BCF values of Fe in addition of Ni 55 µg/g and addition of Cu 70 µg/g and 280 µg/g. The BCF values of metals in stem and leaf tissues in all environments were less than 1. Exception occurred in Control mediums where the BCFs values of Ni and Cu in stem and leaf tissues were greater than 1. The BCF values of Al in all parts seedlings in all different environments were less than 1.

In general, increasing concentrations of Fe and Al in ASS environments due to acidification processes did not cause a significant increase of bioconcentration factor values in all examined seedling tissues. Lower BCF values of Fe apparently occurred in all tissues in ASS mediums compared to non-ASS. The BCF of Al in root tissues was also significantly lesser in ASS conditions than non-ASS mediums. Similarly, the addition of either Ni or Cu in both non-ASS and ASS environments did not significantly raise the bioconcentration values in the tissues.

As mentioned above, the BCF values of major elements Fe and Al in roots were lower (< 1) in ASS mediums when high concentrations of either Ni and Cu was added, suggesting that seedlings excluded Fe from roots in response to the environments with excessive metal concentrations. The experiment results were in agreement with other studies on high metal concentration environments (Kathiresan and Bingham, 2001, MacFarlane et al., 2007, Bayen, 2012, Wang et al., 2012). The development of iron-plaques on the surface of the roots, caused by the leakage of oxygen, plays an important role in mangrove tolerance in high metal environments through exclusion of metal from the root (Machado et al., 2005).

Besides exclusion of metals from roots, metal regulation and excretion through leaves were mechanisms that occurred in to response high metal concentrations in the experimental mediums. Seedlings accumulated metals in stems and leave in steady amounts and increased in certain concentration in ASS mediums where metal concentrations were increased, but its ratio to soil concentrations were less than 1, suggesting the occurrence of tolerance mechanisms. The mechanisms were also observed in control medium, where Ni and Cu naturally existed in small amounts. In this medium, seedlings appeared to take up and accumulate Ni and Cu in leaf tissues, which is higher than soils concentrations. When these metals were added into both non-ASS and ASS mediums in high amounts, the seedlings retained these metals in roots (BCF > 1) and distributed them in limited amount to stem and leaf tissues (BCF < 1). In this experiment, this mechanism was strongly represented by seedlings in the uptake of essential element, Fe. Seedlings distributed Fe to stems and leaves in similar patterns to those accumulated in roots under different mediums. Metal regulation and excretion through leaves by mangroves were also reported by other researchers (MacFarlane and Burchett, 2000, MacFarlane et al., 2007, Bayen, 2012).

The preference of seedlings to take up essential elements and avoid non-essential metal was clearly shown by limited amount of the uptake of non-essential, Al, in both non-ASS and ASS environments. The seedlings strongly excluded Al from the roots, in contrast to high concentrations of Al in soils - including when Al levels in soils significantly increased as a fact of its nature as one of major elements released in acid environments. This was shown by the low BCF values of Al (< 1) in all seedling parts and in all environments with decreasing values in ASS environments. Nickel is also a non-essential element to plants, but its concentrations in soils

after Ni additions in either non-ASS or ASS environments were not as high as Al concentrations. Therefore, the BCF values of Ni in roots were greater (> 1) in all different environments. Low BCF values of metals in the stems and leaves and high BCF values of metals in roots have been reported in other studies (Walsh et al., 1979, Thomas and Eong, 1984, Silva et al., 1990, Zheng, 1997, MacFarlane, 2002, MacFarlane et al., 2003, MacFarlane et al., 2007).

Iron,aluminium, nickel and copper were transported to leaves with low mobility in all different environments (TF < 1). Iron was distributed to leaves in non-ASS mediums slightly mobile compared to those in ASS mediums. In contrast, translocations of Ni were slightly more mobile in ASS than those in non-ASS environments. The mobility of Cu in control medium was slightly higher than in other mediums. The mobility of Al in different mediums was similar, except for the slightly higher in TF value in ASS medium with Ni addition. However, the differences in the values were insignificant. Low values of TF support the BCF results that *R. stylosa* seedlings strongly excluded metals and transported them in limited amounts to prevent excessive metal uptake into the seedlings part, which can cause toxicities. Low TF values of metals found in this study are similar to those previously conducted in both field and laboratory-based studies (Walsh et al., 1979, Thomas and Eong, 1984, Silva et al., 1990, Zheng, 1997, Alongi et al., 2003, MacFarlane et al., 2007).

4.2. Factors influencing metal concentrations in roots and soils

As commonly observed in other studies (Walsh et al., 1979, Thomas and Eong, 1984, MacFarlane and Burchett, 2002, MacFarlane et al., 2003, MacFarlane et al., 2007), the concentrations of examined metals in roots increased as the metal concentrations in the environments increased. However, the relationships of Al in these compartments were not strong (Table 5.6). Negative correlations between the concentrations of Ni and Cu in the mediums as shown in Table 5.5 were also due to the addition of these metals into the different mediums and were not driven by geochemical factors, i.e. artificial acidification.

The Pearson correlation analysis (Table 5.7) shows that the concentrations of metals in roots and soils were influenced by similar geochemical conditions, although correlation between geochemical condition and metal concentrations in roots was not as strong as that in soils. Weak

or opposite correlations shown in the correlation analysis indicated that ion exclusion and selective phenomenon occurred in the experiment environments. This analysis confirms that the uptake of metals by seedlings was restricted to a certain level, at which the concentration then leveled off due to the response of high concentration of metals in soils as explained in subsection 4.1. Weak and opposite correlations between geochemical factors and Al concentrations in roots compared to its correlation to Al levels in soils shown in Pearson matrix correlation (Table 5. 6) also support the argument that mangrove seedlings took the non-essential metals, Al, and Ni to limited levels and excluded from roots. Adsorption of metal complexes with organic matter and sulfide on roots also influenced the correlations.

Strong positive correlations between metal concentrations (particularly the major metals, Fe and Al) in soils and both water-soluble sulfate and HCl extractable sulfur levels, and negative correlation between metal concentration and sulfide concentrations, shown in Table 5.6, describes the experiment conditions, ASS and non-ASS mediums. ASS mediums contained a significant amount of sulfuric acid led to a highly acidic environment. In this low pH and higher redox potential condition, rapid oxidation of soil minerals resulted in high concentrations of Fe and Al in the environments (Dent, 1986, Fitzpatrick et al., 1998) as well as elevation of trace metals (Fitzpatrick et al., 1998, Cook et al., 2000, Macdonald et al., 2004).

Non-ASS environment had the contrast environments. The increase of metal concentrations in soils increased the opportunity for these metals to be adsorbed onto seedling roots, or for uptake by the seedlings. However, the seedlings took metals only to a certain limited concentrations in order to prevent toxicity in such excessive metal environments, which explains the contrasting relationships between the influence of geochemical conditions and the concentration of metals in soils and roots.

The presence of Cu-sulfide precipitation around the root areas is supported by the results of Pearson correlation analysis which show that the concentrations of Cu in the root tissues were correlated to the sulfide concentration and organic content in the soils. In non-ASS environments that had reducing environments (low Eh), precipitation of metal sulfide tends to occur due to reduction of sulfate to sulfide (Xiong and Lu, 1993, Antoniadis et al., 2006, Prasad et al., 2006) by microorganisms through organic decomposition (Berner, 1970) Complexation of Cu with

organic matter tends to occur under reducing condition (MacFarlane et al., 2003). This condition leads to the adsorption of Cu sulfide on the root area (Walsh et al., 1979, Silva et al., 1990, Clark et al., 1997, Saenger et al., 1990, Lacerda, 1998). This argument was also supported by the PCA results presented in the previous chapter (Figure 4.4), where seedlings in non-ASS mediums with addition of Cu 280 µg/g were characterised by high Cu concentration in soil, sulfide level and organic content (Group B). High level sulfide in this medium also encouraged Al sulfide complex to formed and be adsorbed onto the roots, which was confirmed in the Pearson correlation analysis (Table 5.7) with strong relationship, and indicated in the Table A.1.4 in the Appendix A, which shows that the highest concentrations of Al in roots existed in non-ASS medium with addition of Cu 280 µg/g.

5. Conclusions

The concentrations of metals in the soils influenced metal concentrations in the root tissues of *R. stylos*a seedlings. However, in excessive metal concentrations, particularly under ASS environments, *R. stylosa* seedlings retained metals at the root, and limited the uptake of metals to aerial parts to prevent toxicities. This type of response was indicated by the low bioconcentration factor of metals in stem and leaf tissues and high BCF values in root tissues. Low bioconcentration factor values in the stem and leaf tissues were supported by the fact that the translocation factor values of metals were low, which indicates a low mobility as a strategy to avoid excessive uptake of metals into seedling bodies.

High concentrations of metals, particularly major elements Fe and Al, in soils were associated with low pH, oxidative environments, high sulfate and extractable sulfur that were established in ASS mediums. The concentrations of metals in roots and soils were influenced by similar geochemical conditions, but their correlations in roots were not as strong as those in soils. Weak or opposite correlations shown in the correlation analysis suggested that exclusion strategies and selective strategy are in response to excessive metal concentrations and to avoid non-essential elements occurred in the experiment environments. Adsorption of metal complexes with organic matter and sulfide on roots also influenced the metal correlations with geochemical factors.

CHAPTER 6: THE GEOCHEMICAL CHARACTERISTICS OF ACID SULFATE SOILS AND THEIR EFFECTS ON THE ESTABLISHMENT AND GROWTH OF MANGROVE SEEDLINGS IN ABANDONED PONDS

1. Introduction

The restoration of mangrove ecosystems in abandoned ponds that are unsuitable for aquaculture is important to regain mangroves' function. One issue that has been identified as responsible for the failure of some mangrove restoration projects is ASS (Lewis et al., 2006, Wolanski, 2006). Additionally, there is some evidence that mangrove seedlings have been affected by ASS in some abandoned ponds (e.g. in Tiwoho, North Sulawesi, Indonesia). Here, natural revegetation has occurred in abandoned ponds that have had their dikes breached, highlighting the role of suitable hydrology in mangrove restoration (Djamaluddin, 2006, Lewis et al., 2006, Kamali and Hashim, 2011).

Suitable physical conditions are necessary for seedlings to become established (Duke et al., 1998), including depth, frequency, and duration of flooding (McKee, 1993, McKee, 1995a, McKee, 1995b, Field, 1998, Lewis, 2005, Kamali and Hashim, 2011). Hydrology has become the focus of attention for mangrove restoration projects "as it controls the quantity, quality and timing of water entering the site" (Field, 1998), and plays a significant role in self-repair (natural recovery), or secondary succession (Lewis, 2005, Kamali and Hashim, 2011).

Despite widespread knowledge of the role of hydrology in mangrove restoration, there is little research into the geochemistry in ASS areas that may be affected by hydrological factors. Tidal inundation influences many factors, such as redox potential and pH of the soils (McKee, 1995b) and this condition is made even more complex by the oxidation of pyrite, which involves a number of redox reactions and microbial activities (Nordstrom, 1982, Evangelou, 1995, Cook et al., 2004, Ward et al., 2004b). Therefore, knowledge about the geochemistry in ASS areas is important because it influences the establishment of mangrove seedlings.

While the laboratory study identified some geochemical characteristics that mangrove seedlings require in various controlled environments (discussed in Chapter 4), this study seeks to answer

the question: In which geochemical conditions can mangrove seedlings establish naturally, and/or be replanted in abandoned aquaculture ponds?. To obtain a better understanding of the geochemical processes that affect the geochemical conditions and seedling responses in those areas, this study examines the interactions within the geochemical variables.

Understanding the nature of natural revegetation is important, since it has many advantages, such as: providing the most appropriate species to occupy (Macintosh and Ashton, 2002), a low cost establishment in terms of nursery, labour and machinery; less soil disruption; and strong establishment of seedlings (Lewis et al., 2006).

1. Study sites and methods

2.1. Study sites and measurement

The field study was conducted in the rainy season from July to December 2011 in six environments in an abandoned pond complex, and in one control area in Mare ($04^{o}51'S$, $120^{o}18'E$), district of Bone, province of South Sulawesi Selatan, Indonesia. Three seedlings were replanted in Site 1, located at the bank of a creek that was disturbed by ASS, and had no pre-existing seedlings. A seedling was defined as being no more than one metre high, and without branches. Sites 2 and 3 were uninundated or poorly inundated sites that were strongly disturbed by ASS. To have sites that can act as 'AASS control sites' and to allow comparison of a variety of geochemical conditions and mangroves density in other sites, no replantation was performed in these 'unvegetated' sites. Sites 4, 5, 6, and 7 had pre-existing mangrove seedlings (see Table 6.1). The density, establishment, and growth, which involved the height addition and relative growth rate of Rhizophoraceae seedlings, were determined from the replanted seedlings in Site 1 and pre-existing naturally occurring seedlings in other sites.

The density and survival rates of plants were estimated by counting, marking and measuring all individuals in six 1m x 1m plots randomly placed at each site. Plots were revisited at the end of the three-month period trial, and the same plants were examined. The plant height measured was the above ground height. The relative growth rate (RGR) of the seedlings was determined based on the height (Poorter and Garnier, 2007).

Table 6.1. Brief description of the field study sites

Site	Location	Mangrove species present	Description
1	• Abandoned ponds • At the bank of a blocked small creek	Replanted *R. mucronata* seedlings	• Water circulation mainly from creek
2	• Abandoned ponds	One seedling of mangrove fern (*Acrostichum sp*)	• No tidal effect • Jarosite at the surface
3	• Abandoned ponds • Drain	Some seedlings of mangrove ferns (*Acrostichum sp*)	• Limited tidal effect • Jarosite at the surface
4	• Abandoned ponds • Near bank of another creek • Across site 2 of AASS	• Mature (adult) and naturally occuring *R. stylosa* seedlings • Mature (adult), a few naturally occuring, and pre-existing replanted seedlings of *R. mucronata*	Free circulation of creek and seawater tidal water
5	• Abandoned ponds • Near bank of the same creek as at Site 4	• Mature (adult) *R. stylosa, R. mucronata, A. marina, Sonneratia sp* • Naturally occurring *R. stylosa* seedlings, and a few *R. mucronata* seedlings. • Pre-existing replanted seedlings of *R. mucronata*	Free circulation of creek and seawater tidal
6	• Abandoned ponds	• Mature (adult) *R. stylosa* and *R. mucronata* • Naturally occurring *R. stylosa* seedlings and a few *R. mucronata* seedlings	Free seawater tidal effect through broken dykes
7	• At a beach outside abandoned ponds	• Mature (adult) *R. stylosa* and *R. mucronata* • Naturally occurring *R. stylosa* seedlings and pre-existing replanted *R. mucronata* seedlings	Free seawater tidal effect

The study used three replications of porewater and 15 cm soil cores that were collected around mangrove seedlings at each site. The measurement including porewater sulfide, pH, pH_{fox}, redox potential, organic content, water-soluble sulfate, S_{KCl}, S_{POS}, grain size, and the colour of the soil from the soil core at $\pm$ 15 cm depth. Porewater sulfide was analysed using the blue methylene

method, and determined using spectrophotometer (APHA, 1999). pH, pH_{fox} and redox potential of soils were determined using water quality meter. Determination of pH_{fox} was similar to that of pH, but in the case of pH_{fox} the soils were added with H_2O_2 to oxidise sulfides (commonly in pyrite form). Organic content was determined using LOI method (Heiri et al., 2001). The analysis of grain size of the soil was done using wet sieve analysis.

Water soluble sulfate was determined by extracting water from soil samples with deionized water. The samples were shaken for 30 minutes, centrifuged and filtered using 0.45 μm membrane (Page and Steinbock, 2009). The S_P determines the sulfate contained in soils through oxidising the soils to generate maximum acidity from reduced sulfidic material (Ahern et al., 2004). The S_{POS} estimates the net potential acid risk of the soil from the unoxidised sulfur compounds by determining the difference between S_{POS} and S_{KCl} (White and Melville, 1993, Ahern et al., 2004). The analysis of S_{KCl}, S_P, S_{POS} followed Peroxide Oxidisable Combined Acidity and Sulfur (POCAS) method described by White and Melville (1993).

Determination of the pyrite percentages of surface and subsurface (three replications) were measured through Titratable Sulfidic Acidity (TSA) analysis (Konsten and Sarwani, 1990). The estimation of pyrite was based on the calculation:

Pyrite = (TSA: 22,4) x 0,1

2.2. Statistical analysis

This research applied several statistical analyses, using Excel and SPSS 21 to assess the variability of geochemical interactions and their correlations. A normality test was performed, and data transformation was employed for non-normal variables. The transformation types depended on the type of skewness. Kruskal Wallis analysis was used to examine the difference between density and growth values in the study area.

Principal Component Analysis (PCA) was employed to identify biogeochemical trends. Standardised regression was used to examine the relationship between the density, growth and other physical and geochemical variables. Pearson correlation was employed to identify the correlation and interaction between the physical and geochemical variables.

3. Results

3.1. Biological measurement

Table 6.2 shows that the highest seedling densities are in the Control site, as well as in Sites 6 and 5, followed by Site 4 of abandoned ponds. The already existing seedlings in these sites survived during the three month examination period, therefore the density values remained stable (100% establishment), except for those in Site 1. A replantation of three *R. mucronata* seedlings in 3 m^2 of Site 1 ended in a low survival rate, where only one seedling survived for the period (33.33%).

The height of seedlings in the Control site increased about 3.50 cm in average, while the seedling height in Site 4, 5 and 6 increased about 2.00-2.33 cm during the examination period. The height of seedlings in Site 1 increased around 1.00 cm. The average RGR values of seedlings in Sites 4, 5 and 6 were similar (0.0008-0.0009 cm/day) and close to that was the average value in Control site (0.0010 cm/day), which were higher compared to the RGR value in Site 1 (0.0001 cm/day).

3.2. Geochemical conditions

Table 6.3 shows that the average pH and pH_{fox} ranges in the abandoned ponds complex were lower (5.47-7.04, and 3.41 and 6.33, respectively) than those in the Control site (7.37 and 6.14, respectively). The average pH_{fox} values in the study sites were relatively high because of the rainy season and/or tidal effects. In survey time at the end of the dry season, the pH_{fox} values in Site 2 (reservoir), sites at the banks of creeks (1, 4, and 5), and at Site 6 (abandoned ponds) were 2.20, 4 - 6.60, and 4, respectively.

The average redox potential values observed in the ponds complex were between -32 and 201, where Sites 1, 2, and 3 varied widely. The environments in Sites 1, 2, 3, 4, and 5 mostly had oxidised conditions, while Site 6 and the Control site environments were reduced. The average organic content in the abandoned ponds area was high (17.94–23.78%), except in Site 6, which had lower organic content (10.54%). This value was close to the average organic content in the Control site (12.47%).

Table 6.2. The density, establishment, and growth of mangrove seedlings in the study area (n=21). Data are presented as mean +/- standard deviation.

Site	Density (n/m^2)		Establishment (%)		Height (cm)			RGR
	Before	After	Before	After	Before	After	Addition	(cm/day)
1	1.00 ± 0.00	0.33 ± 0.58	100.00 ± 0.00	33.33 ± 57.74	88.67 ± 7.23	89.67 ± 6.66	1.00 ± 1.73	0.0001 ± 0.0002
2	NA	NA	NA	NA	NA	NA	NA	NA
3	NA	NA	NA	NA	NA	NA	NA	NA
4	1.67 ± 0.58	1.67 ± 0.58	100.00 ± 0.00	100.00 ± 0.00	33.00 ± 7.00	35.33 ± 6.35	2.33 ± 1.15	0.0008 ± 0.0005
5	3.67 ± 4.62	3.67 ± 4.62	100.00 ± 0.00	100.00 ± 0.00	26.00 ± 1.00	28.00 ± 1.73	2.00 ± 1.00	0.0008 ± 0.0004
6	4.33 ± 5.77	4.33 ± 5.77	100.00 ± 0.00	100.00 ± 0.00	24.67 ± 4.16	26.67 ± 4.16	2.00 ± 0.00	0.0009 ± 0.0002
7	9.00 ± 3.61	9.00 ± 3.61	100.00 ± 0.00	100.00 ± 0.00	37.17 ± 2.84	40.67 ± 2.08	3.50 ± 1.50	0.0010 ± 0.0005

Table 6.3. Subsurface layer soil properties and porewater sulfide of the study area (n = 21). Data are presented as mean +/- standard deviation.

Site	pH	pH_{fox}	Eh (mV)	LOI (%)	Water-soluble sulfate (%)	S_{KCl} (%)	S_{POS} (%)	Sulfide (mg/L)
1	5.83 ± 0.48	3.54 ± 0.78	71 ± 177	23.78 ± 2.78	1.93 ± 2.19	0.82 ± 0.72	2.38 ± 0.18	0.23 ± 0.08
2	5.78 ± 0.18	4.65 ± 0.16	-32 ± 85	22.08 ± 5.51	1.60 ± 0.20	0.44 ± 0.02	2.58 ± 0.24	0.59 ± 0.06
3	5.47 ± 0.03	3.95 ± 0.16	131 ± 87	17.94 ± 3.14	0.73 ± 0.31	0.36 ± 0.03	0.82 ± 0.51	0.17 ± 0.07
4	5.57 ± 0.13	3.41 ± 0.46	201 ± 14	22.16 ± 4.09	2.47 ± 0.81	0.77 ± 0.34	2.14 ± 0.45	0.22 ± 0.03
5	6.34 ± 0.45	5.36 ± 0.83	28 ± 34	21.88 ± 6.46	1.67 ± 0.23	0.49 ± 0.07	2.07 ± 0.15	0.21 ± 0.02
6	7.04 ± 0.22	6.33 ± 0.04	4 ± 12	10.54 ± 0.72	0.80 ± 0.53	0.32 ± 0.01	0.93 ± 0.34	0.18 ± 0.01
7	7.37 ± 0.09	6.14 ± 0.24	-129 ± 46	12.47 ± 10.07	1.07 ± 0.23	0.29 ± 0.05	1.53 ± 0.19	0.19 ± 0.02

The average values of water-soluble sulfate, KCl extractable sulfur, and peroxide oxidisable sulfur in the ponds complex were higher (0.80-2.47%, 0.32-0.82%, 0.82-2.58%, respectively) compared to the values in the Control site (1.07%, 0.29%, and 1.53%, respectively). Short ranges of average sulfide values found in the complex were between 0.17–0.59 mg/l, and higher values were observed in Site 2. The average sulfide value in the Control site was 0.19 mg/l.

The subsurface soils in the study area consisted of silt, clay and very fine sand texture (60.80–94.47%) with grey to dark soil colours. Orange or yellow combined with the main grey soil colours in the sub layers of Sites 4 and 6, while orange color in dark soils with peat or organic matter existed in Sites 1, 2, and 3 in the abandoned ponds complex (Table 6.4).

In general, the soil environments in the abandoned ponds complex were degraded due to ASS, except Site 6. Site 6, that was largely affected by tidal inundation due to its location, had similar subsurface soil properties to those in the Control site, and was significantly different compared to other sites located in the abandoned ponds complex.

Table 6.4. Average grain size and soil colour (n = 21). Data are presented as mean +/- standard deviation.

Site	Silt, clay, very fine sand (%)	Sand (%)	Description
1	60.80 ± 16.81	39.20 ± 16.81	dark grey, orange + organic
2	80.57 ± 5.36	19.43 ± 5.36	black, orange + organic
3	75.20 ± 7.36	24.80 ± 7.36	dark grey, orange + organic
4	73.90 ± 9.13	26.10 ± 9.13	yellowish grey + fine root
5	81.77 ± 6.93	18.23 ± 6.93	grey, orange + organic
6	94.37 ± 0.40	5.63 ± 0.40	yellowish grey + fine root
7	92.47 ± 2.76	7.53 ± 2.76	light grey

However, the average percentage of pyrite in subsurface soils of Site 6 (3.26%) was different compared to the Control site (1.04%) (Table 6.5), and was closer to those in other sites in the abandoned ponds complex (1.52–5.35%). The average pyrite percentages in surface soils in site 6 were relatively lower (1.04%) and were between the range of the Control site value (0.61%)

and other abandoned pond areas (1.32–3.11%). The characteristics of the sites are described in section 3.4. Principal Component Analysis (PCA).

Table 6.5. Percentage of pyrite in soils (n = 21). Data are presented as mean +/- standard deviation.

	Pyrite (%)	
Site	Top	Sub layer
1	2.53 ± 2.85	2.55 ± 0.41
2	2.01 ± 1.33	3.49 ± 0.53
3	3.11 ± 1.61	1.52 ± 0.43
4	1.80 ± 0.51	4.25 ± 2.50
5	1.32 ± 0.42	5.34 ± 2.00
6	1.04 ± 0.10	3.26 ± 2.87
7	0.61 ± 0.58	1.04 ± 0.20

Noted: The Titratable Actual Acidity, Titratable Potential Acidity, and Titratable Sulfidic Acidity values are presented in Chapter 7.

3.3. Geochemical correlation and interactions

The Kruskal Wallis test shows that the density, establishment and growth values amongst sites are significantly different ($p < 0.05$). There are two groups of density, establishment, and growth in the study area. The first group is Site 1, 2 and 3. The second group is Sites 4, 5, 6 and 7. The characteristics of the groups are presented in the next section.

The physical and geochemical variables in subsurface soils were strongly correlated with each other (Table 6.6). The pH strongly correlated to pH_{fox}. Both pH and pH_{fox} strongly correlated to redox potential, organic content, water-soluble sulfate, KCl extractable sulfur, peroxide sulfur, and silt/clay values. The pH_{fox} also strongly correlated to peroxide oxidisable sulfur.

Water-soluble sulfate, extractable sulfur and peroxide sulfur strongly correlate with each other. The variables also correlate to organic content and redox potential, except for peroxide oxidisable sulfur, which does not correlate to redox potential. Sulfide correlates only to peroxide oxidisable sulfur. The amount of silt/clay strongly correlates to all measured variables, except for peroxide oxidisable sulfur and sulfide.

Table 6.6. Pearson correlation analysis of the geochemical variables (n=21)

	pH	pH_{fox}	Eh	LOI	Water-soluble SO_4	S_{KCl}	S_P	S_{POS}	Sulfide	SiltClay
pH	1	.788**	-.730**	-.609**	-.488**	-.576**	-.434**	-.276	-.148	.659**
pH_{fox}		1	-.731**	-.613**	-.505**	-.597**	-.464**	-.305*	-.084	.625**
Eh			1	.565**	.626**	.645**	.394**	.190	-.037	-.574**
LOI				1	.548**	.608**	.626**	.517**	.133	-.564**
Water-soluble SO_4					1	.763**	.622**	.427**	.181	-.464**
S_{KCl}						1	.742**	.447**	.026	-.559**
S_P							1	.931**	.297	-.432**
S_{POS}								1	.387*	-.278
Sulfide									1	.073
Silt/Clay										1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed

The density, establishment, and growth of mangrove seedlings are positively correlated with both field and oxidisable pH ($p < 0.05$). The density of seedlings have a negative correlation with their Eh ($p < 0.01$) (Table 6.7).

The sulfur species, i.e. water-soluble sulfate, S_{KCl} and S_{POS} do not affect the density, establishment, or growth of the seedlings ($p > 0.05$). However, sulfide negatively correlates to establishment and growth ($p < 0.05$). The growth correlates to silt, clay and very fine sand soil textures ($p=0.051$, $r=0.431$).

Table 6.7. The relationships between density, growth and geochemical factors ($p < 0.05$, $n = 21$)

Variable	p	r
Density		
pH	0.003	0.709
pH_{fox}	0.003	0.609
Eh	0.034	-0.463
Establishment		
pH	0.011	0.541
pH_{fox}	0.057	0.422
Sulfide	0.015	-0.525
Growth		
pH	0.003	0.618
pH_{fox}	0.033	0.466
Sulfide	0.052	-0.429
Silt/clay	0.051	0.431

p : significance values, r : correlation values, * : interaction between the variables

3.4. Principal Component Analysis

The Principal Component Analysis (PCA), shown in Figure 6.1, illustrates that different sites form different groups with particular properties. Comparison of the PCA and Kruskal Wallis test results shows similarities between the sites, which are categorised into two main groups (see Section 3.3). The first group comprises Site 1 that do not have any Rhizophoraceae seedlings, Site 2 and 3 that has low density, establishment, and growth. The second group comprises Sites 4, 5, 6 and 7, that have higher density, establishment, and growth.

Groups 1 is marked by relatively higher sulfides and a wide range of redox potential, but are mainly oxidative, with relatively higher organic content and S_{POS}, and lower pH and pH_{fox}. Site 3 in group 1 had lower water soluble sulfate. In group 2, Site 4 is characterised by oxidative environments with relatively high organic content, water-soluble sulfate, S_{KCl}, and S_{POS}, and lower pH and pH_{fox}. Site 5 has high organic content and a slightly oxidative environment. Sites 6 and 7 have higher pH, pH_{fox}, silt/clay percentages, reductive to low oxidative environments, and lower water-soluble sulfate, S_{KCl}, S_{POS}, and sulfide.

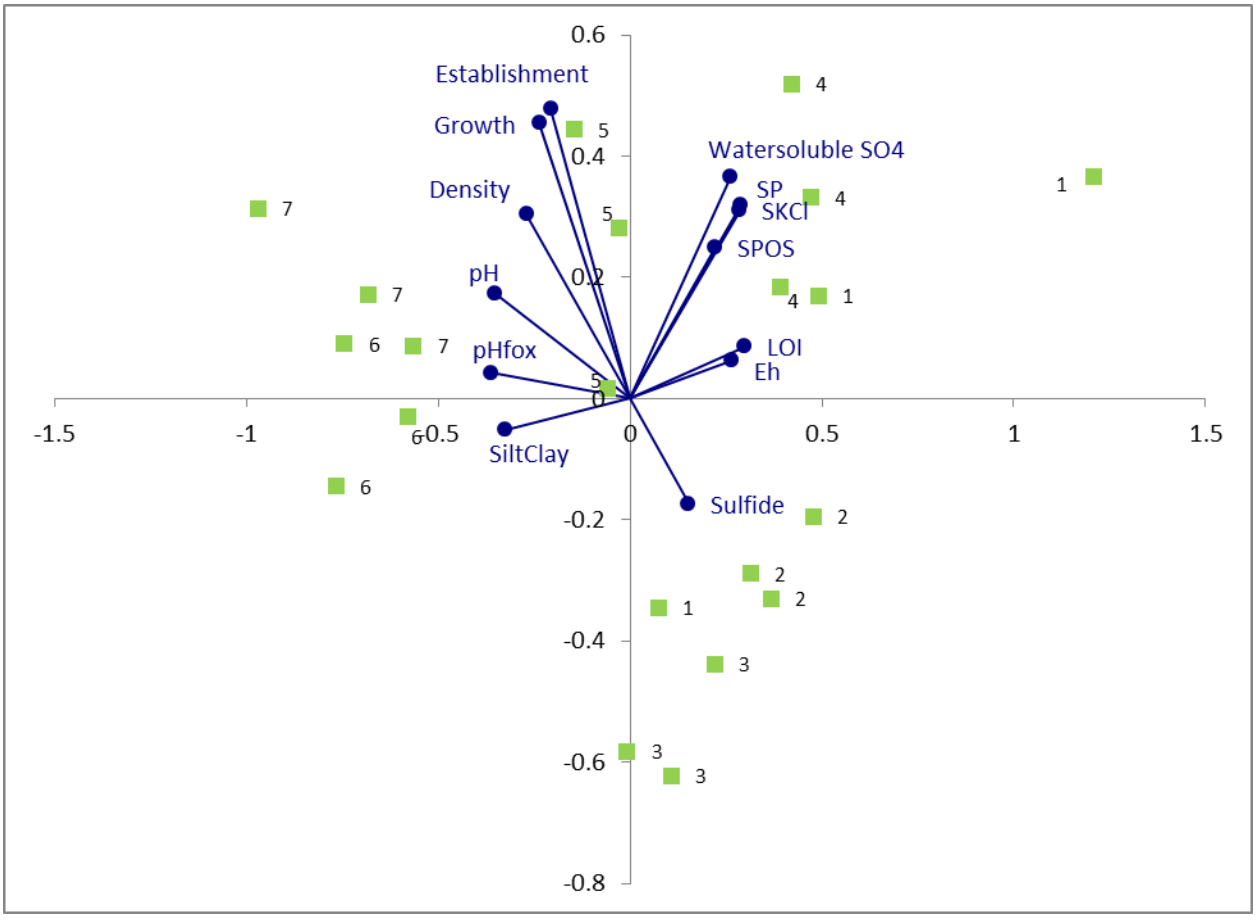


Figure 6.1. The geochemical conditions in the study area, shown by the Principal Component Analysis. The boxes represent the sites

4. Discussion

The higher values of density, establishment, and growth of mangrove seedlings in the study area are associated with freely drained areas with a higher pH (field and oxidisable). Higher density and growth also correlate to lower redox potential. Higher establishment and growth are associated with lower sulfide levels. Seedling growth appears to be higher in soils with greater content of silt/clay and very fine sand textures.

In general, there are two groups of density, establishment, and growth in the study area. The first group consists of Site 1 with no Rhizophoraceae occurrence, and Site 2 and 3 with very low density, establishment, and growth values. The second group comprises Sites 4, 5, 6 and 7 with high density, establishment, and growth values. The first group is characterised by a dry and water logged sites (Site 2), and a site showing a small effect of inundation (Site 1 and 3). The second group is distinguished by their site location and influenced by the water regime.

Tidal seawater inundations at the Site 6 (due to broken dikes) and 7 (Control site) generated lower values of redox potential state. The degree of reducing environment was higher as the tidal inundation effect was higher. The presence of organic matter (Chu et al., 2006) and slow diffusion of oxygen into the water-saturated soil limited oxidation and created reducing condition in the soils (Chu et al., 2006, Baldwin and Fraser, 2009, Reid and Butcher, 2011). In this condition, sulfate reduction occurred through oxidation of organic matter (Armstrong and Armstrong, 2005, Johnston et al., 2009b, Johnston et al., 2010b) by sulfate-reducing bacteria (SRB) such as *Desulfovibrio desulfuricans* (Armstrong and Armstrong, 2005). The sulfate reduction into sulfides consumed H^+ thus increasing pH (Lin et al., 2003). At the same time, iron reduction occurred (van Breemen, 1993, Konsten et al., 1994, Johnston et al., 2009b). Increasing tidal indundation increased pH, due to neutralization of acidic soil by bicarbonate alkalinity of seawater (Indraratna et al., 2002, Johnston et al., 2009b, Wong et al., 2010, Burton et al., 2011). Low acid environments in the second group of sites enabled mangrove seedlings to establish better, which was marked by the higher values of density, establishment, and growth of the Rhizoporaceae seedings, particularly in Site 7 (Control) and Site 6.

In contrast, low pH can lead to H^+ toxicity and effected mangrove seedlings density, establishment and growth in group 1. Aluminium toxicity on plants, which is associated with

acid conditions have been widely reported (Kidd and Proctor, 2000, Samac and Tesfaye, 2003, Kochian et al., 2004, Ma and Ryan, 2010). The toxicity of H^+ depends on plant species, because certain plant species are more sensitive to H^+ than to Al, while the reverse is true for other plant species (Marschner, 1991).

The field study demonstrates that inundation of abandoned ponds, as occurred in Site 6, improves the soil quality of the environment (Chowdhury, 2001), which in turn provides better conditions for mangrove seedlings to establish. Good water circulation encourages good establishment and growth (McKee, 1993, McKee, 1995a, McKee, 1995b, Field, 1998, Lewis, 2005, Kamali and Hashim, 2011, Friess, 2014), as well as high diversity (Azariah et al., 1992) of mangrove species. This result confirms the importance of proper hydrology for the success of mangrove restoration, as emphasised in earlier sections.

High sulfide levels, which generally appear in reducing environments, did not occur in Sites 6 and 7. Seawater inundation from the Gulf of Bone at Sites 6 and 7 contained high levels of oxygen that allowed sulfide oxidation to sulfate. Reformations of sulfide minerals, including pyrite, occur in reducing or in previously oxic-acidic environments (Connell and Patrick, 1968, Johnston et al., 2009b, Johnston et al., 2010b).

Seawater inundation in the abandoned ponds at Site 6 affected the jarosite stability due to the reducing environment (Chu et al., 2006). Seawater circulation also resulted in thinning or disappearance of the peat layer (Anda et al., 2009). Site 6 top surface soils were free from jarosite and the organic content in this site was also low (Table 6.2). An acid sulfate soil glasshouse experiment in Malaysia demonstrated that although palm oil (*Elaieis guineensis*) seedlings showed a moderate tolerance to acidity, the optimal seedling performance of these seedlings occurred in non-jarosite, freely draining soils (Auxtero and Shamshuddin, 1991).

In addition to good inundation that provided a suitable environment for mangroves to establish and grow, the availability of propagules from mature mangroves near the sites was an advantage. The sites in the third group, with high values of density and growth, were all situated in environments that had or were close to mature mangrove stands, which supplied propagules at the sites.

Wide range of redox potential values in group 1 suggests dynamic environments of mix long-term oxidative pedogenesis and short-term seasonal fluctuations that result in dynamic and rapid mineralogical transformation (Johnston et al., 2011). Oxidative conditions occurred in abandoned pond areas at Site 1, and particularly in the dry oxidative environment at Sites 2 and 3. In these sites, pyrite tended to oxidise and produce acid and sulfate as a major form of sulfur (Dent, 1986, Ward et al., 2004a, Ward et al., 2004b), which in turn decreased pH (Dent, 1986, Chu et al., 2006). High concentration of organic matter in these areas allowed sulfate to be adsorbed to organic matter, as indicated by high percentages of total oxidisable sulfur that strongly correlated to organic content values (Table 6.6). Besides adsorption to organic matter, adsorption of sulfates onto hydrated Fe and A1 oxide may also have occurred in these sites, due to high levels of Al and Fe in the study area (Alves and Lavorenti, 2004).

Under oxidative environment, jarosite ($KFe_3(SO_4)_2(OH)_6$) was formed as a result of partial oxidation of the pyrite (van Breemen, 1973, Chu et al., 2006). In rainy season or waterlogged condition, dissolution of jarosite occured and contributed to higher sulfate levels in these sites (Chu et al., 2006). During the rainy season, the redox potential shifted to more reductive environments, which encouraged the reduction of sulfate to sulfide. High organic content and high concentrations of iron existed in these sites stimulated pyrite formation precipitation (Reddy and DeLaune, 2008, Alongi, 2009), which is confirmed by higher pyrite percentages (Table 6.5).

The lack of seedlings established at Sites 2 and 3 could be due to the unavailability of a propagule source nearby. Poor inundation inhibits a route for propagules to enter the sites, and results in poor soil quality. The water flood in Site 2 was mainly from rain water. Stagnant water causes mangroves to grow poorly, or kills seedlings (Gopal and Krishnamurthy, 1993, Wolanski, 2006). In Sites 2 and 3 only mangrove ferns (*Acrostichum sp)* have occurred.

Site 1, which is located near Sites 2 and 3, is furthest from the gulf and does not have a mature mangrove stand nearby. Therefore Site 1 required re-plantation. During the three month evaluation period, one out of three *R. mucronata* seedlings replanted in this site were alive, indicating that the seedlings cannot easily establish in the area. Site 1, as in Sites 2 and 3, was recognised with high acidity properties, which had a negative effect on the density, survival and growth of replanted seedlings.

Site 4 has similar geochemical conditions to Site 1, but it has better values of measured biological indicators. A lower percentage of pyrite on surface soils, and more free water circulating from both seawater and river, distinguish the geochemical conditions and the process in Site 4, and thus affected the establishment and growth of the seedlings (see Table 3.2). The discussion of the factors that lead to high values of acidity in the subsurface soils of Site 4 is presented in Chapter 7.

Overall, better establishment, greater density and growth of mangrove seedlings in higher pH conditions in the field study are in agreement with the findings of the experimental study. As discussed in Chapter 4, the experiment found that higher pH, lower redox potential, lower sulfate, lower sulfide and HCl extractable sulfur in non-ASS environments provided a better environment for mangrove seedlings. This was compared to ASS environments with the opposite geochemical conditions, which inhibited mangrove seedlings from establishing. In the field study, the sulfur species did not affect either the number of surviving seedlings or their growth. Sulfide concentration showed a negative correlation with seedling establishment and growth in the field study (Table 6.6). However, these relationships can be disregarded, as the measured sulfide levels in the study area were low (Table 6.3) compared to the research conducted in Mtoni and Mbeni in coastal areas of Dar es Salaam, Tanzania (0.0025-0.96 mM, or 0.08–3.72 mg/l and 1.5–24.5 mM, or 48–784 mg/l, respectively).

Besides pyrite precipitation, tidal action and active mixing of surface and subsurface soils by burrowing fauna activity (bioturbation) also plays an important role in reducing sulfide levels, by changing the states of elements through introducing oxygen to sub layer and removing toxic sulfide (Kristensen, 2007, Alongi, 2009). Burrowing fauna such as small crabs were often found in the study sites where mature mangroves occurred.

The physical and geochemical variables in the study area were strongly correlated (Table 6.5). Strong correlations between the values of organic content and all other physical and geochemical variables, except sulfide suggest that high organic content is an important factor in the formation of geochemical conditions in the study area. Organic matter provides energy for microorganism activity. Since dissolved oxygen is rapidly consumed by microorganisms after inundation, anaerobes and facultative anaerobes rapidly develop and decompose organic matter (Genon et

al., 1994). Under anaerobic conditions, the microorganisms used sulfate amongst other alternate electron acceptors, such as nitrate, and oxidised forms of Mn and Fe (Bohn et al., 1985, Genon et al., 1994). This decomposition activity leads to low redox potential (Wheeler et al., 1999, Chu et al., 2006), an increase in acidity and the concentration of iron and sulfate following water inundation (Chu et al., 2006).

High organic content also corresponds to the total oxidisable sulfur in the study area (Table 6.5). This is in agreement with (Sokolova and Alekseeva, 2008), who reported that sulfates in the soil can be rapidly adsorbed or transformed to sulfur-containing organic components. A high amount of oxidisable sulfur (0.99–2.10%) compared to the amount of total sulfur extracted by KCl (0.27-0.79%) and water-soluble sulfate (0.03-0.11%) indicates that a high amount of potential sulfur in the study area is retained in organic matter. The highest sulfur content is typical for peat soils and peats (Sokolova and Alekseeva, 2008). Given that sulfur in the study area is retained predominantly in organic matter, it would potentially damage to the environment.

5. Conclusions

The higher values of density, establishment, and growth of mangrove seedlings in the field study are characteristic of areas with free inundation of seawater that have higher pH (field and oxidisable). As the field study shows, greater density also correlates to reduced environments. In the study sites, the growth of mangrove seedlings was also associated with greater portion of silt/clay and very fine sand substrate textures.

Good inundation created a suitable environment for mangroves to establish and grow, as well as access for propagule supply. Inundation of abandoned ponds, as occurred at Site 6, improved soil quality in the environment, which in turn provided a better environment for mangrove seedlings to establish. This study result supports the argument for the importance of correct hydrology, including tidal inundation, for mangrove restoration to be successful.

High organic content plays an important role in geochemical conditions, and correlates to measured variables in this study area, which in turn affected the geochemical process and the

establishment and growth of the seedlings. Availability of propagules from mature mangroves near the sites supported the establishment of mangrove seedlings.

CHAPTER 7: THE INFLUENCE OF POTENTIAL ACIDITY AND PYRITE IN SURFACE SOILS ON ACIDITY CONDITIONS, ESTABLISHMENT AND GROWTH OF RHIZOPHORACEAE SEEDLINGS

1. Introduction

Previous chapters showed that density and growth of Rhizophoraceae seedlings are higher as pH is higher, which demonstrates that acidity inhibits seedling establishment and growth. An increase in H^+ concentration in an environment is an important restraint on plant growth, due to H^+ toxicity (Marschner, 1991).

In highly acidic soils, exchangeable and soluble Al species as well as Fe compounds may act as substantial elements of the existing acidity (McElnea et al., 2002). With a pH below 5, Al becomes soluble and causes severe root elongation, which in turn results in poor plant growth in acid environments, due to reduced capability for water and nutrient uptake (Sumner and Noble, 2003). The effects of Al on plants is usually significantly more severe than H^+ (Menzies, 2003), but this depends on plant species, for some are more sensitive to H^+ than to Al, while the reverse response appears in other plant species (Marschner, 1991).

The oxidation of pyrite in the environment is much more complex than described in theory, where the amount of acidity generated after complete oxidation of pyrite (FeS_2) follows a ratio 1 : 4 (McElnea et al., 2002):

$$FeS_2 + 15/4\ O_2 + 7/2\ H_2O \rightarrow Fe(OH)_3 + 2SO_4^{2-} + 4H^+$$

The amount of net acidity released from soils may vary significantly from that expected from oxidisable sulfur analysis, and from the relationship shown in the above equation (McElnea et al., 2002). Different structures of soils between surface and subsurface layers in ASS affected abandoned ponds can also influence the amount of acidity produced. In turn, these aspects will affect the geochemical processes. Therefore, it is essential to understand the nature of acidity, including those soil layers in an ASS study area, because it may influence the establishment and growth of seedlings. For instance, acid conditions in sub layer soils may affect growth

through nutrient and water uptake (Menzies, 2003). Additionally, soil type and structure as well as the oxidation process affect plants (Marschner, 1991).

This study evaluated the acidity process and its influence on the establishment and development of seedlings. This objective was achieved by examining some acidity properties in the surface and subsurface soils near the roots, their correlations and interactions amongst the variables, and their relationships to the density, establishment and growth of mangrove seedlings. This chapter also evaluates the effect of tidal inundation on the measured acidity properties in the surface and sub layers.

This study applied specific geochemical approach used in ASS areas that allowing tracking of existing, potential, and net amounts of acidity and sulfur in soils (White and Melville, 1993), therefore contributes a more specific understanding on the acidity roles on establishment, density and growth of mangrove seedlings. The study derived from results discussed in the previous chapter discussed the effect of pH and other variables on density, establishment and relative growth rates in the study area, as well as the role of tidal inundation in creating a better soil quality and its effect on those measured biology variables.

2. Study sites and methods

2.1. Study sites, replanting, and measurement

The field study was conducted from July to December 2011 (rainy season) in six abandoned ponds in Mare ($04^{o}51'S$, $120^{o}18'E$), district of Bone, province of South Sulawesi Selatan, Indonesia. A control site (Site 7) was located outside the abandoned pond area. Three seedlings were replanted in Site 1 that had no pre-existing seedlings. This site was located at the bank of a creek that was disturbed by ASS. Sites 2 and 3 were uninundated or poorly inundated sites that were strongly disturbed by ASS. No replantation was performed in these 'unvegetated' site to have sites that can act as 'AASS control sites. Sites 4, 5, 6, and 7 had pre-existing mangrove seedlings (see Table 6.1).

The density, establishment, and growth (height addition and relative growth rate) of Rhizophoraceae seedlings were examined from the replanted seedlings in Site 1 and pre-existing

naturally occurring seedlings in other sites at the early and end of the three-month period study. The densities of seedlings were determined by measuring all individuals in six 1m x 1m plots randomly placed at each site. The plant height measured was the above ground height. The relative growth rate (RGR) of the seedlings was determined based on the height (Poorter and Garnier, 2007).

Three replicates of 15 cm soil cores and porewater were collected around mangrove seedlings at each site. Subsurface soils near root layer (10-15cm) were analyzed for pH, peroxide oxidisable pH (pH_{fox}), redox potential (Eh) using quality meter. Examination of Titratable Peroxide Acidity (TPA), Titratable Actual Acidity (TAA), Titratable Sulfidic Acidity (TSA), S_{KCl}, S_P, S_{POS} were measured using The Peroxide Oxidisable Combined Acidity and Sulfur (POCAS) method described by White and Melville (1993). S_{KCl} determines the adsorbed and soluble sulfate (Ahern et al., 2004). The S_P provides the level of sulfate contained in soils through oxidising the soils to generate maximum acidity from reduced sulfidic material (Ahern et al., 2004). The S_{POS} estimates the net potential acid risk of the soil from the unoxidised sulfur compounds by determining the difference between S_{POS} and S_{KCl} (White and Melville, 1993, Ahern et al., 2004). Pyrite percentage of surface and subsurface soils (three replications) were measured through Titratable Sulfidic Acidity (TSA) analysis (Konsten and Sarwani, 1990).

The water-soluble sulfate levels were determined using the turbidimetry method and measured by a spectrophotometer. The concentration of organic content was determined using the Loss on Ignition (LOI) method (Heiri et al., 2001). The analysis of grain size of the soil was determined using wet sieve analysis.

Exchangeable and organic Fe and Al were measured followed the protocols for the Commission of the European Community Bureau of Reference (BCR) (Davidson et al., 1994). The procedure consists of three steps of sequential extraction to determine metal fractionation. The first step is an acetic acid extraction to determine exchangeable, water and acid soluble forms. The second step uses hydroxylamine hydrochloride to determine reducible forms (iron and manganese oxide bounds). The third step is hydrogen peroxide oxidation coupled with ammonium acetate extraction to determine the oxidisable form (organic matter/sulfide bound) (Davidson et al., 1994). Results from step one and three were used in this Chapter.

2.2. Statistical analysis

Several statistical analyses were applied, using Excel and SPSS 17 to assess the variability of geochemical interactions and their correlations. Three replications of variables were used for statistical analysis. A normality test and transformation were applied for non-normal variables. The types of transformations depended on the type of skewness. Pearson correlation was employed to identify the correlation and interaction between the physical and geochemical variables. Principle Component Analysis (PCA) was also carried out to examine the geochemical characteristics of the sites in the study area.

3. Results

3.1. Biological measurement

As discussed in Chapter 6, the replantation of six *R. mucronata* seedlings in Site 1 ended in a low survival rate, where only one out of 3 seedlings survived in the period, with poor development. On the other hand, 100% of the existing seedlings in other sites survived. This led to stable values of density (Figure 7.1).

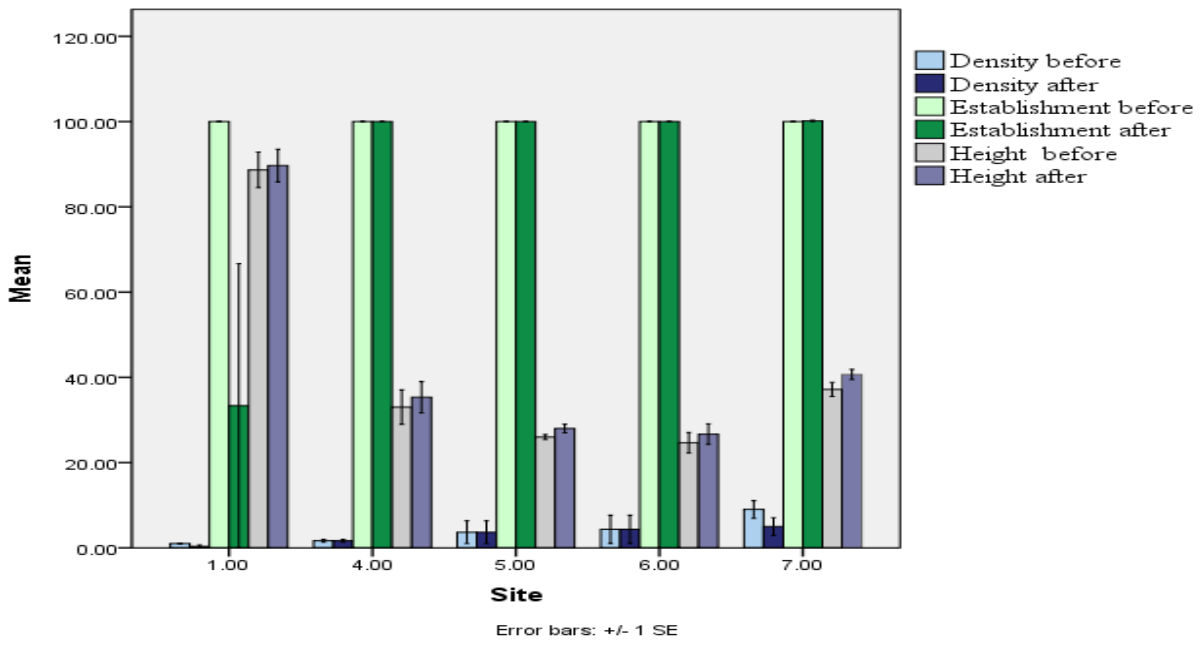
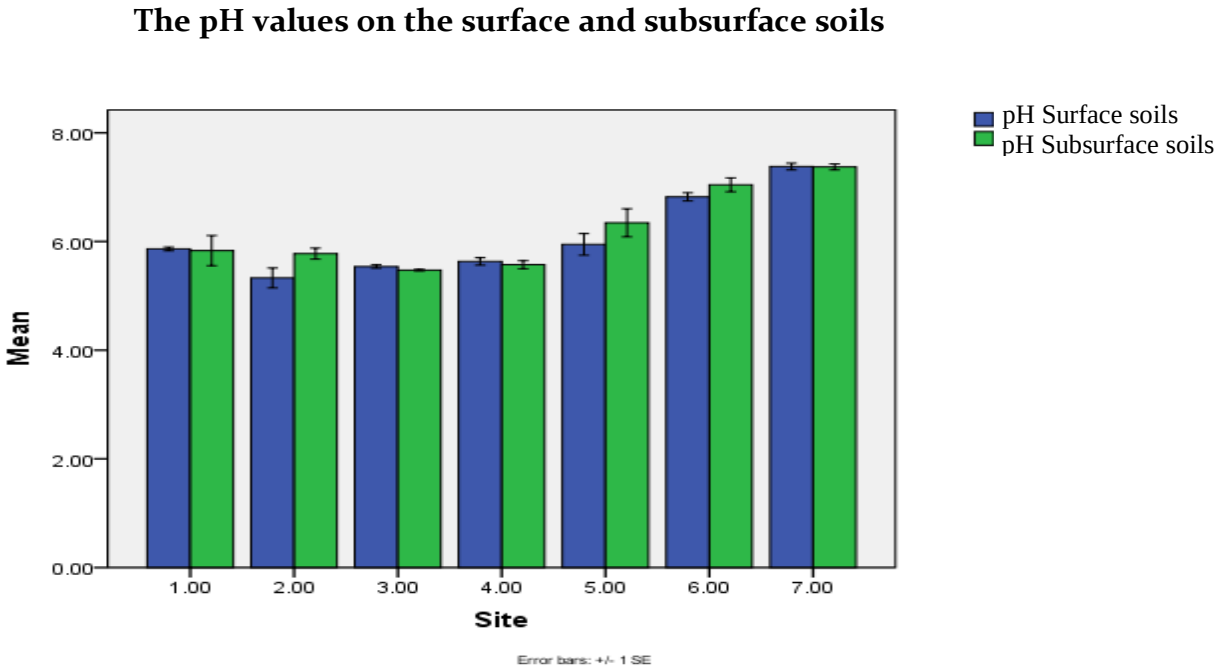


Figure 7.1. The density (number of seedlings/m^2), establishment (number of seedlings), and height addition (cm) of mangrove seedlings in the study area (n=21). Values are mean $\pm$ SE



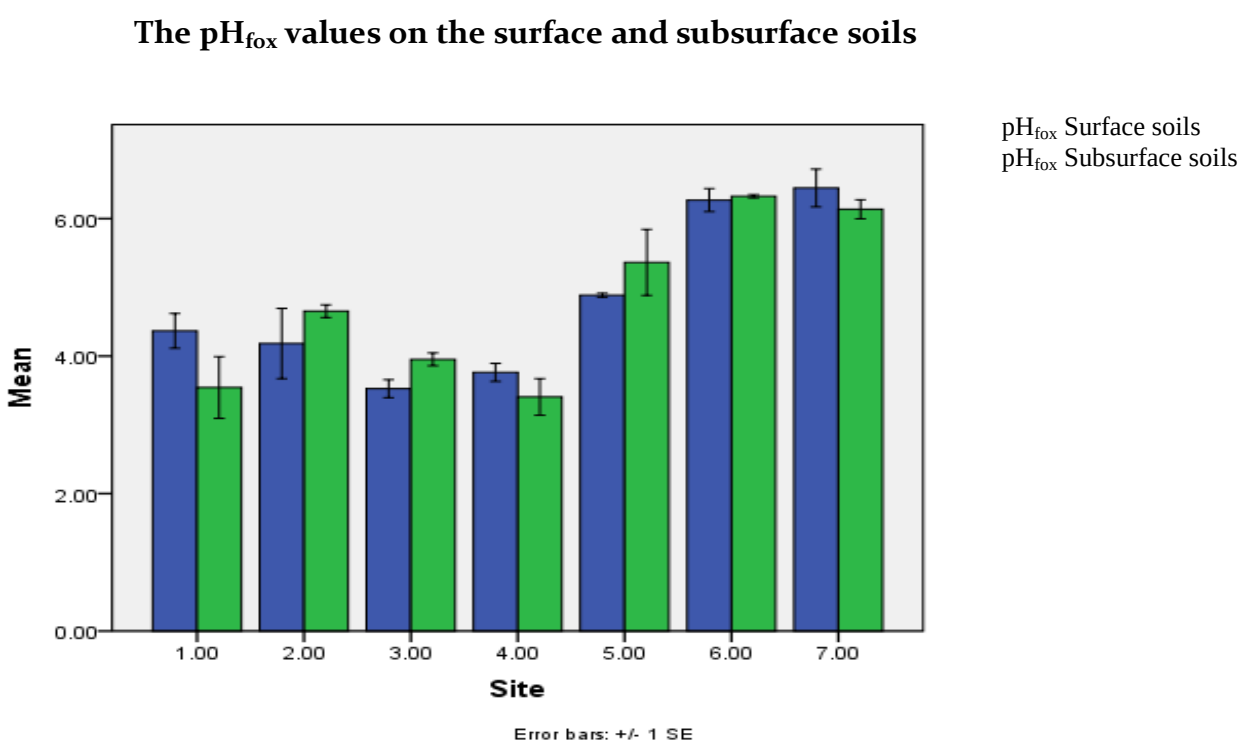


Figure 7.2. Acidity properties on surface and subsurface soils in the field study area (n = 21). Values are mean ± SE

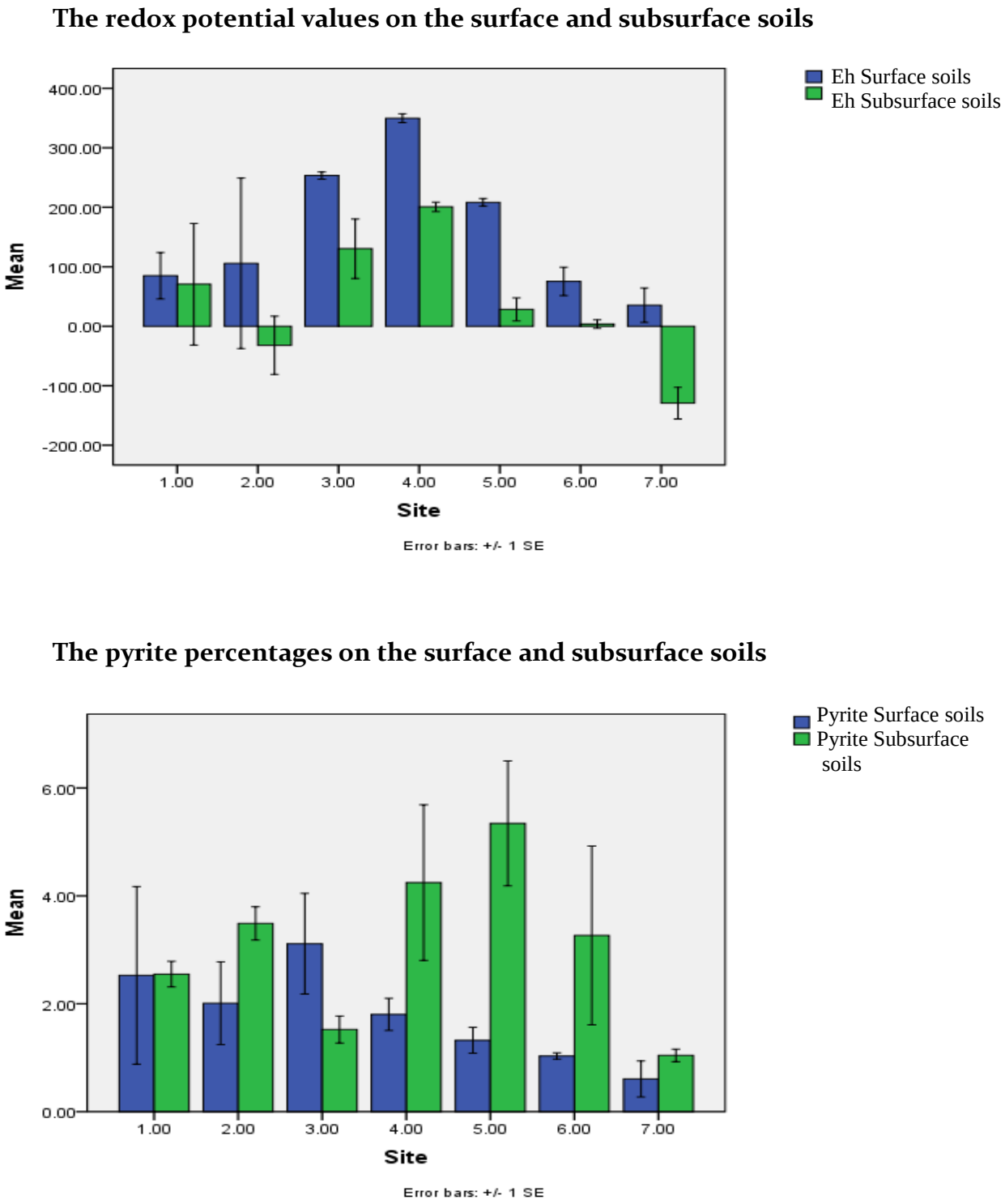


Figure 7.3. Redox potential (mV) and pyrite (%) (n = 21) in surface and subsurface soils. Values are mean ± SE

3.2. Physical properties

Figure 7.2 shows that the average pH values between surface and subsurface soils in all sites in abandoned ponds were similar. The pH in surface soils in abandoned ponds ranged between 5.33 and 6.82, while the pH in subsurface soils ranged 5.47–7.04. Site 6 had higher values than other abandoned pond sites, and was closer to the Control site (Site 7), which had neutral pH in surface and subsurface soils (7.38 and 7.37, respectively). The oxidisable pH values between surface and subsurface soils in abandoned ponds were only slightly different. They ranged between 3.53–6.27 and 3.41–6.33 in surface and subsurface soils, respectively. Site 6 also had higher pH_{fox} compared to other sites in abandoned ponds, but was closer to pH_{fox} values of the Control site. The pH_{fox} values of Control site in surface and subsurface soils were 6.45 and 6.14, respectively.

In general, the surface soil environments in the abandoned pond sites were oxidative, with a wide range of values. For example, Site 1 and 2 had Eh ranges of 35–162 mV and -86–386 mV, respectively. Site 6 had lower positive Eh values (28-103 mV), which was a bit higher than that of Control site (-22-72 mV) (Figure 7.2).

Similarly, subsurface soil environments were generally oxidative. Subsurface soil Eh of Site 2 also had a wide range of values (-32$\pm$85 mV), indicating more complex geochemical processes. Site 6 had low positive average Eh values (4$\pm$12 mV), indicating positive effects of inundation on the formation of reducing conditions. The control site had reducing environments (-129$\pm$46 mV).

High average organic contents (10.54-23.78%) were observed in subsurface soils of abandoned pond sites, where Site 6 had the lowest organic content compared to Control site (12.47%) (Table 7.1).

3.3. Acidity properties and pyrite percentages

Figure 7.4 shows that the values of titratable actual acidity (TAA) in surface and subsurface soils in the study area were very low compared with those of potential acidity (TPA) and therefore sulfidic acidity (TSA). The average existing acidity values in surface soils in the study area had a narrow range, from undetected values in most of the study sites to very low values (5 mol H^+/t and 6.67 mol H^+/t) in inundated/waterlogged sites (Site 2 and 3, respectively). Low TAA values

(6.33 mol H^+/t) were also observed in surface soils in Site 4, located at the edge of a creek. The average values of TAA, TPA and TSA in subsurface soils were higher than those in surface soils.

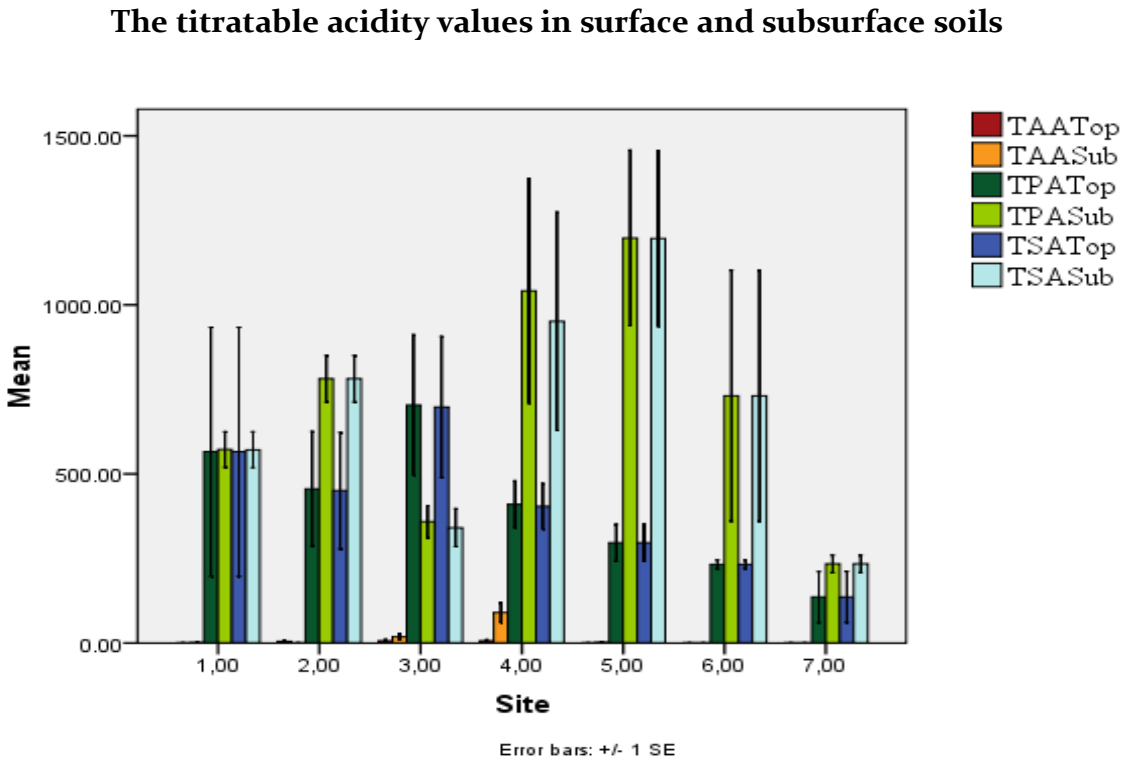


Figure 7.4. Titrable acidity proportions in surface and subsurface soils (n = 21). The data are presented in mol H^+/t. Values are mean ± SE. Top: Surface soils, Sub: Subsurface soils

High average values of TPA (232.17–704 mol H^+/t) in surface soils indicate that potential acidity forms a high proportion of the acidity in the system. Site 6 had the lowest average TPA values in the abandoned pond sites, nearly twice that in the Control site (135.83 mol H^+/t). Compared with average TPA values in surface soils, the average values in subsurface soils were higher (572.33–1198 mol H^+/t), except in Site 3, where the average TPA values in subsurface soils were nearly half those on surface soils. In general, the TSA values, generated from the difference between TPA and TAA, were close to TPA values, given that the TAA values were undetected or very low (Figure 7.4).

Similarly, the pyrite percentage pattern followed the values of TPA and TSA, since pyrite values were obtained from TSA values, where subsurface soils of Site 4 contained more sulfidic acidity

in pyrite (Figure 7.4). The presence of pyrite in soil layers in the study area acted as retained acidity, particularly in the oxidative environments of the abandoned pond sites. The average percentages of pyrite in surface and subsurface soils of the study area are presented in Figure 7.3.

Table 7.1. shows that the average percentage of water soluble sulfate and extractable sulfur in subsurface soils of abandoned pond sites were in a range of 0.73-2.47 % and 0.32 – 0.82 %, respectively. Site 4 and 1 had high percentages of these examined sulfur species. Site 6 had low percentages of both water soluble sulfate and extractable sulfur, which were close to the average values found in Control site (1.07 and 0.29 %, respectively).

Table 7.1. The percentages of organic content, water soluble sulfate and extractable sulfur (%) in subsurface soils (n = 21). Data are presented as mean +/- standard deviation.

Site	Percentages (%)		
	Organic content	Water-soluble SO_4	S_{KCl}
1	23.78 ± 2.78	1.93 ± 2.19	0.82 ± 0.72
2	22.08 ± 5.51	1.60 ± 0.20	0.44 ± 0.02
3	17.94 ± 3.14	0.73 ± 0.31	0.36 ± 0.03
4	22.16 ± 4.09	2.47 ± 0.81	0.77 ± 0.34
5	21.88 ± 6.46	1.67 ± 0.23	0.49 ± 0.07
6	10.54 ± 0.72	0.80 ± 0.53	0.32 ± 0.01
7	12.47 ± 10.07	1.07 ± 0.23	0.29 ± 0.05

Table 7.2. The concentration of exchangeable and organic Al and Fe in subsurface soils (n = 21). Data are presented as mean +/- standard deviation.

	Al (µg/g)		Fe (µg/g)	
Site	Exchangeable	Organic	Exchangeable	Organic
1	231.93 ± 88.62	1590.867 ± 114.57	691.29 ± 599.33	2377.20 ± 460.47
2	498.27 ± 100.95	2556.61 ± 495.17	727.01 ± 209.01	2582.15 ± 96.32
3	353.13 ± 101.20	3013.54 ± 457.16	443.25 ± 508.11	2379.02 ± 288.78
4	408.89 ± 161.55	3459.17 ± 402.22	587.92 ± 571.19	2453.94 ± 233.41
5	132.42 ± 22.99	1645.74 ± 123.21	312.04 ± 120.63	2661.07 ± 38.09
6	155.03 ± 13.83	638.79 ± 162.25	341.55 ± 97.15	1730.90 ± 31.22
7	258.46 ± 35.46	877.29 ± 57.07	132.48 ± 35.92	2385.37 ± 14.79

Table 7.3. Pearson correlation analysis of the existing acidity properties and biological measurements ($p < 0.05$, $n = 21$)

	TAA Top	TAA Sub	pH Top	pH Sub	SO_4 Wtr-sol Sub	S_{KCl} Sub	Exch Al Sub	Exch Fe Sub	Density	Establishment	Growth
TAA Top	1	.341	-.437*	-.530*	.135	.011	.181	-.156	-.335	-.325	-.288
TAA Sub		1	-.281	-.368	.286	.468*	.349	-.189	-.069	.196	.274
pH Top			1	.911**	-.293	-.361	-.493*	.343	.776**	.581**	.627**
pH Sub				1	-.400	-.477*	-.499*	.290	.709**	.541*	.618**
SO_4 Wtr-sol Sub					1	.758**	.222	.096	-.072	.014	-.077
S_{KCl} Sub						1	.297	.131	-.233	-.090	-.065
Exch Al Sub							1	.283	-.307	-.495*	-.376
Exch Fe Sub								1	.342	-.122	-.012
Density									1	.662**	.661**
Establishment										1	.830**
Growth											1

Note: Top: surface soils, Sub: subsurface soils, SO_4 Wtr-sol: water-soluble sulfate, Exch: exchangeable

3.4. Correlations and interactions amongst acidity properties

Pearson correlation analysis (Table 7.3 and Table 7.4) show that the pH and pH_{fox} of both surface and sub layer soils had strong correlations to the density, establishment and growth of mangrove seedlings ($p < 0.01$). The TAA values on the surface soils correlated to the values of pH on surface and subsurface layer soils ($p < 0.05$, $r = -0.437$, $r = -0.530$, respectively). However, TAA values of subsurface soils did not significantly influence the pH values on subsurface layer soils ($p > 0.05$).

The TAA values for the surface soils had no association with the examined sulfur species, nor with exchangeable Al and Fe on the subsurface soils near the roots ($p > 0.05$). The values of TAA in surface and subsurface soils had no direct relationship to either density or growth of the seedlings.

The values of pH_{fox} in surface soils strongly correlated with the density and establishment ($p < 0.01$, $r = 0.590$, $r = 0.556$, respectively), and growth ($p < 0.05$, $r = 0.540$) of seedlings (Table 7.4). The pH_{fox} values in sub layer soils correlated with the density ($p < 0.01$, $r = 0.609$), and growth ($p < 0.05$, $r = 0.466$). The pH_{fox} values in surface and sub layer soils negatively correlated to the TPA values in surface soils ($p < 0.01$, $r = -0.659$, $r = -0.579$, respectively. The values were not shown in the Table). In sequence, the pH_{fox} in surface and subsurface soils also negatively correlated to the TSA and pyrite values in surface soils ($p < 0.01$, $r = -0.655$, $r = -0.575$, respectively) because the values were obtained from the difference between TPA and TAA. See Table 7.5.

Table 7.5 shows that the TPA values of the surface soils correlated with water-soluble sulfate and KCl extractable sulfur in subsurface soil ($p < 0.05$, $r = 0.506$, $r = 0.505$, respectively). The TSA values of surface soils correlated to water-soluble sulfate and KCl extractable sulfur in subsurface soil layers ($p < 0.05$, $r = 0.505$, $r = 0.505$, respectively. The values were not shown in the Table).

Table 7.4. Pearson correlation analysis of the titratable potential and sulfidic acidity and other geochemical variables ($p < 0.05$, $n = 21$)

Variable	p	r
Density:		
pH_{fox} Surface soil	0.005	0.590
pH_{fox} Subsurface soils	0.003	0.609
Eh Subsurface soils	0.034	-0.463
TPA Suface soils	0.006	-0.578
Pyrite Surface soils	0.007	-0.572
Al organic Subsurface soils	0.035	-0.461
Establishment:		
pH_{fox} Surface soils	0.009	0.556
pH_{fox} Subsurface soils	0.057	0.442
TPA Surface soils	0.033	-0.467
TSA Surface soils	0.035	-0.462
Pyrite Surface soils	0.035	-0.462
Al Exchangeable Subsurface soils	0.022	-0.495
Growth:		
pH_{fox} Surface soils	0.012	0.540
pH_{fox} Subsurface soils	0.033	0.466
TPA Surface soils	0.025	-0.489
TSA Surface soils	0.026	-0.485
Pyrite Surface soils	0.026	-0.485

The values of TPA and TSA on surface soils strongly correlated to the establishment ($p < 0.01$, $r = -0.467$, $r = -0.462$, respectively), the density ($p < 0.01$, $r = -0.578$, $r = -0.574$, respectively) and growth ($p < 0.05$, $r = -0.489$, $r = -0.485$, respectively). The average TPA values of pyrite are related to the density of the seedlings ($p = 0.006$, $r = -0.578$). Since the values of pyrite are determined through the TSA values (shown by the positive relationship between TPA and TSA and pyrite values), which in turn relates to TPA values, these variables correlated strongly and had similarity in their interactions with other acidity properties. Organic matter correlated to potential (S_P, S_{POS}) and exchangeable sulfur (S_{KCl}), as well as organic Al in subsurface soils ($p < 0.05$, $r = 0.596$, $r = 0.495$, $r = 0.557$, $r = 0.456$).

Table 7.5. Correlations and interactions amongst properties in study area ($p < 0.055$, $n = 21$)

Variables	P	R
Pyrite, TSA, TPA Surface:		
pH_{fox} Surface	0.001	-0.655
pH_{fox} Sub	0.006	-0.575
Eh Surface	0.000	0.748
S_{KCl} Sub	0.019	0.506
Water-soluble sulfate Sub	0.019	0.505
Al organic Sub	0.054	0.426
Al exchangeable Sub	0.055	0.424
Pyrite, TSA, TPA Sub:		
Eh Sub	0.000	0.748
Fe exchangeable Sub	0.041	-0.449
Al organic Sub:		
Eh surface	0.002	0.632
Eh sub	0.004	0.597
Al exchangeable Sub	0.003	0.609
LOI Sub:		
S_P Sub	0.004	0.596
S_{POS} Sub	0.023	0.495
S_{KCl} Sub	0.009	0.557
Al organic Sub	0.038	0.456
S_P:		
S_{POS} Sub	0.000	0.935
Water-soluble sulfate Sub	0.001	0.668
S_{KCl}	0.001	0.670
Water-soluble sulfate Sub:		
Eh Sub	0.012	0.536
S_{KCl} Sub	0.000	0.758

Notes: Pyrite, TPA and TSA have similar values ($\pm$ 0.04), therefore they were represented by the TPA value.

Table 7.5 shows that the redox condition correlated with many variables, such as: pyrite, water soluble sulfate and extractable sulfur as well organic Al in subsurface soils. These correlations

Table 7.6. Comparison of acidity and related variables in several acid sulfate soil locations in Indonesia

Location	pH	pH_{fox}	pH_{KCl}	pH_{ox}	TAA (mol H^+/ton)	TPA (mol H^+/ton)
Mamuju, West Sulawesi[1]	3.96-7.45	0.01–5.95	2.93–7.4	0.73–1.04	0-106	7–2.36
Pontianak, West Kalimantan[1]		4.34–7.30	0.78–6.85			
Tarakan, East Kalimantan[2]						
initial	6.58–6.74	1.28-1.70			0–33	1337
after reclamation	5.66–6.28	0.26–1.22			0–22	349–995

Notes: 1. (Paena et al., 2010), 2. (Mansur et al., 2008).

Table 7.7. Comparison of acidity and related variables in several acid sulfat soils locations in Indonesia (cont.)

Location	S_{KCl} (%)	S_P (%)	S_{POS} (%)	Pyrite (%)	Fe (µg/g)	Al (µg/g)
Mamuju, West Sulawesi[1]	35.14	0.42-22.07	1.94–18.08	0.03–10.50	26.50–4902	0–953.50
Pontianak, West Kalimantan[1]						
Tarakan, East Kalimantan[2]						
Initial		0-3.70	0–2.66	0–5.97		
after reclamation		2.37–4.13	1.82–3.30	1.56–4.42		

Notes: : 1. (Paena et al., 2010), 2. (Mansur et al., 2008).

and interactions indicate their important roles in geochemical processes, which will be discussed in the next section.

The correlation between TPA and TSA, and between TSA and S_{POS} were not strong or significant ($p > 0.05$, $r = 0.386$, $r = 0.335$, respectively), indicating the existence of neutralising elements, such as carbonates in some sites (i.e. Site 3 and 5) (McElnea et al., 2002).

The comparison of acidity and related variables in several acid sulfate soil locations in Indonesia is presented in Table 7.6 and 7.7.

4. Discussion

In comparison to the other ASS areas in Indonesia, the values of pH, pH_{fox}, titratable acidities, sulfur variables and pyrite in the ASS-affected sites in the study area (Sites 1-5) are in similar ranges. Existing acidity (TAA) in surface soils influenced the pH of surface and subsurface soils, where the pH of soils decreased as the existing acidity increased. However, existing acidity did not directly influence the establishment, density and growth of seedlings. In most study sites, TAA values were low or undetectable, and therefore did not control the establishment, density and growth of the seedlings.

Relatively low or zero values of TAA in surface soils were possibly caused by rainfall and tidal inundation, for substantial rainfall will release existing acidity from the soils, and thus inhibit further pyrite oxidation (McElnea et al., 2002). Zero TAA was also found in several ASS locations in Indonesia, i.e. Mamuju, Pontianak, and Tarakan (Mansur et al., 2008, Paena et al., 2010). Rainfall and tidal inundation caused low sulfate concentrations in surface soils, resulting in insignificant correlation between extractable sulfate and TAA in surface soils compared to that in subsurface layer. Although the correlations between S_{KCl} and either TAA or pH on subsurface soils were strong, as a single variable, S_{KCl} did not correlate directly with either the establishment, density or growth of the seedlings in the study area.

The amount of potential acidity and pyrite in surface soils compared to existing acidity appeared to play an important role in seedling density, establishment and growth. The availability of

pyrite, particularly in surface soils in the abandoned pond sites, affected the pH of surface soils, where greater pyrite amounts created a greater possibility for pyrite to oxidise under oxidative environments.

The oxidation of pyrite under oxidative environments caused leaching of water-soluble sulfate, extractable sulfur, and exchangeable Al to the subsurface soils, and resulted in a decrease of pH in subsurface soils. This in turn affected the density, establishment and growth of the mangrove seedlings, as indicated by the strong positive correlation of pyrite and redox potential in surface soils, as well as water-soluble sulfate and extractable sulfur in subsurface soils, substantial correlation with exchangeable Al, and strong negative correlation of pyrite percentages in surface soils with pH (Table 7.4 and 7.5). A high concentration of water-soluble sulfate was also revealed in an experimental study on sulfide oxidation using ASS material from McLeod Creek, New South Wales, Australia (Ward et al., 2004b). The pyrite oxidation process that produces a high concentration of extractable Al has been reported elsewhere, including in Malaysia (Auxtero and Shamshuddin, 1991, Fitzpatrick et al., 1998, Cook et al., 2000).

Appreciable concentrations of exchangeable Al in subsurface soils that had high organic matter resulted in a high amount of Al-organic matter complex (Alves and Lavorenti, 2004), and this was shown by a strong positive relationship between organic matter and organic bound Al. This complex benefited the environment, as it is less toxic, with its lower activity (Wong and Swift, 2003). However, the presence of high organic matter in the study area did not lead to an increase of pH, as many other experimental studies have revealed, but did lead to a further decrease in pH, as found in some other studies (Wong and Swift, 2003). The decrease of pH soils may have been due to decomposition of less stable organic matter that lead to mineralisation and nitrification of organic compounds (Wong and Swift, 2003), or from the pyrite oxidation process that took place.

Besides being bound to Al, organic matter also played an important role as an acid retaining agent, indicated by strong positive correlations between organic matter and S_P, S_{POS}, and S_{KCl}. High concentrations of sulfate (measured as extractables sulfur) due to pyrite oxidisation were then bound with organic matter that was high in these areas, and resulted in high S_P and S_{POS}. This fact is consistent with many studies reporting that the amount of sulfates adsorbed by soils

is influenced by the presence of organic matter (Sokolova and Alekseeva, 2008, Alves and Lavorenti, 2004).

Strong positive correlation between pyrite percentages and redox potential, and negative correlation between pyrite and exchangeable Fe in subsurface soils, suggesting the role of pyrite oxidation that occurred under oxidative environments on the level of exchangeable Fe. The association of exchangeable Fe with pyrite in subsurface soils was stronger compared to its relationship with Fe organic complex that did not show any significant correlation. This phenomenon contradicts the argument that the presence of high organic matter inhibits pyrite oxidation by adsorption of Fe^{3+} onto organic matter (Morse and Wang, 1997, Morse, 1999, Ward et al., 2004a, Kraal et al., 2013).

High redox potential in Site 4 encouraged pyrite oxidation to occur and resulted in higher TAA in surface and subsurface soils compared to other sites. High TPA, TSA and pyrite in Site 4 also led to high water-soluble sulfate and exchangeable sulfur generated from pyrite oxidation under oxidative environment, and hence a lowering of pH and pH_{fox}. Similar conditions also occurred in other sites, including Site 1, although the existing acidity in Site 1 was undetectable or low. However, mangrove seedlings did establish themselves in these high acid conditions, although with lower levels of density, establishment and growth rates compared to the Control site. This finding indicates their tolerance to acid conditions.

The higher values of TPA, TSA, pyrite, and TAA in subsurface soils of Site 4 and 5, located at the edges of a creek, compared to other sites were possibly caused by several factors. Removal of dredged soils that contained oxidised pyrite soils from nearby, including Site 2, to the area of Site 4 and 5 during the pond preparation may have been responsible for increasing values of associated acidity properties in subsurface soils. Furthermore, the activity of burrowing organisms such as small crabs in Site 4 and 5 introduced oxygen to the subsurface soils, as well as changing the state of elements in this layer (Ferreira et al., 2007, Kristensen, 2007, Alongi, 2009). This process allowed pyrite to oxidise in subsurface soils, and increasing actual acidity and values of associated properties. Tidal action facilitated the leach of the elements produced by pyrite oxidation in surface soil to the subsurface soil layer, which further increased the values of actual acid and associated properties in that layer.

Inhibition of growth cannot be identified as the effect of Al alone, since the association between these two variables was not significant. However, exchangeable Al showed its negative influence on the establishment percentage in the study area, which indicates that Al concentration in the soils affects the early stages of survival. Once the seedlings survived, they would tolerate the Al concentration, which would not significantly affect the growth rate.

Tidal inundation greatly affected acidity conditions in site 6, as shown by the high pH, pH_{fox}, and low existing acidity of both surface and subsurface soils. Tidal action can also wash up organic matter in the site, which caused limited formation of pyrite due to inadequate organic matter that is required by microorganisms to reduce sulfate to sulfide (Berner, 1970, Lin et al., 2000, Jasińska et al., 2012). Low pyrite percentage and potential acidity (TPA and TSA) of surface soils, supported by reducing or low oxidative environments in Site 6, minimised the opportunity for pyrite to oxidise. As a result, the amounts of water-soluble sulfate and extractable sulfur were low. Furthermore, low organic material in this site restricted sulfur from binding with the organic matter, and therefore the amounts of S_P and $S_{POS,}$ which act as retaining agents, were low. The possibility of a further release of sulfur that bonds to organic matter is lower.

Tidal inundation also resulted in relatively lower levels of exchangeable Fe and Al in the study area. This result is similar to the effect of seawater tidal introduction inundation in East Trinity, northern Australia, where there is lower acidity, pH rise, and decrease of exchangeable Al to about 50–400 µg/g (Johnston et al., 2009a, Johnston et al., 2010a). Such soil conditions provide a better environment for mangrove seedlings to establish naturally, or with replantation efforts, and produce long-term healthier environments. The site was also recognised by its high silt/clay percentages that support the establishment of seedlings.

Overall, this study supports the findings discussed in Chapter 6, where the density, establishment and growth were higher in the areas that had higher pH and reducing environments. Higher pH_{fox} in surface and subsurface soils also shows its important roles in achieving higher establishment, density, and growth. Lower pyrite percentages and acidity properties in surface and subsurface soils, as well as lower levels of metals and sulfur species in subsurface soils, supported greater values of density, establishment and growth of seedlings. In contrast, lower establishment, density and growth occurred in the areas that had the opposite geochemical conditions. But

density, establishment and growth still had relatively high values in a mix of such geochemical conditions, underlining the importance of tidal inundation in supporting seedlings to tolerate acid conditions.

5. Conclusions

The existing acidity (TAA) of both surface and subsurface soils did not directly control the density, establishment and growth of the mangrove seedlings in the field study area. Neither did associated existing acidity, such as levels of water-soluble sulfate, extractable sulfur, exchangeable Al and Fe in subsurface soils near roots, correlate to the density, establishment, and growth of seedlings. In contrast, the amount of potential acid (TPA and TSA) and pyrite in the surface soils was strongly associated with the acidity, density, establishment and growth of the seedlings. Exchangeable Al showed a negative correlation with seedling establishment.

The presence of pyrite in surface soils provided a greater opportunity for the oxidation process, which then enhanced the release of water-soluble sulfate, extractable sulfur, and exchangeable Al to subsurface soils, in turn affecting the density and growth of mangrove seedlings. However, mangrove seedlings can still grow and survive in high acidity, as in Site 4 and 1, but with a lower density, establishment, and relative growth rate.

Tidal inundation greatly improved soil quality, particularly in Site 6. Low existing acidity, potential acidity and pyrite percentages in surface soils, reducing or low oxidative environments minimise the opportunity for pyrite to oxidise. As a result, the amounts of water- soluble sulfate, extractable sulfur and exchangeable Fe and Al in subsurface soils were low. Low organic material in this site resulted in a low amount of S_P and S_{POS}. Therefore, the site has a low long-term risk of releasing acid. This condition in turn has provided a better environment for mangrove seedlings to establish and grow.

CHAPTER 8: GENERAL DISCUSSION

The combined experimental and field studies evaluate the response of mangrove seedlings to acid sulfate soil environments, and assess the potential to restore mangroves in such an environment. The results agree with the findings of some researchers: that ASS is one of the stress factors that responsible for the unsuccessful of mangrove restoration, although mangrove seedlings showed their ability to tolerate high metal levels and acidity to some degree.

In the study, mangrove seedlings employed exclusion mechanisms in response to high concentrations of metals. However, negative effects were observed on the density, establishment, and growth of the surviving mangrove seedlings. The seedlings showed similar responses to acid soil conditions. To achieve better survival and development, this study identified that mangrove seedlings require geochemical conditions of higher pH, a reducing environment, and lower potential acidity. Free tidal inundation proved to be a great natural support for improving soil quality, which in turn increased the density, establishment, and growth of naturally occurring and replanted mangrove seedlings in the study area.

This study provides an insight on the researchers' argument who state that a series of remediation programs is required to remove harmful substances before conducting mangrove restoration in an ASS environment. This study is essential because it used geochemical approach, therefore mangrove restoration works effectively.

To provide a better insight into the effect of geochemical conditions on the survival and growth of the mangrove seedlings, major outcomes from the study, based on the research questions: "Which geochemical conditions are required for mangrove seedlings to establish and grow?", and "What is the tolerance of mangrove seedlings to acid conditions and high level of metals?" are discussed below.

1. Major outcomes of the study

1.1. General geochemical conditions required for mangrove seedling establishment

The establishment of mangrove seedlings in this study depended on the type of environment, where the numbers of seedlings survived in non-ASS environments were greater that in ASS environments. Mangrove restoration has better results for density, establishment and growth in areas that have a higher pH and pH_{fox}, reducing environment, and lower potential acidity. Both the laboratory and field studies demonstrated that the establishment of mangrove seedlings is associated significantly with higher pH (including pH_{fox} in the field study). A reducing environment led to a denser seedlings in the field study area.

Lower sulfate and total sulfur provided a good living environment for mangrove seedlings in the non-ASS experimental environments. In contrast, the ASS environments in this study that had opposite geochemical conditions to those in non-ASS environments inhibited mangrove seedlings from establishing. The environment type (non-ASS and ASS) did not significantly affect the value of seedlings' total fresh length or root length.

However, in the field study the measured sulfur species (water-soluble sulfate and extractable sulfur) as a single factor did not significantly affect the density, establishment and growth of the seedlings directly. There was however a negative correlation between sulfide and the establishment and growth of seedlings.

1.2. Response of mangrove seedling to acid environments

Mangrove seedlings can grow and survive in high acid environments, but with lower values of density, establishment, and relative growth rates. One might expect that the density, establishment, and growth of seedlings were primarily influenced by the amount of existing acidity on the environment. However, this study showed that the amount of potential acid (TPA and TSA) and pyrite in surface soils played a key role in the density, establishment, and growth of seedlings. The existing acidity (TAA) on both surface and subsurface soils, as a single variable, did not significantly influence the density, establishment and growth of the mangrove seedlings in the field study area. Other associated existing acidity levels in subsurface soils, such as water-soluble sulfate, exchangeable sulfur, and exchangeable Fe did also not affect those three

biological variables. The exception was a higher level of exchangeable Al in subsurface soils which had a negative effect on seedling establishment. The rainy season and tidal effects reduced some levels of these variables, and might also affect this relationship, and as such should also be taken into consideration (McElnea et al., 2002). Therefore, the expectation is that in a dry season or an area with low tidal impact, the existing acidity might have a negative effect on the density, establishment and growth of the mangrove seedlings.

As in the findings of many studies (Auxtero and Shamshuddin, 1991, Fitzpatrick et al., 1998, Cook et al., 2000, Ward et al., 2004b), the presence of pyrite in surface soils provided a greater opportunity for the oxidation process, which then enhanced the release of water-soluble sulfate, extractable sulfur, and exchangeable Al into subsurface soils in the study area. This process resulted in high acidity of subsurface soils that negatively affected the density, establishment and growth of mangrove seedlings.

1.3. Response of mangrove seedlings to high levels of metal in the environment and the involved geochemical behaviour

In response to very high concentrations of metals under non-ASS experimental mediums, *R. stylosa* seedlings showed a response similar to those observed in other research (Walsh et al., 1979, Thomas and Eong, 1984, Silva et al., 1990, Zheng, 1997, MacFarlane and Burchett, 2002, Alongi et al., 2003, Zhou et al., 2011). As the concentration of metals in the experiment mediums increased, the amount of metals accumulated or adsorbed in roots increased and limitedly distributed the metals to certain amounts to aerial parts. This condition was confirmed by the high BCF metal values in root tissues and low BCF values in stem and leaf tissues, as well as low translocation factor values of metals. Similar condition demonstrated more obviously in ASS experimental mediums, particularly Fe.

The experiment study results also demonstrate another example metal regulation by seedlings under environment with high metal levels, where seedlings tended to accumulate metals based on their function for growth and development. Seedlings roots adsorbed essential metals (Fe and Cu), while non-essential metals were adsorbed to a very limited extent (Al and Ni) in both non-ASS and ASS experimental mediums. Since the concentration of Ni existed in the experimental

mediums were far lower amount compared to Al, it's BCF values in roots were greater than BCF values of Al.

These facts suggest that the exclusion of excessive metals through the roots is a main mechanism employed by seedlings to cope with high concentrations of metals. Similar exclusion mechanisms occurred to those reported elsewhere (Kathiresan and Bingham, 2001, MacFarlane et al., 2007, Bayen, 2012, Wang et al., 2012). Furthermore, high acidity may cause H^+ to compete with metal in the root growth area (Marschner, 1995), therefore reducing metal uptake.

Consideration of the geochemical conditions, such as pH, pH_{fox}, Eh, organic matter, grain size, and concentrations of sulfur species in the mangrove restoration area are very important, because these variables interact with and affect metal concentrations in soils and roots, as shown in this study. In turn, metal concentrations in soils and roots affect the density, establishment and growth of mangrove seedlings. Metal adsorption in the roots was generally controlled by the total concentration of soils in the study area, which was similar to those reported in many studies (MacFarlane et al., 2003, Lyubenova and Schroder, 2010, Yadav et al., 2010).

1.4. The role of tidal inundation and the involved geochemisty behaviour

Using a geochemistry approach, this study supports other research findings on the importance of hydrology in mangrove restoration in acid sulfate soil environments (Djamaluddin, 2006, Lewis et al., 2006, Kamali and Hashim, 2011). In abandoned ponds affected by acid sulfate soils, free inundation of seawater caused an improvement of soils quality in the environment, such as higher pH (field and oxidisable), non-jarosite top soils, and low redox potential. Seawater inundation provides bicarbonates that is capable to neutralise acidic soils, which results in increase of pH and pH_{fox} (Indraratna et al., 2002, Johnston et al., 2009b, Wong et al., 2010, Burton et al., 2011). Similar condition was found in the experiment results, where the pH and pH_{fox} of subsurface soils increased under reducing environment. In addition, the Brighton soils that used as mediums of ASS environments majorly contained sand that rich of bicarbonates and carbonates that have high neutralizing capacity.

Free tidal inundation also created low existing acidity, potential acidity and pyrite percentages on surface soils and reducing environments, thereby reducing the chance for pyrite oxidation. Low

pyrite oxidation resulted in low levels of water-soluble sulfate, extractable sulfur and exchangeable Fe and Al in subsurface soils. Low organic content in these sites led to low amount of S_P and S_{POS}, which in turn provided a suitable environment for mangrove seedlings to establish and grow with higher density.

Improvement of geochemical conditions due to inundation has also been reported in other locations (Chowdhury, 2001, Johnston et al., 2010a). This tidal inundation provided positive advantages, as indicated by properties such as TPA and pyrite values in Site 6 of the abandoned ponds. Relatively low or zero values of TAA in surface soils were also caused by rainfall, where substantial rainfall released existing acidity from the soils, inhibiting further pyrite oxidation (McElnea et al., 2002). Zero TAA values were also found in several ASS locations in Indonesia, in particular Mamuju, Pontianak, and Tarakan (Mansur et al., 2008, Paena et al., 2010).

Tidal inundation also caused a relatively lower level of exchangeable Fe and Al in the study area. Similar results were also reported in East Trinity, northern Australia, demonstrating an improvement of geochemical conditions due to seawater tidal inundation. Here, lower acidity was generated, pH rose, and there was a decrease of exchangeable Al (Johnston et al., 2010a).

Free seawater inundation in the abandoned pond sites affected the jarosite stability, due to the reduced environment (Chu et al., 2006), and resulted in a decreased, or even led to the disappearance of organic matter, including the peat layer (Anda et al., 2009), and the amount of potential sulfur content. The decrease of the amount of organic content and associated variables caused by tidal inundation suggests a low potential risk to contribute acid in the future. Such improvement of soils conditions in turn provided a better environment to establish and grow (McKee, 1993, McKee, 1995a, McKee, 1995b, Field, 1998, Lewis, 2005, Kamali and Hashim, 2011, Friess, 2014).

The free inundation of the ponds in the study area not only supported natural seedling establishment but also provided access for supply. The availability of propagules from mature mangrove stands near the sites is one key requirement for mangrove seedlings to establish naturally around the area.

In contrast, sites with low inundation effects produced oxidative conditions. Under aerobic conditions pyrite tends to oxidise and produce high concentrations of sulfuric acid, which in turn decreases pH (Dent, 1986, Chu et al., 2006), and affects the density, establishment, and growth of mangrove seedlings. In certain sites, jarosite formation occurred at the surface of the soil as a result of partial oxidation of the pyrite (van Breemen, 1973, Chu et al., 2006).

The absence of mangrove seedlings in certain sites was due to poor inundation and resulted in poor soil quality. Such stagnant water is responsible for poor development of mangrove seedlings (Gopal and Krishnamurthy, 1993, Wolanski, 2006) and inhibited a route for propagules to enter the sites. Therefore, replanting is required for this type of environment. However, replanting of mangrove seedlings in such an environment, even at the creek banks, resulted in low survivability and poor seedling growth.

The advantages presented by tidal inundation provide an alternative solution to a liming-assisted restoration project. Despite cost-associated problems faced by developing countries, liming application, particularly for mangrove rehabilitation in damaged zone requires a proper plan, and although liming is widely known to increase soil pH, published research presents contrary results. In three treatments using drainage liming techniques in coastal acid sulfate soil areas in Finland, there was no significant decrease of the discharge of metals and acids during a three year study (Åström et al., 2007). Areas discharging metal and acid can be a problem for seedlings in the restoration area as a result of the higher uptake of bioavailable metal by the seedlings, which in turn increases the possibility of metal toxicity to the plant. However, proper techniques and maintenance of the drainage system may be effective in the long term (Åström et al., 2007).

Other research shows that the effectiveness of liming as a method for decreasing bioavailable metals depends on the type of the metals. For instance, liming does not work effectively on the reduction of bioavailable forms of Cu (do Nascimento et al., 2007), possibly due to Cu's low affinity to soil exchange capacity (Atanassova and Okazaki, 1997). Therefore, Cu strongly binds to organic matter (do Nascimento et al., 2007). However, liming results in a decrease of bioavailable forms of Zn in soils, due to its conversion to the iron oxide form (do Nascimento et al., 2007). Liming also increases organic Zn (do Nascimento et al., 2007), and through this

process the bioavailable form releases slowly (He et al., 1995). Therefore, the availability of organic matter, redox potential and other variables associated with organic decomposition should be examined before liming to prevent any negative impact that might result from the complex geochemical interactions. Thus, the hydrological rehabilitation improves soil quality without, or co-assisted with liming.

2. The implications of the study

While contributions to improved mangrove restoration have been largely conducted in terms of biology and hydrology aspects, this study contributes knowledge to the geochemistry response of mangrove seedlings under ASS environments. This study is therefore important in assessing the feasibility of mangrove restoration in disturbed ASS environments, and associated metal polluted environments. Despite small sample sizes employed, the knowledge generated from both the small-scale experimental study and the field study provides a better understanding of basic and natural responses of mangrove seedlings to the existing geochemical conditions.

The selection of the study field area in Mare, a disturbed complex of ASS abandoned ponds that was previously a mangrove ecosystem, represents the research problem well. This area provides a variety of dynamic geochemical conditions with different mangrove density, which allowed an evaluation of different processes and interactions within the environment A combination of biological measurements with various geochemical and ASS analytical methods provides a strong methodology to apply to other ASS environments.

This research can be applied outside the study area with either similar dominant species, or other species with similar physiological mechanisms. Such possibilities are based on previous research that demonstrated that salt-excreting species (e.g. *A. marina*) tolerate high concentrations of metals. This species excretes excess metals through salt glands on the leaves (MacFarlane and Burchett, 2000). Other research on herbicides revealed a similar condition, where the examined salt-excreting species (i.e. *A. marina* and *Aegiceras corniculatum*) was more vulnerable to herbicides compared to the salt-excluding species (i.e. *R. stylosa* and *Ceriops australis*) (Bell and Duke, 2005). This research suggested that mangroves take up herbicides in a similar manner to salt, indicating therefore that the mechanism could also work in a metal environment.

3. Recommendations for the best strategy

The strategy recommended to achieve effective mangrove restoration in ASS environments, particularly in abandoned ponds, is as follows:

Measurement of the fresh soil pH, pH_{fox} and low redox potential (Eh) are required as the first steps in identifying the general situation of a targeted area. Both pH and pH_{fox} measure the indications of ASS presence through the level of acidity of the soils; the difference is the addition of hydrogen peroxide into the soils for pH_{fox} measurement that indicates the amount of sulfide contained in the examined soils (Watling et al., 2004). Measurement of redox potential of soil provides an indication of reducing power that describes the anaerobic or anoxic condition (Lewis and Brown, 2014). The higher the redox potential value (+), the greater the level of oxidative environment, which indicating the greater chance of pyrite to oxidize and affect the environment.

Due to variety, complexity and dynamic geochemical conditions in a disturbed ASS environment, there is no exact single method to use in order to manage ASS. The Peroxide Oxidisable Combined Acidity and Sulfur (POCAS) method and its improved method, Suspension POCAS (SPOCAS) suite are common suites that are used in analysing ASS (Ahern et al., 2004). By conducting this suite or method, determination of the potential acidification of ASS through acid and sulfur trails can be obtained (Ahern et al., 2004).

As mentioned in previous chapters, POCAS and SPOCAS methods involve several steps, which consist of determining levels of KCl extractable Sulfur (S_{KCl}), peroxide sulfur (S_P), and peroxide oxidisable sulfur (S_{POS}). S_{KCl} measures the adsorbed and soluble sulphate; S_P measures the sulfate presence in soils through oxidising the soils to generate maximum acidity from reduced sulfidic material; and S_{POS} estimates the net potential acid risk of the soil from the unoxidised sulfur compounds by calculating the difference between S_{POS} and S_{KCl}. These suites also measure titratable actual acidity (TAA), titratable peroxide acidity (TPA), and titratable sulfidic acidity (TSA). The TSA value is determined by calculating the difference between the value of TPA and the TAA (White and Melville, 1993, Ahern et al., 2004). The percentage of pyrite can be estimated through TSA values using certain formulation (Konsten and Sarwani, 1990). Analysis of other general geochemical conditions, such as organic matter and grain size, are also required,

as all mentioned variables directly or indirectly influence on geochemistry, density, establishment and growth of mangrove seedlings.

Select an area with high pH, low redox, low in both existing and potential acidity, and the areas with has free tidal inundation as a priority area for mangrove rehabilitation. For instance, choose the furthest seaward free tidal inundation area, or along a creek/river that has good access for tides.

Establishment in the seaward area may provide the advantages of better geochemical conditions, because pyrite becomes stable under the mangroves, therefore reducing the export of acidity and metals to adjacent areas. The existence of mature mangroves in the seaward area provides access for propagules to settle in more landward location. Further restoration to more landward sites with good access inundation may be conducted after mangrove colonisation, or at least once mangrove establishment on the seaward area has succeeded. In the meantime, the established pioneer seedlings may retain concentrations of metals in their roots. Reduction of metal concentrations in soils will allow the next growth of mangroves to develop as healthy plants.

Restoration of mangroves in severe AASS should apply a proper remediation strategy since the restoration may result in a very low establishment and growth rate. Construction of canal networks in severe AASS areas to allow tidal access should be planned carefully, because some evidence shows that the channel establishment itself may result in activation of AASS (Anda et al., 2009). That is, the application of lime to remediate the AASS area requires proper planning, as the activation of AASS can result in higher uptakes of bioavailable metal by the seedlings.

These strategies are particularly important in countries that usually employ direct planting methods in their restoration projects, without considering environmental conditions.

GENERAL CONCLUSIONS

To conclude, this study has generated some major findings:

- The type of experimental environment influenced the survivability of seedlings, where the number of seedlings survived in non-ASS environments was higher than those in ASS mediums. Similar results are also found in the field study. In general, higher pH and pH_{fox}, a reducing environment, and lower potential acidity are the primary geochemical conditions that are required by mangrove seedlings, and are demonstrated to have higher success rates for their density, establishment and growth. Other geochemical variables, such as lower sulfate and total extractable sulfur also provided a good living environment for mangrove seedlings to establish and develop. The environment type (non-ASS or ASS) did not significantly affect on both the value of seedlings' relative growth rate and root length in the experimental study.

- The presence of pyrite in surface soils provided a greater opportunity for the oxidation process, which then enhanced the release of water-soluble sulfate, extractable sulfur, and exchangeable Al into subsurface soils and resulted in high acidity in subsurface soils. In such high acid conditions, mangrove seedlings can grow and survive but with lower values of density, establishment, and relative growth rate. This process emphasises the important effect of the amount of potential acid (TPA and TSA) and pyrite in the surface soils on the acidity, density, establishment and growth of the seedlings in the study area. In contrast, the existing acidity (TAA) in both surface and subsurface soils did not significantly influence the density, establishment and growth of the mangrove seedlings in the field study area. Other associated existing acidity, such as water-soluble sulfate, exchangeable sulfur, and exchangeable Fe in subsurface soils did not affect those biological indicators.

- Mangrove seedlings demonstrated their ability to tolerate high levels of metals in the experimental study by regulating, retaining and excluding them through roots, and excreting them through leaves. The seedlings accumulated and adsorbed high levels of

metal in roots and distributed them in limited amount to the aerial parts, which was confirmed by higher BCF values in roots and low BCF values in stem and leaf tissues, as well as low translocation factors. These conditions were very obvious in ASS experimental mediums. Selective strategy was also clearly shown by the seedlings in the experimental mediums, particularly against the non-essential metal, Al, where the seedlings strongly excluded it from the roots. High levels of exchangeable Al in subsurface soils also had a strong negative effect on the establishment of mangrove seedlings.

- Free inundation of seawater improved the quality of soils in the study area, in terms of higher pH (field and oxidisable), non-jarosite top soils, higher silt/clay percentage, and low organic content. Free tidal inundation also produced low existing acidity, potential acidity and pyrite percentage in surface soils and reducing environments, thereby minimising the opportunity for pyrite to oxidise. As a result, the amount of water-soluble sulfate, extractable sulfur and exchangeable Fe and Al in subsurface soils was low. Low organic material in these sites resulted in a low amount of potential sulfur in the environment. This condition in turn provided a good environment, which led to higher density, establishment and relative growth of mangrove seedlings. This study also demonstrated that metal concentrations in soils and roots were affected by physical and geochemical factors, which highlight the importance of tidal inundation in mangrove restoration projects. Furthermore, free tidal inundation also provided access for propagule supply, which is advantageous to seedlings to establish naturally.

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APPENDICES

APPENDIX A. DATA OF METAL CONCENTRATIONS, BIOCONCENTRATION AND TRANSLOCATION IN THE LABORATORY ENVIRONMENT

Appendix A.1. Concentration of metals in laboratory environment

Table A.1.1. The concentration of metals in soil samples in laboratory environment

Environment	Concentration (µg/g)			
	Fe	Al	Ni	Cu
Control mangrove 1	1400	1237	2	0.5
Control mangrove 2	1300	1134	1	0.5
Control mangrove 3	1200	1037.5	0	1
Ni 25 µg/g 1	1092.5	1756	38	3
Ni 25 µg/g 2	1192.5	1761.5	25	1
Ni 25 µg/g 3	1092.5	1575.5	44.5	1
Ni 55 µg/g 1	1350	1176	67	0.5
Ni 55 µg/g 2	1250	1082	52.5	0
Ni 55 µg/g 3	1350	1187	54	0.5
Cu 70 µg/g 1	1742.5	2054.5	0.2	114
Cu 70 µg/g 2	1442.5	1959.5	5.85	99
Cu 70 µg/g 3	1692.5	2043	6.5	114
Cu 280 µg/g 1	1100	1452	0	340
Cu 280 µg/g 2	950	1222	0	280
Cu 280 µg/g 3	1050	1354.5	0.5	290
ASS 1	16288.35	6225	9.5	6
ASS 2	16997.05	6200	7	6
ASS 3	16806.15	6060	11	5.5
ASS + Ni 25 µg/g 1	10839.15	7015.45	27	12.5
ASS + Ni 25 µg/g 2	12828.75	8200.7	37	8
ASS + Ni 25 µg/g 3	10621.15	6793.05	29.5	11.5
ASS + Ni 55 µg/g 1	17939	6615	49.5	6.5
ASS + Ni 55 µg/g 2	17380.3	6305	48	6
ASS + Ni 55 µg/g 3	17316.5	6215	43.5	5.5
ASS + Cu 70 µg/g 1	12426.6	7042.05	7.5	105
ASS + Cu 70 µg/g 2	12521.9	6969.15	12.5	85
ASS + Cu 70 µg/g 3	12791.25	7094.05	11	235
ASS + Cu 280 µg/g 1	13094.85	7089.85	11.5	245
ASS + Cu 280 µg/g 2	12548.15	6575	7.5	240
ASS + Cu 280 µg/g 3	9854.9	6838.95	16	260

ND = not detected

Table A.1.2. The survival days of mangrove seedlings in laboratory environment

Environment	Survival day
Control mangrove 1	45
Control mangrove 2 (Lived)	80
Control mangrove 3 (Lived)	80
Ni 25 µg/g 1 (Lived)	80
Ni 25 µg/g 2 (Lived)	80
Ni 25 µg/g 3 (Lived)	80
Ni 55 µg/g 1 (Lived)	80
Ni 55 µg/g 2 (Lived)	80
Ni 55 µg/g 3 (Lived)	80
Cu 70 µg/g 1 (Lived)	80
Cu 70 µg/g 2 (Lived)	80
Cu 70 µg/g 3 (Lived)	80
Cu 280 µg/g 1 (Lived)	80
Cu 280 µg/g 2	33
Cu 280 µg/g 3	45
ASS 1 (Lived)	80
ASS 2 (Lived)	80
ASS 3	58
ASS + Ni 25 µg/g 1	27
ASS + Ni 25 µg/g 2 (Lived)	80
ASS + Ni 25 µg/g 3	37
ASS + Ni 55 µg/g 1	54
ASS + Ni 55 µg/g 2	33
ASS + Ni 55 µg/g 3	45
ASS + Cu 70 µg/g 1	27
ASS + Cu 70 µg/g 2	45
ASS + Cu 70 µg/g 3	37
ASS + Cu 280 µg/g 1	64
ASS + Cu 280 µg/g 2	33
ASS + Cu 280 µg/g 3 (Lived)	80

Table A.1.3.The concentration of Fe in mangrove seedling parts in laboratory environment

Environment	Fe (µg/g)		
	Stem	Leaf	Root
Control mangrove 1	12.08	37.50	5985.00
Control mangrove 2 (Lived)	17.10	66.25	2360.00
Control mangrove 3 (Lived)	14.77	20.83	6235.00
Ni 25 µg/g 1 (Lived)	20.11	39.10	8235.00
Ni 25 µg/g 2 (Lived)	7.58	36.25	4630.00
Ni 25 µg/g 3 (Lived)	13.75	112.63	4625.00
Ni 55 µg/g 1 (Lived)	14.58	50.00	4490.00
Ni 55 µg/g 2 (Lived)	15.22	45.00	9132.66
Ni 55 µg/g 3 (Lived)	16.35	27.50	3485.00
Cu 70 µg/g 1 (Lived)	16.35	41.85	1337.50
Cu 70 µg/g 2 (Lived)	15.22	21.46	1537.50
Cu 70 µg/g 3 (Lived)	17.24	32.50	1487.50
Cu 280 µg/g 1 (Lived)	19.08	58.75	2375.00
Cu 280 µg/g 2	9.00	41.25	2925.00
Cu 280 µg/g 3	13.69	53.33	2287.50
ASS 1 (Lived)	18.18	50.00	13235.00
ASS 2 (Lived)	17.14	46.67	18819.83
ASS 3	18.75	91.67	17177.37
ASS + Ni 25 µg/g 1	16.58	57.50	7485.00
ASS + Ni 25 µg/g 2 (Lived)	12.78	81.25	20950.00
ASS + Ni 25 µg/g 3	18.06	79.75	10306.65
ASS + Ni 55 µg/g 1	11.50	280.00	9200.00
ASS + Ni 55 µg/g 2	27.08	120.00	5490.00
ASS + Ni 55 µg/g 3	28.75	133.75	7727.50
ASS + Cu 70 µg/g 1	50.00	38.33	5487.50
ASS + Cu 70 µg/g 2	7.14	20.00	2737.50
ASS + Cu 70 µg/g 3	23.33	65.83	4487.50
ASS + Cu 280 µg/g 1	10.56	66.25	2987.50
ASS + Cu 280 µg/g 2	30.00	62.50	2225.00
ASS + Cu 280 µg/g 3 (Lived)	17.50	30.00	3987.50

Table A.1.4.The concentration of Al in mangrove seedling parts in laboratory environment

Environment	Al (µg/g)		
	Stem	Leaf	Root
Control mangrove 1	ND	ND	237.50
Control mangrove 2 (Lived)	ND	ND	185.00
Control mangrove 3 (Lived)	ND	ND	162.50
Ni 25 µg/g 1 (Lived)	ND	ND	167.50
Ni 25 µg/g 2 (Lived)	ND	ND	550.00
Ni 25 µg/g 3 (Lived)	1.67	0.44	46.25
Ni 55 µg/g 1 (Lived)	ND	8.12	142.50
Ni 55 µg/g 2 (Lived)	ND	10.83	264.42
Ni 55 µg/g 3 (Lived)	ND	2.50	132.50
Cu 70 µg/g 1 (Lived)	ND	10.80	190.00
Cu 70 µg/g 2 (Lived)	ND	ND	190.00
Cu 70 µg/g 3 (Lived)	ND	ND	275.00
Cu 280 µg/g 1 (Lived)	ND	ND	450.00
Cu 280 µg/g 2	ND	ND	355.00
Cu 280 µg/g 3	ND	ND	287.50
ASS 1 (Lived)	ND	8.75	220.00
ASS 2 (Lived)	13.21	42.50	230.00
ASS 3	ND	4.17	247.48
ASS + Ni 25 µg/g 1	ND	ND	240.00
ASS + Ni 25 µg/g 2 (Lived)	14.72	16.25	365.00
ASS + Ni 25 µg/g 3	ND	312.50	387.70
ASS + Ni 55 µg/g 1	ND	170.00	235.00
ASS + Ni 55 µg/g 2	6.25	27.50	242.50
ASS + Ni 55 µg/g 3	9.38	268.75	332.50
ASS + Cu 70 µg/g 1	ND	ND	257.50
ASS + Cu 70 µg/g 2	ND	ND	242.50
ASS + Cu 70 µg/g 3	ND	5	390.00
ASS + Cu 280 µg/g 1	ND	ND	230.00
ASS + Cu 280 µg/g 2	ND	ND	132.50
ASS + Cu 280 µg/g 3 (Lived)	ND	ND	172.50

ND = not detected

Table A.1.5. The concentration of Ni in mangrove seedling parts in laboratory environment

Environment	Ni (µg/g)		
	Stem	Leaf	Root
Control mangrove 1	2.50	5.00	ND
Control mangrove 2 (Lived)	2.10	1.25	10.00
Control mangrove 3 (Lived)	2.50	ND	ND
Ni 25 µg/g 1 (Lived)	3.57	12.75	370.00
Ni 25 µg/g 2 (Lived)	ND	1.25	130.00
Ni 25 µg/g 3 (Lived)	ND	19.88	196.25
Ni 55 µg/g 1 (Lived)	4.58	3.75	637.50
Ni 55 µg/g 2 (Lived)	4.17	0.83	1057.68
Ni 55 µg/g 3 (Lived)	5.50	2.50	255.00
ASS 1 (Lived)	3.00	12.50	15.00
ASS 2 (Lived)	1.10	ND	18.33
ASS 3	3.44	2.50	20.84
ASS + Ni 25 µg/g 1	9.35	32.50	244.87
ASS + Ni 25 µg/g 2 (Lived)	1.11	15.00	35.00
ASS + Ni 25 µg/g 3	6.31	15.00	239.16
ASS + Ni 55 µg/g 1	2.50	25.00	160.00
ASS + Ni 55 µg/g 2	5.83	37.50	145.00
ASS + Ni 55 µg/g 3	6.88	3.75	107.50

ND = not detected

Table A.1.6. The concentration of Cu in mangrove seedling parts in laboratory environment

Environment	Cu (µg/g)		
	Stem	Leaf	Root
Control mangrove 1	3.75	5.83	7.50
Control mangrove 2 (Lived)	2.10	10.00	10.00
Control mangrove 3 (Lived)	1.67	8.33	5.00
Cu 70 µg/g 1 (Lived)	4.58	5.40	397.50
Cu 70 µg/g 2 (Lived)	3.13	10.10	372.50
Cu 70 µg/g 3 (Lived)	2.92	7.50	522.50
Cu 280 µg/g 1 (Lived)	5.36	17.50	8915.0
Cu 280 µg/g 2	3.00	13.75	9926.50
Cu 280 µg/g 3	4.74	10.00	6753.75
ASS 1 (Lived)	2.50	5.00	22.50
ASS 2 (Lived)	2.14	5.83	16.67
ASS 3	2.19	6.67	49.50
ASS + Cu 70 µg/g 1	15.83	6.67	3025.50
ASS + Cu 70 µg/g 2	4.29	3.33	8217.50
ASS + Cu 70 µg/g 3	6.39	5.00	6535.00
ASS + Cu 280 µg/g 1	13.06	20.00	9495.75
ASS + Cu 280 µg/g 2	10.00	27.50	6294.25
ASS + Cu 280 µg/g 3 (Lived)	2.95	10.00	9280.00

Appendix A.2. Bioconcentration factors of metals in the laboratory environment

Table A.2.1. The bioconcentration factors of Fe and Al in mangrove parts in laboratory environment

Environment	Fe			Al		
	Stem	Leaf	Root	Stem	Leaf	Root
Control mangrove 1	0.009	0.027	4.275	ND	ND	0.192
Control mangrove 2 (Lived)	0.013	0.051	1.815	ND	ND	0.163
Control mangrove 3 (Lived)	0.012	0.017	5.196	ND	ND	0.157
Ni 25 µg/g 1 (Lived)	0.018	0.036	7.538	ND	ND	0.095
Ni 25 µg/g 2 (Lived)	0.006	0.030	3.883	ND	ND	0.312
Ni 25 µg/g 3 (Lived)	0.013	0.103	4.233	0.001	0.000	0.029
Ni 55 µg/g 1 (Lived)	0.011	0.037	3.326	ND	0.007	0.121
Ni 55 µg/g 2 (Lived)	0.012	0.036	7.306	ND	0.010	0.244
Ni 55 µg/g 3 (Lived)	0.012	0.020	2.581	ND	0.002	0.112
Cu 70 µg/g 1 (Lived)	0.009	0.024	0.768	ND	0.005	0.092
Cu 70 µg/g 2 (Lived)	0.011	0.015	1.066	ND	ND	0.097
Cu 70 µg/g 3 (Lived)	0.010	0.019	0.879	ND	ND	0.135
Cu 280 µg/g 1 (Lived)	0.017	0.053	2.159	ND	ND	0.310
Cu 280 µg/g 2	0.009	0.043	3.079	ND	ND	0.291
Cu 280 µg/g 3	0.013	0.051	2.179	ND	ND	0.212
ASS 1 (Lived)	0.001	0.003	0.813	ND	0.001	0.035
ASS 2 (Lived)	0.001	0.003	1.107	0.002	0.007	0.037
ASS 3	0.001	0.005	1.022	ND	0.001	0.041
ASS + Ni 25 µg/g 1	0.002	0.005	0.691	ND	ND	0.034
ASS + Ni 25 µg/g 2 (Lived)	0.001	0.006	1.633	0.002	0.002	0.045
ASS + Ni 25 µg/g 3	0.002	0.008	0.970	ND	0.046	0.057
ASS + Ni 55 µg/g 1	0.001	0.016	0.513	ND	0.026	0.036
ASS + Ni 55 µg/g 2	0.002	0.007	0.316	0.001	0.004	0.038
ASS + Ni 55 µg/g 3	0.002	0.008	0.446	0.002	0.043	0.053
ASS + Cu 70 µg/g 1	0.004	0.003	0.442	ND	ND	0.037
ASS + Cu 70 µg/g 2	0.001	0.002	0.219	ND	ND	0.035
ASS + Cu 70 µg/g 3	0.002	0.005	0.351	ND	0.001	0.055
ASS + Cu 280 µg/g 1	0.001	0.005	0.228	ND	ND	0.032
ASS + Cu 280 µg/g 2	0.002	0.005	0.177	ND	ND	0.020
ASS + Cu 280 µg/g 3 (Lived)	0.002	0.003	0.405	ND	ND	0.025

ND = not detected

Table A.2.2. The bioconcentration factors of Ni in mangrove parts in laboratory environment

Environment	Ni		
	Stem	Leaf	Root
Control mangrove 1	1.25	2.50	ND
Control mangrove 2 (Lived)	2.10	1.25	10.00
Control mangrove 3 (Lived)	ND	ND	ND
Ni 25 µg/g 1 (Lived)	0.09	0.34	9.74
Ni 25 µg/g 2 (Lived)	ND	0.05	5.20
Ni 25 µg/g 3 (Lived)	ND	0.45	4.41
Ni 55 µg/g 1 (Lived)	0.07	0.06	9.51
Ni 55 µg/g 2 (Lived)	0.08	0.02	20.15
Ni 55 µg/g 3 (Lived)	0.10	0.05	4.72
ASS 1 (Lived)	0.32	1.32	1.58
ASS 2 (Lived)	0.16	ND	2.62
ASS 3	0.31	0.23	1.89
ASS + Ni 25 µg/g 1	0.35	1.20	9.07
ASS + Ni 25 µg/g 2 (Lived)	0.03	0.41	0.95
ASS + Ni 25 µg/g 3	0.21	0.51	8.11
ASS + Ni 55 µg/g 1	0.05	0.51	3.23
ASS + Ni 55 µg/g 2	0.12	0.78	3.02
ASS + Ni 55 µg/g 3	0.16	0.09	2.47

ND = not detected

Table A.2.3. The bioconcentration factors of Cu in mangrove parts in laboratory environment

Environment	Cu		
	Stem	Leaf	Root
Control mangrove 1	7.50	11.67	15.00
Control mangrove 2 (Lived)	4.20	20.00	20.00
Control mangrove 3 (Lived)	1.67	8.33	5.00
Cu 70 µg/g 1 (Lived)	0.04	0.05	3.49
Cu 70 µg/g 2 (Lived)	0.03	0.10	3.76
Cu 70 µg/g 3 (Lived)	0.03	0.07	4.58
Cu 280 µg/g 1 (Lived)	0.02	0.05	26.22
Cu 280 µg/g 2	0.01	0.05	35.45
Cu 280 µg/g 3	0.02	0.03	23.29
ASS 1 (Lived)	0.42	0.83	3.75
ASS 2 (Lived)	0.36	0.97	2.78
ASS 3	0.40	1.21	9.00
ASS + Cu 70 µg/g 1	0.15	0.06	28.81
ASS + Cu 70 µg/g 2	0.05	0.04	96.68
ASS + Cu 70 µg/g 3	0.03	0.02	27.81
ASS + Cu 280 µg/g 1	0.05	0.08	38.76
ASS + Cu 280 µg/g 2	0.04	0.11	26.23
ASS + Cu 280 µg/g 3 (Lived)	0.01	0.04	35.69

Appendix A.3. Translocation factors of metals in the laboratory environment

Table A.3.1. The translocation factors of Fe and Al in mangrove parts in laboratory environment

Environment	Fe	Al	Ni	Cu
Control mangrove 1	0.0020	ND	ND	0.5000
Control mangrove 2 (Lived)	0.0072	ND	0.2100	0.2100
Control mangrove 3 (Lived)	0.0024	ND	ND	0.3333
Ni 25 µg/g 1 (Lived)	0.0024	ND	0.0097	-
Ni 25 µg/g 2 (Lived)	0.0016	ND	ND	-
Ni 25 µg/g 3 (Lived)	0.0030	0.0360	ND	-
Ni 55 µg/g 1 (Lived)	0.0032	ND	0.0072	-
Ni 55 µg/g 2 (Lived)	0.0017	ND	0.0039	-
Ni 55 µg/g 3 (Lived)	0.0047	ND	0.0216	-
Cu 70 µg/g 1 (Lived)	0.0122	ND	-	0.0115
Cu 70 µg/g 2 (Lived)	0.0099	ND	-	0.0084
Cu 70 µg/g 3 (Lived)	0.0116	ND	-	0.0056
Cu 280 µg/g 1 (Lived)	0.0080	ND	-	0.0006
Cu 280 µg/g 2 (Lived)	0.0031	ND	-	0.0003
Cu 280 µg/g 3 (Lived)	0.0060	ND	-	0.0007
ASS 1 (Lived)	0.0014	ND	0.2000	0.1111
ASS 2 (Lived)	0.0009	0.0575	0.0600	0.1286
ASS 3	0.0011	ND	0.1649	0.0442
ASS + Ni 25 µg/g 1	0.0022	ND	0.0382	-
ASS + Ni 25 µg/g 2 (Lived)	0.0006	0.0403	0.0317	-
ASS + Ni 25 µg/g 3	0.0018	ND	0.0264	-
ASS + Ni 55 µg/g 1	0.0013	ND	0.0156	-
ASS + Ni 55 µg/g 2	0.0049	0.0258	0.0402	-
ASS + Ni 55 µg/g 3	0.0037	0.0282	0.0640	-
ASS + Cu 70 µg/g 1	0.0091	ND	-	0.0052
ASS + Cu 70 µg/g 2	0.0026	ND	-	0.0005
ASS + Cu 70 µg/g 3	0.0052	ND	-	0.0010
ASS + Cu 280 µg/g 1	0.0035	ND	-	0.0014
ASS + Cu 280 µg/g 2	0.0135	ND	-	0.0016
ASS + Cu 280 µg/g 3 (Lived)	0.0044	ND	-	0.0003

ND = not detected

APPENDIX B. ANCOVA RESULTS OF METAL BIOCONCENTRATIONS IN MANGROVE SEEDLING PARTS AND SURVIVAL DAYS IN THE LABORATORY ENVIRONMENTS

Appendix B. 1. ANCOVA results of Fe bioconcentrations in different parts of mangrove seedlings and survival days in laboratory environments.

Table B.1.1. ANCOVA results of Fe bioconcentrations in stem tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:BCF Fe stem

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.001[a]	16	5.336E-5	8.725	.000
Intercept	3.827E-5	1	3.827E-5	6.256	.027
Environment	.000	6	1.718E-5	2.808	.056
Survday	5.262E-6	1	5.262E-6	.860	.371
Environment * Survday	4.091E-5	6	6.818E-6	1.115	.406
Error	7.952E-5	13	6.117E-6		
Total	.002	30			
Corrected Total	.001	29			

a. R Squared = ,915 (Adjusted R Squared = ,810)

Table B.1.2. ANCOVA results of Fe bioconcentrations in leaf tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:BCF Fe leaf

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.011[a]	16	.001	2.111	.090
Intercept	.000	1	.000	.679	.425
Environment	.001	6	.000	.469	.820
Survday	1.731E-6	1	1.731E-6	.006	.942
Environment * Survday	.000	6	2.181E-5	.069	.998
Error	.004	13	.000		
Total	.029	30			
Corrected Total	.015	29			

a. R Squared = ,722 (Adjusted R Squared = ,380)

Table B.1.3. ANCOVA results of Fe bioconcentrations in root tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:BCF Fe root

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	93.717[a]	16	5.857	2.832	.032
Intercept	1.647	1	1.647	.796	.388
Environment	3.753	6	.626	.302	.925
Survday	.013	1	.013	.006	.939
Environment * Survday	1.180	6	.197	.095	.996
Error	26.885	13	2.068		
Total	239.064	30			
Corrected Total	120.601	29			

a. R Squared = ,777 (Adjusted R Squared = ,503)

Appendix B. 2. ANCOVA results of Al bioconcentrations in different parts of mangrove seedlings and survival days in laboratory environments.

Table B.2.1. ANCOVA results of Al bioconcentrations in stem tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:BCF Al stem

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.395E-6[a]	16	3.997E-7	1.353	.294
Intercept	5.101E-9	1	5.101E-9	.017	.897
Environment	1.854E-6	6	3.090E-7	1.046	.440
Survday	9.825E-8	1	9.825E-8	.333	.574
Environment * Survday	2.493E-6	6	4.155E-7	1.407	.284
Error	3.840E-6	13	2.954E-7		
Total	1.391E-5	30			
Corrected Total	1.024E-5	29			

a. R Squared = ,625 (Adjusted R Squared = ,163)

Table B.2.2. ANCOVA results of Al bioconcentrations in leaf tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:BCF Al leaf

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.002[a]	16	.000	1.071	.457
Intercept	2.790E-7	1	2.790E-7	.002	.965
Environment	.000	6	7.609E-5	.561	.754
Survday	5.951E-5	1	5.951E-5	.438	.519
Environment * Survday	.000	6	6.886E-5	.507	.792
Error	.002	13	.000		
Total	.005	30			
Corrected Total	.004	29			

a. R Squared = ,569 (Adjusted R Squared = ,038)

Table B.2.3. ANCOVA results of Al bioconcentrations in root tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:BCF Al root

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.177[a]	16	.011	2.319	.066
Intercept	.010	1	.010	2.118	.169
Environment	.017	6	.003	.607	.721
Survday	4.060E-5	1	4.060E-5	.009	.928
Environment * Survday	.001	6	.000	.026	1.000
Error	.062	13	.005		
Total	.568	30			
Corrected Total	.238	29			

a. R Squared = ,741 (Adjusted R Squared = ,421)

Appendix B. 3. ANCOVA results of Ni bioconcentrations in different parts of mangrove seedlings and survival days in laboratory environments.

Table B.3.1. ANCOVA results of Ni bioconcentrations in stem tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:SQRT BCF Ni stem

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.467[a]	16	.529	.418	.950
Intercept	1.295	1	1.295	1.022	.330
Environment	.877	6	.146	.115	.993
Survday	.644	1	.644	.508	.488
Environment * Survday	.652	6	.109	.086	.997
Error	16.469	13	1.267		
Total	33.985	30			
Corrected Total	24.936	29			

a. R Squared = ,340 (Adjusted R Squared = -,473)

Table B.3.2. ANCOVA results of Ni bioconcentrations in leaf tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:BCF Ni leaf

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	56.073[a]	16	3.505	.336	.979
Intercept	2.717	1	2.717	.260	.619
Environment	3.504	6	.584	.056	.999
Survday	.774	1	.774	.074	.790
Environment * Survday	1.763	6	.294	.028	1.000
Error	135.744	13	10.442		
Total	221.064	30			
Corrected Total	191.817	29			

a. R Squared = ,292 (Adjusted R Squared = -,579)

Table B.3.3. ANCOVA results of Ni bioconcentrations in root tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:BCF Ni root

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	916.711[a]	16	57.294	.416	.951
Intercept	2.608	1	2.608	.019	.893
Environment	82.623	6	13.770	.100	.995
Survday	.001	1	.001	.000	.998
Environment * Survday	52.912	6	8.819	.064	.999
Error	1791.451	13	137.804		
Total	3449.967	30			
Corrected Total	2708.162	29			

a. R Squared = ,338 (Adjusted R Squared = -,476)

Appendix B. 4. ANCOVA results of Cu bioconcentrations in different parts of mangrove seedlings and survival days in laboratory environments.

Table B.4.1. ANCOVA results of Cu bioconcentrations in stem tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:SQRT Cu stem

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.033[a]	16	1.002	2.239	.074
Intercept	1.133	1	1.133	2.533	.136
Environment	2.125	6	.354	.792	.592
Survday	.144	1	.144	.323	.580
Environment * Survday	.607	6	.101	.226	.961
Error	5.817	13	.447		
Total	41.916	30			
Corrected Total	21.851	29			

a. R Squared = ,734 (Adjusted R Squared = ,406)

Table B.4.2. ANCOVA results of Cu bioconcentrations in leaf tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:SQRT BCF Cu leaf

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	42.904[a]	16	2.682	1.919	.121
Intercept	1.464	1	1.464	1.048	.325
Environment	1.187	6	.198	.142	.988
Survday	.001	1	.001	.001	.975
Environment * Survday	.074	6	.012	.009	1.000
Error	18.166	13	1.397		
Total	117.568	30			
Corrected Total	61.071	29			

a. R Squared = ,703 (Adjusted R Squared = ,336)

Table B.4.3. ANCOVA results of Cu bioconcentrations in root tissues and survival days in laboratory environment

Tests of Between-Subjects Effects

Dependent Variable:BCF Cu root

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11230.838[a]	16	701.927	2.222	.076
Intercept	16.374	1	16.374	.052	.823
Environment	1947.346	6	324.558	1.027	.450
Survday	1444.013	1	1444.013	4.571	.052
Environment * Survday	3581.982	6	596.997	1.890	.158
Error	4106.883	13	315.914		
Total	29347.411	30			
Corrected Total	15337.721	29			

a. R Squared = ,732 (Adjusted R Squared = ,403)

APPENDIX C. ANOVA RESULTS OF METAL BIOCONCENTRATION IN THE LABORATORY ENVIRONMENT

Appendix C. 1. ANOVA results for Fe bioconcentrations in mangrove seedlings parts.

Table C.1.1. ANOVA result of Fe bioconcentrations in stem tissues of mangrove seedlings.

ANOVA

Fe stem

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	9	.000	14.296	.000
Within Groups	.000	20	.000		
Total	.001	29			

Table C.1.2. ANOVA result of Fe bioconcentrations in leaf tissues of mangrove seedlings.

ANOVA

Fe leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.010	9	.001	5.522	.001
Within Groups	.004	20	.000		
Total	.015	29			

Table C.1.3. ANOVA result of Fe bioconcentrations in root tissues of mangrove seedlings.

ANOVA

Fe root

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	92.267	9	10.252	7.236	.000
Within Groups	28.334	20	1.417		
Total	120.601	29			

Appendix C. 2. Post hoc and homogenous subsets results for Fe bioconcentrations in stem tissues of mangrove seedlings in different environments.

Table C.2.1. Post hoc results for Fe bioconcentrations in stem tissues of mangrove seedlings in different environments.

(I) Environment	(J) Environment	p
1	6	0.002
	7	0.003
	8	0.003
	9	0.006
	10	0.004
2	6	0.001
	7	0.001
	8	0.001
	9	0.002
	10	0.001
3	6	0.001
	7	0.002
	8	0.002
	9	0.004
	10	0.003
4	6	0.009
	7	0.012
	8	0.011
	9	0.026
	10	0.016
5	6	0.000
	7	0.000
	8	0.000
	9	0.001
	10	0.000

Table C.2.2. Groups in homogenous subsets for Fe bioconcentrations in stem tissues of mangrove seedlings.

Fe stem

	Environment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	6	3	.001083	
	8	3	.001287	
	7	3	.001410	
	10	3	.001660	
	9	3	.002137	
	4	3		.010040
	1	3		.011363
	3	3		.011693
	2	3		.012450
	5	3		.013283
	Sig.		1.000	.840

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Appendix C. 3. Post hoc and homogenous subsets results for Fe bioconcentrations in leaf tissues of mangrove seedlings in different environments.

Table C.3.1. Post hoc results for Fe bioconcentrations in leaf tissues of mangrove seedlings in different environments.

(I) Environment	(J) Environment	p
2	6	0.007
	7	0.012
	8	0.023
	9	0.007
	10	0.008
5	6	0.027
	7	0.043
	9	0.025
	10	0.030

Table C.3.2. Groups in homogenous subsets for Fe bioconcentrations in leaf tissues of mangrove seedlings.

Fe leaf

	Environment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	9	3	.003267		
	6	3	.003767		
	10	3	.004367		
	7	3	.006367		
	8	3	.010067	.010067	
	4	3	.019367	.019367	.019367
	3	3	.031133	.031133	.031133
	1	3	.031733	.031733	.031733
	5	3		.049200	.049200
	2	3			.056433
	Sig.		.374	.080	.112

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Appendix C. 4. Post hoc and homogenous subsets results for Fe bioconcentrations in root tissues of mangrove seedlings in different environments.

Table C.4.1. Post hoc results for Fe bioconcentrations in root tissues of mangrove seedlings in different environments.

(I) Environment	(J) Environment	p
1	9	0.052
	10	0.045
2	4	0.007
	6	0.009
	7	0.011
	8	0.003
	9	0.002
	10	0.002
3	4	0.044
	6	0.052
	8	0.016
	9	0.013
	10	0.011
4	2	0.007
	3	0.044

Table C.4.2. Groups in homogenous subsets for Fe bioconcentrations in root tissues of mangrove seedlings.

Fe root

	Environment	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	10	3	.270000			
	9	3	.337000	.337000		
	8	3	.425000	.425000		
	4	3	.904133	.904133		
	6	3	.980600	.980600	.980600	
	7	3	1.098033E0	1.098033E0	1.098033E0	
	5	3	2.472200E0	2.472200E0	2.472200E0	2.472200E0
	1	3		3.762067E0	3.762067E0	3.762067E0
	3	3			4.404500E0	4.404500E0
	2	3				5.217933E0
	Sig.		.449	.052	.052	.193

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Appendix C. 5. Anova results for Al bioconcentrations in mangrove seedlings parts.

Table C.5.1. ANOVA result of Al bioconcentrations in stem tissues of mangrove seedlings.

ANOVA

BCF Al stem

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	9	.000	.993	.476
Within Groups	.000	20	.000		
Total	.000	29			

Table C.5.2. ANOVA result of Al bioconcentrations in leaf tissues of mangrove seedlings.

ANOVA

BCF Al leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	9	.000	1.945	.103
Within Groups	.002	20	.000		
Total	.004	29			

Table C.5.3. ANOVA result of Al bioconcentrations in root tissues of mangrove seedlings.

ANOVA

BCF Al root

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.176	9	.020	6.236	.000
Within Groups	.063	20	.003		
Total	.238	29			

Appendix C. 6. Post hoc and homogenous subsets results for Al bioconcentrations in root tissues of mangrove seedlings in different environments.

Table C.6.1. Post hoc results for Al bioconcentrations in root tissues of mangrove seedlings in different environments.

(I) Environment	(J) Environment	p
4	5	0.048
5	6	0.002
	7	0.003
	8	0.002
	9	0.002
	10	0.001

Table C.6.2. Groups in homogenous subsets for Al bioconcentrations in root tissues of mangrove seedlings.

Alroot

	Environment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	10	3	.025933	
	6	3	.037733	
	9	3	.042133	
	8	3	.042500	
	7	3	.045267	
	4	3	.108033	
	2	3	.145667	.145667
	3	3	.159067	.159067
	1	3	.170567	.170567
	5	3		.270900
	Sig.		.105	.223

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Appendix C. 7. ANOVA results for Ni bioconcentrations in mangrove seedlings parts.

Table C.7.1. ANOVA result of Ni bioconcentrations in stem tissues of mangrove seedlings.

ANOVA

SQRTBCF Ni stem

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.971	5	.194	1.764	.195
Within Groups	1.321	12	.110		
Total	2.292	17			

Table C.7.2. ANOVA result of Ni bioconcentrations in leaf tissues of mangrove seedlings.

ANOVA

BCF Ni leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.576	5	.515	1.282	.334
Within Groups	4.821	12	.402		
Total	7.397	17			

Table C.7.3. ANOVA result of Ni bioconcentrations in root tissues of mangrove seedlings.

ANOVA

BCF Ni root

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	180.225	5	36.045	1.743	.199
Within Groups	248.104	12	20.675		
Total	428.329	17			

Appendix C. 8. ANOVA results for Cu bioconcentrations in mangrove seedlings parts.

Table C.8.1. ANOVA result of Cu bioconcentrations in stem tissues of mangrove seedlings.

ANOVA

SQRT BCF Custem

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.183	5	1.637	18.080	.000
Within Groups	1.086	12	.091		
Total	9.270	17			

Table C.8.2. ANOVA result of Cu bioconcentrations in leaf tissues of mangrove seedlings.

ANOVA

SQRT BCF Cu leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	27.037	5	5.407	48.305	.000
Within Groups	1.343	12	.112		
Total	28.380	17			

Table C.8.3. ANOVA result of Cu bioconcentrations in root tissues of mangrove seedlings.

ANOVA

BCF Cu root

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5107.705	5	1021.541	3.582	.033
Within Groups	3422.021	12	285.168		
Total	8529.725	17			

Appendix C. 9. Post hoc and homogenous subsets results for Cu bioconcentrations in stem tissues of mangrove seedlings in different environments.

Table C.9.1. Post hoc results for Cu bioconcentrations in stem tissues of mangrove seedlings in different environments.

(I) Environment	(J) Environment	p
1	4	0.000
	5	0.000
	6	0.001
	9	0.000
	10	0.000

Table C.9.2. Groups in homogenous subsets for Cu bioconcentrations in stem tissues of mangrove seedlings.

SQRTBCFCustem

	Environment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	5	3	.1191	
	4	3	.1794	
	10	3	.1806	
	9	3	.2593	
	6	3	.6246	
	1	3		2.0263
	Sig.		.368	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Appendix C. 10. Post hoc and homogenous subsets results for Cu bioconcentrations in leaf tissues of mangrove seedlings in different environments.

Table C.10.1. Post hoc results for Cu bioconcentrations in leaf tissues of mangrove seedlings in different environments.

(I) Environment	(J) Environment	p
1	4	0.000
	5	0.000
	6	0.000
	9	0.000
	10	0.000

Table C.10.2. Groups in homogenous subsets for Cu bioconcentrations in leaf tissues of mangrove seedlings.

SQRTBCFCuleaf

	Environment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	9	3	.1989	
	5	3	.2117	
	4	3	.2647	
	10	3	.2737	
	6	3	1.0000	
	1	3		3.5915
	Sig.		.101	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Appendix C. 11. Post hoc and homogenous subsets results for Cu bioconcentrations in root tissues of mangrove seedlings in different environments.

Table C.11.1. Post hoc results for Cu bioconcentrations in root tissues of mangrove seedlings in different environments.

(I) Environment	(J) Environment	p
4	9	0.045
6	9	0.052

Table C.11.2. Groups in homogenous subsets for Cu bioconcentrations in root tissues of mangrove seedlings.

Cu root

	Environment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	4	3	3.944333	
	6	3	5.175733	5.175733
	1	3	1.333343E1	1.333343E1
	5	3	2.832050E1	2.832050E1
	10	3	3.355893E1	3.355893E1
	9	3		5.109987E1
	Sig.		.327	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

APPENDIX D. DATA OF ELEMENT CONCENTRATIONS IN THE FIELD STUDY ENVIRONMENT

Appendix D.1. Speciation of metals in the field study area

Table D.1.1. Speciation of Fe in soils and total concentration of root samples in the field study area

Site	Fe (µg/g)				
	Total	Exchangeable	Fe Mn oxy	Organic	Root
1	38923.69	1271.66	1407.42	1852.87	19316.50
1	35580.19	74.64	1383.96	2715.79	19053.00
1	45382.89	727.58	929.18	2562.95	19371.50
4	36055.89	108.30	1119.88	2701.48	7222.50
4	58048.89	435.64	1352.02	2422.53	3233.75
4	43844.59	1219.82	1483.46	2237.83	4629.13
5	30236.89	435.96	1483.04	2705.05	7956.63
5	24652.26	195.00	1551.88	2638.44	6217.92
5	25458.14	305.16	1541.54	2639.74	9696.50
6	8225.74	239.22	987.68	1719.24	6756.63
6	9909.36	352.92	1366.22	1766.28	7553.25
6	9116.29	432.52	1228.32	1707.20	9624.63
7	16946.14	1370.96	1495.32	2368.31	5586.42
7	19328.59	1154.08	1490.02	2758.70	1557.00
7	15642.21	1326.64	1496.18	2394.63	3103.25

Table D.1.2. Speciation of Al in soils and total concentration of root samples in the field study area

Site	Al (µg/g)				
	Total	Exchangeable	Fe Mn oxy	Organic	Root
1	48625.47	333.74	1712.62	1592.85	3180.25
1	45423.37	189.96	3041.16	1704.43	4053.75
1	30955.47	172.10	2258.66	1475.32	3376.75
4	24150.39	293.40	3704.74	3776.40	1814.38
4	31445.19	339.76	2978.24	3006.78	2975.00
4	25628.61	593.50	2915.30	3594.33	5117.69
5	22974.04	127.66	2452.10	1713.22	2042.75
5	22820.62	157.42	3086.04	1720.46	2488.67
5	23420.94	112.18	2723.26	1503.53	3048.88
6	8575.02	170.32	1647.84	459.55	1111.25
6	9582.77	151.40	1987.50	681.17	1365.50
6	9814.74	143.38	1503.46	775.64	1599.25
7	14290.32	284.02	3190.18	929.53	1215.83
7	15420.89	181.98	3103.64	1044.82	1429.25
7	14155.52	217.98	3069.40	885.96	1639.13

APPENDIX E. PROCEDURES USED IN THE STUDY

Appendix E.1. Field pH peroxide test (pH_{fox})

Equipment

Plastic container, stirrer, pH probemeter.

Reagent

30% hydrogen peroxide (H_2O_2) adjusted to pH4.5 – 5.5 with NaOH.

Procedure

1. Transfer ½ teaspoon of soils to a container and add few drops of 30% H_2O_2 and stir the mixture.
2. Wait for about 15 min to complete the reaction and record the pH with pH probemeter.

(Ahern et al., 2004).

Appendix E.2. Analysis of organic content

Equipment

Ceramic dish, combustion, balance.

Procedure

1. Oven-dry 4 g of soil samples at 105^{o}C for 24 h.
2. Measure the weight of the sample.
3. Heat the soils at 550^{o}C for 4 h.
4. Weigh the soils.

Calculation

The LOI is measured using the following equation:

$$LOI_{550} = ((DW_{105} - DW_{550})/DW_{105}) \times 100$$

Where: LOI_{550} = LOI at 550^{o}C (in percentage)

DW_{105} = the dry weight of the sample before combustion (in g)

DW_{550} = the dry weight of the sample after heating to 550^{o}C (in g)

(Heiri et al., 2001).

Appendix E.3. Analysis of water-soluble sulfate

Equipment

Shaker, centrifuge, spectrophotometer, 50 ml graduated cylinder, 15 ml plastic centrifuge tubes, 0.45 μm membrane filter.

Reagents

Deionised water

Sample handling and preservation

Store extracted samples at 4°C. The holding time for the extracted samples is 28 days.

Procedure

1. Shake 2.5 g soils with 50 ml deionised water in a mechanical shaker for 30 min at 200 rpm. Include a blank in each of the series.
2. Centrifuge 25 ml of the suspension for 20 min at 5,800 x g, and filter using 0.45 μm membrane.
3. Measure the soil extract using spectrophotometer.

(Page and Steinbock, 2009).

Appendix E.4. Analysis of HCl extractable sulfur

Equipment

Electronic balance (100 ± 0.01 g), fume hood, plastic extraction bottle, sample shaker, thick medium speed high retention filter paper (eg. Whatman #3 paper).

Reagent

4 M HCl
Add about 97.5 ml of concentrated (31.5–33% w/V) HCl to 100 ml deionised water and dilute to 250 ml at 20 °C.

Procedure

1. Weigh 1g of oven-dried finely ground soil samples into a plastic extraction bottle.
2. Make a 1:40 soil suspension by adding 40 ml of 4 M HCl and stopper bottle in a fume hood. This addition can cause a strong reaction. Wait until reaction settles before closing the sample bottle lid. Include a solution blank with each analysis batch.

(Ahern et al., 2004).

Appendix E.5. Determination of sulfate using turbidimetric method

Equipment

Magnetic stirrer, measuring spoon with capacity 0.2 to 0.3 ml, stopwatch, spectrophotometer.

Reagents

a. Buffer solution

Dissolve 15 g magnesium chloride, $MgCl_2 \cdot 6H_2O$, 2.5 g sodium acetate,$CH_3COONa \cdot 3H_2O$, 0.5 g potassium nitrate, KNO_3, and 10 ml acetic acid, CH_3COOH (99%), in 250 ml distilled water and make up to 500 ml.

c. Barium chloride, $BaCl_2$, crystals, 20 to 30 mesh. In standardisation, uniform turbidity is produced with this mesh range and the appropriate buffer.

d. Standard sulfate solution: Prepare a standard sulfate solution by dissolving 0.0739 g

Procedure

1. Measure 25 ml sample into a 150 ml erlenmeyer flask. A suitable portion made up to 25 ml can also be made, for instance 2.5 ml into 25 ml.
2. Add 5 ml buffer solution and mix with a stirrer. Add half spoon of $BaCl_2$ crystals while stirring the solution, and begin timing immediately. Stir for 60 ± 2 s at constant speed.
4. Transfer solution into the spectrophotometer tube, and wait for about 5 ± 0.5 min before determining turbidity.
5. Determine SO_4^{2-} concentration in sample by comparing turbidity reading with a calibration curve of $SO4^{2-}$ standards. Prepare standard in a range of 0 to 40 mg/L SO_4^{2-} with interval standards at 5 mg/l. Run a standard every three or four samples to check reliability of calibration curve.
6. Run blanks to which $BaCl_2$ is not added to correct sample color and turbidity.

Calculation

Determine SO_4^{2-} concentration directly from the calibration curve after subtracting sample absorbance before adding $BaCl_2$.
(APHA, 1999).

Appendix E.6. Methylene blue method

Equipment

Test tubes, droppers, delivering 20 drops/ml methylene blue solution, dark glass bottle 500 ml, spectrophotometer.

Reagents

a. Amine-sulfuric acid stock solution:

1. Dissolve 27 g *N*,*N*-dimethyl-*p*-phenylenediamine oxalate in an iced mixture of 50 ml concentrated H_2SO_4 and 20 ml distilled water.

2. Cool and dilute to 100 ml with distilled water. Use fresh oxalate to prevent oxidation resulting from old supply.

3. Store in a dark glass bottle.

Note: When this stock solution is diluted and used in the procedure with a sulfide-free sample, the color will change from pink to colorless within 3 min.

b. Amine-sulfuric acid reagent:

Dilute 12.5 ml amine-sulfuric acid stock solution with 487.5 ml 1 + 1 H_2SO_4. Store in a dark glass bottle.

c. *Ferric chloride solution:*

Dissolve 50 g $FeCl_3 \cdot 6H_2O$ in 20 ml water.

d. Sulfuric acid solution,

H_2SO_4, 1 + 1.

e. Diammonium hydrogen phosphate solution:

Dissolve 25 g $(NH_4)_2HPO_4$ in 50 ml distilled water.

Preparation of sulfide standards

1. Prepare sulfide standards from sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) crystals. Use reagent water to prepare sulfide standards and sample dilutions.
2. Boil and degas with argon while cooling.
3. Remove single crystals of $Na_2S \cdot 9H_2O$ from reagent bottle with plastic tweezers; rinse it in degassed reagent water as soon as possible to remove surface contamination, and blot crystal dry with a tissue for the excess water present at the surface of the crystal.
4. Tranfer the crystal to a tared immediately to 3.750 g $Na_2S \cdot 9H_2O$ and dilute it up to 500 mL to give a stock solution of which 1.00 mL = 1.00 mg S^{2-}. Prepare a range of sulfide concentrations between 1 and 8 mg/L.

Procedure

1. Transfer 7.5 ml sample to each of two matched test tubes. As sample has been preserved with zinc acetate, shake the tubes briskly before splitting the sample.
2. Add 0.5 ml amine-sulfuric acid reagent and 0.15 ml (3 drops) $FeCl_3$ solution to Tube A.
3. Invert the tube once slowly to prevent low results due to the loss of H_2S.
4. Add 0.5 ml 1 + 1 H_2SO_4 and 0.15 ml (3 drops) $FeCl_3$ solution to Tube B and shake gently to mix. Appereance of blue color in Tube A indicates the presence of S^{2-}. The complete color development usually appears in around 1 min or longer.
5. Wait between 3 and 5 min and add 1.6 ml $(NH_4)_2HPO_4$ solution to each tube.
6. Wait at least 10 min before making color comparisons.
7. Determine the absorbance of sample on spectrophotometer at 664 nm. Read sulfide concentration from calibration curve by plotting the concentration vs. absorbance obtained.

(APHA, 1999).

Appendix E.7. Total Actual Acidity (TAA) and pH_{KCl} method

Equipment

Extraction plastic bottle, sample shaker, pH probemeter.

Reagent

a. 1 M KCl
Dissolve 74,55 g KCl and dilute to 1000 ml distilled water.

b. 0,05 N NaOH
Dissolve 2 g NaOH in 1000 ml distilled water and standardised with 0,1 HCl.

Procedure

1. Weigh 2.5 g of oven dried 0.5 µm sieved sample and transfer to the extraction plastic bottle.
2. Add the soils with 25 mL of 1M KCl. Prepare a blank for each series.
3. Extract the solution on a shaker for 30 min and allow to stand overnight.
4. Record the pH. If pH is more than 5.5 then TAA is zero. If the pH is less than 5.5, 0.05 N NaOH should be added to the solution to reach pH 5.5.

Calculation

$TAA = (V_1/V_2) \times (T_1-T_2) \times M \times (1000/W)$

Where:
V_1 = Volume of extracted sample
V_2 = Volume of sample that is used
T_1 = Amount of sample titrated
T_2 = Amount of blank titrated
M = concentration of NaOH
W = mass of sample
(White and Melville, 1993).

Appendix E.8. KCl extractable sulfur (S_{KCl} %) method

Equipment

Spectrophotometer

Reagent

a. $BaCl_2$
Weight 366 g of $BaCl_2$ to 1000 ml distilled water.

b. Conditioning Reagent
1. Dissolve 150 g NaCl to 550 ml distilled water.
2. Add 60 ml concentrated HCl, 200 ml absolute ethanol, 100 ml glycerine, and make to 1000 ml with distilled water.

Procedure

1. Drop 0.5 ml of KCl extract obtained from TAA measurement to tube and add 4.5 ml of distilled water.
2. Add 1 ml conditioning reagent and 1 ml of $BaCl_2$.
3. Prepare blank from distilled water for each of series.
4. Shake to mix the solution and read the absorbance of sample on the spectrophotometer at 520 nm. Determine sulfide concentration from calibration curve by plotting the concentration vs. absorbance obtained.
5. Report KCl extractable S value as S_{KCl} %.

(White and Melville, 1993).

Appendix E.9. Total Potential Acidity (TPA) dan pH_{OX} method

Equipment

Extraction plastic bottle, hot plate, shaker, pH probemeter

Reagent

a. 1 M KCl
Dissolve 74.55 g KCl to 1000 ml distilled water

b. 0,05 N NaOH
Dissolve 2 g NaOH to 1000 ml distilled water and standarised with 0.1 HCl.

c. 30 % H_2O_2

Procedure

1. Weigh 2.5 g of 0.5 µm oven-dried sample to 100 ml erlemeyer flask.
2. Titrate 25 ml of 1 M KCl.
3. Weigh the erlenmeyer, sample, and KCl to obtain the initial weight.
4. Add 5 ml of H_2O_2 and gently heat the suspension on a hot plate at 55 – 60^{o}C until oxidaton is complete. Spray the suspension with distilled water before the sample froths over.
5. Remove the sample from the hot plate and allow the suspension to cool.
6. Weigh and add distilled water to obtain the same initial weight.
7. Remove the extraction bottle, shake for 30 min and allow to stand overnight.
8. Record the pH to obtain pHox. If the pH is less than 5.5, add 0.05 N NaOH to reach pH 5.5.

Calculation

$TPA = (V_1/V_2) \times (T_1-T_2) \times M \times (1000/W)$

Where:
V_1 = Volume of extracted sample
V_2 = Volume of sample that is used
T_1 = Amount of sample titrated
T_2 = Amount of blank titrated
M = concentration of NaOH
W = mass of sample
(White and Melville, 1993).

Appendix E.10. Peroxide sulfur (S_P) method

Equipment

Spectrophotometer

Reagent

a. $BaCl_2$
Dissolve 366 g $BaCl_2$ to 1000 ml distilled water

b. Conditioning Reagent
Dissolve 150 g NaCl to 550 ml distilled water, add 60 ml concentrated HCl, 200 ml absolute ethanol, 100 ml glycerine, extract of H_2O_2 from the TPA step, and make up to 1000 ml with distilled water.

Procedure

1. Drop 0.5 ml H_2O_2 extract obtained from the TPA measurement to a tube and add 4.5 ml distilled water.
2. Add 1 ml of conditioning reagent, 1 ml $BaCl_2$ and shake to mix.
3. Prepare blank from distilled water and run in each batch.
4. Read the absorbance on the spectraphotometer at 520 nm. Determine sulfide concentration from calibration curve by plotting the concentration vs. absorbance obtained.
5. Report KCl extractable S value as S_P %.

(White and Melville, 1993).

Appendix E.11. Determination of Total Sulfate Acidity, Peroxide Oxidisable Sulfur, and Pyrite

Total Sulfate Acidity (TSA) is obtained by subtracting the TAA from the TPA.

Peroxide Oxidisable Sulfur is obtained by subtracting the S_{KCl} from the S_P.

Estimation of pyrite is conducted using the formula below:

Pyrite = (TSA : 22.4) x 0.1

Where:
TSA unit is in mmol H^+/100g = mmol H^+/0.0001t
= 0.001 mol H^+/0.0001t
= mol H^+/0.1t
Therefore:
% Pyrit (% FeS_2) = (TSA : 22.4) x 0.1

(Konsten and Sarwani, 1990, Sabang et al., 2005, White and Melville, 1993).

Appendix E.12. Total metal analysis

Equipment

Digester tube or glass container, glass cylinder, digester block or hot plate, flask, filter paper, ICP OES, or AAS.

Reagent

Distilled water, concentrated nitric acid, 30% hydrogen peroxide.

Procedure

1. Transfer 1 g oven-dried soils, or available amount of mangrove tissues to a digester tube or glass container, slurry it with 1 ml distilled water, and cover with glass cylinder. Prepare blank each of series.
2. Digest the sample with 10 ml of concentrated nitric acid on a digester block, or hot plate at approximately 100^{o}C for two hours.
3. Heat the extraction mixture again for another hour after the addition of 3 ml of 30% H_2O_2.
4. The digestates were filtered and made to the volume of a 50 ml flask for soils and root, and 25 ml for mangrove stem and leaf.
5. Detect the metal concentration using using ICP OES, or AAS.

(Defew et al., 2005, Khrisnamurty et al., 1976, MacFarlane and Burchett, 2001, Ramos e Silva et al., 2006)

Appendix E.13. Sequential extraction metal analysis

Equipment

Extraction plastic bottle, shaker, centrifuge tube, centrifuge, AAS.

Reagent

a. Ammonium acetate
b. Nitric acid
c. Hydrochloric acid,
d. 0 .11 mol/1 acetic acid
e. 30% w/v hydrogen peroxide (8.8 mol/1)
f. 0 .1 mol/Hydroxylammonium chloride, adjusted to pH 2 with nitric acid
g. ammonium acetate

Procedure

a. Exchangeable metal extraction

1. Transfer 1 g of dry soils (< 63 μm fraction) in a 100 ml polypropylene centrifuge tube and add 40 ml volume of acetic acid.
2. Shake sample overnight at around 20°C at speed of 40 rpm.
3. Centrifuge the suspension at 8000 rpm for 40 min, transfer the clean liquid into a sample bottle and store at 4°C for analysis.
4. Wash the residue with 20 ml distilled water, shake for 15 min, centrifuge, and discard the washings.

b. Reducible metal extraction

1. Transfer the residue from step 1 to an extraction plastic bottle and add 40 ml volume of hydroxylammonium chloride to the residue from step one (exchangeable metal extraction).
2. Perform the same procedure as described in step 1, i.e . shake the sample overnight, centrifuge to separate the clean liquid, and and wash the residue with distilled water.

c. Oxidisable metal extraction

1. Transfer the residue from step 2 to a container, and add carefully in small aliquots 10 ml of hydrogen peroxide, and cover with a watch glass.
2. Digest the sample at room temperature for about one hour and shake intermittently.
3. Continue to digest the sample by transferring it to a water bath at 85°C for around one hour.
4. Remove the watch glass to reduce the sample volume to about 1-2 ml.
5. Add a second 10 ml H_20_2 and cover the container with the watch glass, and heat to 85°C for about one hour.
6. Remove the cover to reduce the volume as performed before.
7. Add 50 ml volume of ammonium acetate, and allow the sample to cool to a moist residue.
8. Shake the sample, centrifuge and separate the extract as mentioned in step one.

(Davidson et al., 1994).

Appendix E. 14. Lillie's method for ferric and ferrous iron microscopic analysis

Fixation

Submerge sample with10% buffered neutral formalin

Technique

Cut paraffin section at 6 microns

Reagent

a. Potassium Ferrocyanide solution
- 0.4 g Potassium ferrocyanide ($K_4Fe(CN)_6.3H_2O$)
- 40 ml 0.06 N Hydrochloric acid

Add 1 ml concentrated HCl to 500 ml distilled water and make up to 200 ml with distilled water.

b. Potassium Ferricyanide solution
40 ml 0.6 N Hydrochloric acid

Note: Prepare all fresh solutions before use.

c. Basic Fuchsin solution
- 0.5 g Basic fuchsin
- 100 ml distilled water
-1 ml Glacial acetic acid

Procedure

Use control slide. Use chemically clean glassware.

1. Deparaffinise and hydrate sample with distilled water.
2. Place sample section for ferric iron analysis in potassium ferrocyanide solution for one hour. And place sample section for ferrous iron analysis in potassium ferricyanide solution for one hour.
3. Wash well in 1% aqueous glacial acetic acid.
4. Immerse sample with Basic fuchsin solution for 10 minutes and rinse it in distilled water.
5. Dehydrate sample in 95% alcohol, then absolute alcohol, then clear in xylene, change two times each.
6. Mount sample with permount or Histoclad.

Result

Ferric iron appears as a dark Prussian blue
Ferrous iron appears as a dark turnbull's blue
The background is light red
(Luna, 1968).

APPENDIX F. ABSTRACT SUBMITTED TO THE ASIAN CONFERENCE ON SUSTAINABILITY, ENERGY, AND THE ENVIRONMENT

Appendix F.1. The abstract submitted to the Asian Conference on Sustainability, Energy, and the Environment in Osaka, Japan (3 – 6 May, 2012).

Natural improvements of geochemical conditions of acid sulfate soils caused by free tidal inundation and its effects on the mangrove seedlings

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Abstract

Acid sulfate soils (ASS) are one of the stressor factors that cause many mangrove restoration projects to fail. Achieving successful rehabilitation in an ASS affected area requires an understanding of the geochemical conditions that influence the establishment and growth of mangrove seedlings.

Tidal inundation influences many physic and geochemical factors, and this condition are made even more complex by the oxidation of pyrite. This study evaluated the effect of tidal inundation on geochemical conditions in subsurface soils near roots and their impacts on the density, establishment, and growth of mangrove seedlings. This study is also to seek the answer the question: In which geochemical conditions can mangrove seedlings establish naturally, and/or be replanted in abandoned aquaculture ponds?

The study area was in abandoned ponds complex situated in the Mare District, adjacent to Bone Bay, South Sulawesi, Indonesia. The study used six replications of pH, pH_{fox}, redox potential, organic content, water-soluble sulfate, KCl extractable sulfur, peroxide oxididable sulfur, and grain size of subsurface soils near roots (10 - 15 cm) of soil cores were measured. Three replications of pyrite analysis were conducted for the surface and subsurface soils. The density, establishment and the growth of Rhizophoraceae were also determined.

Free tidal inundation at abandoned pond site improved the soil quality. High density, establishment, and growth of mangrove seedlings were characterized by freely drained areas with a higher pH (field and oxidisable), lower organic content, and high proportion of silt/clay. Higher density and growth also correlated to reduced environments. Sulfur species did not influence the density, establishment, and growth of the seedlings directly. A supply of propagules from the mangrove stands, or access to good waterways were also important for seedlings to establish naturally.

APPENDIX G. MAPS OF COLLECTION SITES FOR THE LABORATORY STUDY

Appendix G.1. Maps of sample collection sites for laboratory study

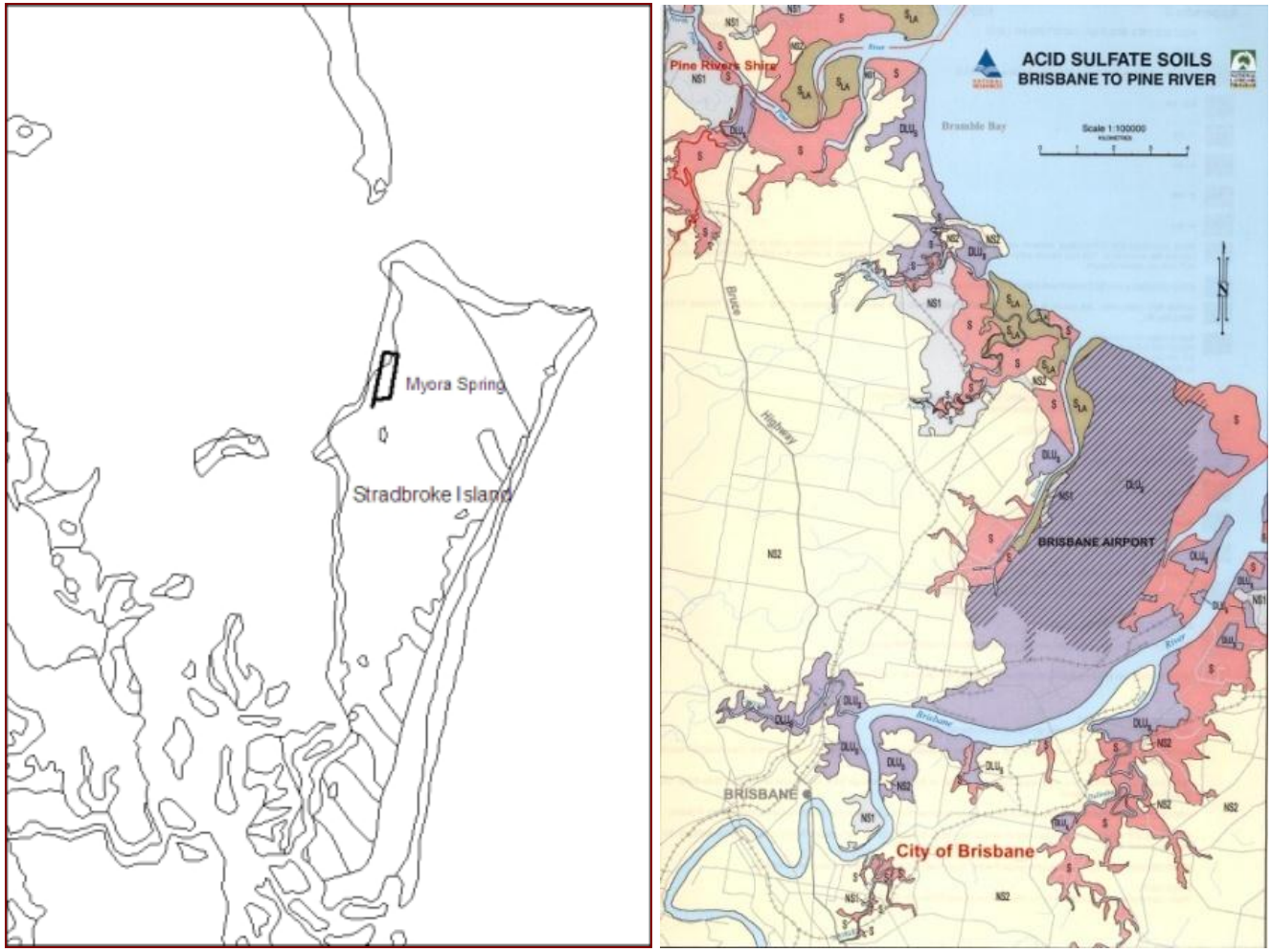

Figure G.1. Map of Myora Springs, Stradbroke Island, Queensland (left side), and Brighton, Queensland (right side)