ABSTRACT

EFFECT OF HEAVY METALS ON SALT MARSH BIOTA

by

Kallaya Suntornvongsagul

Environmental restoration of disturbed, degraded and potentially contaminated wetland sites aims for the persistent and sustainable re-establishment of biological communities and important wetland functions. An ecologically informed restoration strategy presupposes some understanding of these system processes, but also of potential impacts on contaminants in the environment for risk assessment, long-term management, and potential cost-effective mitigative measures. In this study, potential effects of moderate heavy metal contamination in sediments of the urban salt marsh Harrier Meadow, NJ, were evaluated on growth performance of the common salt marsh plant *S. patens* and associated arbuscular mycorrhizal fungi (AMF) colonizing its roots. Growth performance as well as uptake of heavy metals into roots and translocation from below- into above-ground parts was studied as a function of basic environmental conditions such as pH and redox potential, absence or presence of AMF, as well as additional contamination with nickel (Ni) at selected times during the first growing season, and after the third growing season.

The experimental setup in the greenhouse with soil cores containing *S. patens* from Harrier Meadow resulted in consistent environmental conditions in cores within and between treatments, with values for most physicochemical parameters being not significantly different at comparable depths. Concentrations and spectra of total

hydrolysable amino acids (THAA) in the organic material analyzed after the third growing season as measure for organic matter quality and thus metabolic processes were similar in cores of all treatments and thus indicated no significant biotic environmental differences that might have developed in time as a function of treatments. Measures for AMF were similar on plants from all treatments even though small, statistically significant differences were obtained for percent root length colonized, and arbuscular or vesicular colonization. Since none of the treatments eliminated AMF, these differences could not be related to treatment effects, but were suggested to be the result of potential shifts in AMF community structure, that, however, were not analyzed within the scope of the thesis.

Plant growth performance assessment based on leaf nitrogen (N) content, stable isotope ratios ($\delta^{13}C$), carboxylation efficiency (CE), CO_2 saturated photosynthetic capacity (A_{max}), estimations of optimal transfer efficiency of open photosynthesis II (PSII) reaction centers (F_v/F_m), sprout numbers, and shoot and root biomass generally showed seasonal variation in these characteristics only. However, additional variation was displayed for parameters such as A_{max}, F_v/F_m, and shoot and root biomass where significant effects of Ni amendment were found during "reproduction". These effects were no longer significant at the end of the growing season, and were not obtained when Ni amendment was combined with AMF suppression.

Sediment of Harrier Meadow was found to be moderately contaminated with heavy metals (Cd, Cr, Cu, Ni, Zn). Calculations using MineQL+, a data driven, chemical equilibrium modeling program suggested that, under suboxic and anoxic conditions found in all cores, only Cd, and under anoxic conditions, Ni would not form precipitates,

while Cd, Cr, Cu, Pb and Zn would mainly form precipitates. 70–95% of the total concentration of the dissolved forms of all heavy metals could also be adsorbed to organic compounds. None of the heavy metals nor the additional Ni application resulted in uptake or translocation values typical for hyperaccumulating plants, but rather values in the typical range found for many agricultural plants. Since all values in plant tissues were above the critical deficiency contents and below the critical toxicity contents, effects on plant growth performance should not be expected. Ni amendment did not increase uptake and translocation in most cases although effects of Ni amendment were displayed for root uptake of certain heavy metals (i.e., Cu, Pb, Zn and Fe) at the depth of 7.5 cm.

These results demonstrate that moderate heavy metal contamination found in sediments of Harrier Meadow did not affect growth performance of the common salt marsh plant *S. patens* and associated arbuscular mycorrhizal fungi (AMF) colonizing its roots during key points of the growing season. Although considerable uptake of all heavy metals into or adsorption to roots was encountered, translocation from below- into above-ground parts of *S. patens* was not significant, and always below regulatory action limits (maximum limit levels for vegetative leaves of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn are 0.2, 2.30, 73.3, 425.50, 500, 67.90, 0.3 and 99.40 $mg.kg^{-1}$). Thus, the risks associated with the restoration of moderately contaminated salt marshes with *S. patens*, i.e., the potential of *S. patens* to act as a conduit for the movement of toxic metals into the food web, are minimal and thus of low public health concern.

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by
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To Jengsun Suntornvongsagul and Dr. Suthirak Sujarittanonta

สองบุรุษผู้เป็นแบบอย่างในการดำเนินชีวิต...อย่างสง่างาม

พ่อสอนเราว่าคนเราต้องมีศักดิ์ศรี ขยันทำงานอาชีพสุจริต

และมีความกตัญญูรู้คุณคน จึงจะเจริญรุ่งเรือง

อาจารย์กล่าวว่าเขาจะไร้ศักดิ์ศรี หากเขาโกงเงินจากผู้อื่น

และเห็นว่าการให้เกียรติผู้ร่วมงานเป็นกลยุทธ์สำคัญ

เพื่อทำให้งานบรรลุผล แม้ว่าเงินจะเป็นฐานสำคัญก็ตาม

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LIST OF SYMBOLS

$\delta^{13}C$	discriminate carbon isotope
AC	arbuscular colonization
A_{max}	CO_2 saturated photosynthetic rate
AMF	Vesicular-arbuscular mycorrhizal fungi
As	arsenic
B	benomyl
Cd	cadmium
CE	carboxylation efficiency
Cr	chromium
Cu	copper
DET	diffusive equilibration in thin films
DOC	dissolved organic carbon
DPPDS	N,N-dimethyl-p-phenylenediamine sulfate
EPA	Environmental Protection Agency
FA	fulvic acids
Fe	iron
F_v/F_m	photochemical efficiency
HA	humic acids
HC	hyphal colonization
Hg	mercury
HPLC	high performance liquid chromatography

Mn	manganese
MTBE	methyl tertiary-butyl ether
Ni	nickel
NJDEP	New Jersey Department of Environmental Protection
NJMC	New Jersey Meadowlands Commission
OPA	*o*-phthaldialdehyde
PAHs	polyaromatic hydrocarbons
Pb	lead
PC	phytochelatins
PCA	photosynthetic carbon assimilation
PHCs	petroleum hydrocarbons
POC	particulate organic carbon
PSII	photosynthesis II
SOC	suspended organic carbon
TCE	trichloroethylene
THAA	total hydrolysable amino acids
TN	total nitrogen
TOC	total organic carbon
USEPA	United States Environmental Protection Agency
VC	vesicular colonization
Zn	zinc

CHAPTER 1

INTRODUCTION

1.1 Coastal Salt Marshes

Coastal salt marshes are among the most productive ecosystems in North America, and tidal exports from these systems are important sources of carbon (C) and energy for estuarine waters (Peterson & Howarth 1987, Currin et al. 1995). These marshes serve as an interface between terrestrial and aquatic ecosystems, and act as sulfate-sulfide and nitrogen (N) transformers, absorbing inorganic forms of N and exporting organic N. As such, salt marsh plant communities have a profound effect on estuarine productivity and on plant and animal community structure (Steever et al. 1976). The salt marsh vegetation is dominated by halophytic flowering plants, often by one or a few species of grass, perfectly adapted to the harsh environmental conditions that include high salinity and soil water saturation accompanying permanent or tidal inundation (Valiela & Teal 1974, Adam 1990). In addition to limited water availability and as a consequence of soil water saturation, salt marsh vegetation generally experiences hypoxia and a functional N deficiency (Valiela & Teal 1974, Chalmers 1979).

The vegetation of salt marshes can be divided into zones (i.e. into upper and lower marsh) generally depending on hydrology and salinity. Characteristic patterns found in the North American Atlantic coastal salt marshes include the salt tolerant tall form of cordgrass (*Spartina alterniflora* Loisel) dominating in the intertidal zone or the lower marsh. In the high marsh, the tall form of *S. alterniflora* is succeeded by its short form and by salt-meadow grass (*S. patens*) mixed with spike grass (*Distichlis spicata*) (Figure

1.1). Beyond this zone and at normal high tide, *Juncus geradi* forms pure stands. At the upper edge of the marsh that is inundated only by spring tides and thus characterized by lower salinity, *Panicum virgatum*, *Phragmites australis*, and others become more prominent (Mitsch & Gosselink 1993).

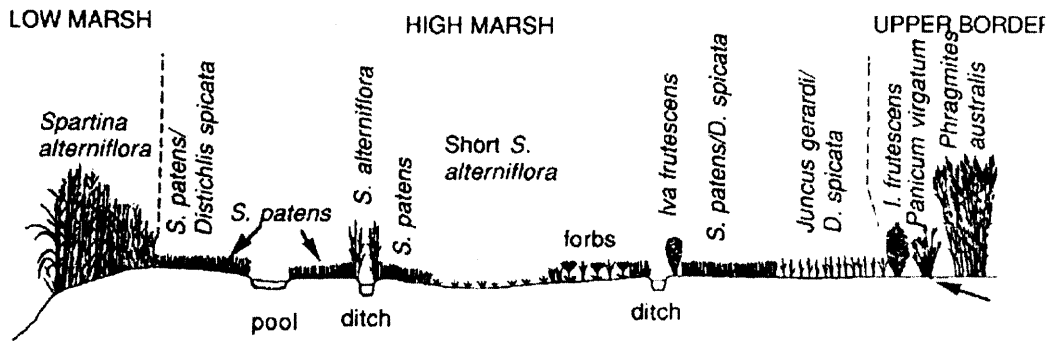

Figure 1.1 Zonation of vegetation in a typical North American Atlantic coastal salt marsh.

(Source: Mitsch & Gosselink 1993)

The development and the zonation of vegetation in salt marshes are influenced by sediment biogeochemistry mainly determined by soil water salinity linked with tidal flooding frequency, the availability of nutrients such as nitrogen (N) and phosphorus (P), and the degree of anaerobiosis, which determines the pathways of decomposition as well as nutrient availability. The salinity depends on many factors such as tidal flooding, runoff, drainage slopes, soil textures, vegetation, groundwater levels and fossil salt deposits (Mitsch & Gosselink 1993). Tidal flooding affects soil chemistry by creating and stabilizing an anoxic environment with low redox potential and high pH (Portnoy 1999). The salt marshes frequently inundated by the tidal flow are characterized by a high

salinity of up to 34 ppt with sodium chloride and sulfate as most abundant seawater constituents, and anoxic conditions in most of the sediments with sulfate- and iron-reduction as major processes (Luther & Church 1988).

Many salt marsh plants are adapted to these environmental conditions in a way similar to plants in other stressed environments; for example, by using the C_4 biochemical pathway of photosynthesis (Mitsch & Gosselink 1993). In this pathway, the first product of CO_2 incorporation is a four-carbon compound, oxaloacetic acid, formed by carboxylation of phosphoenolpyruvate. Phosphoenolpyruvate has a four-fold higher affinity for CO_2 than ribulose diphosphate, the CO_2 acceptor in the C_3 pathway. This and the fact that malate formed by the C_4 pathway can be stored and decarboxylated, and the released CO_2 fixed through the normal C_3 pathway, which essentially provides C_4 plants with a tool for CO_2 recycling from cell respiration, allows C_4 plants to fix CO_2 more efficiently than C_3 plants. This mechanism provides an adaptive advantage to C_4 plants in saline environments in which water availability is restricted similar to arid areas because the water potential is very low. Since stomata must be open for plants to take up CO_2, water loss would be excessive. The more efficient fixation and use of CO_2 by C_4 plants therefore allows them to grow faster and reduce water loss compared to C_3 plants.

The danger of high salt concentrations is osmotic stress that results in the fast dehydration of the cell cytoplasm, and the direct toxic effect of high concentration of, for example, Na^+ which is the major ion in seawater, inhibiting enzyme activity in cytoplasms. The adaptation mechanisms of plants include those on the cellular level such as maintenance of high osmotic concentrations in the cell, the active transport mechanisms that excrete ions across cell membranes or the steric configurations of

enzymes that enable them to become or stay active at high salt concentrations. On the whole plant level, barriers such as the endodermis of the roots as well as the periderm and exodermis prevent or control the entry of salts, which might also be restricted to third- and fourth-order roots. Salts entering the plants might be excreted through secretory organs, i.e. specialized salt glands embedded in the leaf. In *Spartina* sp., for example, the surface of the leaves is usually covered with crystalline salt particles, enriched in Na^+ relative to K^+ which was used to propose an active and selective excretion process (Figure 1.2). Salt exclusion and secretion protect the shoot and leaf cells of the plant from high concentrations of salt and maintain an optimum ionic balance between mono- and bivalent cations and between sodium and potassium (Mitsch & Gosselink 1993).

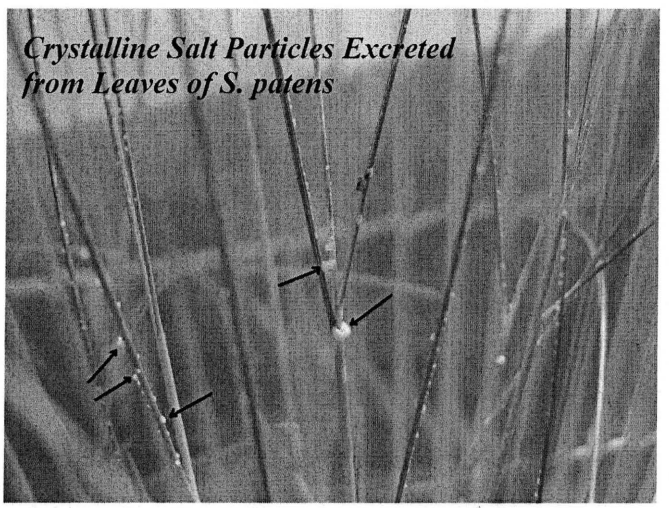

Figure 1.2 Crystalline salt particle excretions on the leaves of *S. patens*.

Hypoxia effects of the environment are countered by wetland plants through structural mechanisms that avoid root anoxia. Aerenchyma systems, i.e. air spaces in roots and stems, allow the diffusion of air from aerial parts of the plants into the root systems which makes them independent of potential O_2 diffusion from surrounding soil (Figure 1.3[1]). The presence of aerenchymal tissue, however, is often not sufficient for the effective transport of O_2 to the roots (Mitsch & Gosselink 1993). Under conditions of O_2 deprivation, plants respire anaerobically, with pyruvate being decarboxylated to acetaldehyde, which is then reduced to ethanol. Since both compounds are potentially toxic to plant tissue, plants such as *S. alterniflora* can increase alcohol dehydrogenase activity under anaerobic conditions, enhancing the reduction to ethanol. Ethanol does not accumulate, but diffuses into the environment. The additional mechanisms of detoxification include the formation of nontoxic organic acids such as malate instead of alcohol.

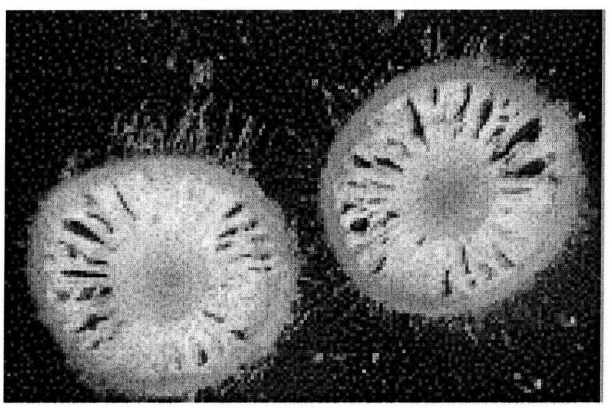

Figure 1.3 Dark openings in this cross section of eastern gamagrass root are air passages that form in aerenchyma tissue and enable plants to grow in flooded conditions.

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[1] **Source: www.sprrs.usda.gov**

One of the primary limitations to salt marsh productivity is a functional N deficiency (Valiela & Teal 1974, Chalmers 1979) brought about by soil saturation accompanying tidal inundation (Valiela & Teal 1974, Adam 1990). However, tidal waters also import N to the marsh surface, and are an important source of available N to marsh plant communities. The magnitude of the tidal N subsidy decreases with increasing distance from tidal creeks, and thus, the high salt marsh usually receives less tidally imported N than the low salt marsh (Chalmers 1979, Adam 1990, Bertness 1992). The N budget of high marsh plants; therefore, depends more on N recycled within the plant and N mineralized from soil organic matter than on tidal import (DeLaune et al. 1989, White & Howes 1994). Fertilizer amendment studies have shown improved plant growth following N-application, suggesting that this salt marsh vegetation is N-limited (Valiela & Teal 1974, Chalmers 1979).

For the N budget of high marsh systems N-fixation might be an important component since it may provide as much N to the system as tidal import (DeLaune et al. 1989, White & Howes 1994). Diazotrophic bacteria, however, have a high demand for P that can easily exceed the requirements of the plants with which they associate. Although salt marshes are not considered P-limited (Valiela & Teal 1974, Chalmers 1979, Valiela & Teal 1979, Adam 1990), a functional P limitation may exist for N-fixing bacteria, and this may underlie the apparent N limitation in these systems. Since salt marsh grasses do not form mutualistic relationships with symbiotic diazotrophs, N-fixation in salt marshes might be entirely due to free-living or root-associated diazotrophs provided with energy via root exudates (Patriquin & Keddy 1978, McClung et al. 1983, Whiting et al. 1986, Bagwell et al. 1998, Bagwell & Lovell 2000).

Synergistic relationships between plants and microorganisms are known to be an important mechanism influencing plant succession and tolerance of abiotic conditions in physically harsh environments such as salt marshes (Bertness 1992, Bertness & Callaway 1994, Callaway 1997). The prominence of such interactions for plant success grows with the severity of the abiotic environment (Walker & Chapin 1987). Mycorrhizal root networks are often encountered in plant communities of this type. Vesicular-arbuscular mycorrhizal fungi (AMF) are commonly found in most terrestrial and aquatic plant communities, and as many as two-thirds of extant plant families contain species that form some type of AMF association (Smith & Read 1997). These relationships are generally mutualistic and characterized by the bi-directional transfer of carbon and nutrients between fungus and plant host (Smith & Read 1997). The plant receives benefit principally from the increased soil absorptive strength of an extensive soil hyphal network, which either improves nutrient gain or facilitates water uptake as in the case of plants from arid or saline environments (Rozema et al. 1986, Allen 1991, Smith & Read 1997). AMF may be especially important in marginal or stressful environments (Walker & Chapin 1987) and can act as keystone mutualists by affecting the outcome of community interactions (Boucher 1985).

Many salt marsh plants contain AMF, especially in the more N-limited, infrequently flooded high marsh (Van Duin et al. 1989, Cooke & Lefor 1990), and the absence of AMF has been attributed to reduced vigor of important high marsh species such as *S. patens* (Cooke & Lefor 1990). Previous work has suggested that AMF improve water uptake in salt marsh plants (Rozema et al. 1986), and can increase early season growth and N acquisition possibly through reduced stomatal limitations to photosynthesis

(Burke et al. 2002b). High salt marsh plants such as *S. patens* and *D. spicata* commonly form associations with AMF within their roots (Figure 1.4) (Patriquin & Keddy 1978, Cooke et al. 1993). AMF are highly effective at acquiring and supplying limiting nutrients such as P and N from the soil to the host plant (Ames et al. 1983, Hayman 1986). In low salt marshes, where tidal N import is high, dominant species such as *S. alterniflora* are typically found without AMF (Van Duin et al. 1989, Hoefnagels et al. 1993). The greater competitive ability of *S. patens* over *S. alterniflora* in high marsh environments (Bertness 1991) may; therefore, depend in part on the ability of *S. patens* to form associations with AMF to increase N acquisition when tidal N subsidy is low.

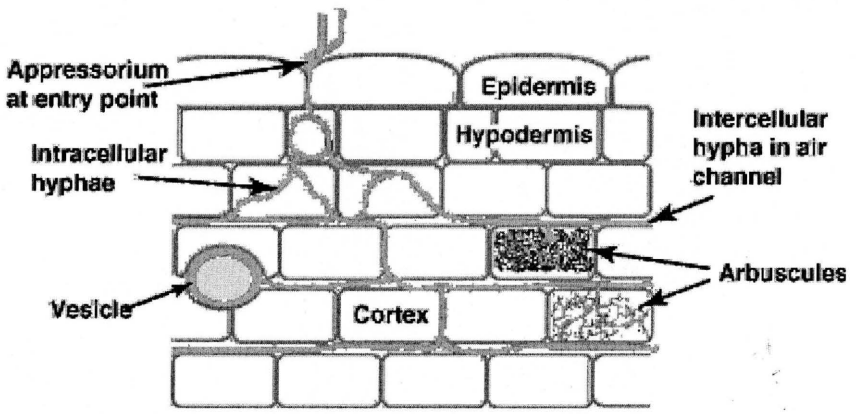

Figure 1.4 Schematic presentation of key structures of vesicular-arbuscular mycorrhizae in plant roots.

(Source: Mark Brundrett, CSIRO Forestry and Forest Products, (http://www.ffp.csiro.au/research/mycorrhiza/vam.html 2004))

AMF are known to alter root architecture (Hetrick et al. 1991) and reduce the production of extra-cellular mucilage around plant roots (Ling-lee et al. 1977) which can affect root sheath development in grasses (Peterson 1992). They can also alter both the distribution and quality of carbon (C) excreted into the environment by plant roots

(Giddens & Todd 1984, Reid 1984). Since C availability is limited in salt marsh sediments (Moriarty et al. 1986, Whiting et al. 1986) and AMF act as C sinks within the plant-fungal system (Hodge 2000), their presence could affect C availability in both the rhizosphere as well as in surrounding bulk soil. Specific rhizosphere microbial populations could be affected by changes in root exudation engendered by AMF colonization (Linderman 1988), as could bulk soil microbial populations by the reacquisition of excreted substances by the extra-radical mycelium (Sun et al. 1999). The presence of AMF could therefore intensify microbial competition leading to smaller microbial populations within the rhizosphere. This assumption is supported by studies that showed that the presence of certain AMF belonging to the genus *Glomus* reduced the biomass of N_2-fixing microorganisms, caused by the competition of root symbionts for C-containing plant exudates (Hamel et al. 1991, Paul & Clark 1996).

The interception of root exudates at the source by AMF might be analogous to flowering onset, where plant source-sink relationships change, resulting in less C transport to root tissue and reduced root exudation (Reekie & Bazzaz 1987). Phenological changes in *S. alterniflora* have been linked to reductions in rhizosphere populations of sulfate-reducing bacteria, the consequence of reduced C flow to roots during flowering onset (Hines et al. 1989, Rooney-Varga et al. 1997, Hines et al. 1999). The reduction in excretable C that accompanies flowering may be analogous to AMF colonization, with the effect of both processes changing the populations of soil microorganisms. These examples demonstrate that plant growth performance under natural conditions in salt marshes is a function of complex interactions between physicochemical parameters of the soil and the synergistic relationships among plants and microorganisms. Changes in the

populations of functional groups such as N_2-fixing bacteria and AMF, could impact plant resource acquisition, plant nutrient status and performance. The disturbance of these natural sites might therefore impact a complex system by affecting single components (plants, AMF, or bacteria), and subsequently their interaction which will reduce the stability of the system and eventually result in habitat degradation or even destruction.

1.2 Coastal Salt Marsh Degradation and Destruction

Anthropogenic activities can produce and add further environmental stresses to the already harsh environmental conditions that plant communities naturally experience in salt marshes. Initial human impacts on salt marshes were based on the historical misconception that regarded them as wastelands. This misconception led to the destruction of more than half of the total salt marshes in the lower 48 states of the U.S.A. Most of them were converted for agricultural use (Mitsch & Gosselink 1993). The alterations that generally resulted in the destruction or the degradation of the salt marshes were water drainage, soil dredging and land filling, highway construction, mining and mineral extraction, and water pollution (Mitsch & Gosselink 1993). In coastal salt marshes, for example, engineering projects that included diking, drainage, and land filling restricted seawater flow to the marshes and resulted in the reduction of tidal range and salinity, and eventually altered sediment chemistry and bulk composition (Portnoy 1999). This in turn had consequences for the pattern of plant zonation especially that of salt marsh plant communities that were dominated by salt-tolerant *S. alterniflora* and *S. patens,* but then replaced with less salt-tolerant and fast-growing *P. australis* (Dame 1989).

In addition to their deliberate destruction by human activities, salt marshes were and still are disturbed, degraded or destructed more or less indirectly. In densely populated areas, urban settings and current or past industrial sites are generally found in or adjacent to natural habitats (Figure 1.5) (Mason & Sullivan 1998, Nedeau et al. 2003).

Figure 1.5 Harrier Meadow with New York City in close proximity.

Urban settings are complex entities characterized by intense human activity that generate multiple waste streams. Some streams are diffuse but ubiquitous (e.g. heat, noise), while others are diffuse but periodic (e.g. surface runoff, rush hour smog), concentrated and periodic (e.g. trash collection, sewage flow, industrial effluents), or intense and episodic (e.g. combined storm-sewer overflow, park use on sunny weekends). Additional waste streams are generated by a large variety of industries that are located in the urban agglomeration. Consequently, sediments in waterfront sites and salt marshes have often intercepted these waste streams and become repositories of accumulated contamination that affect plant and animal communities.

In New York-New Jersey estuaries, the widespread use of petroleum as heating and motor fuel, for example, has contributed to the presence of petroleum hydrocarbons (PHCs) and polyaromatic hydrocarbons (PAHs) in stream and wetland sediments, where it has been transported via storm drainage from roadways and industrial areas. Methyl tertiary-butyl ether (MTBE) has recently become an issue of groundwater contamination, while chlorinated solvents such as trichloroethylene (TCE) remain a major issue at specific sites. In New Jersey, lead (Pb), cadmium (Cd), and arsenic (As) in groundwater threaten drinking water, to the point that over 50% of the groundwater is considered potentially contaminated. Many aboveground areas in the Northeast of the US are contaminated with moderate to elevated concentrations of heavy metals such as mercury (Hg), chromium (Cr), copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn). One example is Berry's Creek, an estuary that is part of the larger New York/New Jersey Harbor Estuary where mercury (Hg) concentrations as high as 89,000 ppm have been found at an effluent discharge point and as high as 8,500 ppm in the sediments downstream of the discharge point of a Hg processing facility even though Hg processing ceased in 1974 (Jack McCormick and Association 1977). Because of its Hg contamination, Berry's Creek cannot be developed and provides no economic benefits to the community.

Contamination with organic or inorganic compounds creates profound effects on ecosystem functions. For example, slightly increased N availability in high salt marsh areas by agricultural or urban emissions might facilitate the success of invasive plant species such as *P. australis*, a highly competitive C_3 grass, that is capable of exploiting enhanced nutrient resources (Amsberry et al. 2000) and result in the displacement of native plant species like *S. patens* (Roman et al. 1984). The effect of increased N levels

might be mediated by impacts on microbial populations associated with *S. patens* such as AMF and associated bacteria. Although increased N availability (though N-fixation might be reduced concomitantly) is not expected to affect the vigor of *S. patens* in salt marshes, but rather to increase the competitive ability of the invasive plant species *P. australis*, indirect effects might occur from the greater availability of N. Enhanced N availability was found to result in shifts of AMF community composition (Egerton-Warburton & Allen 2000), which could have large impacts on AMF colonization and the effectiveness of plant-AMF associations (Francis & Read 1994, van der Heijden et al. 1998). The reduction of these fungi could decrease water or P uptake, and thus result in lower productivity and growth of *S. patens*, which suggested an increase in the success of *P. australis* (Levine et al. 1998, Amsberry et al. 2000). AMF composition, however, was also found to change seasonally (Daniell et al. 2001), in addition to the changes of their colonization (Daniell et al. 2001, Burke et al. 2003). Changes in populations of functional groups such as N_2-fixing bacteria, or of AMF could therefore impact plant resource acquisition, plant nutrient status and performance.

Heavy metals have large effects on processes important for maintaining fertility and the primary production of soils (Giller et al. 1998). Their accumulation leads to the reduction of total microbial biomass (Fliessbach et al. 1994), to a decrease in numbers of specific populations such as infecting rhizobia (Chaudri et al. 1993) or mycorrhizae (Koomen et al. 1990), or to shifts in microbial community structure and a reduction in total microbial diversity (Griffiths et al. 1997). Key functions in soil processes such as N mineralization and immobilization, mineralization of organic material, and N_2-fixation are inhibited even by metal concentrations below regulatory action levels (Chaudri et al.

1993). Many salt marsh plant species have been shown to take up heavy metals with similar uptake patterns that result in concentrating metals primarily in roots (Weis & Weis 2004). However, there is a high degree of variability between metals and between various plant species (Fitzgerald et al. 2003). In general, dicotyledonous species tend to have similar metal concentrations in shoots and roots, while monocotyledonous species have higher concentrations in roots (Otte et al. 1991). Other factors affecting the uptake of heavy metals include the variation of plant species, age and growth stages, the change of environmental factors such as the seasonal variations, the presence of iron plaques on the roots, redox potential and salinity, the level of metal contamination, and the properties of soil. Metal transport is either passive, only driven by the concentration gradient across the membrane, or inducible substrate-specific and energy-dependent (Nies 1999, Schuetzenduebel & Polle 2002).

Basic abiotic environmental conditions such as the mineralogy of soil and sediment particles, salinity, pH, organic matter content, tidal inundation and thus aeration form the framework of environmental determinants underlying the fate of heavy metals in estuarine environments. This fate may be directly affected by the biota, but also indirectly by their ability to change environmental conditions. Environmental conditions essentially determine the mobility of heavy metals, which is a function of complexation and sorption interactions between the aqueous, biological, and various mineral phases in contact with that aqueous phase. Thus, the bioavailability of metals to plants and their resulting toxicity depend on complex interactions between metals and soil/sediments, plants, and the microbial community.

Based on their different chemical and physical properties, heavy metal toxicity in plants can be due to a) the production of reactive oxygen species, a reaction typical for iron (Fe) and copper (Cu), b) the blocking of essential functional groups in biomolecules, a reaction typical for non-redox-active metals such as cadmium (Cd) and mercury (Hg), and c) the displacement of essential metal ions from biomolecules as found with different metals (Schuetzenduebel & Polle 2002). The increased uptake of heavy metals can result in the reduction of plant biomass, which is often coincident with the decrease of plant nutrient uptake (Jones & Hutchinson 1988, Díaz et al. 1996). For instance, elevated levels of Al in plants can interfere with cell wall extensibility and mitosis (Marschner 1995), and elevated levels of Ni may reduce the concentrations of photosynthetic pigments such as chlorophyll a and b (Jones & Hutchinson 1988), and inhibit activity of phosphoenolpyruvate (Morgutti et al. 1984), a key enzyme in the photosynthetic carbon assimilation (PCA) cycle of C_4 plants such as *S. patens*. Most studies of direct physiological effects of metals on plants have been made *in vitro*, which can amplify direct effects of metals due to the lack of cellular isolation or biochemical complexation mechanisms (van Assche & Clijsters 1985). Recent studies show that *in vivo* effects of metals are modulated and rapidly attenuated by antioxidative defences, phytochelatins (PC) and cellular compartmentalization (Rauser 1995, Haag-Kewer et al. 1999, Schuetzenduebel & Polle 2002).

Both the toxic effects of heavy metals and negative effects on nutrient availability for plants may be ameliorated by mycorrhizal symbionts (Schuetzenduebel & Polle 2002). AMF have been shown to improve plant tolerance to heavy metal stress in polluted environments and to bind heavy metals (Weissenhorn et al. 1995a, Joner &

Leyval 1997, Tonin et al. 2001). They may increase heavy metal sequestration in root tissue and greatly reduce translocation to leaf and stem, possibly by increasing the root endodermis barrier to metal uptake. They probably also assist plants in overcoming nutrient deficiency associated with increased metal concentrations, either through increases in P (Jones & Hutchinson 1988) or Fe (Caris et al. 1998) uptake which could help to overcome the negative effects of, for example, Ni on photosynthetic pigments (Jones & Hutchinson 1988). Thus, previously documented reductions in plant growth may have been demonstrating indirect effects of below-ground processes rather than direct effects on plant physiological performance (Haag-Kewer et al. 1999). A better understanding of the effective protection of salt marsh plants like *S. patens* from damages by the toxicity of heavy metals is needed for the proper management of salt marsh systems and restoration efforts aimed at restoring marshes in urban environments.

1.3 Salt Marsh Restoration

Since wetlands including salt marshes and surface waters are identified as environmentally sensitive areas by federal and state governmental agencies like the United States Environmental Protection Agency (USEPA) or the New Jersey Department of Environmental Protection (NJDEP), and are also recognized as important links to complex wildlife food webs, that involve endangered species and commercially important marine fisheries, state and federal laws and regulations require replacement of wetlands lost in development such as highway construction, coastal drainage and filling, or commercial development (Mitsch & Gosselink 1993). Contaminants such as hydrocarbon substances and heavy metals in these areas must be cleaned up or

remediated. Therefore, and since destruction and degradation of wetlands has tremendous impacts on their quality as habitat for wildlife, e.g., as wildlife sanctuary or as living and breeding ground for aquatic life (Boesch & Turnar 1984, Rozas et al. 1988, Kneib 1997), as well as for human life, e.g. as sites maintaining water quality or controlling water drainage and soil erosion (Whitaker & Terrell 1992), many efforts are currently under way to restore these sensitive ecosystems (Broome et al. 1988, Whitaker & Terrell 1992). The restoration includes the improvement of hydrologic conditions, the creation of specific habitats, the control of invasive plant species, and the reintroduction of indigenous marsh vegetation (Bijlmakers & de Swart 1995, Teal & Weinstein 2002). Habitat enhancement activities in the salt marshes may therefore include the eradication of invasive organisms such as the invasive and highly competitive plant species, *P. australis*, the replanting of native plant species such as *Spartina* sp., and the restoration of the tidal flow (Bijlmakers & de Swart 1995, Teal & Weinstein 2002).

Since ecological restoration projects aim for the robust re-establishment of biological and ecological communities, especially with respect to their persistence and sustainability, an ecologically informed restoration strategy presupposes some understanding of system processes that determine the re-establishment of important wetland functions (Portnoy 1999). Although restoration efforts have focused primarily on vegetation over the past decades, with little attention to sediment biogeochemistry, re-establishment of site-specific hydrology and thus of related biogeochemical determinants in the sediment is of considerable, if not major, interest in sustainable restoration activities (Sinicrope et al. 1990). Restoring tidal flow to salt marshes increases the tidal range, inundation and salinity, and can be expected to change sediment chemistry from

oxic environments with high redox potential and low pH to anoxic environments with low redox potential and higher pH (Portnoy 1999). Salt marshes frequently inundated by tidal flow, are characterized by high salinity of up to 34 ppt (parts per thousand) with sodium chloride and sulfate as most abundant seawater constituents, and anoxic conditions in most of the sediments with sulfate- and iron-reduction as major processes (Luther & Church 1988).

Restoration projects that change the environmental conditions may result in the alteration of contaminant mobility and bio-availability. The mobility and the bio-availability of heavy metals, for example, depends on their speciation that is determined by various physicochemical and biological parameters such as the nature of the soil and sediment particles, the amount of organic matter, the pH, salinity, redox potential of the sediment, and biological parameters such as the vegetation and the presence and the activity of microorganisms (Bryan & Langston 1992, Gambrell 1994, Doyle & Otte 1997, Carbonell-Barrachina et al. 1998). The fate of heavy metals in the environment may be directly affected by the biota (e.g. by plant uptake) (Burke et al. 2000, Weis & Weis 2004), and probably indirectly affected by their ability to change environmental conditions (e.g. by the release of protons or oxygen, or by iron- or sulfate reduction) (Gambrell et al. 1991, Otte et al. 1995, Doyle & Otte 1997, Wright & Otte 1999). Abiotic and biotic determinants are generally interacting and therefore provide environments with variable or changing conditions in time and space. The bioavailability of metals is therefore the consequence of complex interactions between metals and soil/sediments, plants, and the microbial community. Although estuarine sediments are generally considered a sink for metals due to the reducing conditions (Fitzgerald et al. 2003, Weis

& Weis 2004), the effects of changes in the biogeochemical characteristics of soil on the metal complexation and adsorption in the environment must be determined and well clarified for potential risks, and sustainable management with proper technology tools (e.g. remediation approaches).

1.4 Salt Marshes of the Hackensack Meadowlands District

The urban salt marshes of the Hackensack Meadowlands District in NJ, which is the part of the larger New York/New Jersey Harbor Estuary (Figure 1.6), provide examples for impacted and partially or fully degraded environments as well as for moderately to heavily contaminated areas with several EPA superfund sites (e.g. Berry's Creek, Kearny Marsh). Within the Hackensack Meadowlands District, the New Jersey Meadowlands Commission (NJMC) is responsible for land use planning, the implementation of zoning controls, subdivision and site plan reviews, regional solid waste management, and the protection of the environment.

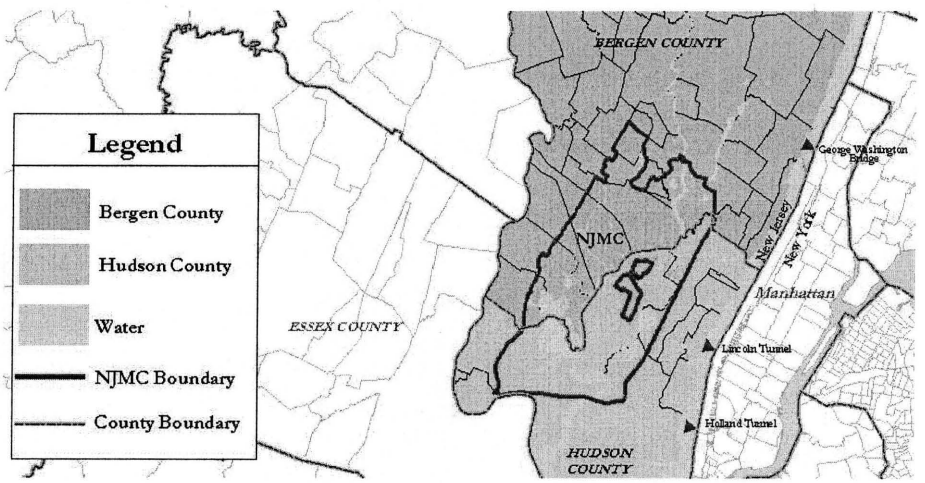

Figure 1.6 Boundary and location of the Hackensack Meadowlands District, New Jersey.

From the original thousands of acres of wetlands in the area that is now the District only 32 square miles are still considered undisturbed or intact wetlands; these remnant and unique habitats that have been identified by the EPA in the 1989 "Functional Assessment of Wetlands in New Jersey's Hackensack Meadowlands", include forested wetlands found near Teterboro Airport and Losen Slote (approximately 165 acres), freshwater meadows near Losen Slote and Moonachie Creek, Kingsland Marsh, and Kearny Marsh (approximately 605 acres), rock outcropping with hardwood forest at Snake Hill (Laurel Hill, or Fraternity Rock) and Little Snake Hill (approximately 35 acres), and high salt marsh (dominated by *S. patens* and/or *D. spicata*) found near the Hackensack River in two locations: (1) the west of the Hackensack River, the south of Berry's Creek, and the east of the NJ Turnpike's western spur; and (2) the east and north of the Hackensack River, and the south of NJ Transit's Boonton Line (approximately 175 acres).

The remaining wetlands of the Meadowlands District have been destroyed or degraded by human activities such as excavating, dredging, draining, mosquito control, land filling, or industrial pollution. Destruction and degradation are the direct consequences of human activities with the aim to change environmental conditions for their purposes. Dikes were built to control flooding of tidal wetlands, the transitional zone between upland and marine environments, or swamps and wetlands used as landfills. In addition to industrial or household waste, the landfills received dredged sediments removed from the New York/New Jersey Harbor. About 5 million cubic yards of sediments needs to be dredged annually to keep the port economically active. About 60% of this dredged material, however, does not meet ocean disposal standards

(Environmental Protection Agency, EPA) because it is heavily contaminated with metals, petroleum hydrocarbons, PAHs and PCBs, and thus has to be treated or placed upland in the landfills.

In the year 1969, about 2,500 acres in the Meadowlands District were used as official sites for landfills. Around 500 acres of them were still active in 1987, until these landfills finally reached their capacity and stopped accepting waste. Today, only one landfill (ERIE Landfill) with a size around 20 acres located in Kearny and North Arlington remains active. In addition to these landfills regulated by the New Jersey Meadowlands Commission (NJMC), hundreds of acres were used as landfills that were not controlled by NJMC. These landfills ceased operations in 1970's and were usually abandoned by their operators without escrow funds for the proper post closure of the landfills. These landfills, therefore, remain contamination threats to nearby areas, especially the wetlands and the aquatic resources in the Hackensack estuary. Similar threats exist from many sites that, in addition to the official landfill sites in the Hackensack Meadowlands, were used as unregulated disposal sites for solid and industrial waste for more than 150 years; from these sites pollutants continue to leach into the watercourses (Fox & York 1995). Point source pollution tends to come from activities such as sewage treatment plants and industrial discharges while non-point sources of pollution cannot be pinpointed to the initial discharging sources including storm sewers, landfills, leachate, and surface runoff. As a result of the existing point and non-point sources, soil and water quality in these areas has been and still is significantly impacted. A characteristic that makes the wetlands of the Meadowlands District especially

susceptible to these impacts is that the lower Hackensack River is not as well flushed as other estuaries, due to its limited freshwater inflow and indirect link with the open sea.

1.5 Salt Marsh Restoration of the Hackensack Meadowlands District

The activities of NJMC in the Hackensack Meadowlands District focus on twelve wetlands sites that are in various phases of development at the moment (Figure 1.7).

Wetland restoration as proposed and implemented by NJMC in the Hackensack Meadowlands District involves five basic steps:

- Baseline studies - data collection to evaluate existing conditions, and to determine the best enhancement design for the site.
- Planning and specifications - development of the overall plan for the site development based on the results of the baseline studies.
- Permitting and jurisdictional agency approvals - securing all authorizations required prior to the implementation of any enhancement plan.
- Construction - implementation of the design plans.
- Monitoring and maintenance - follow-up work required to ensure that the goals of the enhancement plan have been met.

Restoration work still in the planning phase deals with Berry's Creek Marsh, while baseline studies are performed for Eastern Brackish Marsh, Oritani Marsh, Riverbend Wetland Preserve, and Secaucus High School Marsh. Awaiting baseline studies are Anderson Creek Marsh, Kearny Freshwater Marsh, and Kearny Brackish Marsh. Nearly completed sites include Skeetkill Creek and Mill Creek Marsh, and Harrier Meadow (www.meadowlands.state.nj.us/eip/wetlands.html).

Figure 1.7 Map of the Hackensack Meadowlands District, highlighting current restorations sites managed by NJMC.

NJMCs current wetland restoration work in the Meadowlands involves mitigation and cleanup of moderately contaminated salt marshes such as Harrier Meadow (North Arlington). Adjacent to Harrier Meadow, just north of the site, is ERIE Landfill, the only active landfill in the Meadowlands District that receives non-hazardous waste, including bulky wastes, construction and demolition wastes, vegetative wastes, and non-hazardous industrial waste (Figure 1.8) (http://www.meadowlands.state.nj.us/eip/wl-harrier.html).

Harrier Meadow was used as disposal site for shot rock from the construction of U.S Route 280 in the past. At the Harrier Meadow salt marsh mitigation site, construction work was completed during September 1998. At the moment, portions of the area support

a mixture of native high salt marsh vegetation, largely dominated by C_4 grasses such as salt-meadow grass (*S. patens*), cordgrass (*S. alterniflora*), or spike grass (*D. spicata*), common salt marsh plants native to the northeastern US that form different vegetation zones depending on the environmental conditions such as salinity, soil anoxia and N availability (Bertness 1991, Levine et al. 1998). Other areas consist of dense monocultures of invasive C_3 species, common reed (*P. australis*) and purple loosestrife (*Lythrum salicaria*), that are rapidly replacing native wetland species throughout the northeastern US (Figure 1.8).

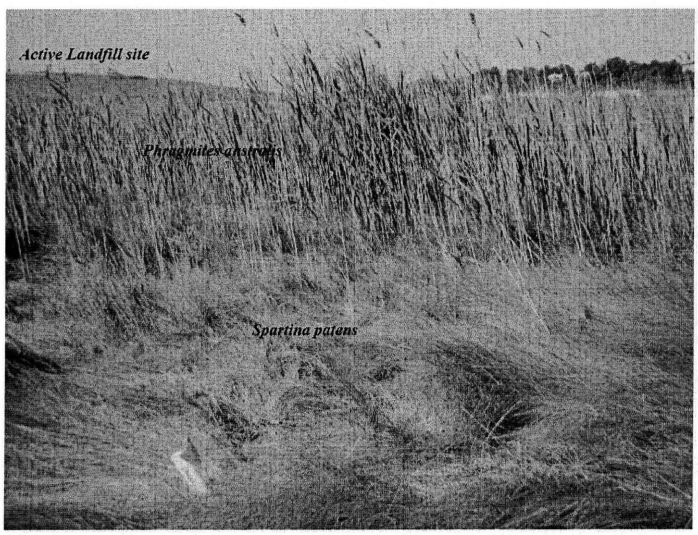

Figure 1.8 Sampling site in Harrier Meadow with indigenous *S. patens* and invasive *P. australis*.

Sediments of Harrier Meadow are moderately contaminated with heavy metals at concentrations close to NJ regulatory action levels (25 ppm Ni, 45 ppm Cr, 3 ppm Cd, 72 ppm Cu, 270 ppm Pb, 670 ppm Zn). *S. alterniflora* and *P. australis* have been shown to accumulate heavy metals, though at different rates with differing modes of uptake (Carbonell-Barrachina et al. 1998, Burke et al. 2000, Weis et al. 2002). Similar to *S.*

patens, they are also known to change environmental conditions in bulk soil and rhizosphere directly through oxygen translocation via their aerenchyma system (Sundby et al. 1998), or indirectly by the stimulation of microbial activities by releasing chelating root exudates, or salts and organic material through leaf litter deposition (Burke et al. 2000, Kraus, 1988, Kraus et al. 1986) which in turn could change environmental conditions.

The activity of microorganisms in salt marshes, however, also depends on interrelationships with other organisms. In contrast to *S. alterniflora* and *P. australis*, *S. patens* is usually found to harbor the AMF (Van Duin et al. 1989, Hoefnagels et al. 1993) that are effective at acquiring and supplying N and P, as well as trace elements from the sediment to the host plant (Hayman 1986, Davies et al. 2001, Ryan & Angus 2003). AMF have been shown to improve plant tolerance to heavy metal stress in polluted environments and to bind heavy metals (Weissenhorn et al. 1995a, Joner & Leyval 1997, Tonin et al. 2001). However, in highly contaminated environments, the presence of AMF might result in the opposite effect, i.e. a decrease in plant biomass due to increased metal toxicity (Kilham & Firestone 1983, Pawlowska et al. 1996). Heavy metals might also impact AMF colonization and diversity. However, few and variable results exist on the significance of AMF in soils contaminated with heavy metals (Weissenhorn et al. 1995b). Reduction in AMF could result in the lower productivity and growth of native salt marsh grasses, a situation found to increase the success of invasive plant species such as *P. australis* (Levine et al. 1998, Amsberry et al. 2000). However, the maintenance of plant biodiversity in many environments is not only dependent on the overall colonization of roots by AMF, but is also affected by the diversity of AMF (van der Heijden et al. 1998).

Reductions in AMF and thus plant growth performance could have broad implications for ecosystem functioning in general and the cycling of nutrients and energy in salt marsh ecosystems in particular because the functioning and the stability of terrestrial ecosystems are basically determined by plant biodiversity. However, contamination poses not only a threat to plants and animals in the environment, but also to human health through groundwater infiltration and exposure through the food web (see (Galloway et al. 1982, Giller et al. 1998) for review). Since environmental conditions essentially determine the mobility and bioavailability of heavy metals, factors affecting or altering metal speciation must be fundamentally investigated and understood for risk assessment and long-term management of the restoration site.

1.6 Aim of This Study

Environmental restoration and management of contaminated urban settings, of urban salt marshes as well as of shore-front properties are major problems facing states and industries. Although ecological restoration can generally be viewed as positive, its impact on inorganic and organic contaminants in the environment must be understood for risk assessment, long-term management, and potential cost-effective mitigative measures. The risks associated with accumulated contamination are especially pronounced if pollutants are recalcitrant to degradation or persistent and bioaccumulative. Based on economic considerations as well as public health concerns, these types of sites are difficult to be developed or redeveloped. Under such circumstances, restoration with e.g. *Spartina* sp. might be viewed as a cost-effective phytoremediation strategy if heavy metals can be concentrated and removed in leaves, or biodegradation of organic contaminants can be

enhanced. Since bioavailability of heavy metals depends on complex interactions of abiotic and biotic factors in salt marsh sediments and might be enhanced in the presence of plants that take up and translocate these contaminants, the risks associated with the restoration of such contaminated sites must be assessed, especially their interaction with plants, that have the potential to act as a conduit for the movement of toxic metals into the food web.

The aim of this study was to evaluate the potential effects of heavy metal contamination in the sediments of Harrier Meadow on the growth performance of the common salt marsh plant *S. patens* and associated AMF colonizing its roots. These studies were performed on field-collected sediment cores with *S. patens*. Half of these cores were artificially amended with nickel (Ni) in order to increase its availability. Since the availability of root exudates varies during the growing season and corresponds to major plant phenological stages previously linked to changes in microbial populations (Moriarty et al. 1986, Welsh et al. 1996, Rooney-Varga et al. 1997), these studies were performed during key points of the growing season coinciding with major plant phenological stages, i.e. vegetative growth, reproduction, senescence. In the beginning of the growing season at the end of April, largest root exudation is correlated to fastest plant growth (further referred to as "vegetative growth"), while reduced exudation correlated to flowering has been found in the middle of June ("reproduction"). At the end of the growing season in September, no exudates are available due to plant senescence ("senescence").

Since contamination might not only affect microorganisms and plants in the environment (Carbonell-Barrachina et al. 1998), but also poses a threat to animals and

finally human health through exposure through the food web (see (Gambrell 1994, Giller et al. 1998) for review), heavy metal uptake into the roots of *S. patens* and translocation from below- into above-ground parts was evaluated at the same time as an indication for the potential risks of release through the food web or through leaching, excretion or mineralization (Weis & Weis 2004).

Since AMF have been shown to affect plant growth performance in heavy metal contaminated sites, either improving plant tolerance to heavy metal stress (Weissenhorn et al. 1995a, Joner & Leyval 1997, Davies et al. 2001, Tonin et al. 2001), or resulting in the opposite effect by decreasing plant biomass due to increased metal toxicity (Kilham & Firestone 1983, Pawlowska et al. 1996), studies included comparative analyses with samples treated with the systemic fungicide benomyl in an attempt to create AMF-free plants (Kahiluoto et al. 2000, Burke et al. 2003). In order to exclude as many variables as possible from the system under study, sediment cores densely covered with *S. patens* were kept in the greenhouse under standardized conditions over the growing season (Burke et al. 2003).

The study included two time frames: an analysis coinciding with different plant phenological stages during the first year (further referred to as "short-term experiment") using small core samples from the sediment cores, and a final destructive analysis of all cores after the third growing season at "senescence" ("long-term experiment").

While the "short-term experiment" was aimed at investigating potential direct (and thus short term) effects of heavy metals on plant growth performance and microbial interactions at different plant phenological stages, the "long-term experiment" was meant to investigate potential acclimation of plants and microorganisms to the environmental

conditions that were supposed to be a function of the level of heavy contamination, soil/sediment characteristics and the associated organisms (Giller et al. 1998, Kelly et al. 2003). Analyses of total hydrolysable amino acids (THAA) as well as of specific amino acid composition at the end of the long-term experiment were finally used as an indication of changes in environmental quality, i.e., the degradation state of the organic matter (Dauwe & Middelburg 1998), due to the long-term application of Ni, benomyl or benomyl and Ni onto the cores, compared to non-treated control cores.

CHAPTER 2

MATERIAL AND METHODS

2.1 Experimental Setup

Soil cores covered with *S. patens* plants (13 x 13 x 13 cm, n = 20) weighing approximately 2 kg fresh weight, were collected from Harrier Meadow at the end of February similar to a previous study (Burke et al. 2002a). Cores were transported back to a greenhouse at Rutgers University-Newark, fitted into plastic pots and placed in individual pails containing 5 ppt artificial seawater (Instant Ocean® Mentor, OH, USA) amended with 5 ppm NH_4^+ and PO_4^{3-}, respectively. Pails containing potted cores were arranged randomly on a greenhouse bench and artificial seawater was added to each pail to adjust the standing seawater which was finally present at 5 cm below the top of the core (Figure 2.1). This correlated to the level of water observed in holes remaining in the field after core removal. This level was roughly maintained during the experiment through daily adjustments with amended 5 ppt artificial seawater. The water in the pails was completely exchanged once a week to maintain this salinity. This setup generates large redox gradients between surface and bottom of the cores, in a similar range as found under natural conditions in the field (Burke & Hahn 2000, Burke et al. 2002b). After a 1-week acclimation period, the concentration of Ni was increased in half of the cores (n=10) through amendments with $NiSO_4$ (50 mg kg^{-1} core material) dissolved in water and poured onto the surface of the core. The second half of the cores received Na_2SO_4 (50 mg kg^{-1} core material) dissolved in water, and served as controls for heavy metal contaminated cores.

Half of the Ni- and Na_2SO_4-amended cores received an additional treatment with the systemic fungicide benomyl (50% WP; Bonide Products, Inc. Yorkville, NY, USA; 0.2 g per kg of soil dissolved in 100 ml water and applied to the core surface) to suppress AMF colonization (Wilson et al. 2001).

Thus, the experimental setup consisted of four treatments:

- Five field cores amended with Na_2SO_4, that served as "control treatment"
- Five cores amended with $NiSO_4$, referred to as "Ni treatment"
- Five cores amended Na_2SO_4 and the systemic fungicide benomyl, referred to as "benomyl treatment", and
- Five cores amended with both $NiSO_4$ and benomyl, referred to as "Ni/benomyl treatment".

Figure 2.1 Experimental setup with sediment cores and *S. patens* in the green house.

The pots of all treatments were randomly placed on a bench in the greenhouse and moved once a week during the growth period for 6 months (March to September) under ambient light (750 μmol m^{-2} s^{-1}) and temperature conditions (22-25°C) to minimize

bench effects. Between October and March, plants from which all senescent above-ground biomass was harvested, were kept at 4°C in a cold room. In March, seawater was renewed and plants transported back into the greenhouse for the next growing season. At this time, benomyl treatments were repeated, without additional $NiSO_4$ amendments. This procedure was repeated for three years until cores were sampled destructively at the end of the third growing season (September 2003).

2.2 Sampling

During the first growing season (March 2 to August 28, 2000), each core and treatment was sampled with a cork corer (2 cm diameter) at three sampling events that were meant to coincide with major plant phenological stages: at the beginning of the vegetation period (middle of May, largest root exudation correlated to fastest plant growth, further referred to as "vegetative growth" period), the second in the middle (end of June, reduced exudation correlated to flowering, referred to as "reproduction" period) and the third at the end (end of August, no exudation due to senescence, referred to as "senescence" period) (Burke et al. 2002a). Because growth conditions were enhanced in the greenhouse, these periods were found about 6 weeks later in the field. For comparative analyses, samples were thus taken from the field site about 6 weeks later than from cores in the greenhouse. Samples taken from *S. patens* sites at the end of February from the field served as samples corresponding to the dormancy stage.

Soil and root samples were separated. Samples (n=3) were collected between 1.5 - 3.5 cm (further referred to as 2.5 cm depth), 4.0 - 6.0 cm (5 cm depth), and 6.5 - 8.5 cm (7.5 cm depth) below the surface of each core as in a previous study where these samples

demarcated significant changes in redox potential and sulfate/sulfide profiles (Burke et al. 2003). Each of these samples was divided into five 1-g portions. From each of the samples one portion was retained for root and soil dry weight determination and one used for the analysis of mycorrhizae. The other three portions were evaluated for heavy metal concentrations in plant roots, and associated soil.

At the end of the third growing season (September 3, 2003), each core and treatment was sampled destructively by cutting the whole cores into slices resembling the upper 1.5 cm (referred to as 0.7 cm), and the following 2.5 cm, 5 cm, and 7.5 cm depths as described above.

2.3 Environmental Analysis

During sample retrieval, pH and redox potential were measured using an Oakton® pH/mV meter (Cole-Parmer Instrument Company, Vernon Hills, IL, USA), and temperature with a digital thermometer. Pore water profiles were obtained using DET (diffusive equilibration in thin films) gel probes (Krom et al. 1994, Mortimer et al. 1998, Mortimer et al. 1999). Gel probes were initially cast in 5 mL plastic pipettes which were subsequently cut and perforated. Prior to deployment into soil cores (placed in the middle of the cores laterally and extended vertically throughout the whole core (10 cm)), gel probes were equilibrated in a 5 ppt artificial seawater solution for 4 hours. Probes were permitted to adjust to soil conditions for one week before they were harvested and the three depths retained for analysis. Immediately after collection, gels were split in two longitudinal half-pieces. The first piece was immediately immersed in the 1 mL of 2% ZnAcetate solution and the second one was in distilled water. Both were placed on ice,

then centrifuged for 1 minute at 14,000 x g to insure complete gel immersion and subsequently back equilibrated for 20 hours at 4°C.

The concentrations of NH_4^+ were measured using UV-visible spectrophotometry employing the phenate method for seawater samples (Eaton et al. 1995). 100 µL of pore water was mixed with 900 µL of artificial seawater in a 1.5-mL Eppendorf tube before adding 40 µL of phenol solution, 40 µL of 0.5% sodium nitroprusside, and 100 µL of oxidizing solution (4 mL of alkaline citrate (i.e. 20 g of tri sodium citrate, 1 g NaOH, adjusted to 100 mL with de-ionized water) mixed with 1 mL of 5% NaOCl). After incubation for at least 2 hours at room temperature, absorbance of color development in the mixed sample was measured at a wavelength of 640 nm using a spectrophotometer (Spectronic 1210&1001 Plus, Milton Roy, NY), and the concentration of NH_4^+ calculated based on values from a standard curve derived from dilutions of NH_4Cl solutions.

Total dissolved sulfide concentrations were also determined by UV-visible spectrophotometry and colorimetric methods (EPA 1986, Kelly & Wood 1998). 250 µL of ZnAcetate-preserved samples was mixed with 0.5 mL of 2% ZnAcetate and 1 mL of de-ionized water before 250 µl of 0.2% N,N-dimethyl-p-phenylenediamine sulfate (DPPDS) solution was added. Into the mixed solution, 12.5 µL of 1% ferric ammonium sulfate solution was added, mixed and made up to 2.5 ml with de-ionized water (Eaton et al. 1995). After incubation for at least 15 minutes at room temperature, absorbance of color development in the mixed sample was measured at a wavelength of 670 nm using a spectrophotometer (Spectronic 1210&1001 Plus, Milton Roy, NY), and the concentration of sulfide calculated based on values from a standard curve (i.e. dilutions of 3% solution of NaS).

For the analysis of total organic carbon (TOC) and total nitrogen (TN), soil separated from roots was dried at 80°C until the dry weight was constant. Dry samples were ground by using a mortar and a pestle, and sieved (<74 μm, 200 mesh) to ensure sample homogeneity with respect to particle size and distribution. The TOC and TN were determined on freeze-dried soil samples by a Elementar's high temperature TOC/TN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Freeze-dried soil samples that had been finely powdered and homogenized were cured of carbonate by the addition of 1-2 mL 10% HCl, and subsequently dried at 80°C. Approximately 10 to 40 mg of sample was weigthed into a tin sample cup and dropped into a feeding tube of the Elementar's high temperature TOC/TN analyzer for sample combustion.

For the analysis of total hydrolysable amino acids (THAA) (Jones & Gilligan 1983, Dauwe & Middelburg 1998), soils cleared of roots were dried at 80°C until the dry weight was constant. Dry samples were ground with a mortar and pestle, and sieved (<74 μm, 200 mesh) to ensure sample homogeneity with respect to particle size and distribution. To hydrolyze the soil, 100 mg of homogenized, freeze-dried samples were hydrolyzed in 5 mL of 6 N HCL solution in a vial, that was flushed with N_2 before, at 110°C for 24 hours. Afterwards, the hydrolyzed sample was centrifuged at 5,000 rpm for 10 minutes, and the supernatant stored at –20°C or neutralized.

For the neutralization, 100 μL of the hydrolyzed supernatant was added into 1,800 μL of 0.1 N HCl which contained 70 μmol of Internal Standard (aminoadipic acid), and subsequently neutralized with 100 μL of 6 N NaOH. To measure total hydrolysable amino acids, 100 μL of clear neutralized solution was added into 2 mL of 25 mM sodium phosphate buffer, pH 8.0, in a disposable fluorescence cuvette. 100 μL of *o*-

phthaldialdehyde (OPA)-reagent freshly prepared by mixing 100 mL OPA-reagent with 50 µL 2-mercaptoethanol, was added and mixed well. The OPA-Reagent was a mixture of 50 mg of OPA, 500 µL of HPLC-grade methanol, 50 µL of 30% Brij35 and 4500 µL of borate buffer, pH 10.5 (0.4 M boric acid adjusted to pH 10.5 with NaOH). The mixed solution was incubated in the dark for 5 minutes at 25°C before measuring the total hydrolysable amino acids by fluorescence spectrophotometer (Cary Eclipse e10205 6462) at the wavelength of 340/455 nm.

Amino acid analysis was performed by the HPLC using a Jasco (Tokyo, Japan) system consisting of a pump (Model PU-980), a low-pressure gradient valve unit (Model LG 980-02), and an autosampler (Model AS2057 plus). The system was equipped with a Nova-Pak C18, 60 A, 4 µm, 3.9 x 150 mm HPLC Cartridge Column (WAT036975, Waters, Bedford, MA)), a precolumn Adsorbosphere OPA 5µm, 150mm x 4.6 mm (96078, Alltech, Deerfield, IL), and a thermostat-controlled oven (TECHLAB, Erkerode, Germany). The concentration of the amino acids was determined by a spectrofluorometer detector (821-FP, Jasco). Both HPLC and detector were controlled by a computer-based data system and a HPLC controller (Browin Chromatograpy-Software Version 1.5, Jasco).

The mixing gradient was prepared with two degassed solvents: Solvent A was tetrahydrofuran-sodium phosphate buffer (25 mM, pH 7.2) (7.5:1000, v/v), and solvent B was methanol-acetonitrile-sodium phosphate buffer (25 mM, pH 7.2) (35:15:50). At different times, flow rates and A:B gradients varied: at 0 minutes, the flow rate was 1 mL/min and the A:B gradient was 100:0%. At 3 minutes, the flow rate was 1.1 mL/min

and the gradient was 100: 0%, and at 10 to 53 minutes, the flow rate was 1.5 mL/min and the A:B gradient was changing from 100:0% to 0:100%.

Fluorescent amino acid derivates were prepared by mixing 20 µL of neutralized sample (see THAA analysis) with 10 µL of OPA-Reagent in a needle with no air intermediary (Cowie & Hedges 1992). The reagent was prepared by dissolving 50 mg of *o*-phthaldialdehyde in 500 µL of HPLC-grade methanol in a 15 mL Falcontube. 50 µL of 30% Brij35 and 4,500 µL of borate buffer (pH 10.5, 0.4 M boric acid adjusted to pH 10.5 with NaOH) were added, the solution mixed and kept in the dark at 4°C until further use. Before use 10 µL of 2-mercaptoethanol were added to 400 µL of OPA-Reagent. The needle with sample content was then transferred to a holding loop to mix the content 10 times with a reaction time of 1.5 minutes before injecting 10 µL of the derivate into the column.

To measure hydrolysable amino acids by HPLC (Lindroth & Mopper 1979), 360 µL of the neutralized solution was added in a HPLC-vial and then mixed with the 90 µL of borate buffer, pH 10.5. These derivatives were then separated by reverse-phase HPLC; with detection of individual amino acid peaks by a flow-through fluorometer (excitation wavelength = 340 nm; emission wavelength = 455 nm). The concentrations of amino acids were calculated from a chromatographic peak based on the amino acid standards which were D-alanine, arginine, asparagines, aspartic acid, cysteine, amino-n-butyic acid, L-glutamine, glutamic acid, glycine, histidine, isoleucine, L-alanine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, galactosamine-HCl, glucosamine-HCl, taurin, ß-alanine, hydroxylysine-HCl, L-citrulline,

ammonium chloride, ornithine-2HCl, and L-2-aminobutyric acid. Internal standards were aminoadipic acid and methylthreonine.

2.4 Chemical Equilibrium Modeling

In order to get an indication about metal speciation in the soil under suboxic (redox potential of -50 mV) and anoxic (redox potential of -200 mV) conditions as exhibited in the short-term experiment, calculations were performed at both redox potentials, transient temperature (i.e. 25°C) and a pH range from 5 to 9, using MineQL+, a data driven, chemical equilibrium modeling program that can be used in aqueous systems with low to moderate ionic strength (<0.5 M). The oxidation state of these metals were selected based on the Pourbaix diagram and their oxidation states commonly found in soils (McBride 1994). Additional components required for the calculations were basic ionic concentrations of the artificial seawater (Atkinson & Bingman 1998), of the additions in the experimental set up (i.e. NH_4^+ and PO_4^{3-}), of humic and fulvic acids, and of the heavy metals present and/or added (Table 2.1). Concentrations of most of these components were either known (Atkinson & Bingman 1998) or could be measured; however, the concentration of humic and fulvic acids was estimated based on the TOC content.

The TOC is the sum of dissolved organic carbon (DOC, i.e. the organic carbon passing though a 0.45 μm filter) and suspended organic carbon (SOC), or the sum of DOC and particulate organic carbon (POC) (Thurman 1986). Since the SOC in many lakes, small streams and the open ocean usually accounts for less than 10% of the TOC (Thurman 1986), the average TOC of 20% measured in the cores at a depth of 2.5 cm was assumed to entirely represent the DOC.

Table 2.1 Components of Seawater Used for Chemical Equilibrium Modeling

Component	In MineQL+ (M)
Ca^{2+}	1.37×10^{-3}
Cl^-	7.89×10^{-2}
K^+	1.90×10^{-3}
Mg^{2+}	7.60×10^{-3}
Na^+	6.75×10^{-2}
NH_4^+	2.77×10^{-3}
NO_3^-	1.46×10^{-7}
PO_4^{3-}	5.26×10^{-4}
SO_4^{2-}	5.33×10^{-3}
HA	4.62×10^{-4}
FA	3.70×10^{-2}
Cd^{2+}	4.11×10^{-6}
Cr^{3+}	4.53×10^{-4}
Cu^{2+}	3.63×10^{-4}
Fe^{2+}	1.24×10^{-1}
Mn^{2+}	8.41×10^{-3}
Ni^{2+}	1.97×10^{-3}
Pb^{2+}	1.11×10^{-4}
Zn^{2+}	1.06×10^{-3}

Since the DOC in wetlands along the West coast of the United States have been shown to consist of about 15% humic acids with estimated molecular weights between 0.8×10^4 and 8.0×10^4 and 60% fulvic acids with estimated molecular weights between

1.25 x 10^3 and 20 x 10^3 (Thurman 1986), the averaged DOC retrieved from the 1.5 and 2.5 cm depths in all cores was estimated to consist of about 3% humic (HA) and 12% fulvic (FA) acids. Since the high molecular weight fraction (> 3 x 10^4) of humic acids accounts for about 50% of the total humic acids, and the dominant fraction of fulvic acids has a molecular weight of 1,500 or smaller (Khan & Schnitzer 1971) cited by (Tan 2003), the average molecular weight of humic and fulvic acids was assumed to be 3.0 x 10^4 and 1.5 x 10^3, respectively, which resulted in concentrations of humic and fulvic acids in the cores of 4.62 x 10^{-4} and 3.70 x 10^{-2}, respectively (Table 2.1).

2.5 AMF Analysis

Roots for the AMF analysis were stained with Trypan Blue in lactic acid using a modification of the procedure outlined by Kormanick and McGraw (Kormanick & McGraw 1982) that employed an incubation in 5% KOH for 6 hours at room temperature instead of heating for root clearing. The counts of AMF colonized roots were made using a slide mount method (McGonigle et al. 1990, Burke et al. 2002a, Burke et al. 2002b). A total of 100 intersections were scored for each slide (slides=24). The root length colonized by mycorrhizal hyphae (hyphal colonization, HC), arbuscules (arbuscular colonization, AC) and vesicles (vesicular colonization, VC) was determined according to the method of Brundrett et al. (Brundrett et al. 1994) where hyphae were considered mycorrhizal only if visually connected to arbuscules, i.e. intricately branched haustoria that formed within a root cortex cell and are considered the major site of exchange between the fungus and host, or to vesicles, i.e. hyphal swellings in the root cortex that accumulate storage products.

2.6 Plant Growth Performance Analysis

At each sampling interval in the first growing season, photosynthetic gas exchange was measured using a portable open-flow infrared gas exchange system (LiCOR 6400, LiCOR Instruments, Lincoln, NE) with a full environmentally-controlled cuvette.

Net assimilation responses to internal CO_2 concentration (A/C_i curves) were generated to calculate carboxylation efficiency (CE) of the mesophyll (the linear portion of the curve = CE) and CO_2 saturated photosynthetic rate (A_{max}) with least-squares non-linear regression analysis using an exponential model (Jacob et al. 1995a). Control software was used to maintain leaf-to-air vapor pressure deficit at 2.5 kPa, with leaf temperatures adjusted to seasonal mean by altering power to a Peltier-cooling block attached to the cuvette. Cuvette CO_2 concentrations were reduced to 50 ppm, then raised incrementally to saturating levels (2,000 ppm) by mixing ambient air with CO_2 introduced from an internal CO_2 source. The leaf was allowed to equilibrate for at least 3 minutes at each CO_2 concentration before logging data.

The physiological status of the plants was assessed by measuring the optimal transfer efficiency of opened photosynthesis II (PSII) reaction centers using a pulse amplitude modulated fluorometer (Hansatech FMS-2, Kings Lynn, UK). Five to six attached individual leaves were dark-adapted in a leaf-clip for a minimum of 15 minutes prior to estimation of photochemical efficiency (F_v/F_m). Baseline fluorescence (F_0) was established by exposing the leaves to a weak modulating beam of 5 μmol $m^{-2}s^{-1}$ of 680 nm light for 5 seconds. Maximum fluorescence yield (F_m) was established with a saturating 0.85 μmol $m^{-2}s^{-1}$ flash of 8500 white light. F_v/F_m was calculated as $F_v/F_m = (F_m - F_0)/F_m$ (Schreiber et al. 1998).

Plant leaves were harvested at the same time. A portion of the above-ground biomass was covered in a bag before clipped to ground level. The fresh plant material was then dried for at least 24 hours at 80°C, and subsequently weighted to determine biomass (g dry weight per core). To correct for variation in clone size between pots, projected basal area of the clone was determined prior to harvesting. C, N, and discriminate carbon isotope ($\delta^{13}C$) concentrations of these leaves were determined by the Stable Isotope/Soil Biology Laboratory (University of Georgia, Athens, USA) using a NA1500 CHN analyzer (Carlo Erba Instruments, Milan, Italy).

2.7 Heavy Metal Analysis

For heavy metal analyses, shoots, and separated roots and soil were dried at 80°C until the dry weight was constant. Dry samples were ground by using a mortar and a pestle, and sieved (<74 μm, 200 mesh) to ensure sample homogeneity with respect to particle size and distribution. 0.5 g of ground samples were digested by using the OI Analytical Microwave Digestion System including OI Analytical PFA Teflon® vessels (CMS Field Products Group, City, Texas). Soil samples were digested in 10 mL of 70% HNO_3, while plant material was digested in 5 mL of 70% HNO_3 and 2 mL of 30% H_2O_2 based on the United States Environmental Protection Agency (US EPA) Method 3051 (EPA 1983).

Heavy metal concentrations for Cd, Cu, Fe, Mn, Ni, Pb and Zn in plant leaves, roots, and associated soil was determined after digestion by Atomic Absorption Spectrometry (Varian SpctrAA 220FS, Walnut Creek, CA) using flame or graphite tube atomizer detection depending on the concentration of the metals in the samples (EPA 1983, Allen et al. 1991).

2.8 Statistical Analysis

All data are presented as means ± standard error (X ± SE). The significance of differences was calculated with an alpha level of 0.05 by using SPSS 11.0.1 (LEAD Technologies Inc., Chicago, IL). Univariate Analysis of Variance or Test of Between-Subjects Effects was used to assess the effects of phenological stages, depths of soil and experimental treatments on environmental parameters such as temperature, redox potential and pH. One-way ANOVA was conducted to clarify the significant effect of different treatments and then a post hoc analysis, Duncan's test was used to indicate significance among ordered means (Duncan 1995).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Environmental Analysis

In order to extract the effects of Ni and/or benomyl addition on AMF, plant growth performance and heavy metal uptake and translocation, soil cores densely covered with plants of *S. patens* and treated with Ni and/or benomyl were kept in the greenhouse under standardized conditions over the growing season to exclude as many variables as possible from the system under study. Since intact cores from the field site were used in this study, the basic composition of the cores (matrix, root and water content) and the pore water chemistry (salinity, SO_4^{2-}, Cl^-) resembled that found in the field (unpublished data). Temperature, pH, and redox potential monitored during the sampling events in the short-term experiment displayed only small differences between treatments, although several differences were statistically significant. Temperature varied between treatments by up to 4°C in spring (i.e. during "vegetative growth") with benomyl-treated cores displaying higher temperatures than untreated cores (Figure 3.1). These differences were not found during "reproduction" and "senescence", and were therefore attributed to sampling artifacts. Benomyl-treated cores were sampled in the afternoon, while untreated cores were sampled in the morning. Temperature profiles were generally comparable at the depth of 2.5, 5.0, and 7.5 cm, and followed seasonal patterns with higher temperatures during summer with the average 27 ± 1°C than that measured in spring (22 ± 2°C) and in Fall (25 ± 2°C).

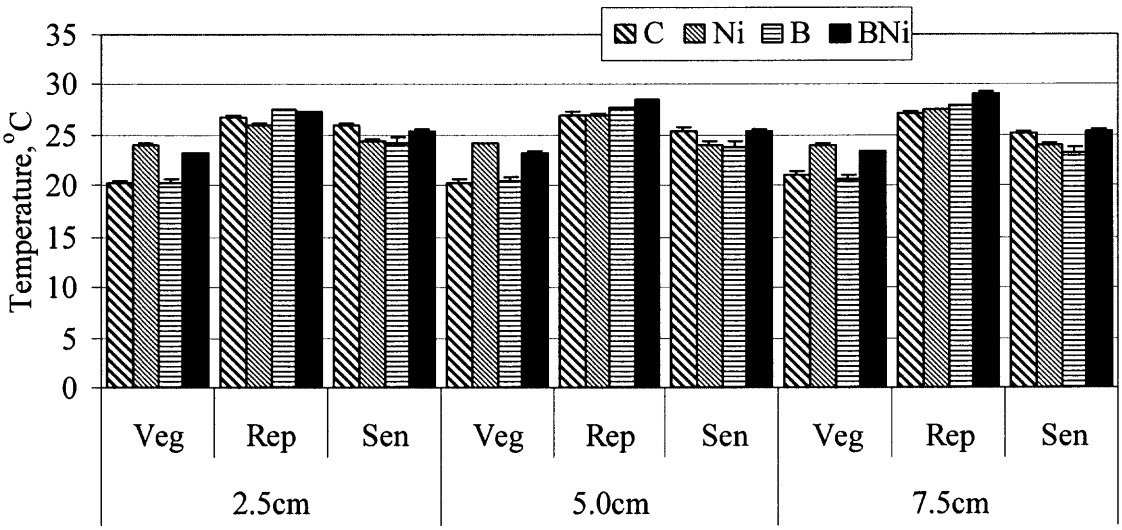

Figure 3.1 Temperature depth profiles (2.5, 5.0 and 7.5 cm) in soil cores vegetated with *S. patens* and without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

These profiles are generally consistent with those found in previous studies on benomyl-effects on the AMF in cores with *S. patens* from Piermont Marsh, an organic, relatively undisturbed 500 ha salt marsh located along the western bank of the Hudson River approximately 18 km north of New York City (Burke et al. 2002a, Burke et al. 2003). Although soil temperature is an important environmental parameter that might affect processes such as water and nutrient uptake, plant and AMF metabolisms, AMF formation and function, as well as root and shoot growth (Dong et al. 201, Cooper & Tinker 1981, Hogue & Neilson 1986, Tagliavini et al. 1991, Engels & Marschner 1992, Wright & Millner 1994, McMichael & Burke 1998, Toselli et al. 1999), it is not considered to be an additional variable in both the short-term and the long-term experiment.

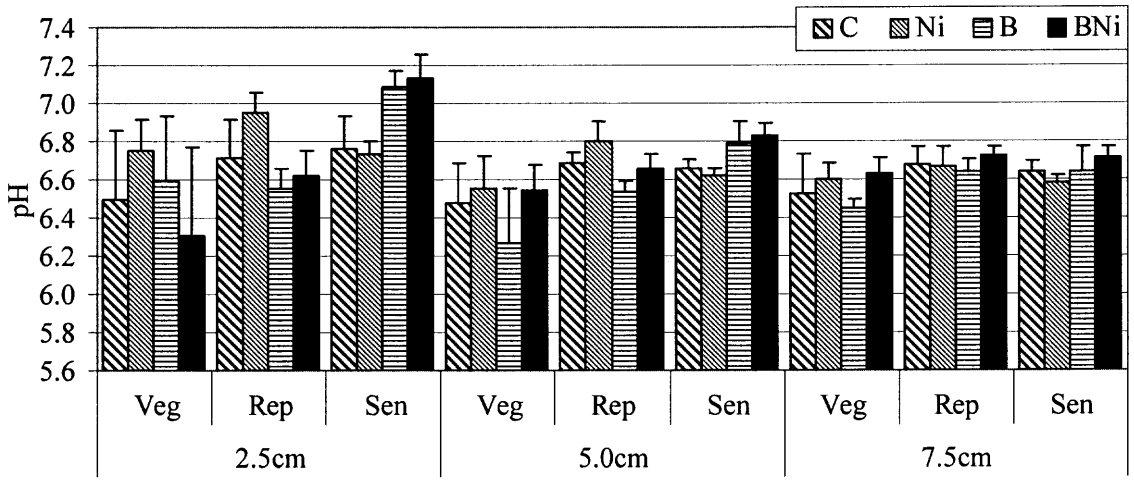

Figure 3.2 pH depth profiles (2.5, 5.0 and 7.5 cm) in soil cores vegetated with *S. patens* and without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

The pH was very consistent among treatments and depths with values generally between 6.5 and 6.8 throughout the season (Figure 3.2). This basic trend was also observed for redox potential that was not significantly different between treatments. Differences were observed during the season with decreasing redox potential in time, and lower redox potential with depth (Figure 3.3).

In contrast to temperature, pH and redox potential are considered more important environmental determinants because both have significant impacts on solubility of minerals or nutrient availability for plants (Van Cleve et al. 1971, Borman et al. 1993, McBride 1994, Larcher 1995, Sparks 1995). Because pH is relatively constant in depth and during the season, redox potential is considered the major environmental variable in the experimental setup.

During the growing seasons, all treatments in the short- and the long-term experiments essentially experience oxic to suboxic conditions in the upper 2.5 cm with

the average redox potential of about -50 ± 105 mV, while the remaining depths at 5.0 and 7.5 cm are anoxic with average values of about -200 ± 80 mV.

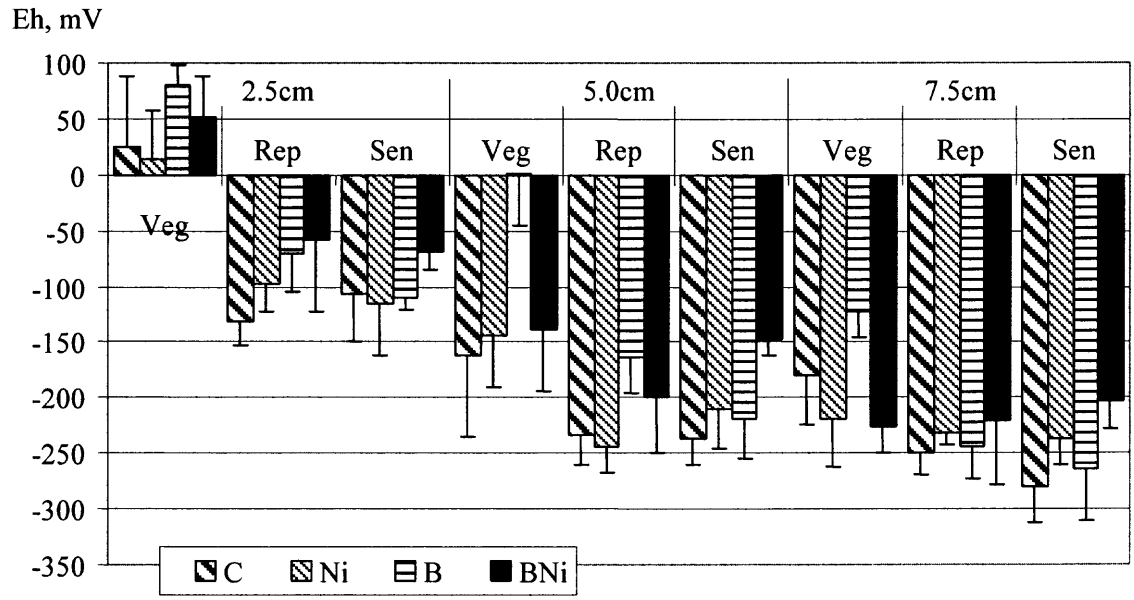

Figure 3.3 Redox potential depth profiles (2.5, 5.0 and 7.5 cm) in soil cores vegetated with *S. patens* and without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

A decrease in redox potential from positive to negative values can be caused by prolonged tidal inundation which results in a reduction of O_2 due to microbial activity (Pezeshki & DeLaune 1996). In the absence of O_2, anaerobic microorganisms can use oxidized compounds such as sulfate as terminal electron acceptors for respiration which results in the transformation of these compounds to reduced forms that can be toxic to the plants (e.g. hydrogen sulfide) (DeLaune & Pezeshki 1991). Thus, at a low redox potential (i.e. at large negative values) plant stress might be higher than at more positive redox potentials (Pezeshki et al. 1991, Naidoo et al. 1992, Bandyopadhyay et al. 1993, Pezeshki & DeLaune 1996). A more negative redox potential, for example, can result in the lower photosynthetic rates in *S. patens* (Pezeshki & DeLaune 1996). Although *S. patens* can

adapt to frequent inundation, it still requires periods of drainage or other means in which higher redox potentials are experienced for optimal growth (Pezeshki et al. 1991). In contrast to *S. alterniflora*, *S. patens* does not have an aerenchyma tissue that provides internal gas spaces that extend from leaves to root tips and function as conduits for gas exchange between plants and their rhizosphere (Bertness 1991). However, even the presence of aerenchymal tissue is often not sufficient for the effective transport of O_2 to the roots (Mitsch & Gosselink 1993).

For sulfide or NH_4^+ concentrations, no significant differences were found between treatments in pore water. Values for these ions, however, changed during the growing season and with the depth of the cores. The sulfide concentrations ranged between 0.3 ± 0.1 and 0.7 ± 0.1 mg L^{-1} during the vegetative growth at the depth of 2.5 cm. The highest levels were obtained during reproduction with concentrations between 2.0 ± 0.5 to 3.2 ± 1.0 mg L^{-1}. The sulfide concentrations generally increased with depth of the cores with a maximum value of 4.5 ± 1.0 mg L^{-1} at 7.5 cm. Values for NH_4^+ ranged from 4.5 ± 0.5 to 5.4 ± 0.5 mg L^{-1} during vegetative growth and 7.4 ± 1.0 to 9.8 ± 2.4 mg L^{-1} during senescence. The NH_4^+ level was generally lower at 2.5 cm and increased with depth, but these differences were not significant. These values support the assumption of suboxic conditions in the upper 2.5 cm and anoxic conditions in the lower parts of the cores (Burke et al. 2002a, Burke et al. 2002b, 2003). Under anoxic conditions, mineralization of organic material by microorganisms results in an accumulation of NH_4^+ due to the lack of nitrification, and of sulfide by the use of sulfate as an alternative terminal electron acceptor to oxygen during mineralization. In the upper part, diffusion of sulfide or sulfide oxidation might be responsible for the smaller values, in addition to its production by

sulfate-reducing bacteria. Proof for the latter assumption, however, requires additional measurements (e.g. that of oxygen or nitrate and nitrite). All values are comparable with those obtained in previous greenhouse and field studies although the matrix material was different (i.e. mainly organic *versus* inorganic matrix found in Harrier Meadow soils) (Burke et al. 2002a, Burke et al. 2002b). In order to eliminate potential nutritional stress, phosphate and NH_4^+ were added periodically at the concentration that had been shown previously to be effective (i.e. no detectable phosphorus and nitrogen limitation on the plant growth performance) (Burke, Hamerlynck, Hahn, unpublished results). Although no data on phosphate are available, the presence of NH_4^+ at all sampling times showed that the plants were not nitrogen-limited at any time during both short- and long-term experiments.

The consistency of the environmental conditions in the cores is also reflected in TOC and %N values among treatments where no significant differences were obtained (Figure 3.4 and Figure 3.5). The TOC values decreased with depth from about 20% at the depth of 0.7 cm to 12% at the depth of 2.5 cm, 5% at the depth of 5.0 cm and 2% at the depth of 7.5 cm. The %N values also deceased with soil depth from about 1.6% at the depth of 0.7 cm to about 0.1% at the depth of 7.5 cm.

Thus, the experimental setup resulted in quite consistent environmental conditions in the cores within and among treatments, with values for most physicochemical parameters being not significantly different at comparable depths which confirms expectations based on previous studies (Burke & Hahn 2000, Burke et al. 2002a).

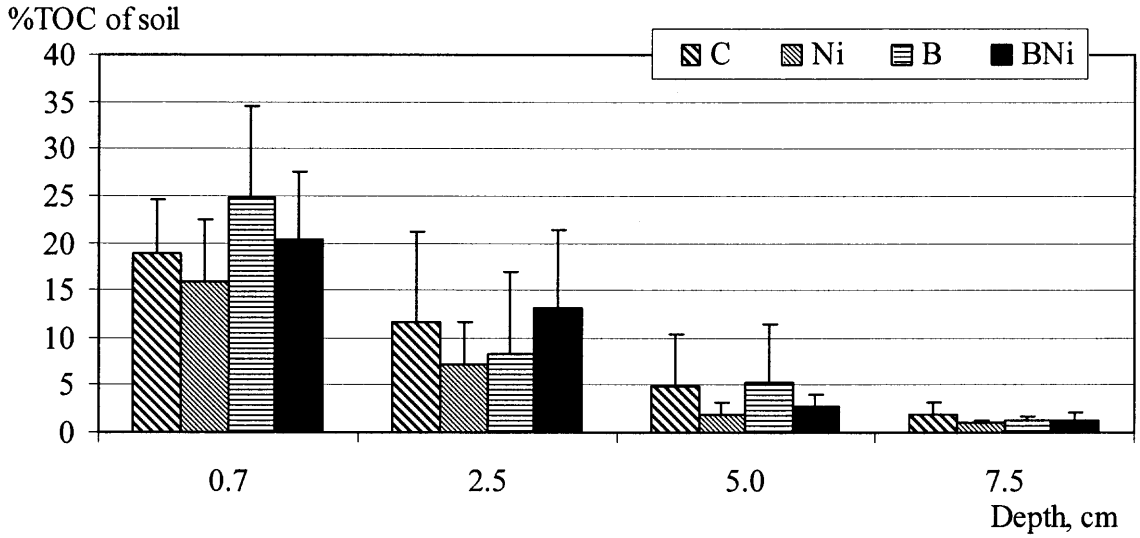

Figure 3.4 Total organic carbon (TOC) depth profiles (0.7, 2.5, 5.0 and 7.5 cm) in soil cores vegetated with *S. patens* and without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after three growing season. Bars and error bars represent mean and standard error values.

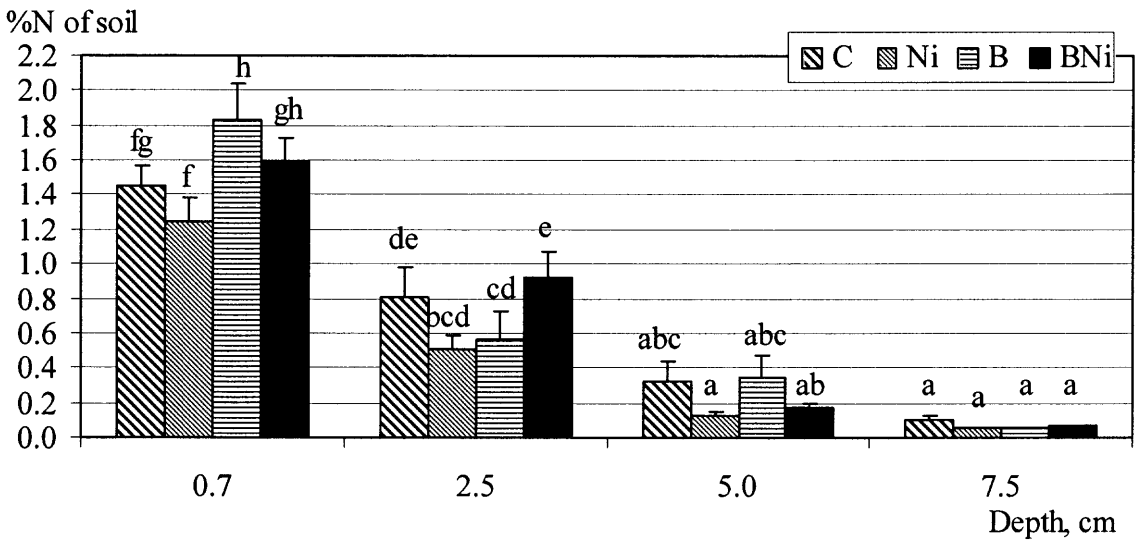

Figure 3.5 Nitrogen in soil at different depths (0.7, 2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences among treatments ($p > 0.05$) determined by Duncan's Test.

Concentrations and spectra of total hydrolysable amino acids (THAA) in the organic material analyzed after the third growing season as a measure for organic matter quality and thus metabolic processes were similar in cores of all treatments and thus indicated no significant biotic environmental differences that might have developed in time as a function of treatments (Figure 3.6). THAA may contribute from 30% to 50% to the soil organic N pool (Friedel & Scheller 2002), and are therefore the major form of the characterized organic N (Chen et al. 2004). Proteins and their constituents in living or dead organisms represent the most labile fractions of bulk organic matter, and thus amino acids play a key role in the biogeochemical cycle of organic matter. THAA analysis therefore provides a means to characterize a specific condition of an environment, i.e. the degradation of the organic matter (Dauwe & Middelburg 1998). The concentration of THAA in soil cores was significantly different with respect to depths of soil cores ($F(3,224) = 153.84$, $p < 0.05$), but not to treatments ($F(3,224) = 2.48$, $p > 0.05$). However, Duncan's test for ordered mean comparison indicated a significantly higher concentration of THAA in benomyl/Ni-amended cores than in control, Ni- and benomyl-treated cores.

THAA concentrations are generally higher than those of amino acids measured by the HPLC since NH_4Cl and amino sugars are also detected. The total concentration of amino acids was decreasing with depth, essentially following the pattern of TOC reduction from about 20% in the upper 0.7 cm to 2% in the lower 7.5 cm (Figure 3.7).

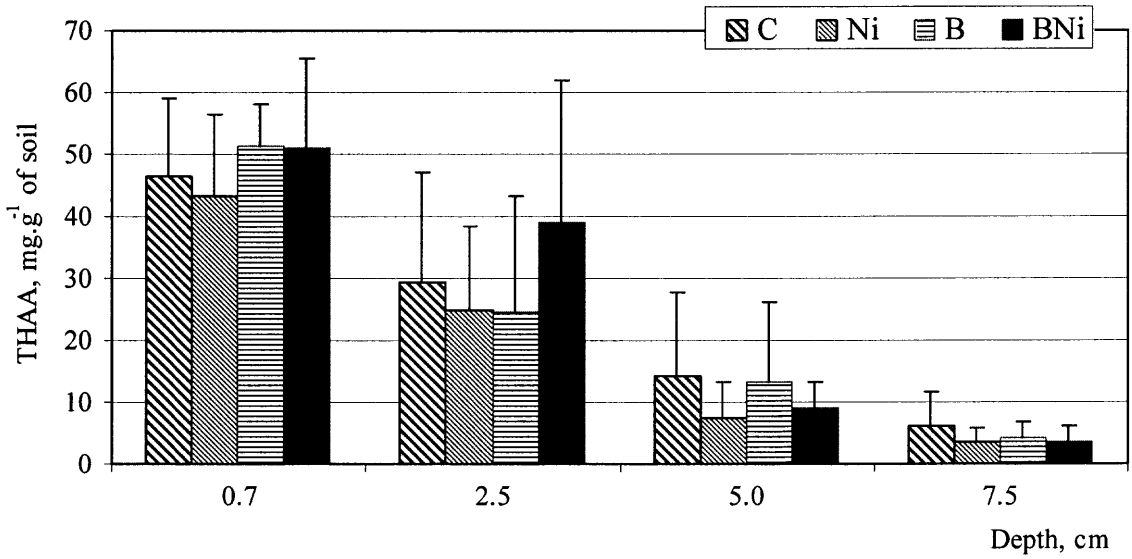

Figure 3.6 Total hydrolysable amino acids in soil at different depths (0.7, 2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values.

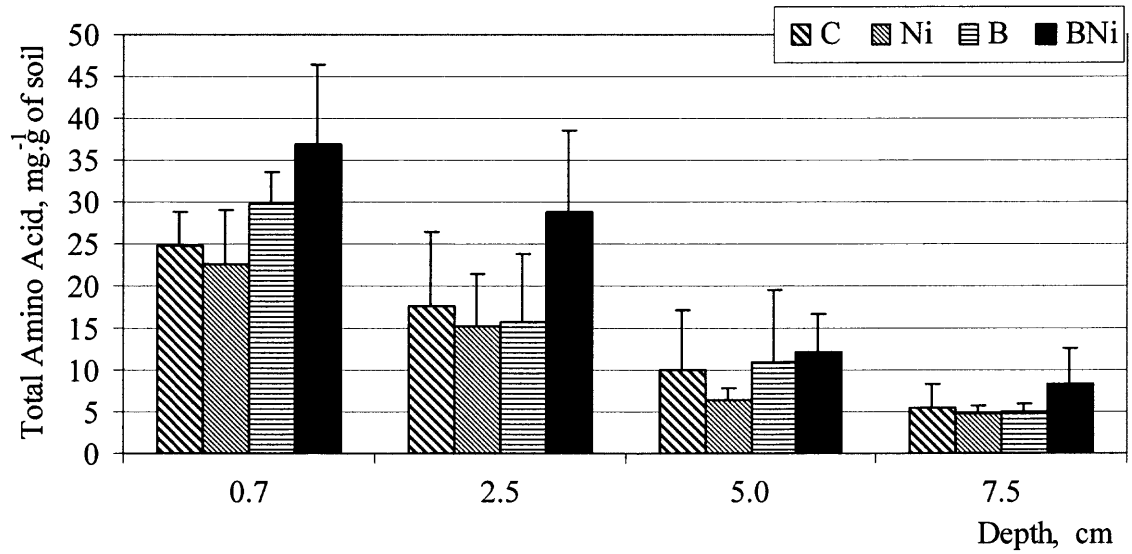

Figure 3.7 Total amino acid concentration in soil at different depths (0.7, 2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values.

With the average values of 28.5 ± 1.1, 19.3 ± 1.3, 9.9 ± 0.8, and 5.8 ± 0.4 mg g^{-1} of soil for the depths of 0.7, 2.5, 5.0 and 7.5cm, respectively, the relative importance of

amino acids within the TOC is slightly increasing with depth which suggests different environmental conditions in depth as suggested by the environmental analyses, e.g. that of the redox potential. These environmental conditions control the degradation processes of organic material and therefore affect the quantity and quality of THAA (Yamashita & Tanoue 2003).

The TOC concentrations decreased with depth about 10-fold from the depth of 0.7 to 7.5 cm, while amino acids decrease only about 5-fold between depths of 0.7 and 7.5 cm. The relative importance of THAA within the TOC increased from about 14% at the depth of 0.7 cm, to 16%, 20% and 29% at the depths of 2.5, 5.0 and 7.5 cm. These values reflect the large variability of THAA concentrations found for different environments and environmental conditions where percentages between 3 and 50% have been reported (Behrends & Liebezeit 1999, Haugen & Lichtentaler 1999). Since microorganisms are supposed to contribute only small amounts to the TOC with up to 5% (Friedel & Scheller 2002), their generally high variability in the THAA composition (Friedel & Scheller 2002) should remain undetected in the general analysis of amino acid composition of the organic material.

Major amino acids found at all depths were alanine (ALA), glycine (GLY), lysine (LYS), aspartic acid (ASP), glutamic acid (GLU), and valine (VAL), while serine (SER), histidine (HIS), threonine (THR), isoleucine (ILE), leucine (LEU) and phenylalanine (PHE) were detected only in small amounts. These data are in agreement with others that reported ALA, ASP, GLU and GLY as the major contributors to the pool of THAA in different environments (Andersson et al. 2000, Medernach et al. 2001, Friedel & Scheller 2002). LYS, VAL and SER were found in relatively high concentrations as well;

however, mean concentrations of these amino acids as well as of all remaining amino acids displayed high standard deviations. LYS, VAL and SER are regarded as easily degradable THAA, while ALA and GLY are relatively recalcitrant and thus might increase in relative abundance with decreasing THAA concentrations in dissolved organic matter (Yamashita & Tanoue 2003).

TYR, PHE and GLU were reported to be easily degradable as well (Dauwe et al. 1999) which supports the absence or very low values for TYR and PHE in the samples, but not the major contribution to the THAA pool of GLU. At the same time, THR is reported to be recalcitrant and thus should accumulate, similar to ALA and GLY (Dauwe et al. 1999) which was also not the case in the study. Other studies report the enrichment of GLY, SER and THR in more degraded material, while TYR, PHE, GLU, LEU and ILE were depleted (Dauwe & Middelburg 1998). This discrepancy as well as the large variability of values, and thus the large standard deviations of the measurements, might be caused by analytical problems since the concentration of most amino acids was close to the detection limit of the analytical system which was in the lower "pmol" range. This was the case for all measurements at the lower depths in the cores (5.0 and 7.5 cm).

Therefore, quantitative measurements at these depths were considered unreliable, and -though documented- not included into any statistical comparison. These analytical limitations also impacted further evaluations on the relative importance of these specific amino acids, i.e. relation to the TOC content at the different depths rather than to soil dry weight as documented. Although major differences in concentrations for both TOC and specific amino acids were indicated between the upper, oxic and suboxic part (0.7 and 2.5 cm), and the lower anoxic part (5.0 and 7.5 cm) of the cores, the close proximity of

concentration values for specific amino acids to the analytical detection limit prevented detailed in-depth analysis on the importance of these amino acids in the anoxic part of the cores.

At the depth of 0.7 cm, concentrations for ALA were about 2 mg g^{-1} of soil for all, but the benomyl/Ni treatment which was close to 3 mg g^{-1} of soil (Figure 3.8). Thus, only this treatment exhibited significant differences in the ALA concentration from the others. The same result was obtained for the depth at 2.5 cm, though at lower concentrations with about 2 mg g^{-1} of soil for the cores treated with benomyl/Ni and 1 mg g^{-1} of soil for the remaining treatments. The same patterns were obtained for amino acids GLY (Figure 3.9) and LYS (Figure 3.10).

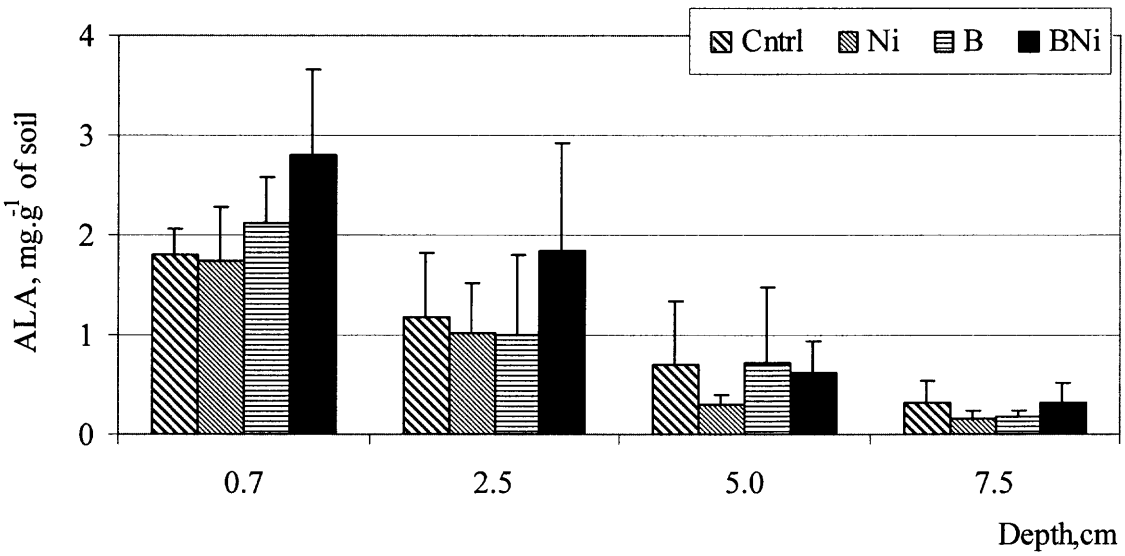

Figure 3.8 Alanine (ALA) concentration in soil at different depths (0.7, 2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values.

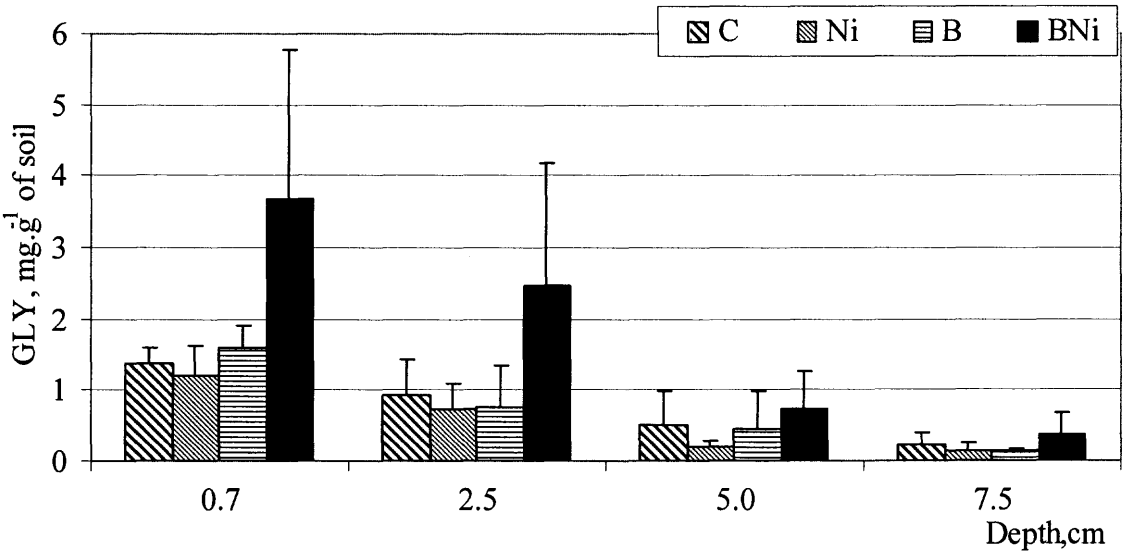

Figure 3.9 Glycine (GLY) concentration in soil at different depths (0.7, 2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values.

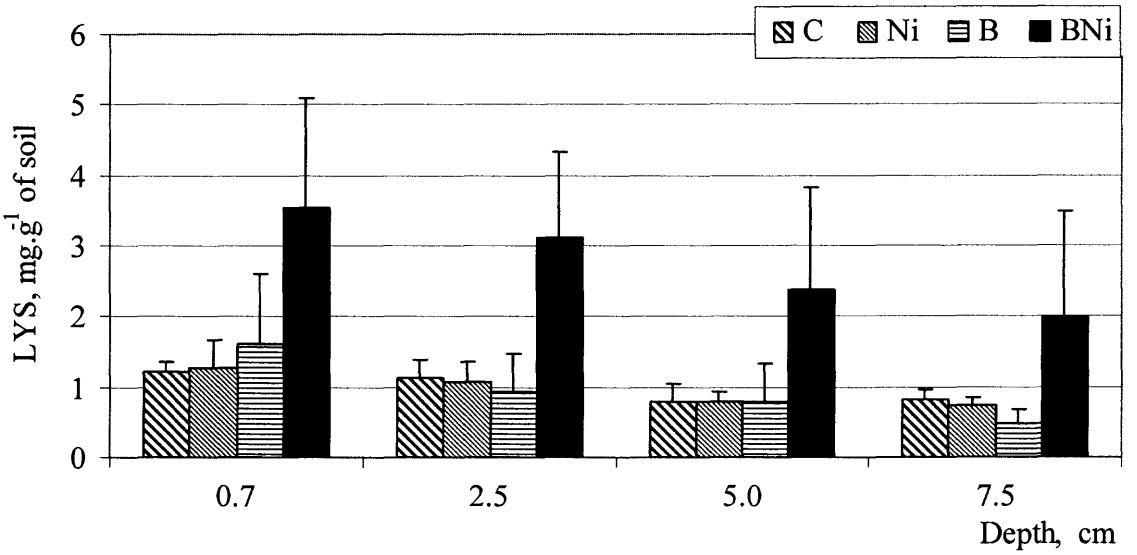

Figure 3.10 Lycine (LYS) concentration in soil at different depths (0.7, 2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values.

In contrast to ALA, GLY and LYS, the remaining three amino acids that were most prominent with respect to concentration in soil, ASP (Figure 3.11), GLU (Figure 3.12) and VAL (Figure 3.13), did not show significant differences among treatments; although, a small reduction of concentration with depth was observed.

Thus, the pattern of total hydrolyzable amino acids as well as of specific amino acids was rather uniform among treatments (except for benomyl/Ni-amendment), even though the importance of THAA or specific amino acids changed with depth and thus with changing environmental conditions. Only benomyl/Ni-amended cores differed slightly with higher THAA concentration and higher values for ALA, GLY and LYS that are supposedly more recalcitrant than others (Yamashita & Tanoue 2003), and thus accumulate during organic matter degradation. Based on absolute concentrations, their higher values in benomyl/Ni-amended cores would suggest more degraded organic material than in the other cores and thus other processes occurring in these cores (Yamashita & Tanoue 2003).

These significant differences between treatments become non-significant when higher THAA concentrations and higher concentrations for ALA, GLY and LYS are used to determine relative abundance. This procedure, however, would change the non-significant differences in ASP, GLU and VAL concentrations and thus suggest their faster degradation in benomyl/Ni-amended cores, and therefore different processes in benomyl/Ni-amended cores.

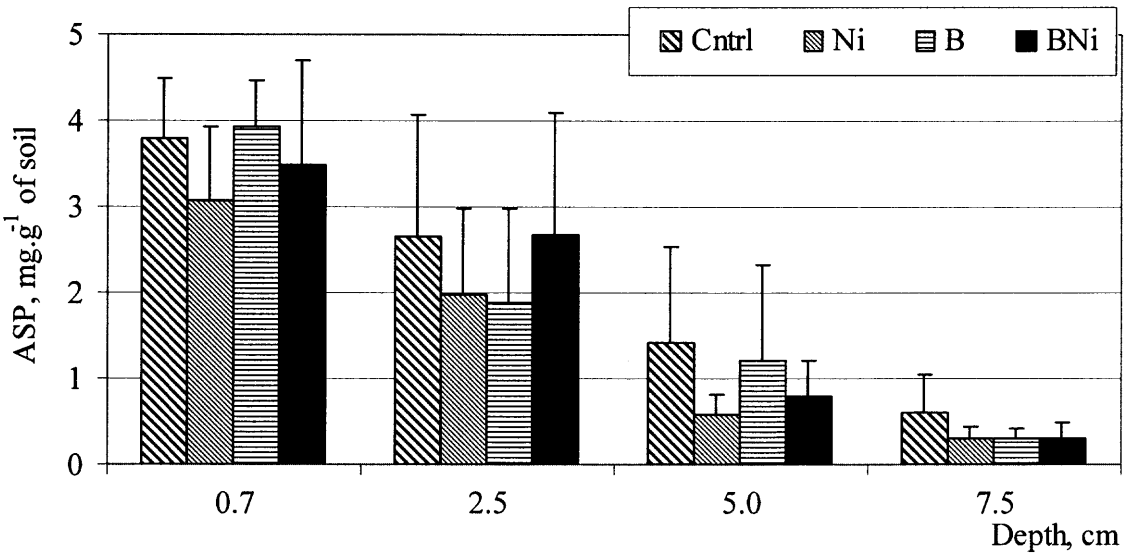

Figure 3.11 Aspartic acid (ASP) concentration in soil at different depths (0.7, 2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values.

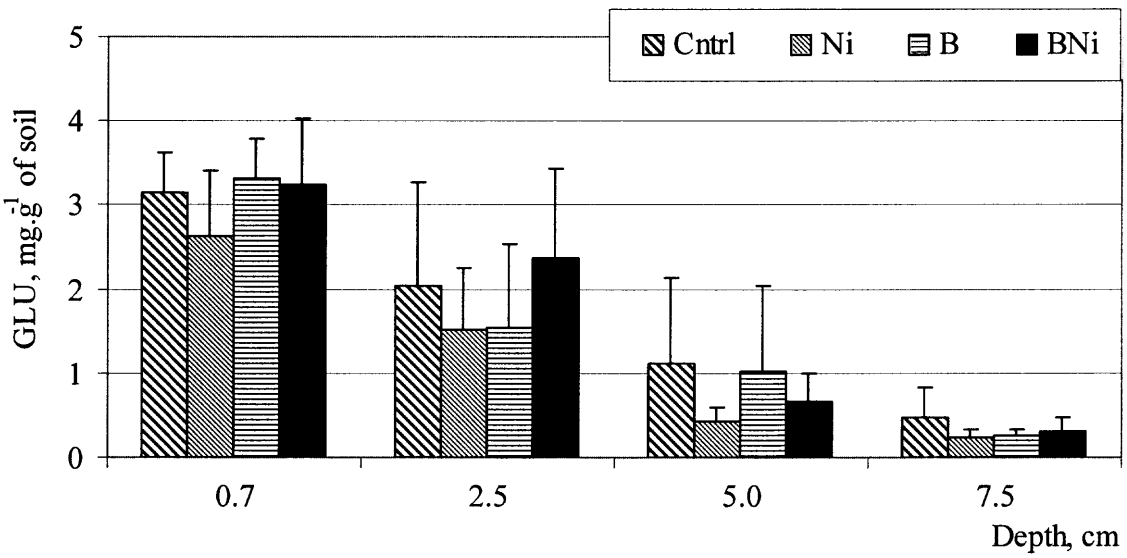

Figure 3.12 Glutamic acid (GLU) concentration in soil at different depths (0.7, 2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values.

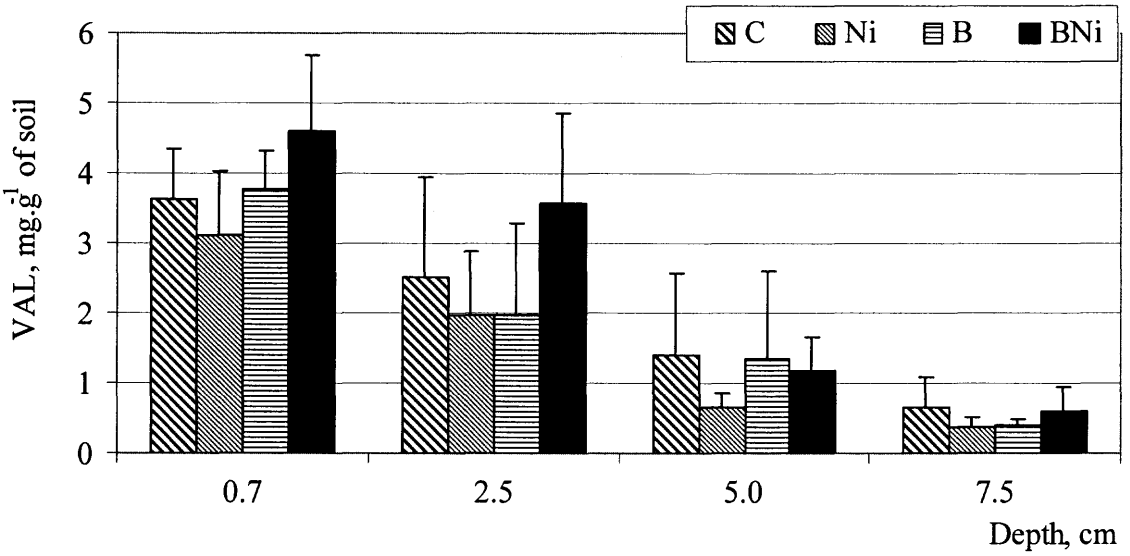

Figure 3.13 Valine (VAL) concentration in soil at different depths (0.7, 2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values.

3.2 Chemical Equilibrium Modeling

The most pronounced variable of the physicochemical parameters in the experimental setup is the redox potential. The redox potential is not only a vital parameter that influences the health of *S. patens* (Anastasiou & Brooks 2003), but also determines bioavailability and thus the toxicity of heavy metals. The bioavailability depends on the chemical speciation, and not on the total concentration of these metals in the environment (Förstner & Wittmann 1981, Morrison & Wei 1991, Pagencopf 1993). Free (i.e. hydrated) metal ions are regarded as the most toxic forms. Hydrated metal ions can interact with inorganic and organic substances (e.g. humic substances, proteins, polypeptides, carbohydrates, etc.) and form complexes that often decrease or completely suppress their toxicity (Luoma 1983, Flemming & Trevors 1989, Linnik 2003).

The importance of humic substances (humin, humic and fulvic acids) (Tan 2003) in the chemical equilibrium modeling is due to their substantial potential to complex dissolved species such as metal ions and cationic organic molecules and to interact with mineral surfaces. Since both major and minor elements take part in chemical interactions involving dissolved and particulate phases in estuarine environments, the complexation process may affect the solubility of both the humic ligand and the species bound. In addition, scavenging by organic coating has been found to remove a range of metal ions efficiently from solution (Zhang et al. 1996).

Trace elements are introduced into estuaries in two principal forms, one associated with solids and colloids, and another one in solution. When trace elements associated with solid material are considered, it is convenient to make a fundamental distinction between those held on the surface and those in inter-sheet (i.e. non-lattice) positions. The elements existing in inter-sheet position are particularly susceptible to processes involving reactions between dissolved and particulate forms, with changes in physicochemical parameters such as pH, redox potential, and ion concentrations, that estuarine systems experience. Depending on the environmental conditions, they are able to dissolve from the particulates into the aqueous phase and, in turn, those in aqueous phase can bind to solid phases. Using the chemical equilibrium modeling of trace metals in pore water is a way to estimate the availability of trace metals in the aqueous phase and a recent approach to determine the hazardous potential of soils containing heavy metals (Campbell & Tessier 1996).

In order to get an indication about metal speciation in the soil under suboxic (i.e. redox potential of -50 mV) and anoxic (i.e. redox potential of -200 mV) conditions,

calculations were performed at both redox potentials, transient temperature (i.e. 25°C) and a pH range from 5 to 9, using MineQL+, a chemical equilibrium modeling program. The data-driven program required the input of basic ionic concentrations of the artificial seawater (Atkinson & Bingman 1998), the concentrations of the additions in the experimental set up (i.e. NH_4^+ and PO_4^{3-}), that of humic and fulvic acids and that of the heavy metals (Table 2.1).

The initial calculations of solubility focused on components of the artificial seawater alone (i.e. without heavy metals) in order to determine their speciation and potential precipitation. The total concentration of aqueous speciation and the solubility diagrams of these calculations are presented in Appendix. The environmental conditions used (i.e. the salinity of 5 ppt) suggested the precipitation of hydroxylapatite ($Ca_5(PO_4)_3(OH)$) since the concentration of Ca^{2+}, a major ion in the seawater, is higher than 1.26×10^{-3} M (or 5.05×10^{1} mg L^{-1}) at pH 7.0. Subsequently, the speciation of the major components including heavy metals were modeled without (Table 3.1 and Table 3.2) and finally with (Table 3.3 and Table 3.4) consideration of potential mineral precipitation (see Appendix for "solubility diagrams"). Without consideration of precipitation, the speciation in water is shown at thermodynamic equilibrium under suboxic (Table 3.1) and anoxic (Table 3.2) conditions. Considering precipitation, speciation in water is shown with one mineral precipitating under suboxic (Table 3.3) and anoxic (Table 3.4) conditions. These data show similar speciation for all metals under suboxic and anoxic conditions in the pH range from 5.0 to 9.0, except for ferric (Fe(III)) and ferrous (Fe(II)) iron.

Table 3.1 Speciation of Elements in Artificial Seawater under Suboxic Conditions without Mineral Precipitation at pH 7.0

Component	Species*	Formula Weight (g)	Total Conc. (%)	Conc. (M)	Conc. (mg L^{-1})
Cd(II)					
	Cd^{2+}	112.41	29	1.17×10^{-6}	3.33×10^{-1}
	$CdCl^{+}$	147.86	59	2.41×10^{-6}	3.56×10^{-1}
	$CdCl_2^{0}$	183.32	10	4.13×10^{-7}	7.57×10^{-2}
Cr(III)					
	$Cr(OH)_2^{+}$	86.01	80	3.60×10^{-4}	3.10×10^{1}
	$Cr(OH)^{2+}$	69.00	17	7.75×10^{-5}	5.35×10^{0}
Cu(II)					
	Cu^{2+}	63.55	60	2.16×10^{-4}	1.37×10^{1}
	$Cu_2(OH)_2^{2+}$	161.11	18	3.27×10^{-5}	5.27×10^{0}
	$CuOH^{+}$	80.55	7	2.61×10^{-5}	2.10×10^{0}
	$CuNH_3^{2+}$	81.56	7	2.46×10^{-5}	2.01×10^{0}
Fe(III)					
	$Fe(OH)_2^{+}$	89.87	93	1.15×10^{-1}	1.03×10^{4}
	$Fe(OH)_3^{0}$	106.87	7	8.99×10^{-3}	9.61×10^{1}
Mn(II)					
	Mn^{2+}	54.94	93	7.78×10^{-3}	4.27×10^{4}
	$MnSO_4^{0}$	151.00	5	4.01×10^{-4}	6.06×10^{2}
Ni(II)					
	Ni^{2+}	58.69	89	1.76×10^{-3}	1.03×10^{2}
	$NiCl^{+}$	94.15	5	9.67×10^{-5}	9.10×10^{0}
	$NiSO_4^{0}$	154.75	5	1.02×10^{-4}	1.58×10^{1}
Pb(II)					
	Pb^{2+}	207.20	46	5.08×10^{-5}	1.05×10^{1}
	$PbCl_2^{0}$	278.11	6	7.13×10^{-6}	1.98×10^{0}
	$PbCl^{+}$	242.65	35	3.88×10^{-5}	9.41×10^{0}
	$PbSO_4^{0}$	303.26	7	7.22×10^{-6}	2.19×10^{0}
Zn(II)					
	Zn^{2+}	65.41	88	9.35×10^{-4}	6.12×10^{1}
	$ZnCl^{+}$	100.86	5	5.06×10^{-5}	5.10×10^{0}
	$ZnSO_4^{0}$	161.47	6	5.94×10^{-5}	9.59×10^{0}

* only species accounting for more than 5% of the total concentration are shown

Table 3.2 Speciation of Elements in Artificial Seawater under Anoxic Conditions without Mineral Precipitation at pH 7.0

Component	Species*	Formula Weight (g)	Total Conc. (%)	Conc. (M)	Conc. (mg L^{-1})
Cd(II)					
	Cd^{2+}	112.41	31	1.29×10^{-6}	1.45×10^{-1}
	$CdCl^{+}$	147.86	58	2.38×10^{-6}	3.53×10^{-1}
	$CdCl_2^{0}$	183.32	9	3.83×10^{-7}	7.02×10^{-2}
Cr(III)					
	$Cr(OH)_2^{+}$	86.01	79	3.59×10^{-4}	3.09×10^{1}
	$Cr(OH)^{2+}$	69.00	18	8.24×10^{-5}	5.69×10^{0}
Cu(II)					
	Cu^{2+}	63.55	62	2.26×10^{-4}	1.44×10^{1}
	$Cu_2(OH)_2^{2+}$	161.11	18	3.27×10^{-5}	1.27×10^{0}
	$CuOH^{+}$	80.55	7	2.56×10^{-5}	2.06×10^{0}
	$CuNH_3^{2+}$	81.56	7	2.53×10^{-5}	2.06×10^{0}
Fe(II)					
	Fe^{2+}	55.85	96	1.19×10^{-1}	6.65×10^{3}
Mn(II)					
	Mn^{2+}	54.94	96	8.05×10^{-3}	4.42×10^{2}
Ni(II)					
	Ni^{2+}	58.69	93	1.83×10^{-3}	1.07×10^{2}
	$NiCl^{+}$	94.15	5	9.05×10^{-5}	8.53×10^{0}
Pb(II)					
	Pb^{2+}	207.20	50	5.60×10^{-5}	$1.16 \times 10^{+1}$
	$PbOH^{+}$	224.21	5	5.03×10^{-6}	1.13×10^{0}
	$PbCl_2^{0}$	278.11	6	6.64×10^{-6}	1.85×10^{0}
	$PbCl^{+}$	242.65	35	3.85×10^{-5}	9.34×10^{0}
Zn(II)					
	Zn^{2+}	65.41	92	9.78×10^{-4}	6.40×10^{1}
	$ZnCl^{+}$	100.86	5	4.76×10^{-5}	4.80×10^{0}

* only species accounting for more than 5% of the total concentration are shown

Table 3.3 Speciation of Elements in Artificial Seawater under Suboxic Conditions with Major Mineral Precipitation at pH 7.0

Components	Precipitated Mineral	Species*	Formula Weight (g)	Total Conc. (%)	Conc. (M)	Conc. (mg L^{-1})
H^+					1.24×10^{-7}	
Ca(II)	Hydroxylapatite					
	($Ca_5(PO_4)_3(OH)$)	Ca^{2+}	40.08	94	5.24×10^{-4}	2.10×10^{1}
		$CaSO_4^0$	136.14	6	3.52×10^{-5}	4.79×10^{0}
Cd(II)	Non-precipitation					
		Cd^{2+}	112.41	29	1.17×10^{-6}	1.32×10^{-1}
		$CdCl^+$	147.86	59	2.41×10^{-6}	3.56×10^{-1}
		$CdCl_2^0$	183.32	10	4.13×10^{-7}	7.57×10^{-2}
Cr(III)	$FeCr_2O_4$					
		$Cr(OH)_2^+$	86.01	80	9.15×10^{-9}	7.87×10^{-4}
		$Cr(OH)^{2+}$	69.00	17	1.97×10^{-9}	1.36×10^{-4}
Cu(II)	Cupric Ferrite					
	($CuFe_2O_4$)	Cu^{2+}	63.55	73	3.36×10^{-29}	2.14×10^{-24}
		$CuOH^+$	80.55	9	4.06×10^{-30}	3.27×10^{-25}
		$CuNH_3^{2+}$	81.56	8	3.86×10^{-30}	3.15×10^{-25}
		$CuSO_4^0$	159.61	5	2.24×10^{-30}	3.58×10^{-25}
Fe(III)	Ferrihydrite					
	(Fe_2O_3)	$Fe(OH)_2^+$	89.87	93	5.37×10^{-9}	4.83×10^{-4}
		$Fe(OH)_3^0$	106.87	7	4.28×10^{-10}	4.57×10^{-5}
Mn(II)	Rhodochrosite					
	($MnCO_3$)	Mn^{2+}	54.94	92	4.22×10^{-3}	2.32×10^{2}
		$MnSO_4^0$	151.00	5	2.28×10^{-4}	3.44×10^{1}
Ni(II)	$Ni_3(PO_4)_2$					
		Ni^{2+}	58.69	89	1.16×10^{-3}	6.81×10^{1}
		$NiCl^+$	94.15	5	6.40×10^{-3}	6.03×10^{0}
		$NiSO_4^0$	151.00	5	6.77×10^{-5}	1.02×10^{1}
Pb(II)	Pyromorphite					
	($Pb_5(PO_4)Cl$)	Pb^{2+}	207.20	46	5.17×10^{-11}	1.07×10^{-5}
		$PbCl_2^0$	278.11	6	7.26×10^{-12}	2.02×10^{-6}
		$PbCl^+$	242.65	35	3.95×10^{-11}	9.58×10^{-6}
		$PbSO_4^0$	303.26	7	7.36×10^{-12}	2.23×10^{-6}
Zn(II)	$Zn_3(PO_4)_2.4H_2O$					
		Zn^{2+}	65.41	88	2.48×10^{-4}	1.62×10^{1}
		$ZnCl^+$	100.86	5	1.35×10^{-5}	1.36×10^{0}
		$ZnSO_4^0$	161.47	6	1.59×10^{-5}	2.57×10^{0}

* only species accounting for more than 5% of the total concentration are shown

Table 3.4 Speciation of Elements in Artificial Seawater under Anoxic Conditions with Major Mineral Precipitation at pH 7.0

Components	Precipitated Mineral	Species	Formula Weight (g)	Total Conc. (%)	Conc. (M)	Conc. ($mg.L^{-1}$)
H^+					1.24×10^{-7}	
Ca(II)	Hydroxylapatite					
	($Ca_5(PO_4)_3(OH)$)	Ca^{2+}	40.08	98	9.79×10^{-4}	3.92×10^{1}
Cd(II)	Non-precipitation					
		Cd^{2+}	112.41	31	1.29×10^{-6}	1.45×10^{-1}
		$CdCl^+$	147.86	58	2.38×10^{-6}	3.52×10^{-1}
		$CdCl_2^0$	183.32	9	3.83×10^{-7}	7.02×10^{-2}
Cr(III)	$FeCr_2O_4$					
		$Cr(OH)_2^+$	86.01	79	3.26×10^{-10}	2.80×10^{-5}
		$Cr(OH)^{2+}$	69.00	18	7.48×10^{-11}	5.16×10^{-6}
Cu(II)	$Cu_3(PO_4)_2$					
		Cu^{2+}	63.55	72	4.92×10^{-5}	3.13×10^{0}
		$Cu_2(OH)_2^{2+}$	161.11	5	1.55×10^{-6}	2.50×10^{-1}
		$CuOH^+$	80.55	8	5.57×10^{-6}	4.49×10^{-1}
		$CuNH_3^{2+}$	81.56	8	5.56×10^{-6}	4.53×10^{-1}
Fe(II)	Siderite					
	($FeCO_3$)	Fe^{2+}	55.85	89	8.90×10^{-3}	4.97×10^{2}
		$FeSO_4^0$	151.92	6	6.01×10^{-4}	9.13×10^{1}
Mn(II)	Rhodochrosite					
	($MnCO_3$)	Mn^{2+}	54.94	96	4.62×10^{-4}	2.54×10^{1}
Ni(II)	Non-precipitation					
		Ni^{2+}	58.69	93	1.83×10^{-3}	1.07×10^{2}
		$NiCl^+$	94.15	5	9.05×10^{-5}	8.52×10^{0}
Pb(II)	Pyromorphite					
	($Pb_5(PO_4)Cl$)	Pb^{2+}	207.20	50	3.29×10^{-10}	6.82×10^{-5}
		$PbOH^+$	224.21	5	2.96×10^{-11}	6.64×10^{-6}
		$PbCl_2^0$	278.11	6	3.91×10^{-11}	1.09×10^{-5}
		$PbCl^+$	242.65	35	2.26×10^{-10}	5.48×10^{-5}
Zn(II)	$Zn_3(PO_4)_2.4H_2O$					
		Zn^{2+}	65.41	92	3.67×10^{-4}	2.40×10^{1}
		$ZnCl^+$	100.86	5	1.78×10^{-5}	1.80×10^{0}

* only species accounting for more than 5% of the total concentration are shown

With respect to metals analyzed (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in the experimental system, Cd was the only metal not exhibiting any precipitation under both suboxic and anoxic conditions according to the calculations in MineQL+. The most prominent species of Cd in the seawater under both suboxic and anoxic conditions in the pH range of pH from 5.0 to 9.0 is $CdCl^+$, followed by Cd^{2+} and $CdCl_2$. At pH 7.0, $CdCl^+$ accounts for about 60% of total aqueous Cd which corresponds to about 3.56×10^{-1} and 3.52×10^{-1} mg L^{-1} under suboxic (Table 3.1 and Table 3.3) and anoxic conditions (Table 3.2 and Table 3.4), respectively. Cd^{2+} and $CdCl_2$ represent about 30 and 10% of all Cd, respectively.

The calculations also indicated that Ni should precipitate only under suboxic conditions (Table 3.3), but not under anoxic conditions (Table 3.4). In aqueous phase, Ni^{2+} and $NiCO_3^0$ are the principal species under both suboxic (Table 3.1) and anoxic (Table 3.2) conditions at the pH range between 5.0 and 9.0. Ni^{2+} is most prominent at a pH > 5.0 to 8.8, with $NiCO_3^0$ becoming more important at a pH > 8.8. At pH 7.0, Ni^{2+} represents about 90% of all Ni under suboxic conditions which corresponds to 1.03×10^2 mg L^{-1}. This concentration is reduced to 6.81×10^1 mg L^{-1} when precipitation as Ni_3PO_4 occurs (Table 3.1 and Table 3.3). Precipitation of Ni_3PO_4 is only expected to happen under suboxic, but not under anoxic conditions. Under anoxic conditions (Table 3.4), the concentration of Ni^{2+} is 1.07×10^2 mg L^{-1} of seawater.

All other metals analyzed show precipitation under both suboxic and anoxic conditions according to the calculations in MineQL+ (Table 3.3 and Table 3.4). The speciation of Cr under suboxic and anoxic conditions is a strong function of pH. $Cr(OH)^{2+}$ is the dominant species in the pH range between pH > 5.0 to 6.3. At higher pH

(i.e. pH >6.3), $Cr(OH)_2^+$ becomes dominant; however, its importance decreases with increasing pH, until at pH 8.5 $Cr(OH)_2^0$ becomes the most prominent species. At pH 7.0, $Cr(OH)_2^+$ and $Cr(OH)^{2+}$ represent about 80 and 10% of the total Cr, respectively, under suboxic (Table 3.1) and anoxic (Table 3.2) conditions. Under suboxic conditions, Cr precipitation should reduce $Cr(OH)_2^+$ and $Cr(OH)^{2+}$ from 3.10×10^1 and 5.35×10^0 mg L^{-1} (Table 3.1), respectively, to 7.87×10^{-4} and 1.36×10^{-4} mg L^{-1} Table 3.3). Under anoxic conditions, the precipitation of $Cr(OH)_2^+$ and $Cr(OH)^{2+}$ will reduce their concentrations in the water phase from 3.09×10^1 mg L^{-1} and 5.69×10^0 mg L^{-1}, respectively, to 2.80×10^{-5} and 5.16×10^{-6} mg L^{-1} (Table 3.4). Cr_2O_3 should be a stable precipitate under suboxic conditions within the pH range from 5.0 to 9.0, whereas under anoxic conditions, Cr_2O_3 precipitates at pH>5.0 to 5.5, and $FeCr_2O_4$ when the pH > 5.5.

Similar to Cr, the speciation of Cu is a strong function of the pH. At a pH between > 5.0 to 7.4, Cu^{2+} is the dominant species, followed by $Cu_2(OH)_2^{2+}$ when the pH is > 7.4 to 8.0, and then by $CuCO_3^0$ and $Cu(CO_3)^{2+}$ at pH 8.4 and 8.8, respectively. At pH 7.0, Cu^{2+} accounts for about 60% of all Cu under both suboxic (Table 3.1) and anoxic (Table 3.2) conditions. $Cu_2(OH)_2^{2+}$ represents < 20% and $CuOH^+$ and $CuNH_3^{2+}$ less than 10% each. Under suboxic conditions, precipitation reduces Cu^{2+}, $CuOH^+$, and $CuNH_3^{2+}$ concentrations in the water phase from 1.37×10^1, 2.10×10^0, and 2.01×10^0 mg L^{-1} (Table 3.1) to 2.14×10^{-24}, 3.27×10^{-25}, and 3.15×10^{-25} mg L^{-1} (Table 3.3), respectively. The results showed that Cu should be present mostly in the solid phase under the suboxic condition. Under the anoxic condition, Cu^{2+}, $Cu_2(OH)_2^{2+}$, $CuOH^+$, and $CuNH_3^{2+}$ in the water phase are reduced from 1.44×10^1, 5.27×10^0, 2.06×10^0 and 2.06×10^0 mg L^{-1} (Table 3.2) to 3.13×10^0, 2.50×10^{-1}, 4.49×10^{-1} and 4.53×10^{-1} mg L^{-1}, respectively

(Table 3.4). Cupric ferrite ($CuFe_2O_4$) is the most stable precipitate under suboxic conditions within the pH range from pH 5.0 to 9.0, while under anoxic conditions tsumebite ($PbCu(PO_4)(SO_4)(OH)$) and $Cu_3(PO_4)_2$ are most prominent precipitates at pH 5.0 to 6.1, with $Cu_3(PO_4)_2$ becoming more prominent at a pH > 6.1 to 8.1. Between pH 8.1 to 9.0, tsumebite is the major precipitate again.

As shown for precipitation of Cu in form of tsumebite ($PbCu(PO_4)(SO_4)(OH)$), Pb in solution might co-precipitate with other heavy metals as sulfides, carbonates or phosphates depending on pH and redox potential. According to the calculations in MineQL+, Pb is generally present as Pb^{2+} in a pH range from pH > 5.0 to 7.8 under both suboxic and anoxic conditions. At a pH from pH 7.8 to 8.8, $PbCO_3^0$ and at pH > 8.8, $Pb(CO_3)_2^{2-}$ become more important species. At pH 7.0 and suboxic conditions, Pb^{2+} and $PbCl^+$ represent 46 and 35% of all Pb species in the water phase, which is equivalent to 1.05×10^1 and 9.41×10^0 mg L^{-1}, respectively (Table 3.1). These species are reduced by precipitation to 1.07×10^{-5} and 9.58×10^{-6} mg L^{-1}, respectively (Table 3.3). Under anoxic conditions, Pb^{2+} and $PbCl^+$ account for 50 and 35% of all Pb species in the water phase which is equivalent to 1.16×10^1 and 9.34×10^0 mg L^{-1}, respectively (Table 3.2). The precipitation reduces their concentrations to 6.82×10^{-5} and 5.48×10^{-5} mg L^{-1}, respectively (Table 3.4). Under both suboxic and anoxic conditions, pyromorphyte ($Pb_5(PO)_3Cl$) is the major precipitate depending on the concentration of Pb^{2+}. Depending on pH and redox potential, $PbCO_3$ might also be formed as demonstrated for river soils (Moors & Ramamoorthy 1984). $PbCO_3$ is almost insoluble, similar to PbS and $PbCl_2$ (Odum 2000).

In the water phase under both suboxic and anoxic conditions, Zn is mainly present as Zn^{2+} at pH > 5.0 to 8.8, and as $ZnCO_3^0$ when the pH is > 8.8. $Zn_3(PO_4)_2.4H_20$ precipitates first when the concentration of Zn^{2+} is higher than 1.62 x 10^1 mg L^{-1} under suboxic conditions (Table 3.3) and 2.40 x 10^1 mg L^{-1} under anoxic conditions (Table 3.4).

Similar to Zn, Mn generally occurs as Mn^{2+}, a stable reduced form in the water phase in soils under both suboxic and anoxic conditions and a pH range of 5.0 to 9.0. At pH 7.0 and suboxic conditions, Mn^{2+} accounts for about 93% of all Mn, equivalent to 4,27 x 10^2 mg L^{-1} (Table 3.1). Mn^{2+} concentrations are reduced to 2.32 x 10^2 mg L^{-1} when rhodochrosite ($MnCO_3$) is precipitated (Table 3.3). Under anoxic conditions, 96% of all Mn or 7.30 x 10^2 mg L^{-1} (Table 3.2) is represented by Mn^{2+}, which is reduced to 4.41 x 10^1 mg L^{-1} when rhodochrosite is precipitated (Table 3.4). In addition to rhodochrosite, which precipitates between pH 6.8 to 9.0, $MnHPO_4$ can be found as precipitate when the pH is >5.0 to 6.8. Precipitates might also include the various MnO_2 phases such as MnOOH that appears to be stable in the deep sea (Brookins 1988, Geoffrey & Horst 1999).

In estuarine soils, Fe mainly appears in ferrous (II) or ferric (III) form. Under suboxic conditions the ferric form is dominant in the water phase whereas the ferrous form is dominant under anoxic conditions. Under suboxic conditions and a pH from 5.0 to 9.0, the calculation shows Fe(III) prominently occurring as $Fe(OH)_2^+$, $Fe(OH)_3^0$ and $Fe(OH)_4^-$. $Fe(OH)_2^+$ is dominant at a pH of > 5.0 to 8.1, $Fe(OH)_3^0$ at a pH 8.1 to 8.5, and $Fe(OH)_4^-$ at a > pH 8.5. At pH 7.0, $Fe(OH)_2^+$ and $Fe(OH)_3^0$ account for 93 and 7% of the total Fe in the water phase which is 1.03 x 10^4 and 9.61 x 10^2 mg L^{-1} (Table 3.1) Both concentrations are reduced to 4.83 x 10^{-4} and 4.57 x 10^{-5} mg L^{-1}, respectively, when

ferrihydrite (Fe_2O_3) is precipitating (Table 3.3). Ferrihydrite precipitation is controlled by the concentration of $Fe(OH)_2^+$. Under anoxic conditions, Fe(II) generally appears as Fe^{2+} in the water phase. At pH 7.0, Fe^{2+} represents about 96% of the Fe in the aqueous phase (Table 3.2). This resembles the concentration of 6.65×10^3 mg L^{-1} that is reduced to 4.97×10^2 mg L^{-1} by precipitation of siderite ($FeCO_3$) (Table 3.4). Siderite precipitation is controlled by the concentration of Fe^{2+} and a pH > 6.5. Below that pH (i.e. from pH 5.0 to almost 6.5), $FeCr_2O_3$ might be the major precipitate.

The valance state of +2 is the most stable for most heavy metals in the environments. Using MineQL+ to calculate their chemical equilibrium, they mainly occur in the form of free ions (Mn^{2+}, Ni^{2+}, Zn^{2+}, and Fe^{2+}), as chloride complexes ($CdCl^+$, $PbCl^+$) and as hydroxyl complexes ($Cr(OH)_2^+$ and $Fe(OH)_2^+$). The presence of oxygen (i.e. under suboxic conditions) seems to affect the formation and concentration of some species in the water phase, since their concentration is generally lower than that of the same species in the anoxic seawater. Changes in redox potential therefore result in changes of speciation and mineral formation

Since Cd (under both suboxic and anoxic conditions) and Ni (under anoxic conditions) do not form precipitates under the environmental conditions in the cores, both remain soluble and may thus have a higher distribution efficiency within the cores, a higher uptake chance by the plant, but also the possibility to leach out of the cores and be removed from the test system through the watering regimen applied. The mobility of heavy metals in cores, however, also depends on other factors in the soil systems, especially the concentration of organic material in the soil cores. The proportion of Cd, Cr, Cu, Pb and Zn adsorbed to organic compounds, for example, can reach 70–95% of

the total concentration of their dissolved forms. For Mn much lower percentages were reported with an average not higher than 30% indicating that adsorption or complexation with organic matter of natural origin is not very high (Linnik 2003). Cd on the contrary tends to adsorb to a high degree to complex compounds with natural organic ligands according to the molecular weight determination of Cd complexes and their chemical analyses (Linnik 2003). Absorption to organic material can have consequence on the fate of heavy metals. Absorption might result in an immobilization of heavy metals, and thus decrease mobility and availability. In contrast to immobilization, adsorbed heavy metals might become more mobile and bioavailable if, for example, complexed to mobile organic material such as dissolved organic material or living roots. Changing environmental conditions through microbial activities degrading the organic material, changing pH or redox potential, might subsequently result in a release or precipitation of the metals, with potential consequences for microorganisms or on plant growth performance.

3.3 AMF Analysis

Since previous studies found the highest level of AMF colonization at the more aerobic 2.5-cm soil depth (Burke et al. 2002b), AMF colonization of roots was only analyzed at this level during the first growing season. *S. patens* was found to form AMF with hyphal coils, with few arbuscules and few vesicles detected. Roots of *S. patens* grown in the original, non-treated cores displayed a slight decrease in root length colonized by AMF between vegetative and reproductive growth phases (17.2 % to 15.1 %) and these values further decreased during the senescent growth phase (13.8 %) (Figure 3.14).

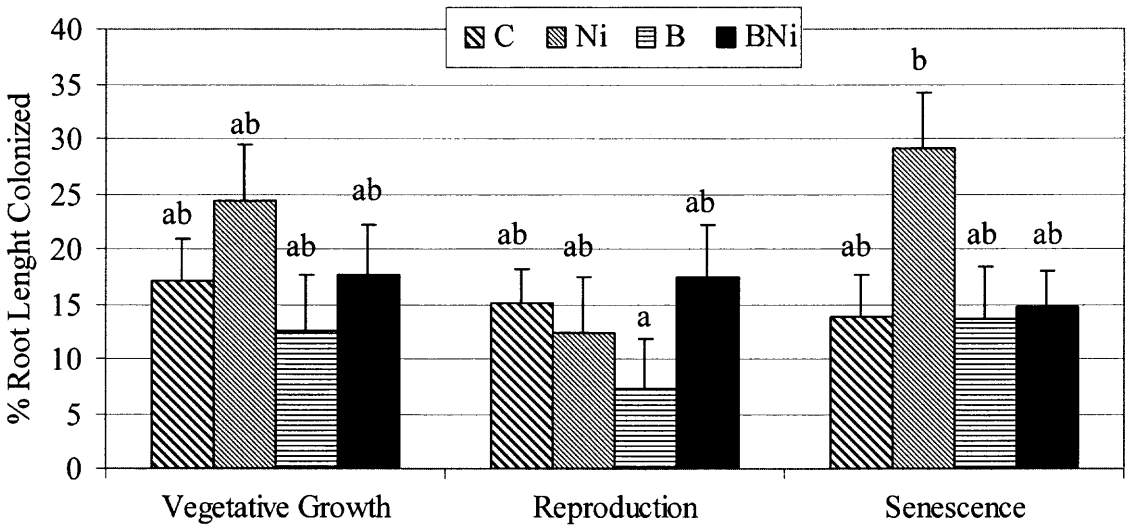

Figure 3.14 Root length colonized in *S. patens* roots at 2.5 cm soil depth of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences between treatments ($p > 0.05$) determined by Duncan's Test.

However, these changes over the course of the season were not significant. These values approximated those found in the field where AMF colonization rose from 7.5% during vegetative growth to 17.2% during reproduction and 10.1% during senescence (data not shown). The overall values as well as the seasonal pattern are similar to those of greenhouse and field evaluations described in previous studies (Burke & Hahn 2000, Burke et al. 2002a, Burke et al. 2002b, 2003).

The colonization of *S. patens* roots by AMF in the study was consistent with the level of colonization previously obtained by other authors (Cooke et al. 1993, Hoefnagels et al. 1993). In North Carolina marshes, the root length colonized typically ranged from

26-52%, but levels as low as 5% have been reported from marshes in Connecticut (Cooke et al. 1993, Hoefnagels et al. 1993). However, similar as discussed in those studies, the root length colonized cannot be ultimately assigned to AMF alone since saprophytic fungi are common on the surface of estuarine environments accounting for up to 10% of the microbial biomass (Rublee 1982a).

Percent root length colonized in Ni-, benomyl- or benomyl/Ni-treated cores showed the same seasonal pattern as in non-treated cores and AMF colonization seemed greater, but was not significant ($F(11,57) = 0.93$, $p > 0.05$) in the Ni-treated cores as compared to the non-treated cores (Figure 3.14). Colonization in Ni-treated cores declined from 24.4 % to 12.4 % during reproduction before increasing to 29.1 % during the senescent growth stage. Benomyl/Ni-treated cores showed insignificant differences with the non-treated cores.

The levels of arbuscular colonization were relatively low, ranging from a high of 5.5% during senescence to a low of 0.4 % during reproduction (Figure 3.15). Significant differences between Ni-treated and non-treated soil cores was observed ($F(11,57) = 2.20$, $p < 0.05$). In contrast to data on Ni-treated and non-treated cores, arbuscular colonization in benomyl-treated cores (i.e. both benomyl- and benomyl/Ni-treated cores) increased in time and was highest during senescence. Data, however, were quite variable with high standard errors. An evaluation of this finding and its ecological significance is therefore required before definite assumptions or statements on the accuracy of these data can be made. Since arbuscules are short-lived and begin to collapse after a few days, it was not surprising that hyphae and vesicles that can remain in roots for months or years, display larger colonization values.

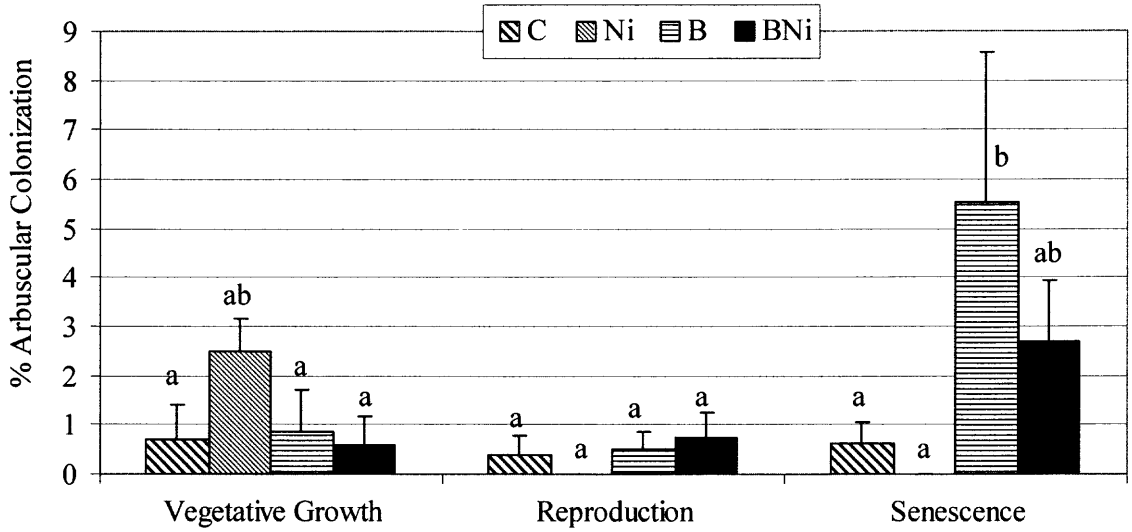

Figure 3.15 Arbuscular colonization in *S. patens* roots at 2.5 cm soil depth of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences between treatments ($p > 0.05$) determined by Duncan's Test.

Vesicular colonization did not significantly vary over the growing season ($F(2,57) = 0.97$, $p > 0.05$), but Ni-treated cores had significantly greater vesicular colonization during vegetative growth than benomyl-treated cores; however, these differences disappeared during the growing season (Figure 3.16). The vesicular colonization declined in Ni-amended cores from a high of 4.7% during vegetative growth to a low of 1.70 % during senescence.

In non-treated cores, vesicular colonization rose from 2.6 % during the vegetative growth to 4.3% during reproduction before declining to the level originally observed (i.e. 2.3%). This pattern for vesicular colonization was also observed in field samples where vesicular colonization rose from 1.4% to 2.4% during reproduction before declining to 1.4% during the plant senescence (data not shown).

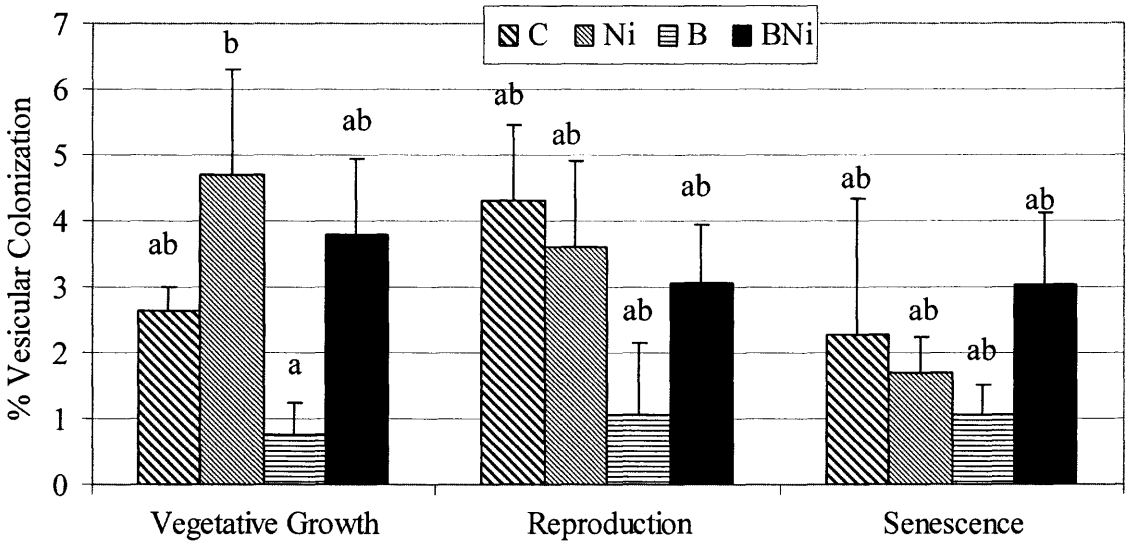

Figure 3.16 Vesicular colonization in *S. patens* roots at 2.5 cm soil depth of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences between treatments ($p > 0.05$) determined by Duncan's Test.

Benomyl treatment resulted in lower vesicular colonization than without treatment ($t(6) = 3.14$, $p < 0.05$). This result is consistent with previous studies in which the suppression of AMF was achieved by applying benomyl as a soil drench at the same or similar rate as in this study (Harnett et al. 1993, Hartnett & Wilson 1999, Smith et al. 1999, O'Connor et al. 2002). Similar to these studies and others (Newsham et al. 1995, Pedesen & Sulvia 1997, Kahiluoto et al. 2000, Kahiluoto & Vestberg 2000), previous studies in the laboratory have shown similar AMF suppression without achieving complete elimination (Burke et al. 2002a, Burke et al. 2002b).

Benomyl has been found to be an effective fungicide reducing mycorrhizal activity (Kahiluoto et al. 2000, Kahiluoto & Vestberg 2000) without negatively affecting the host plant species, and thus has been used in many studies on the role of AMF

communities on plant growth, competition and resilience to abiotic stress (Hetrick et al. 1994, Smith et al. 2000, Burke et al. 2002a).

The dose of benomyl that sufficiently suppresses AMF depends on many factors such as the rate of application, soil characteristic (% organic matter, cation exchange capacity, particle size and compound degradation), and the method of application. Benomyl has a very high adsorption coefficient (K_a = 50 and 90 $\mu g\ g^{-1}$ in two different silt loam soils) which causes very low leaching with K_{om} up to 1000 in soils. Especially the active intermediate carbendazim is mostly absorbed in soil (Hershberger & Arce 1993). Most of metabolites were found in the uppermost (0-12.7 cm) soil layer. They are less mobile in soil with high organic content and low pH (Chang 1985). In rice paddy soil, around 94% were found in the top 5 cm (Ryan 1989). The half-life of benomyl (carbendazim) is 320 days in mesocosms (Marsh & Arthur 1989) and 6-12 months in the field (Baude et al. 1974). Thus, benomyl application into a soil system might not reach concentrations necessary to eliminate AMF, but be sufficient to suppress their function.

Suppression, but not elimination of AMF can thus be a result of potential adsorption and thus inactivation of benomyl to soil minerals (O'Connor et al. 2002), but could also reflect the presence of functional AMF from groups that are resistant to this fungicide (Smith et al. 2000). Benomyl is also effective against fungi other than the AMF and repeated application might also inhibit non-mycorrhizal species (West et al. 1993). Saprophytic fungi can contribute up to 10% of the microbial biomass at the surface of estuarine soils (Rublee 1982b, Rublee 1982a, Hobbie & Fletcher 1988); however, reducing conditions below the surface layer are expected to significantly limit the role of

fungi in marsh soils below a depth of 1-cm (Rublee 1982b, Rublee 1982a, Hobbie & Fletcher 1988, Adam 1990, Mansfield & Barlocher 1993).

In contrast to benomyl application alone, additional Ni amendment resulted in vesicular colonization with average values similar to those on plants in non-treated cores (Figure 3.16). These values were more than twice as high as those after benomyl treatment alone. Similar to speculations about the incomplete elimination of AMF, the presence of functional AMF from groups that are resistant to benomyl and at the same time to higher Ni concentrations could be cause for these results. Shifts in microbial composition as well as a reduction in abundance have been suggested in other studies in which toxic effects of Ni were analyzed (Frostegård et al. 1993), (Scott-Fordsmand 1997). Mycorrhizal fungi in moderately contaminated soil have been shown to acclimate to higher concentrations of heavy metals (Merharg 2003). Since the soil cores in the studies have been contaminated with moderate concentrations of heavy metals, the acclimation of AMF to the added Ni may occur.

However, further investigations are needed in order to support any of these speculations. These investigations need to include studies on AMF diversity, both in soil and roots which would potentially allow us to differentiate between a higher diversity of dormant stages in soil, and a lower diversity of AMF actively infecting roots. A similar outcome, though with functional AMF belonging to different groups, could be obtained in comparative analyses of non-treated and Ni-treated cores. Plants on these cores did not show significant differences in root length colonized, or arbuscular and vesicular colonization. Thus, although no treatment effect (i.e. that of Ni amendment) was detected, such effects could still be established with respect to diversity of AMF. Shifts in

AMF community composition have been shown to impact on AMF colonization and the effectiveness of plant-AMF associations (Francis & Read 1994, van der Heijden et al. 1998), and thus potentially affect plant growth performance.

3.4 Plant Growth Performance Analysis

Foliar nitrogen content plays a central role as a parameter in determining the effect of environmental parameters on plants in an ecosystem. N is found as a major component in leaves in chlorophylls, thylakoid proteins, and associated cofactors and enzymes. The N is obtained through uptake and reduction of nitrate, and the synthesis of proteins represents major respiratory costs to plants (Penning de Vries et al. 1974). The foliar N and photosynthetic rates are positively correlated, and high-nitrogen shoots had significantly greater rates of photosynthesis and transpiration than low-nitrogen shoots (Mitchell & Hinckley 1993). The foliar N content is therefore generally a good predictor of photosynthetic capacity in C_4 plants (Sage & Pearcy 1987).

Leaves sampled in the study were of the same approximate age and grown under the same conditions, except for any differences in leaf attributes related to Ni-, benomyl-, or benomyl/Ni-application. None of the treatments affected foliar N concentrations on a dry weight basis as compared to non-treated cores ($p > 0.05$) but foliar N concentrations did change significantly over time ($p < 0.05$) (Figure 3.17). Foliar N declined from 1.5 ± 0.1 and 1.4 ± 0.1 g N (100 g leaf [dry wt.])$^{-1}$ during vegetative growth to 0.93 ± 0.05 g N and 0.88 ± 0.04 g (100 g leaf [dry wt.])$^{-1}$ during senescence in non-treated and Ni-treated cores, respectively.

Benomyl application did not change this pattern significantly, nor did the combination of benomyl/Ni (Figure 3.17). These seasonal foliar N content declines likely reflected dilution as biomass increases with time. Declines of the magnitude in the study have been previously recorded for *S. alterniflora* (Dai & Wiegert 1997); yet the declines in foliar N content in the study were never so great as to approach the estimated critical N leaf concentration for *Spartina* of 0.7% (Smart & Barko 1980). Thus, data on foliar N content do not show differences between treatments and do not indicate any adverse treatment effects.

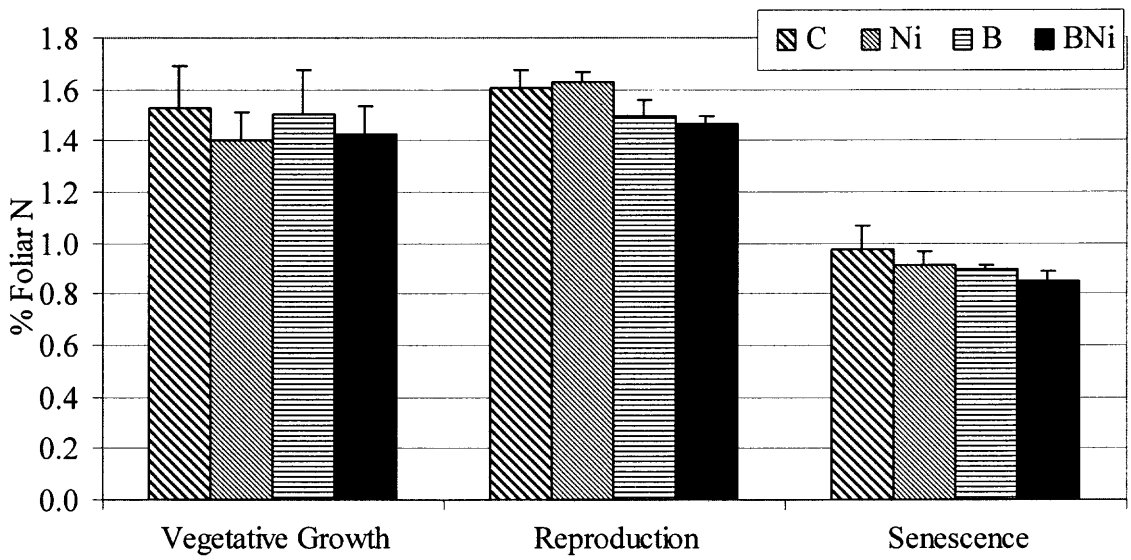

Figure 3.17 Foliar nitrogen (N) in *S. patens* leaves of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence in the short-term experiment. Bars and error bars represent mean and standard error values.

C_4 plants have been shown to discriminate carbon isotopes in response to environmental variables such as water and light availability (Buchmann et al. 1996). However, $\delta^{13}C$ did not differ during the course of the experiment between treatments,

though values for all treatment were slightly higher at the end of the season during senescence (Figure 3.18). The $\delta^{13}C$ values at the time of senescence provide an integrated estimation of water use efficiency (WUE) over the course of the season (Ehleringer & Osmond 1989, Damesin et al. 1998). The values were less negative in cores from all treatments than during vegetative growth and reproduction (Figure 3.18). The higher $\delta^{13}C$ suggests that stomatal limitations to photosynthesis were more frequent in these plants, possibly reflecting periods of stomatal closure, either within or between days, which occurred between sampling periods.

Similar to $\delta^{13}C$ values, values for carboxylation efficiency (CE) were not significantly different ($p > 0.05$). The CE declined significantly across the study period seasonally (Figure 3.19), with the highest levels generally observed for plants in all treatments during the vegetative growth compared to levels during the reproduction and the senescence, with no treatment differences observed. The CE is the reflection of the kinematic constraints imposed by the carboxylation enzymes (Von Cammerere & Farquhar 1981). Thus, the seasonal decreases in the *CE* likely reflect higher total concentration of PEP-carboxylase and Rubisco in the earlier portions of the growing period, and an associated down-regulation of enzyme activity during the latter. The $\delta^{13}C$ data showed that the seasonal down-regulation of CE reflects changes in the seasonal water status and supply of CO_2 to the leaf. However, without additional biochemical data, this assumption remains uncertain.

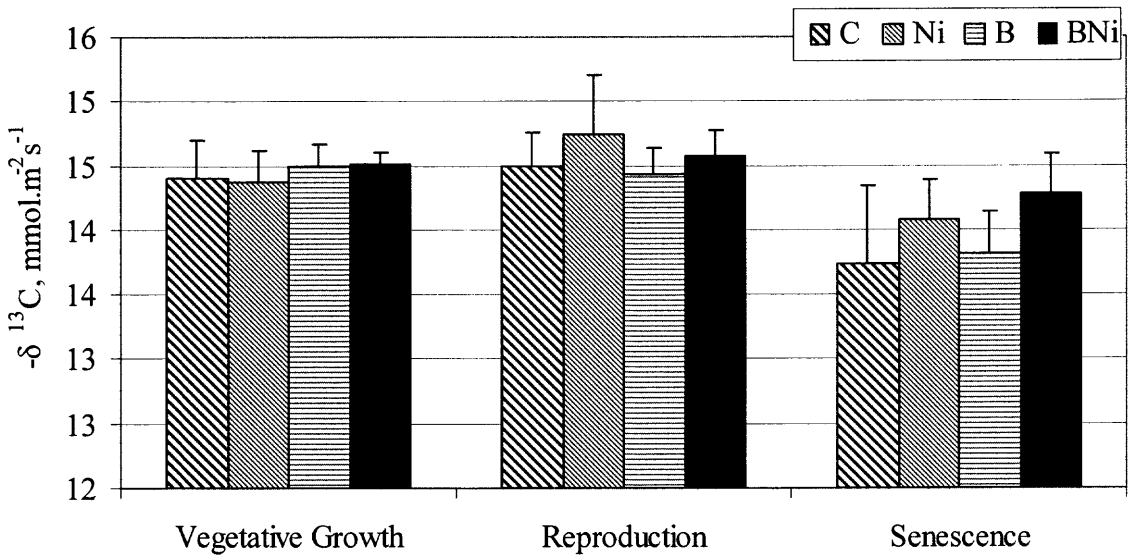

Figure 3.18 Stable isotope ratios ($\delta^{13}C$) in *S. patens* leaves of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence in the short-term experiment. Bars and error bars represent mean and standard error values.

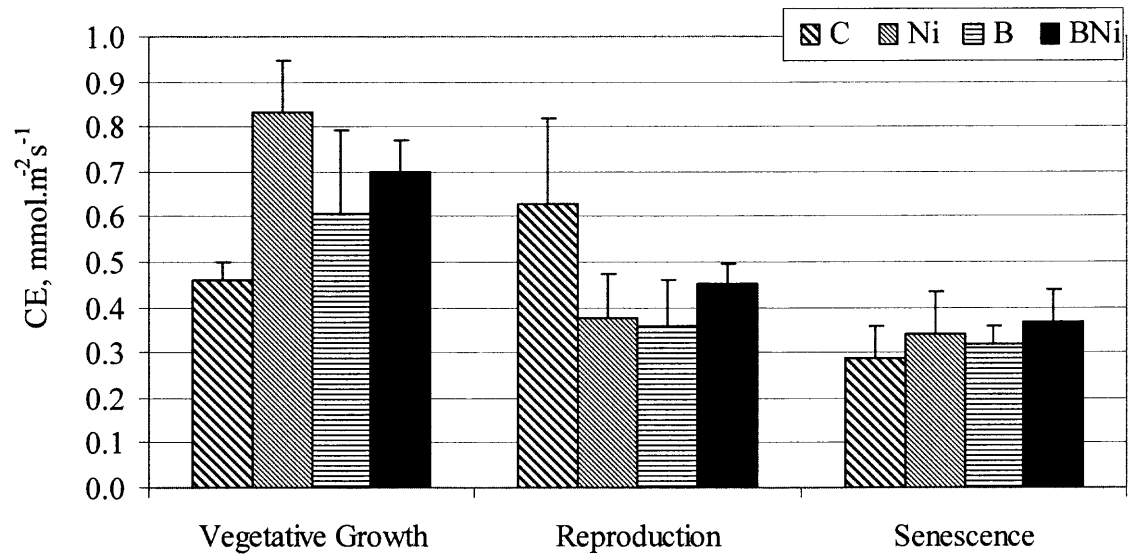

Figure 3.19 Carboxylation efficiency (CE) in *S. patens* leaves of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence in the short-term experiment. Bars and error bars represent mean and standard error values.

Analysis of A/C_i curves indicated that benomyl-treatment and Ni-amendment affected patterns of photosynthetic regulation in *S. patens*. CO_2 saturated photosynthetic capacity (A_{max}) varied significantly with time, being lowest during vegetative growth, and higher during reproduction before declining during senescence to levels initially encountered during vegetative growth (Figure 3.20). The A_{max} is usually associated with an increase in the total concentration of carboxylation sites (Jacob et al. 1995b, Huxman et al. 1998), and thus seasonal increases in A_{max} likely reflect an increase and subsequent decrease in the total concentration of phospho-enol-pyruvate (PEP) carboxylase and ribulose-1,5-bisphosphate carboxylase (Rubisco) during the growing season.

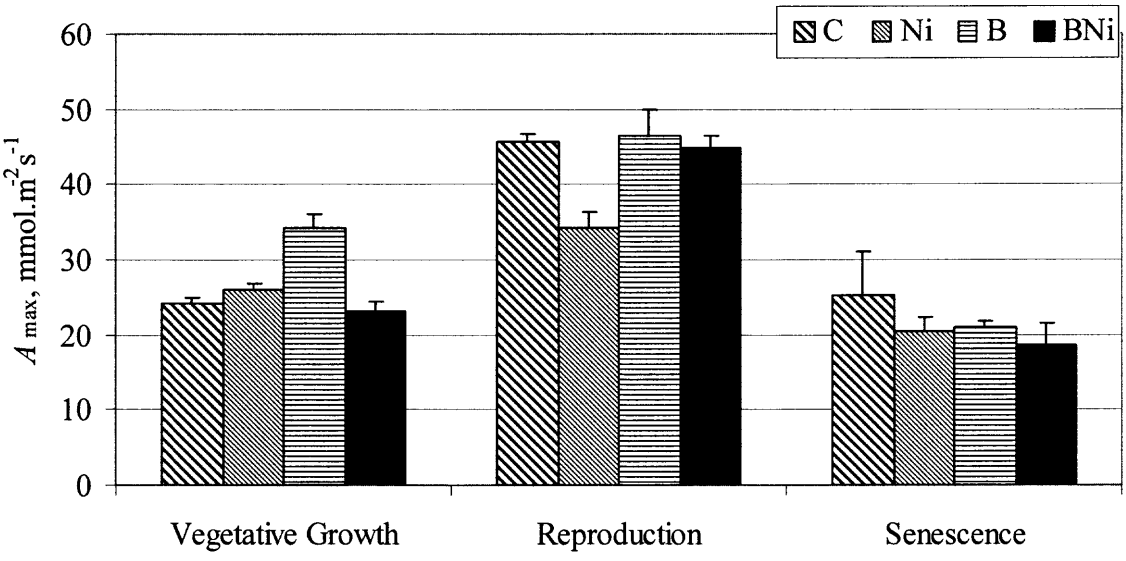

Figure 3.20 CO_2 saturated photosynthetic capacity (A_{max}) in *S. patens* leaves of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence in the short-term experiment. Bars and error bars represent mean and standard error values.

However, when A_{max} of all treatments by each plant stage were compared, during the vegetative growth significantly higher A_{max} in plants from benomyl-treated cores

compared to those in non-, Ni- or benomyl/Ni-treated cores (F (3,11) = 13.392, $p < 0.05$) (Figure 3.21), and during the reproduction, significantly lower A_{max} in plants from Ni-treated cores (F (3,11) = 6.800, $p < 0.05$) compared to those in non-, benomyl- or benomyl/Ni-treated cores (Figure 3.22) drove a significant treatment by time interaction.

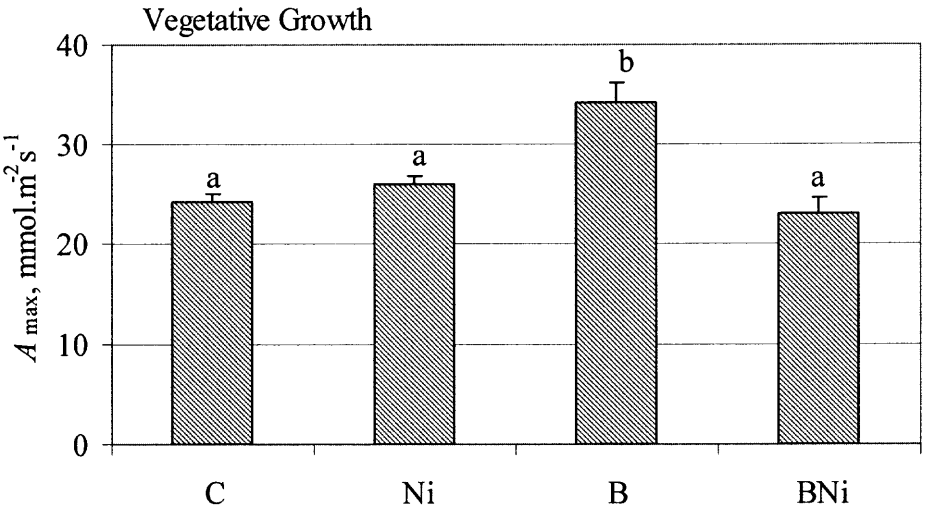

Figure 3.21 CO_2 saturated photosynthetic capacity (A_{max}) in *S. patens* leaves of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during vegetative growth in the short-term experiment. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

Estimations of optimal transfer efficiency of open photosynthesis II (PSII) reaction centers (F_v/F_m) declined with time, being highest in plants from both Ni- and non-treated cores during vegetative growth and these declines were significant (Figure 3.23). Plants from Ni-treated cores experienced significantly lower F_v/F_m chlorophyll fluorescence than did plants from non-treated cores during reproduction (F(3,19) = 6.88, $p < 0.05$) suggesting that there was greater photo-oxidative stress in these plants (Figure 3.23).

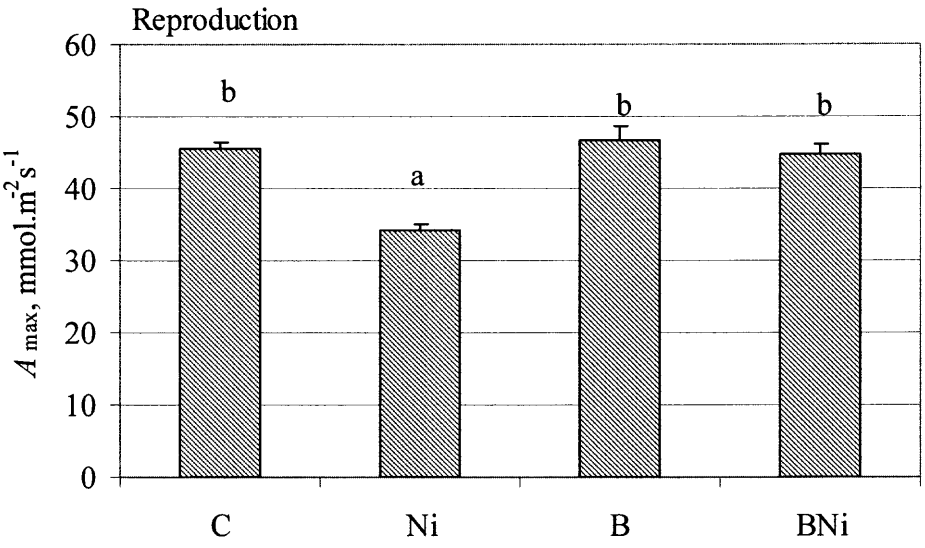

Figure 3.22 Saturated photosynthetic capacity (A_{max}) in *S. patens* leaves of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during reproduction in the short-term experiment. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

The F_v/F_m ratios were fairly high during the course of the study and declines in time are probably indicative of a low-level stress being imposed on the plants over the 6-month growth period. Thus, application of Ni resulted in significant seasonal effects on photosynthetic regulation on *S. patens*. This is in contrast to results on the foliar N content, the $\delta^{13}C$ and the CE that were not affected by the addition of Ni and/or benomyl.

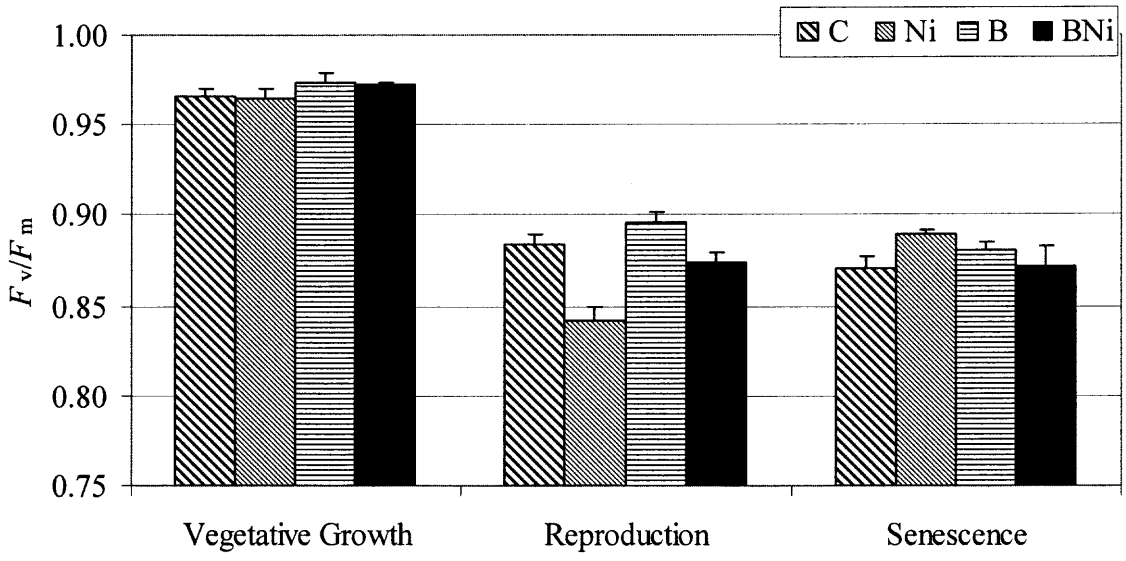

Figure 3.23 Optimal transfer efficiency of open photosynthesis II (PSII) reaction centers (F_v/F_m) in *S. patens* leaves of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence in the short-term experiment. Bars and error bars represent mean and standard error values.

Similar to F_v/F_m, however, above-ground biomass of *S. patens* in Ni-treated cores was significantly reduced compared to non-treated cores during vegetative and reproductive growth (F (3,19) = 2.98, $p < 0.05$), but these differences disappeared by senescence when plants in non-treated cores had an averaged above-ground biomass of 1.69 ± 0.58 kg m^{-2} compared to biomass in Ni-treated cores of 1.34 ± 0.55 kg m^{-2} (Table 3.5). These data are comparable to field measurements of *S. patens* biomass at Harrier Meadow, where a maximum biomass during reproductive growth of 1.1 ± 0.2 kg m^{-2} was observed (data not shown).

Table 3.5 Average Shoot Biomass of *S. patens* Growing in Four Treatments and Its Growth Rate

Treatment	Phenological stage	Shoot biomass kg m^{-2} (X ± SE)	Growth rate kg m^{-2} $month^{-1}$
C	Vegetative Growth	0.73 ± 0.55	0.37
	Reproduction	1.00 ± 0.32	0.13
	Senescence	1.69 ± 0.58	0.34
	Total	1.14 ± 0.62	
Ni	Vegetative Growth	0.44 ± 0.15	0.22
	Reproduction	0.52 ± 0.09	0.04
	Senescence	1.34 ± 0.55	0.41
	Total	0.77 ± 0.52	
B	Vegetative Growth	0.56 ± 0.16	0.28
	Reproduction	0.83 ± 0.26	0.13
	Senescence	2.38 ± 1.80	0.78
	Total	1.26 ± 1.28	
BNi	Vegetative Growth	0.50 ± 0.19	0.25
	Reproduction	1.08 ± 0.49	0.29
	Senescence	1.71 ± 0.23	0.32
	Total	1.10 ± 0.59	
Total	Vegetative Growth	0.56 ± 0.31	0.28
	Reproduction	0.86 ± 0.37	0.15
	Senescence	1.78 ± 0.99	0.46
	Total	1.06 ± 0.81	

Seasonal differences in shoot biomass between treatments were also reflected in growth rates. The rate of shoot growth revealed that Ni addition caused a reduction of

shoot growth (0.04 kg m^{-2} $month^{-1}$) in the reproduction stage, which was three times less than the control (0.13 kg m^{-2} $month^{-1}$) in the same period. Due to the large standard errors of growth yields and the relatively small sizes of the samples (i.e. one quarter of the core surface), these estimates, however, are very prone to errors.

Similar to the results of the short-term experiment at the senescence stage, shoot biomass of *S. patens* was not significantly different between treatments at the end of the third year study (long-term experiment) (Figure 3.24). These results indicate small seasonal variation, but no major effect on the plant growth performance over the season.

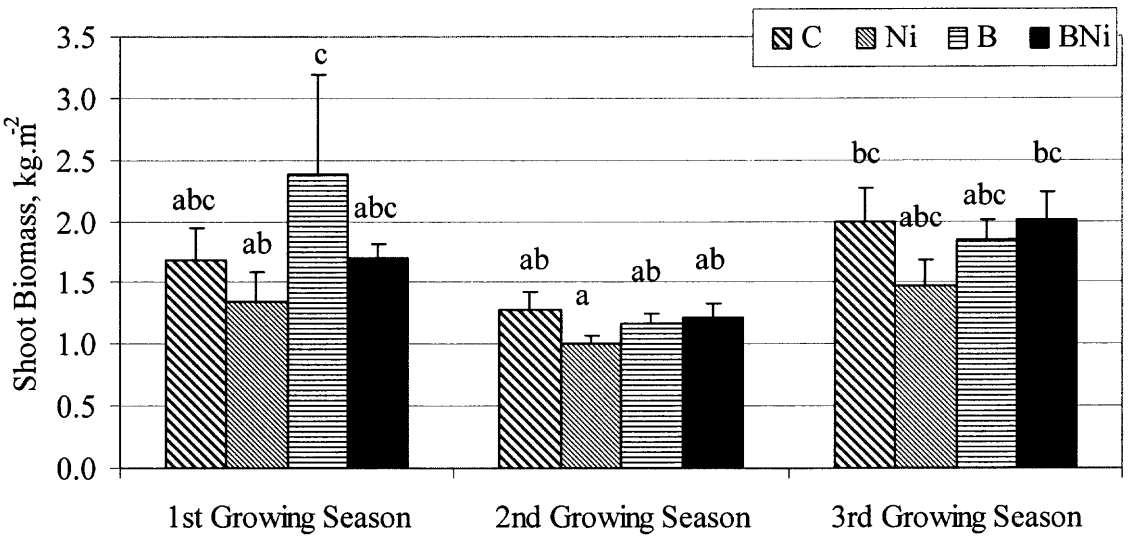

Figure 3.24 Shoot biomass of *S. patens* grown in soil cores of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) after senescence of 3 growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

Table 3.6 Numbers of Sprouts of *S. patens* Growing in Four Treatments and Its Rate of Increase

Treatment	Phenological stage	Number of sprouts (X ± SE)	Rate of increase # m^{-2} $month^{-1}$
C	Vegetative Growth	21133 ± 15303	10567
	Reproduction	15559 ± 6810	7779
	Senescence	26428 ± 8145	13214
	Total	21040 ± 10964	10520
Ni	Vegetative Growth	13405 ± 4919	6703
	Reproduction	11010 ± 2088	5505
	Senescence	22838 ± 8319	11419
	Total	15751 ± 7474	7875
B	Vegetative Growth	14162 ± 3584	7081
	Reproduction	12194 ± 2639	6097
	Senescence	33271 ± 23954	16635
	Total	19876 ± 16322	9938
BNi	Vegetative Growth	16859 ± 8186	8429
	Reproduction	19779 ± 9615	9889
	Senescence	28970 ± 8415	14485
	Total	21869 ± 9712	10935
Total	Vegetative Growth	16390 ± 8991	8195
	Reproduction	14635 ± 6620	7318
	Senescence	27876 ± 13396	13938
	Total	19634 ± 11533	9817

This statement is supported by additional analyses that included the determination of numbers of sprouts of *S. patens*. The number of sprouts of *S. patens* increased

significantly during the season with higher numbers at senescence than for vegetation and reproduction (Table 3.6). An analysis of variance, however, showed that there were no treatment effects ($p > 0.05$).

Although the root biomass was collected only after the third growing season, the results were similar to the results of shoot biomass harvested at the same time. Differences between the control and Ni or Ni/benomyl treatments were not obtained in all depths. The effect of Ni and/or benomyl addition was not significantly different. However, root biomass generally decreased with increasing depth, independent of the treatment (F (3,224) = 116.41 $p < 0.05$). These results are summarized in the final sampling of the long-term experiment (Figure 3.25). The root biomass generally declined with depth from average values of around 0.06 g cm^{-3} at the 0.7 cm depth to 0.015 g cm^{-3} at the 7.5 cm depth.

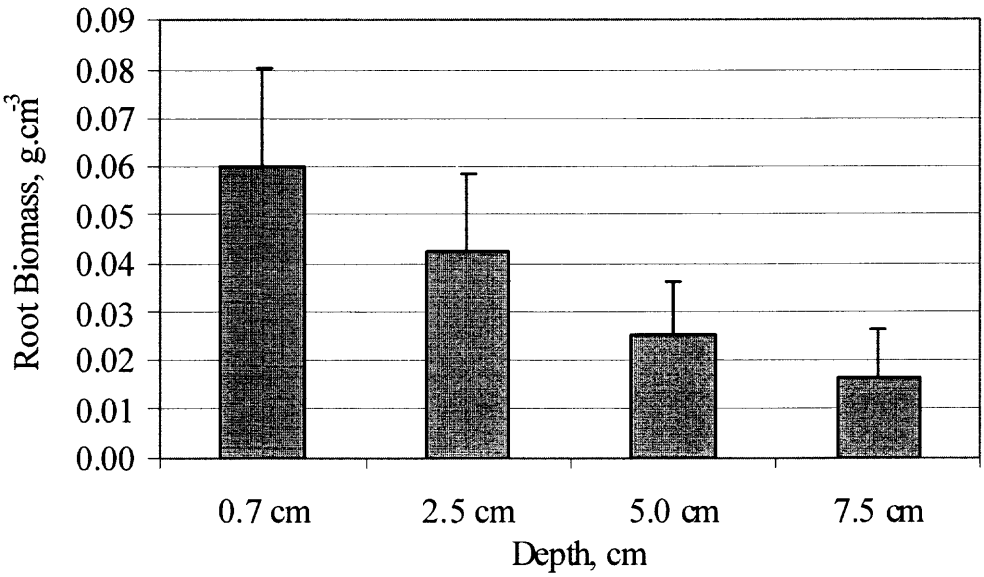

Figure 3.25 Root biomass (g cm^{-3}) at different depths of the cores, at the end of the long-term experiment (senescence). Bars and error bars represent mean and standard error values.

Thus, although the plant growth performance assessment based on the foliar N content, the $\delta^{13}C$, the CE, the A_{max}, the F_v/F_m, the sprout numbers, and the shoot and root biomass generally showed seasonal variation in these characteristics only. However, additional variation was displayed for parameters such as A_{max}, F_v/F_m, and shoot biomass where significant effects of Ni amendment were found during the reproduction. These effects were no longer significant at the end of the growing season, and were not obtained in the benomyl/Ni treatments. Since environmental characteristics such as temperature, pH, redox potential, NH_4^+, sulfide and TOC as well as the analysis of AMF do also not provide significant differences among treatments that could be specifically related to the lower plant growth performance in the Ni-amended cores during reproduction, additional evidence for potential effects of Ni amendment was sought in Ni uptake and translocation patterns in plants of all four treatments.

3.5 Heavy Metal Analysis

3.5.1 Analysis of Nickel (Ni)

In soil, the +2 oxidation state is the only stable form of Ni (McBride 1994). Ni^{2+} favors bonding to organic ligands containing nitrogen and sulfur, and thus accumulation of Ni in humus can be pronounced (McBride 1994). High organic matter levels in Ni-rich soils, however, can solubilize Ni^{2+} as organic complexes. Under reducing conditions, Ni^{2+} is incorporated into sulfides that restrict mobility to very low levels. Soil Ni concentrations generally range from 4 to 55 ppm, with values commonly found in the US between 13 and 30 ppm (McBride 1994).

In the short-term experiment, Ni concentrations in soil revealed significant differences with respect to experimental conditions ($F(3,132) = 22.23$), the depth of the soil profiles ($F(2,132) = 55.73$) and the sampling times during the growing season ($F(2,132) = 5.69$), $p < 0.05$) (Figure 3.26). Ni-amendment, i.e. treatments with Ni alone (Ni) and with benomyl/Ni (BNi), resulted in about 10-fold higher Ni concentrations in soil than in the non (C)- and benomyl (B)-treated cores. Since $NiSO_4$ had been applied to the core surface, higher concentrations of Ni were generally more pronounced in the upper centimeters of the cores, i.e. at a depth of 2.5 cm than in the lower parts at a depth of 5.0 and 7.5 cm (Figure 3.26). This pattern was relatively stable during the season with comparable values for Ni concentrations during vegetative growth and senescence, but about 50% lower values during reproduction (Figure 3.26). An analysis of variance showed that this reduction caused by different phenological stages at 2.5 cm depth was significant ($F(11, 51) = 5.74$, $p < 0.05$). Since at the depth of 2.5 cm about 5-times higher TOC was found than in the lower parts of the cores, absorption of Ni to organic material might have resulted in a relative accumulation of Ni in the upper part of the cores. This Ni could gradually leach out into the lower depths. Potential absorption and release of Ni from organic material might result in losses of Ni from the cores studied through leaching, or be responsible for enhanced Ni uptake by plants in time.

Compared to values for Ni concentrations in soil from the long-term experiment (i.e. 3 years after Ni amendment in the senescence stage), a statistically significant reduction of Ni concentrations was found at the depth of 2.5 cm in the Ni-treated cores, while concentrations at depths of 5.0 and 7.5 cm remained comparable to those in the short-term experiment (Figure 3.27).

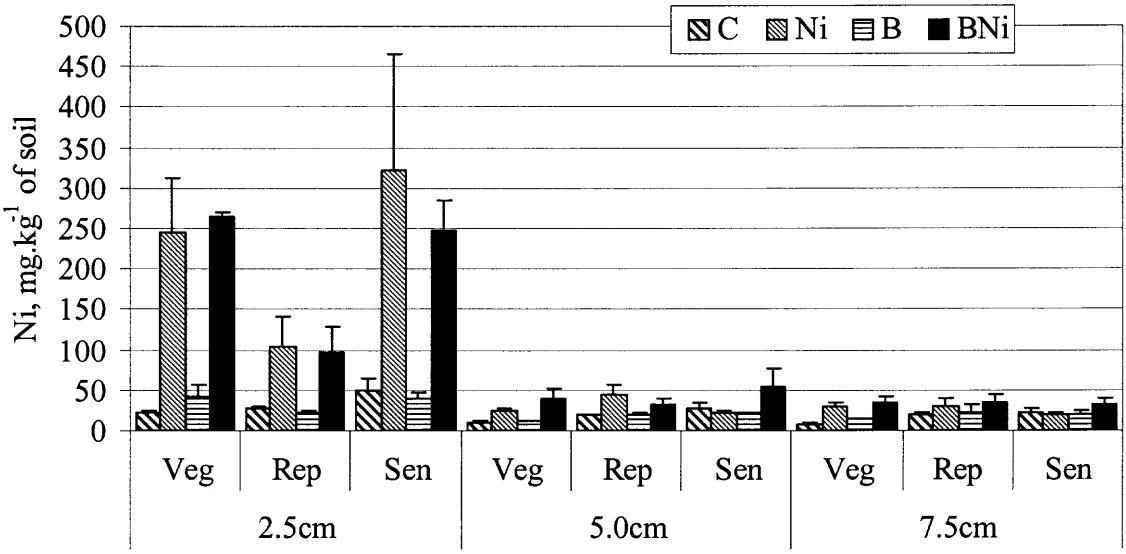

Figure 3.26 Ni concentrations in soil depth profiles (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen) after one growing season. Bars and error bars represent mean and standard error values.

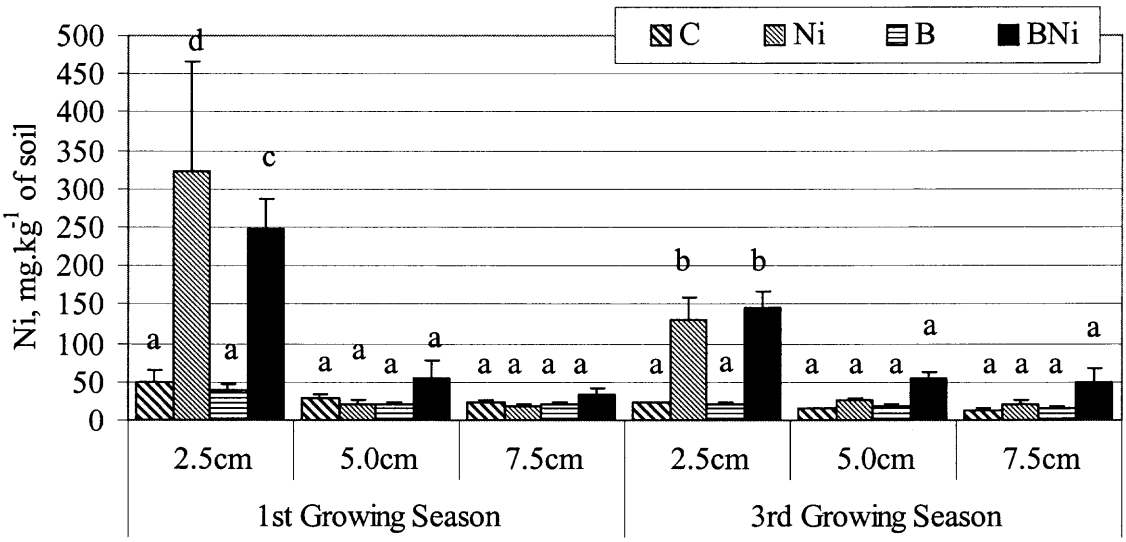

Figure 3.27 Ni concentrations in soil depth profiles (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

Although at lower concentration with about 50% of the values found in the short-term experiment, these values at the depth of 2.5 cm were still significantly higher than those in the lower parts of the cores (Figure 3.27). Highest concentrations of Ni were observed in the upper 1.5 cm (i.e. the 0.7-cm-depth), which resembled values for Ni at the depth of 2.5 cm of the short-term experiment (data not shown). Unfortunately, comparative data are not available from the short-term experiment since this depth was not used for any analyses. Since TOC at the 0.7 cm depth is very high (up to 20%) similar speculations on absorption and release in time might come up as for the short-term experiment. Ni might have been released in time and either taken up by the plants, or leached out of the topsoil layer into the lower parts and from there into the artificial seawater, and thus out of the system that was monitored. The latter case might significantly hamper potential estimates on Ni concentration balances between soil and plants, and thus on estimates on Ni uptake potential of *S. patens*, and subsequent removal from the soil system.

Ni is a trace element essential as mineral nutrient for higher plants (i.e. a micronutrient), (Marschner 1995). Seven Ni-containing enzymes, i.e., urease, hydrogenase, CO-dehydrogenase, methyl-coenzyme M reductase, Ni-superoxide dismutase, glyoxalase I and cis-trans isomerase have been reported, but only five enzymes were characterized (Wattt & Ludden 1999). In plants, urease is the only enzyme known so far where Ni is the metal component required for the activity (Marschner 1995). Ni deficiency would impact nitrogen supply since, regardless of the form of the nitrogen source (i.e. urea, NH_4-N, NO_3-N, N_2 fixation), large amounts of urea would accumulate in the leaves and symptoms of leaf tip necrosis would occur (Eskew et al.

1984). Ni is mainly present in biological systems as Ni^{2+} (Cammack et al. 1988), readily mobile in the xylem and phloem (Kochian 1991), and in some plant species preferentially translocated into the seeds, similar to molybdenum (Marschner 1995). Thus, Ni is assumed to be taken up by the roots of *S. patens* and translocated into the leaves.

In this study, Ni amendment (Ni, and BNi) resulted in significantly higher uptake into roots compared to plants grown on non-amended cores (C- and B) ($F(3,109) = 50.67$, $p < 0.05$), in the short-term experiment at all sampling times and depths (Figure 3.28).

Values for non- and benomyl-treated cores remained relatively constant and did not show any significant differences in time or with depth. These results demonstrate that additional Ni can be taken up into the roots and accumulate when more Ni is available. Effects of benomyl on Ni uptake capability, however, were not obtained (Table 3.7).

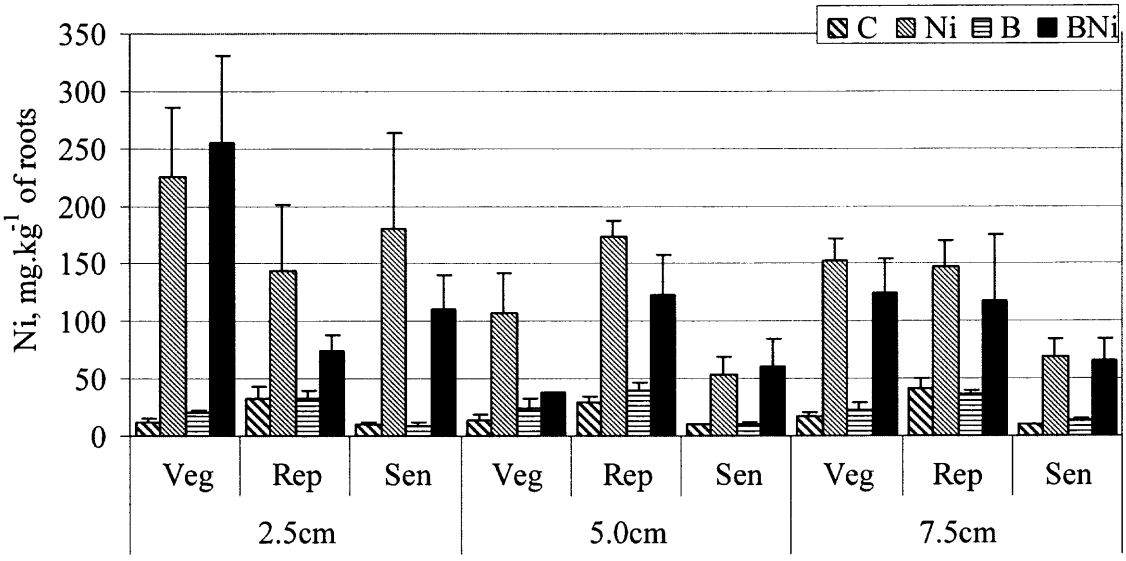

Figure 3.28 Ni concentrations in roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

Changes in Ni concentrations were also obtained during the growing season ($F(3,109) = 6.05$, $p < 0.05$). Ni concentrations were usually higher in roots of the upper depth (2.5 cm) than in roots of the lowers part of the cores (5.0 and 7.5 cm) ($F(3,109) = 7.38$), $p < 0.05$). These differences, however, did not reflect the 10-fold decreases in concentrations with depth as demonstrated for soil, but rather moderate decreases with a factor of up to 2 (Figure 3.28).

Table 3.7 Ability to Take up Ni into Roots in Ni- and BNi-treatments

Depth	Season	Ratio Between		
		Ni/C	BNi/C	BNi/B
2.5 cm				
	Vegetative Growth	19	21	13
	Reproduction	4	2	2
	Senescence	18	11	12
5.0 cm				
	Vegetative Growth	8	3	2
	Reproduction	6	4	3
	Senescence	6	6	6
7.5 cm				
	Vegetative Growth	8	7	6
	Reproduction	4	3	3
	Senescence	7	7	5

At the same depths such as at 2.5 cm depth, highest Ni concentrations were usually found during vegetative growth, lowest during reproduction and slightly increased values during senescence (Figure 3.29). A similar pattern was obtained for Ni concentrations in roots in the long-term experiment (Figure 3.30) when plants growing on cores amended with Ni only were analyzed with values comparable to those obtained in

the short-term experiment. Additional benomyl-treatment, however, resulted in a different pattern. At the depth of 2.5 cm, about 60% lower Ni concentrations compared to those in the short-term experiment were measured, while values at the depths of 5.0 and 7.5 cm were about 10- and 20-fold higher than those in the short-term experiment (Figure 3.30). Calculations of distribution coefficient values (Kd = [mg in root/kg soil]/[mg in solution/L soil]) for these samples indicated a higher availability of Ni^{2+} in deeper soil under anoxic conditions after Ni- and benomyl-amendment, but not after Ni-amendment only. The highest distribution coefficient with 1 L kg^{-1} was calculated for the 7.5-cm-depth in Ni- and benomyl-amended cores which was approximately 7 times higher than distribution coefficient of the same depth in Ni-treated cores (0.27 L kg^{-1}). The latter values were similar at all depths in Ni-treated cores and the 2.5-cm -depth of the Ni- and benomyl-amended cores.

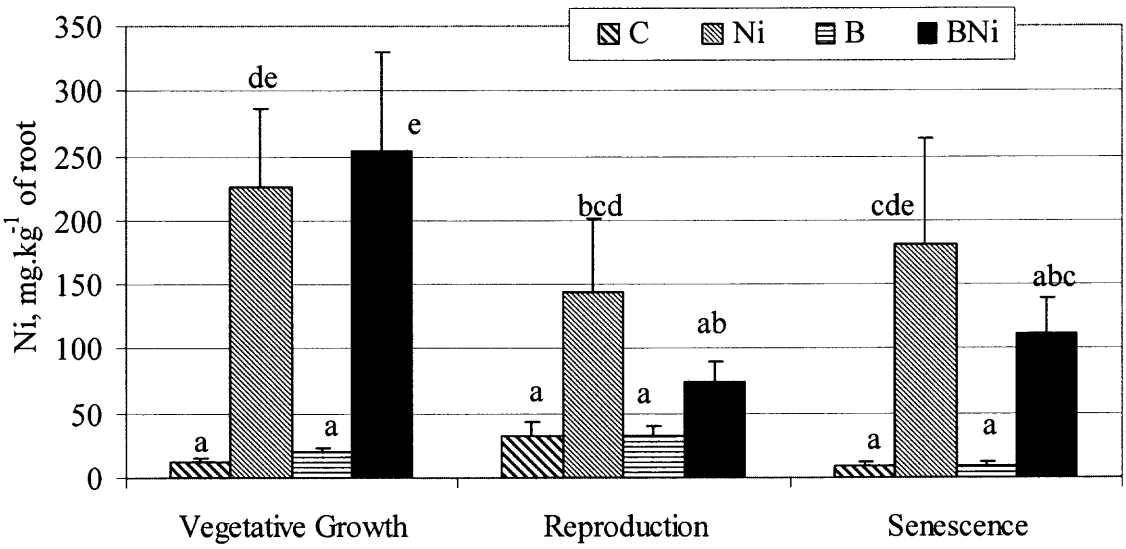

Figure 3.29 Ni concentrations in roots at a depth of 2.5 cm of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence after one growing season. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

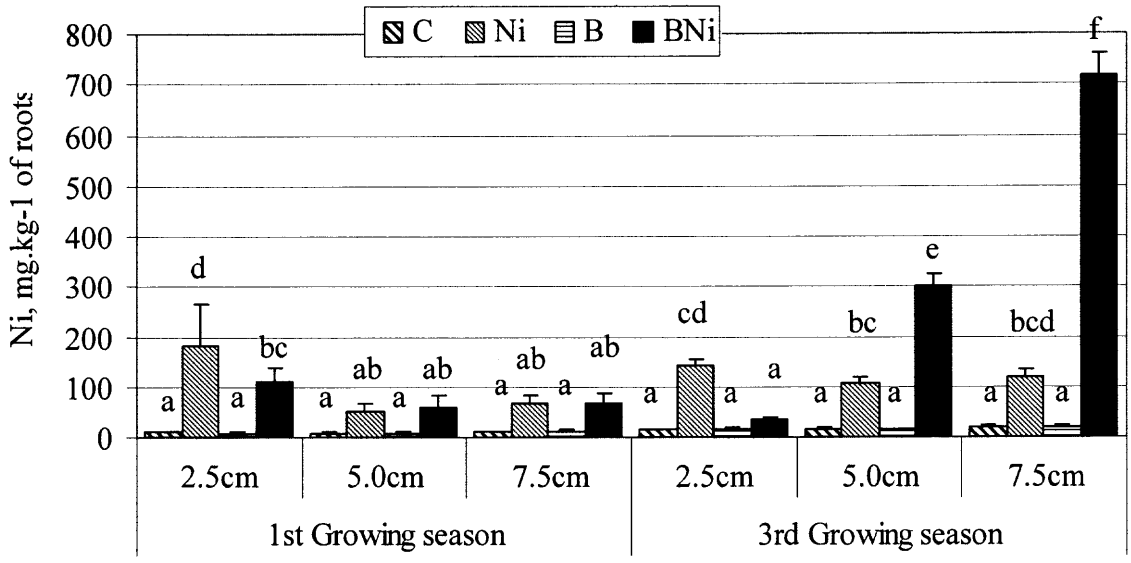

Figure 3.30 Ni concentrations in roots taken at different depths (2.5, 5.0 and 7.5 cm) in soil cores with *S. patens* of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

About the causes for the seasonal variations obtained during the short-term study and the different patterns for the BNi-treatment in the long-term study can only be speculated. Since the concentrations of Ni in roots did not follow the same pattern as concentrations in soil, one aspect would include uptake of Ni in root tips by newly developing roots, which should be highest in the upper part of the core during vegetative growth. Subsequent root growth could result in downward movement of Ni taken up, or adsorbed, and thus translocation in time following root growth from upper parts of the core to lower parts. Since root growth, Ni uptake and adsorption patterns were not analyzed, these thoughts remain highly speculative and require additional studies.

The same is warranted for the increasing Ni concentrations at depths of 5.0 and 7.5 cm in roots of *S. patens* grown in the benomyl/Ni treated cores. Although benomyl-

treatment suggests a relationship between enhanced long-term Ni uptake and potentially reduced AMF, two findings in this study contradict this suggestion: (1) AMF are obviously not reduced at the depth of 2.5 cm (Figure 3.16), and (2) the increase is displayed at depths 5.0 and 7.5 cm where AMF generally do not play a significant role (Burke & Hahn 2000, Burke et al. 2002a, Burke et al. 2002b, 2003). Both findings suggest that environmental conditions with respect to presence or absence of AMF should be comparable to those for Ni-amended cores, but without benomyl-treatment, which, however, is not the case. An additional factor becoming important in the long-term experiment could be the formulation of benomyl, i.e. the carrier material of unknown specificity for benomyl that accounts for about 50% of the total weight of the fungicide. The repeated application of benomyl could have resulted in different environmental conditions after the third growing season compared to those after the first. Still, this potential change does not influence Ni concentrations in soil, or in roots only treated with benomyl but not with Ni, and thus does not provide additional clues for the higher Ni concentrations in roots of cores treated with benomyl/Ni.

In most plants the Ni content in the plant parts is in the range of 1 to 10 g kg^{-1} dry weight. This range reveals significant differences between plant species in uptake and translocation processes (Marschner 1995), but also reflects Ni availability in the environment. In the experimental setup, Ni concentrations in shoots were generally around 1 g kg^{-1} in non-amended cores, but about 10-fold higher when Ni was amended to the cores ($F(3,44) = 34.75$, $p < 0.05$). Seasonal differences were small, and statistically not significant ($F(2,44) = 2.32$, $p > 0.05$). Ni uptake and translocation was highest during vegetative growth and declined slightly during the season. Thus, Ni amendment into soil

that increased the Ni concentration about 10-fold results in a comparable increase in root and shoot Ni concentration compared to non-amended controls (Figure 3.31).

When Ni concentrations in shoots were determined during senescence after one and three growing seasons, the Ni concentration in the shoots was significantly higher after the third year when Ni was amended to the cores (Figure 3.32). Plants growing on non-amended cores did not show statistically significant differences. This increase might be a function of adaptation processes by the plants which allowed them to tolerate and potentially translocate more heavy metals (McCabe & Otte 2000, Stoltz & Greger 2002) or a function of innate metal tolerance (Ye et al. 2003).

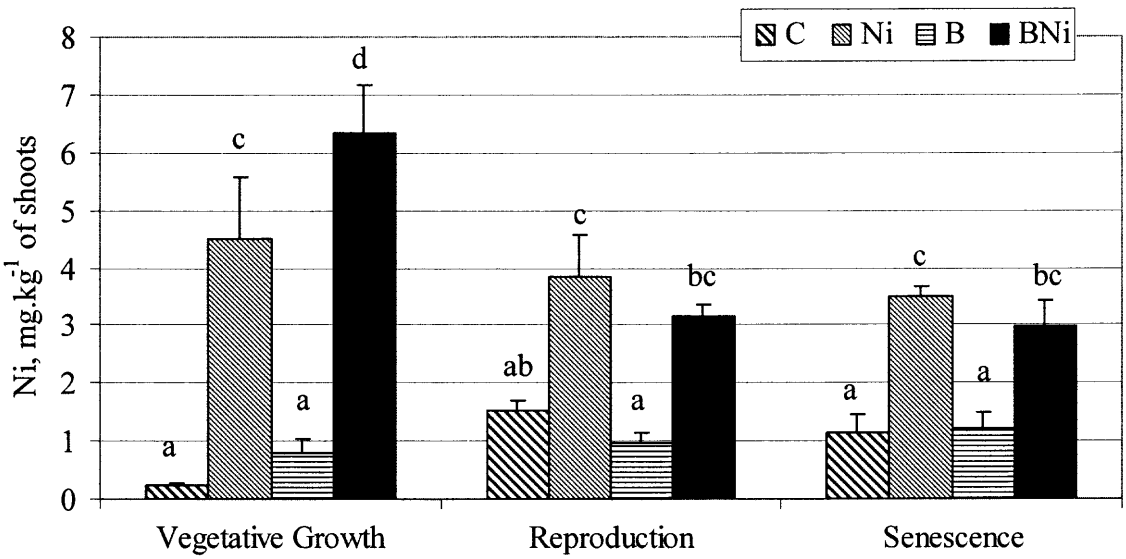

Figure 3.31 Ni concentrations in *S. patens* shoots of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during vegetative growth, reproduction, and senescence. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences between treatments ($p > 0.05$) as determined by Duncan's Test.

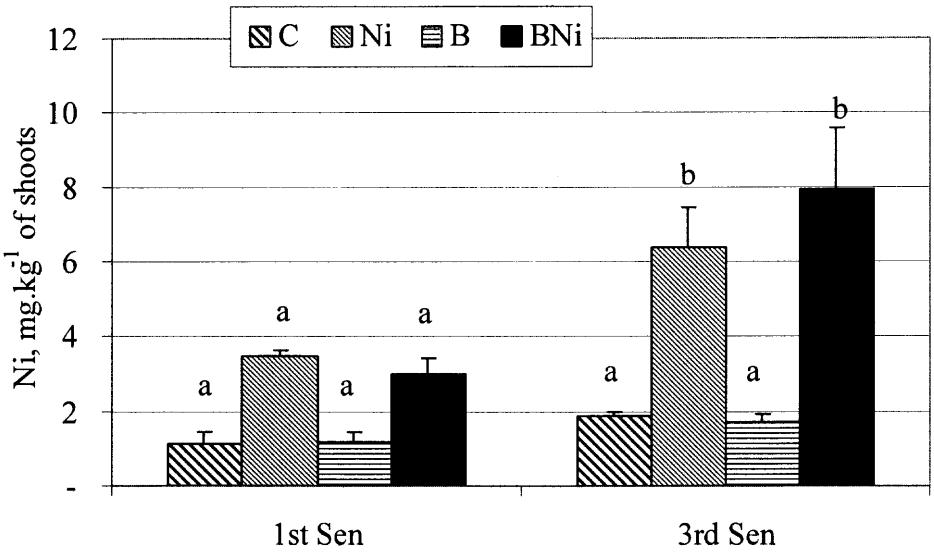

Figure 3.32 Ni concentrations in *S. patens* shoots of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences between treatments ($p > 0.05$) as determined by Duncan's Test.

Since samples had been collected from Harrier Meadow which is contaminated with moderate levels of metals, the higher Ni concentration may have selected for more resistant microbes or plants with higher transformation or uptake capabilities supporting the accumulation of Ni in both roots and shoots in Ni amended treatments after the third growing season. However, the higher concentration could also be a consequence of Ni already accumulated in the root cortex of *S. patens* after the third year, providing a larger pool for translocation into the shoots, and thus reflect a time relationship rather than plant physiological adaptations.

3.5.2 Analysis of Heavy Metals other than Ni (Cd, Cr, Cu, Fe, Mn, Pb, Zn)

Since soil cores of Harrier Meadow used in short- and long-term experiments were moderately contaminated with other heavy metals than Ni, additional analyses dealt with Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), and Zinc (Zn), and their fate in soil cores vegetated with *S. patens* and without treatment (C), and Ni addition (Ni), during senescence after one or three growing seasons. The analyses of Iron (Fe) and Manganese (Mn) were not available for the short-term experiment, but added in the long-term study.

3.5.2.1 Cadmium (Cd). In soil, Cd is a chalcophile, associating geochemically with Zn in sulfide minerals (McBride 1994). It has a high mobility in acidic soil in form of Cd^{2+}. Above a pH of 7.0, Cd^{2+} may co-precipitate with $CaCO_3$ or precipitate as $CdCO_3$ and Cd phosphates. However, in waterlogged soil, it may form CdS which displays a low mobility (McBride 1994). Generally, soils with Cd concentrations exceeding 0.5 g kg^{-1} are considered contaminated. Average concentrations of Cd in soil are reported to range from 0.06 to 1.1 ppm (McBride 1994). The concentration of Cd of experimental soil was from 1.0 to 6.5 $mg.k^{-1}$ which was moderately contaminated.

Cd concentrations in soil were found to be affected by depth ($F(2,117) = 13.12$, $p < 0.05$), treatment ($F(2,117) = 3.12$, $p < 0.05$) as well as plant phenological stages during the growing season ($F(2,117) = 20.86$, $p < 0.05$). With depth, concentrations generally declined significantly during vegetative growth only, and were not significantly different during the other phenological stages (Figure 3.33).

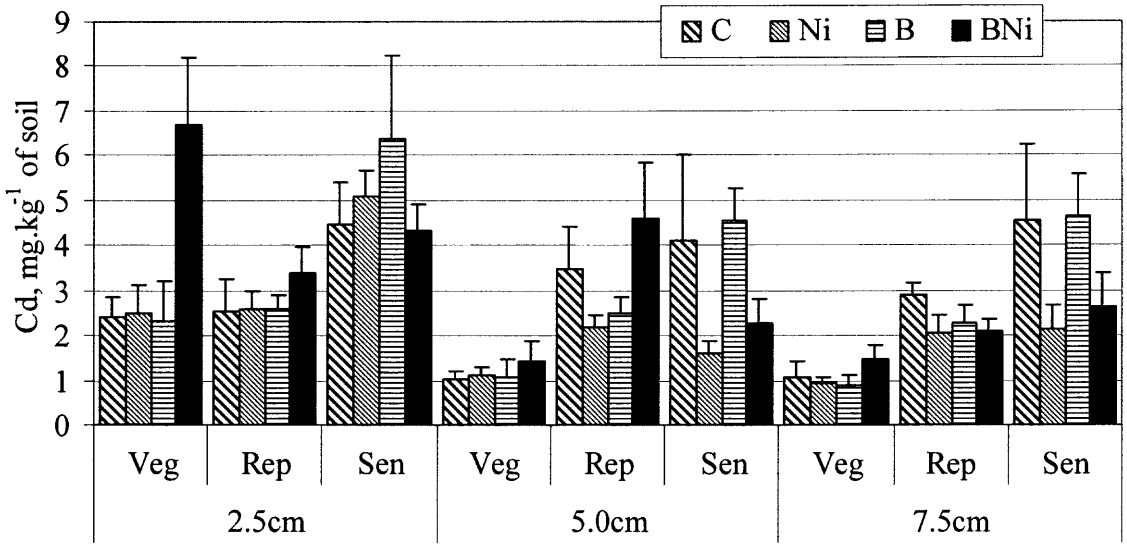

Figure 3.33 Cd concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

Compared to the untreated control, neither benomyl- nor Ni-amendment alone resulted in large and thus significant differences in the Cd concentrations, except for senescence where lower Cd concentrations were found in Ni-amended cores at lower depths, i.e. the depths of 5.0 and 7.5 cm (Figure 3.33). At this time, similarly low Cd concentrations were observed in benomyl/Ni-amended cores. During vegetative growth, benomyl/Ni-amended cores showed significantly higher Cd concentrations compared to those of the other treatments that were similar. These differences were only obtained at the upper depth of 2.5 cm, and reduced during the growing season. During the growing season, Cd concentrations increased in all treatments at all depths. These differences with treatment were no longer obtained after 3 years when Cd concentrations in soil were reduced to about 25% of the original values at all depths (Figure 3.34). These results

indicate that Ni amendment has an impact on Cd under reducing conditions during the first growing season, and that Cd can be removed from the system in time.

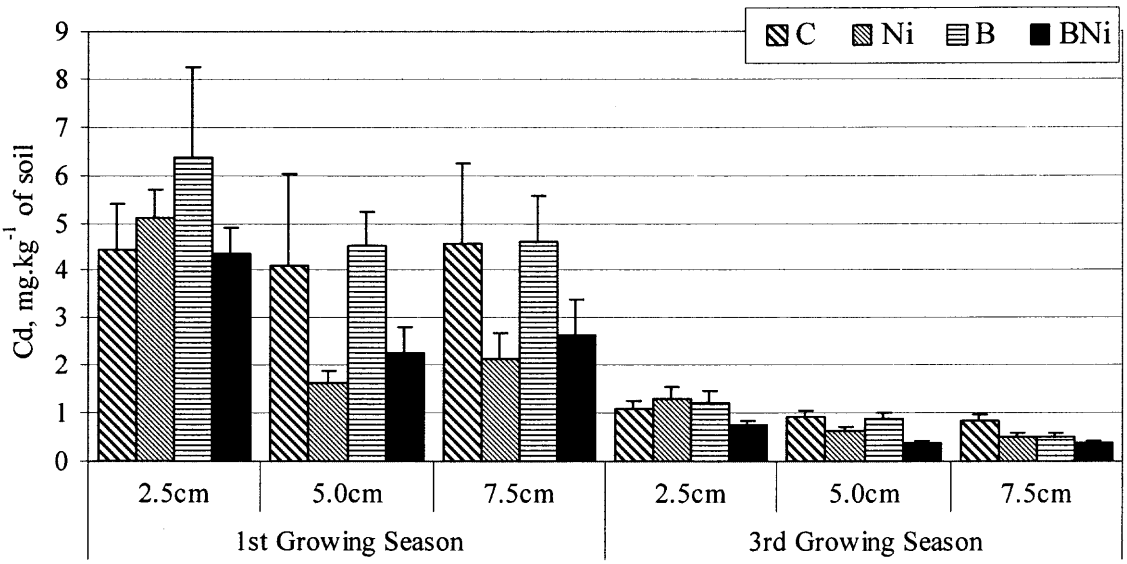

Figure 3.34 Cd concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

In the first growing season, lower Cd concentrations at lower depths in Ni-amended cores might be a function of low contents of organic material, and competition for adsorption sites on organic material of Ni and Cd that are supposed to be both completely available in solution based on MineQL+ calculations. In marine environments Cd generally exist as positively charged divalent ions, and thus can be adsorbed onto solid surfaces with negative electrical charges. The most important of these solids are organic matter, hydroxides of Fe and Mn, clays, and particles of biogenic carbonate minerals (Sadiq 1992). However, organic complexes of Cd in seawater are only moderately stable (Sadiq 1992). The binding of Cd on humic substances for seawater

displayed relatively low affinities compared to other ions such as e.g., Mg<Ca<Cd = Mn<Co<Zn = Ni<Cu<Hg (Mantoura et al. 1978).

Although in this study some Ni may precipitate in the suboxic layer, it might compete with Cd in the anoxic portion of the cores, and thus Cd mobility in the cores likely affected by Ni addition at the lower depths. This phenomenon would be less pronounced at higher organic matter content at a depth of 2.5 cm or at 0.7 cm where organic matter content is highest. The latter could be cause for the high Cd concentrations during vegetative growth at the depth of 2.5 cm where cumulative effects of benomyl- (and its formulation) and Ni-amendment to the top of the core could have resulted in a Cd release, downward movement and thus relative accumulation at the 2.5 cm depth. Removal in time, i.e. during the first growing season and after the third growing season, could then be caused by either uptake through the plant, but more likely by accelerated leaching due to changes in mobility.

None of the treatments had an effect on Cd uptake into the roots ($F(2,101) = 1.44$, $p > 0.05$), but depth ($F(2,101) = 7.35$, $p < 0.05$) and phenological stages ($F(2,101) = 36.25$, $p < 0.05$) influenced Cd concentration (Figure 3.35). At lower depths (i.e. at depths of 5.0 and 7.5 cm) generally higher concentrations of Cd were found in the roots, and values declined to about 50% in the long-term experiment (Figure 3.36). During reproduction, Cd uptake was generally highest, with slightly lower values found initially during vegetative growth and later during senescence (Figure 3.35).

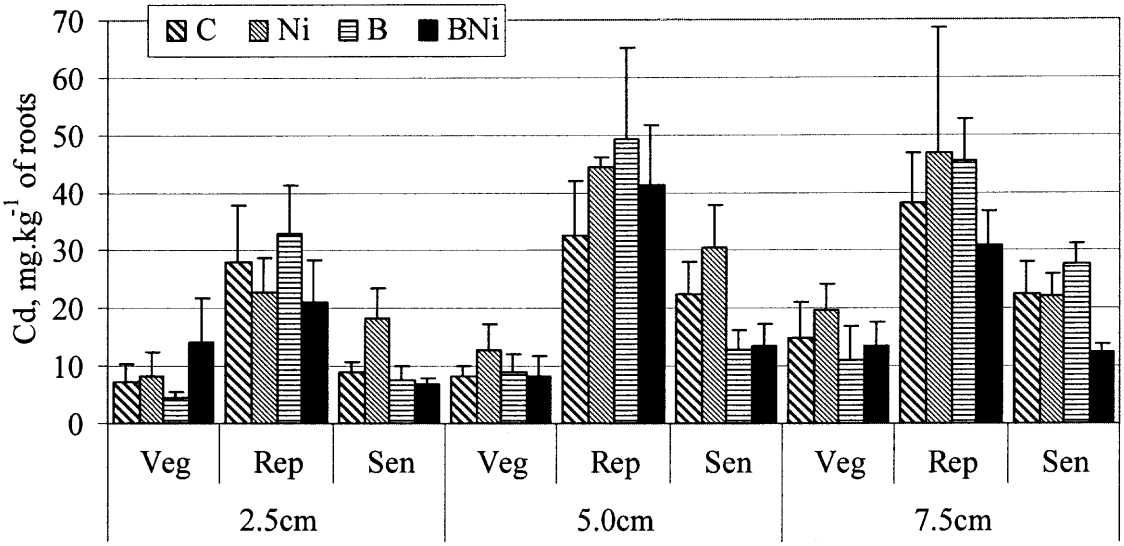

Figure 3.35 Cd concentrations in roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

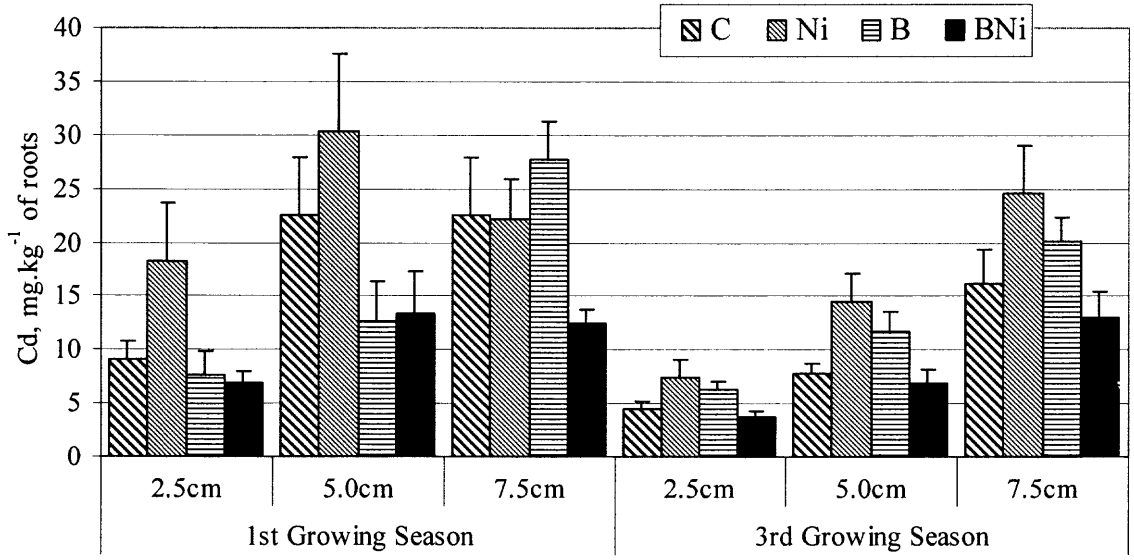

Figure 3.36 Cd concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

Although none of the treatments showed a significant effect on the Cd uptake, the concentrations in roots at the 7.5 cm depth of the benomyl/Ni-treatment was lower than that of the other treatments at senescence, both after short- and long-term observations. Lower Cd concentrations in roots at this time are related to lower concentrations in soil, thus potentially a consequence of lower Cd availability.

Overall, the addition of Ni, and even more pronounced, the addition of benomyl and Ni together, might have resulted in increased release of adsorbed Cd in these treatments, and thus increased uptake into plant roots. In time, however, leaching due to increased mobility resulted in lower Cd concentrations in soil, and as a consequence lower Cd uptake through the roots.

Translocation of Cd from roots to shoots was not significantly different between treatments during vegetative growth and reproduction, but lower in Ni-amended cores during senescence in the short-term experiment (Figure 3.37). After the third growing season, values in all treatments had declined to about 20% and less of the original values. Thus, although Cd is removed from the soil, taken up and translocated in the plant, it seems unlikely that plant uptake is the major cause for the reduction in Cd concentrations in soil. Since Cd does not thermodynamically precipitate, it is more likely that a significant part has leached out of the soil cores. An accurate confirmation of this statement, however, requires more attention in future research.

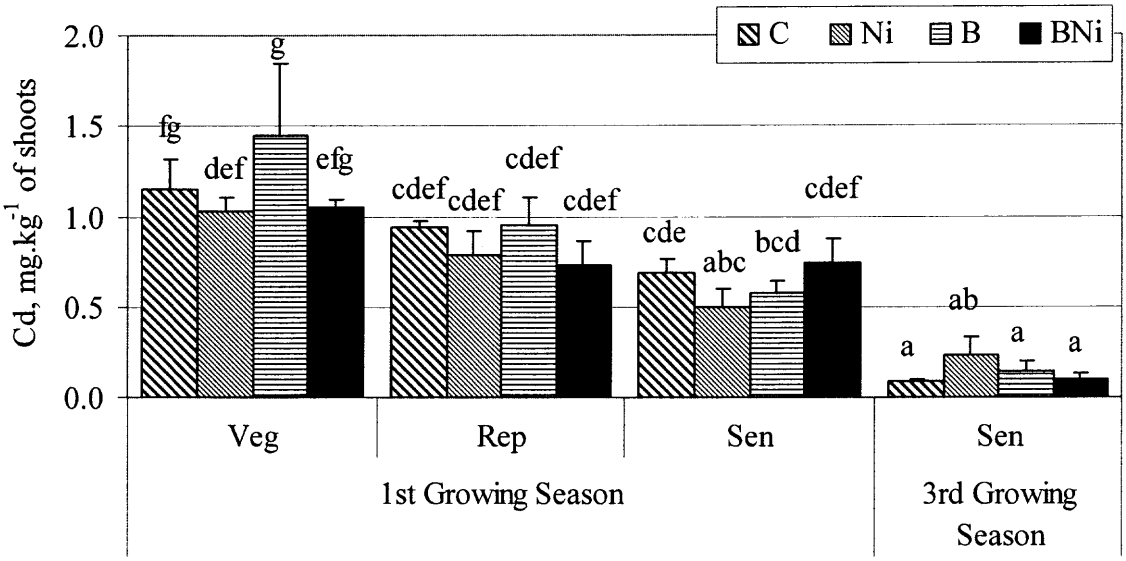

Figure 3.37 Cd concentrations in *S. patens* shoots of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences between treatments ($p > 0.05$) determined by Duncan's Test.

Cd can be delivered to plants cells by metal transporters with broad substrate specificitites and probably also via Ca channels (Polle & Schützendübel 2003). Cd uptake at the root surface has been characterized in a number of plant species, including wheat (Smeyers-Verbeke et al. 1978), corn (*Zea mays*) (Florijn & Van Beusichem 1993), and barley (*Hordeum vulgare*) (Cutler & Rains 1974) as concentration-dependent process exhibiting saturable kinetics controling influx of Cd (Mullins & Sommers 1986). The saturable nature of Cd uptake in these studies suggested that Cd might be taken up via a carrier-mediated system. In most plant species Cd is accumulated in the roots, even though translocation to the shoot may vary considerably depending on the plant species (Larsson et al. 2002). Translocation of Cd from roots to shoots is likely to occur via the xylem and to be driven by transpiration from the leaves (Salt et al. 1995). Values accounting for 2 to 20% of the Cd concentration in the roots were found to be

translocated to the shoots in different plant species (Larsson et al. 2002) with shoot Cd levels usually smaller than 1 mg kg^{-1} (Baker et al. 1994). This value essentially represents those found on average in shoots of *S. patens* in all treatments and accounts for about 5% of the Cd accumulated in roots. Thus, Cd levels in roots and shoots are in a comparable range as those of other non-hyperaccumulative plants.

3.5.2.2 Chromium (Cr). In soils, Cr commonly occurs in the +3 (chromic) oxidation state as the Cr^{3+} cation, and in the +6 oxidation state as chromate, CrO_4^{2-}. However, soil conditions generally favor the Cr^{3+} form, a very immobile cation that complexes strongly with organic matter and chemisorbs on oxides and silicate clays, even at quite low pH (McBride 1994). Soil Cr concentrations range worldwide from 7 to 221 ppm, with lower concentrations generally depicted in soils from the US (20 to 85 ppm).

In the experimental setup, the Test of Between-Subject Effects shows that phenological stages ($F(2,117) = 4.57$, $p < 0.05$), depth ($F(2,117) = 20.23$, $p < 0.05$) and treatment ($F(3,117) = 3.70$, $p < 0.05$) influence Cr concentrations in soil (Figure 3.38). Similar to Cd, Cr concentrations in soil significantly declined with the depth, and were significantly lower after the third growing season (Figure 3.39). Ni amended cores usually displayed lower Cr concentrations in soil than non-treated cores, with largest differences under anoxic conditions (i.e. at depths of 5.0 and 7.5 cm) with values about 50% lower than in the non-treated cores ($F(3,117) = 3.70$, $p < 0.05$). Studies on the fate and transport of metals in the Dnieper water body showed that dissolved forms of heavy metals (Cd, Cr, Zn, Cu and Pb) occurred mainly absorbed to DOM. The proportion of adsorbed metals reached 70% to 90% of the total concentration of their dissolved forms (Linnik 2003). Since at these depths low organic carbon concentrations (3% and 1%,

respectively) which is believed to play an important role in the adsorption of Cr (Sadiq 1992) were present, competition for adsorption between Ni and Cr, similar to Cd, might be a prominent reason for this effect.

Additional Ni essentially replaces adsorbed Cr on organic material and releases it so that it can leach out. Declines in Cr concentrations in time, however, were much lower than concentrations of Cd. Benomyl application did not change this pattern. Cr can also be adsorbed onto sediment surfaces in marine environments. (Sadiq 1992) postulated that $FeCr_2O_4$ is the most stable and insoluble solid phase of Cr that may regulate Cr concentrations in seawaters and interstitial waters.

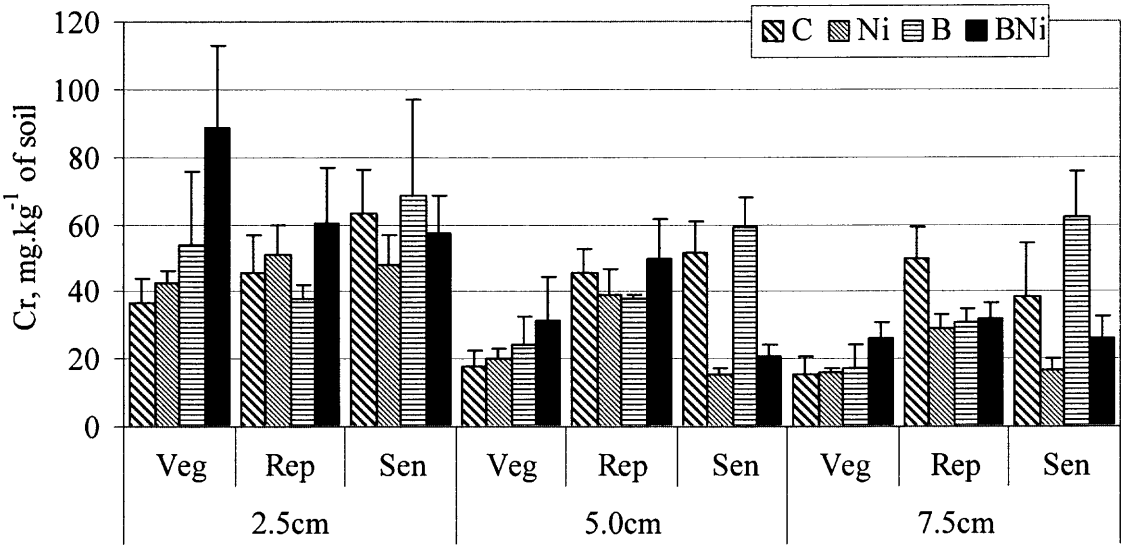

Figure 3.38 Cr concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

After the third growing season, differences between treatments in soil concentrations of Cr had essentially disappeared (Figure 3.39). However, concentrations

had decreased in the upper 2.5 cm to about 30% of that found after the first season. Ni amended cores displayed slightly higher concentrations at the lower depths than after the first season, while those of the controls had decreased to a similar level. Ni amendment resulted in higher Cr uptake in the roots (Figure 3.40). However, these differences were often not statistically significant. Higher uptake was also obtained with depth, and after the third growing season where concentrations in the roots reached about 3-4 times higher concentrations than after the first growing season (Figure 3.41).

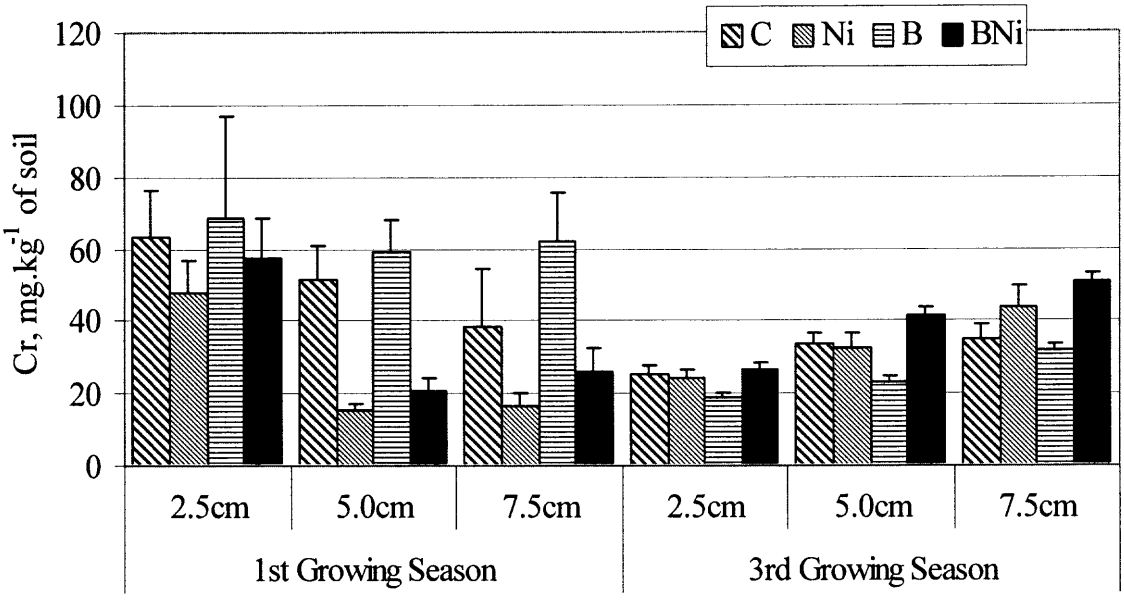

Figure 3.39 Cr concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

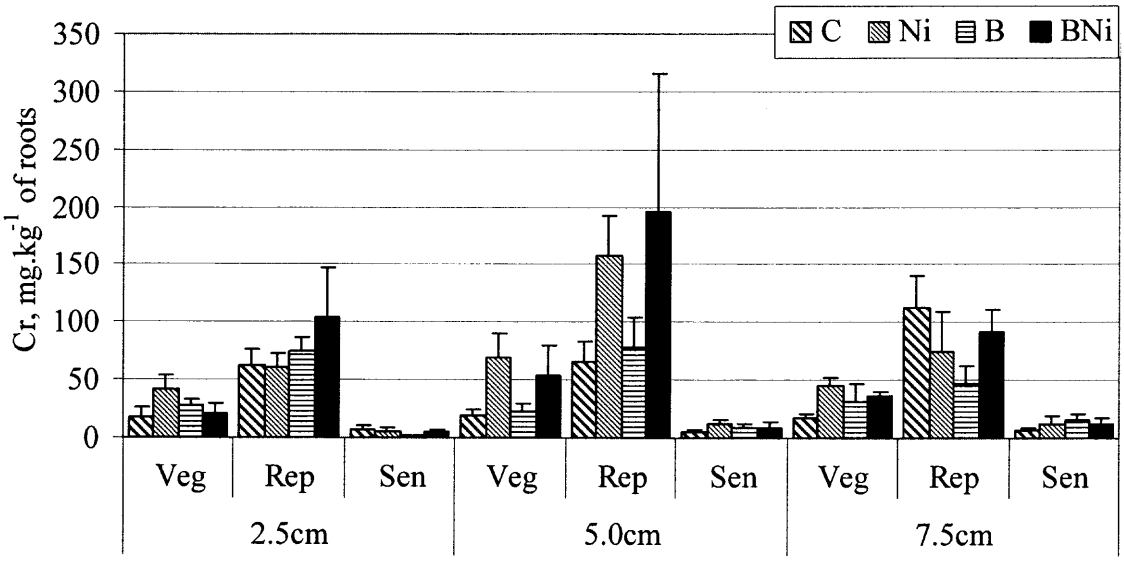

Figure 3.40 Cr concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

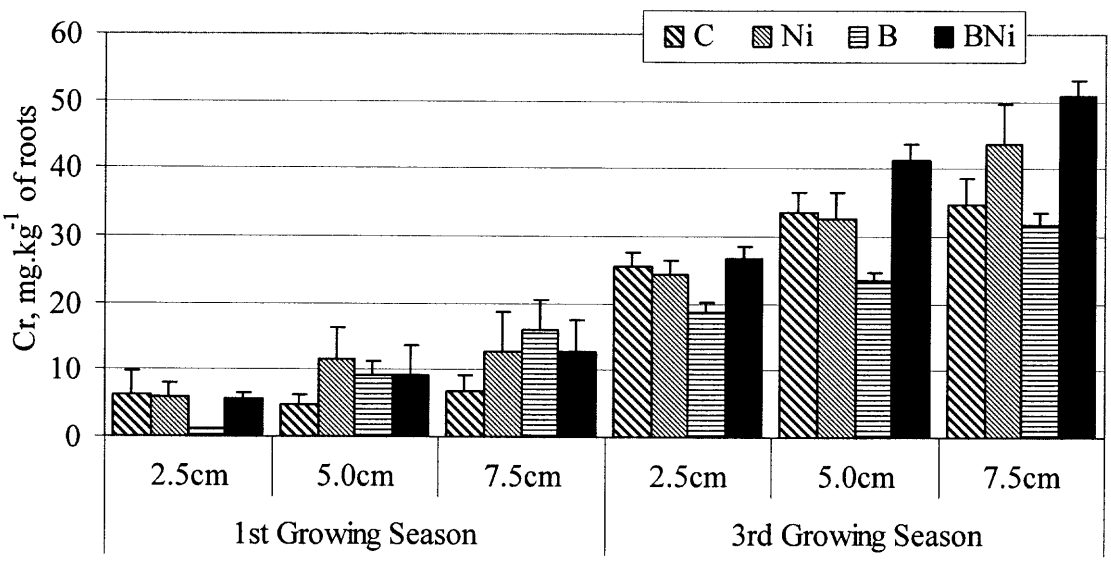

Figure 3.41 Cr concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

Large differences between treatments were not found after the third growing season, suggesting no long-term effect of Ni amendment on Cr uptake and accumulation in the roots. Since Cr accumulation in roots increased in time with decreasing Cr concentration in soil, active uptake mechanisms by the plant similar to those suggested for Cd might be postulated. Cr translocation across cell walls and plasma membranes has been reported to be performed via sulfate/phosphate channels and translocation is concomitant with reduction reactions in which Cr(VI) is reduced to Cr(III) and thus to a considerably less toxic form (Howe et al. 2003).

Detoxification involves immobilization and compartmentalization of Cr(III) by processes such as precipitation of Cr(III) hydroxides at the root, the complexation of Cr(III) with organic material and its storage, and the transport of Cr(III)-organic complexes to leaf margins where Cr is meant to be less disruptive to plant metabolic processes (Howe et al. 2003). However, most of the Cr in the cores is meant to be present as Cr(III) according to MINEQL+ calculations, and thus not really available for plant uptake (Han et al. 2004).

Translocation of Cr into shoots was small at all times, and generally not significantly different between treatments (Figure 3.42). After the third growing season, Cr concentrations were insignificantly higher in shoots than after the first season, corresponding to higher concentrations in roots at that time. Retention of Cr in roots, and only small translocation into shoots has been reported previously (Cary et al. 1977). These results were independent of speciation and complexation with organic material (Cary et al. 1977). Although there are significant differences in the degree of tolerance, uptake and translocation of Cr among plants (Shahandeh & Hossner 2000), Cr

concentrations in shoots and leaves of many plants are below 1 mg kg^{-1} over a wide range of Cr concentrations in soils (Losi et al. 1994).

For Indian mustard (*Brassica juncea*), a hyperaccumulator which generally accumulate metals at levels 100-fold greater than those tropically contain in shoots of the common nonaccumulator (Lasat 2002), leaf and stem concentrations of Cr reach up to 12 mg kg^{-1} when Cr concentrations in soil are 500 mg kg^{-1} (Han et al. 2004).

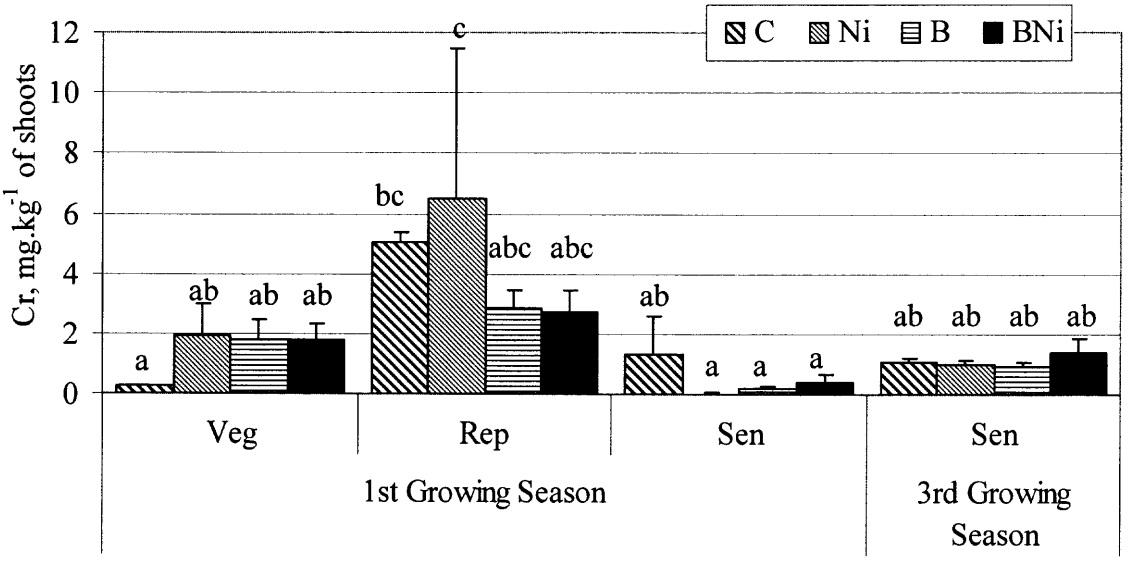

Figure 3.42 Cr concentrations in *S. patens* shoots of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent Insignificant differences between treatments ($p > 0.05$) as determined by Duncan's Test.

At lower soil Cr concentrations, Cr concentrations in leaves essentially resemble those in shoots of *S. patens* grown in the soil cores with an average Cr concentration of 50 mg kg^{-1}. The values of 3-4 mg Cr kg^{-1} shoot material, however, are comparable to those obtained with *B. juncea* on non-contaminated soil (Han et al. 2004). Uptake into roots generally resulted in 2-3 times higher concentrations of Cr (10-20 mg kg^{-1}) which was in the same range as detected in the study. Thus, *S. patens* cannot be considered as

hyperaccumulator of Cr, at least not at the relatively low concentrations of Cr found in the cores. At higher concentrations; however, significantly more accumulation might potentially be detected, similar as with Cr in *Brassica juncea* (Han et al. 2004).

3.5.2.3 Copper (Cu). Cu occurs in soil solids and solutions almost exclusively as the divalent cation Cu^{2+}. However, reduction of Cu^{2+} (cupric) to Cu^{+} (cuprous) and Cu^{0} (metallic copper) is possible under reducing conditions, especially if halide or sulfide ions are present to stabilize Cu^{+} (McBride 1994). For soils with high Cu concentrations, precipitation as cupric hydroxide, oxide, or hydroxyl-carbonates has been observed. Soil Cu concentrations range worldwide from 6 to 80 ppm, with concentrations in the US ranging from 14 to 29 ppm. Similar to Cd and Cr in the setup, Cu concentrations in soil changed in time ($F(2,117) = 3.32$, $p < 0.05$) and with depth ($F(2,117) = 9.34$, $p < 0.05$), but were not affected by Ni amendment ($F(3,117) = 0.08$, $p > 0.05$) (Figure 3.43).

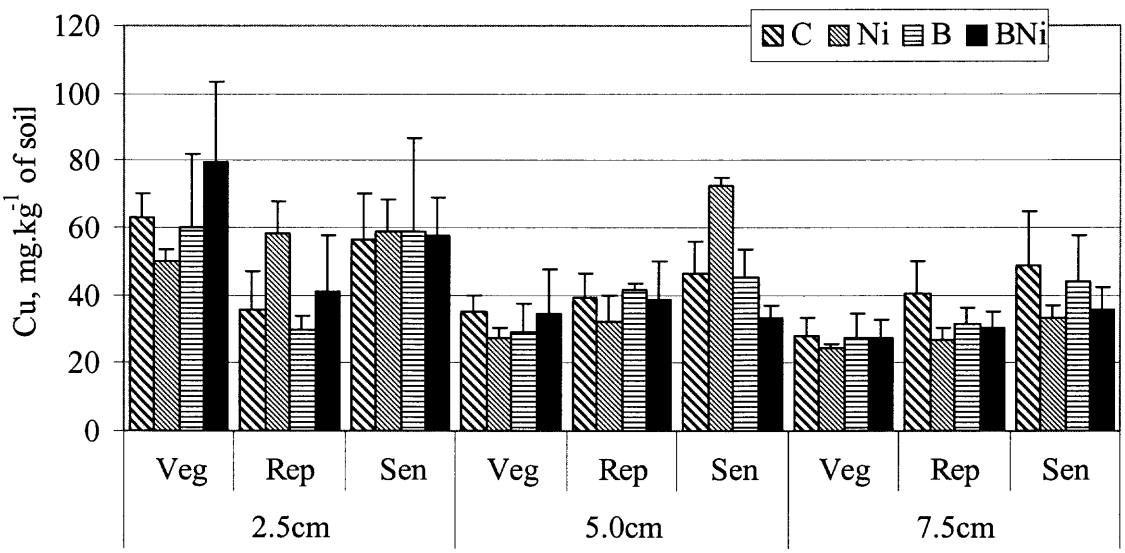

Figure 3.43 Cu concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

According to the MineQL+ calculations, most of Cu should precipitate under suboxic conditions as cupric ferrite and under anoxic conditions as tsumebite and $Cu_3(PO_4)_2$ depending on the pH, and thus be present in sparingly soluble forms under the conditions applied. Since cupric ferrite ($CuFe_2O_4$) contains Fe(III), its solubility will be influenced by the redox conditions of marine systems (Sadiq 1992). Moreover, binding of Cu on humic substances should reduce potential ions as suggested for seawater due to its high binding affinity to organic material in the sequence Mg<Ca<Cd = Mn<Co<Zn = Ni<Cu<Hg (Mantoura et al. 1978). This expectation is supported by the only slight, statistically non-significant differences for Cu concentrations in soil after the third growing season (Figure 3.44).

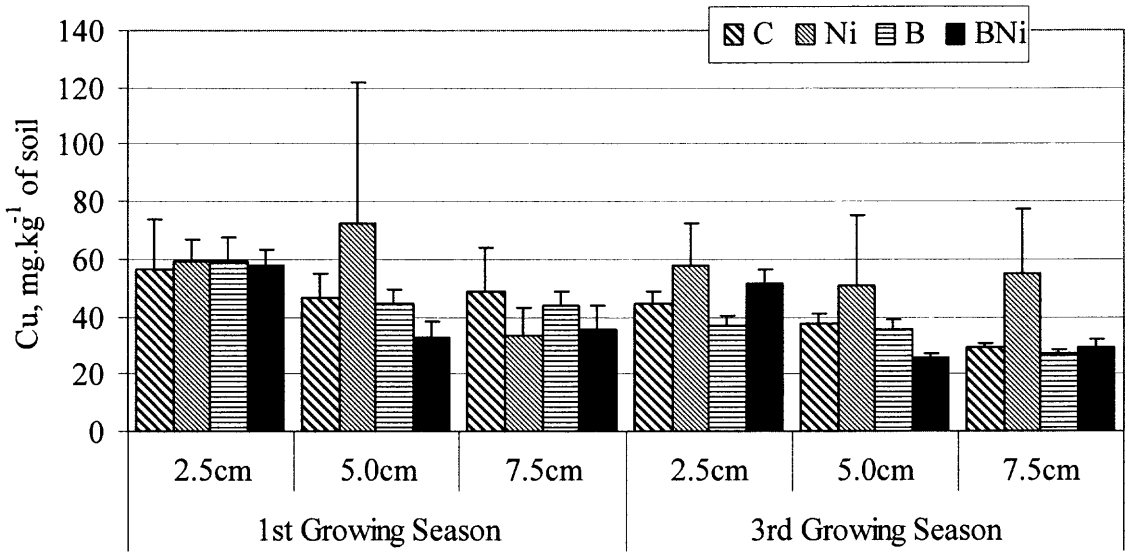

Figure 3.44 Cu concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

A similar pattern of Cu concentrations was found for roots with only small, statistically usually non-significant differences between treatments with depth and in time (Figure 3.45). However, seasonal differences with highest uptake during reproduction were noted ($F(2,98) = 86.71$, $p < 0.05$) (Figure 3.45). An exception with higher Cu concentrations was found in the roots at the depth of 7.5 cm after Ni amendment compared to that of the control after the third growing season ($F(11,112) = 11.03$, $p < 0.05$) (Figure 3.46). After the third growing season (i.e. at the plant phenological stage of senescence), Cu translocation into shoots is significantly higher than after the first growing season (Figure 3.47).

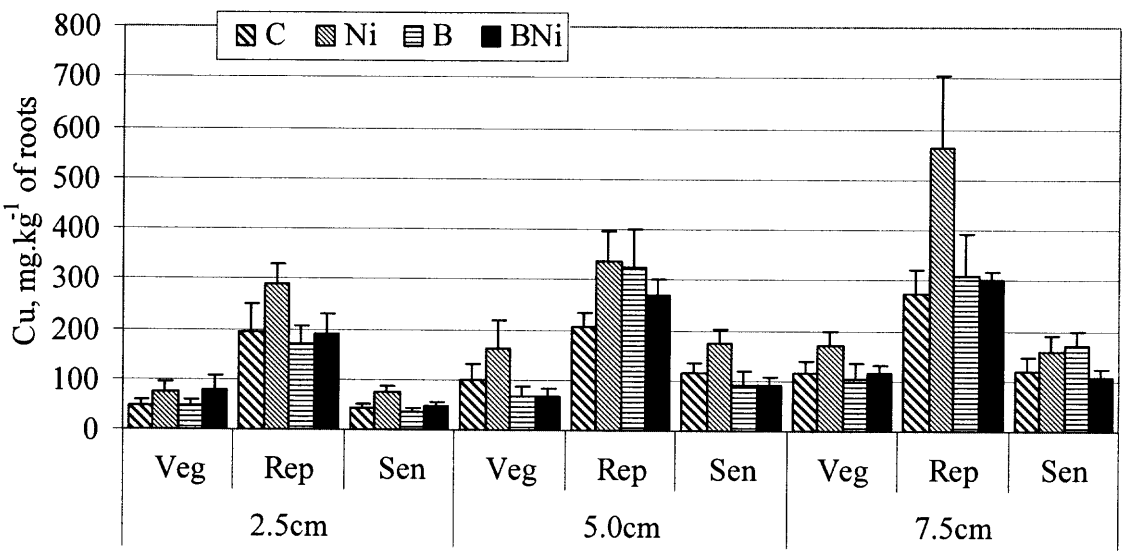

Figure 3.45 Cu concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

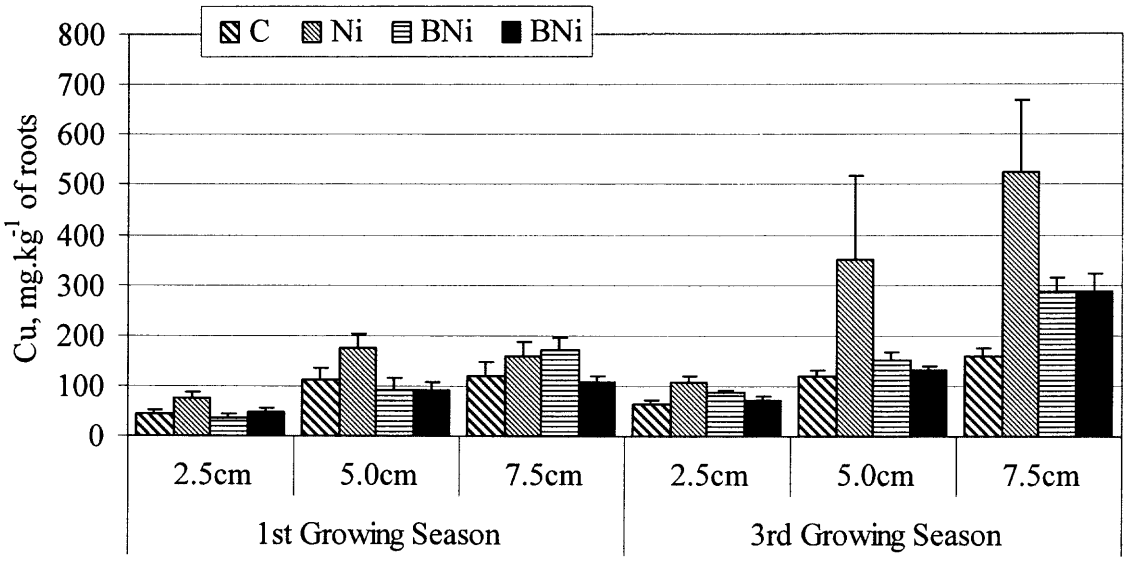

Figure 3.46 Cu concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

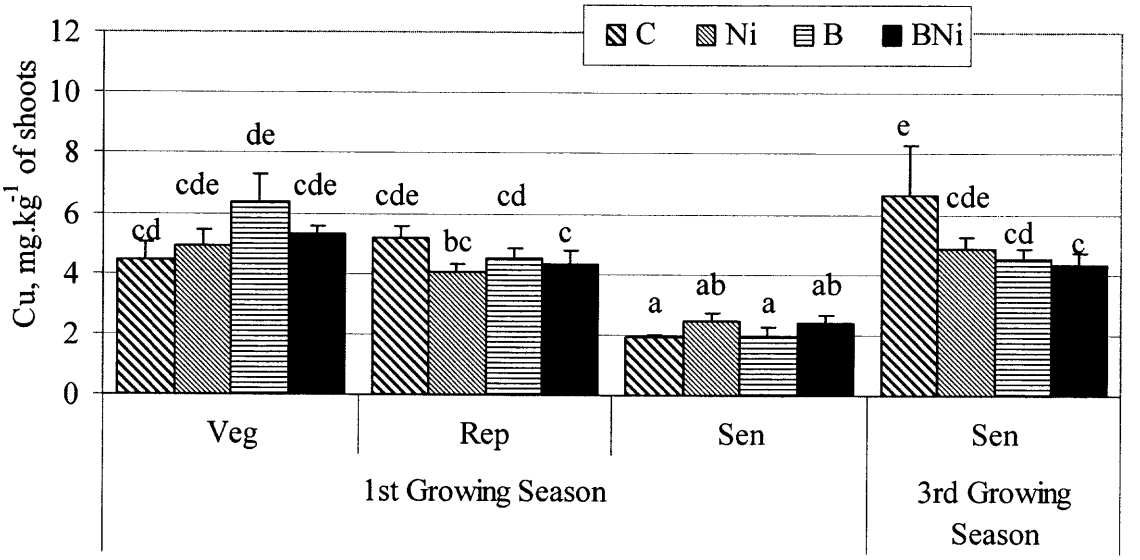

Figure 3.47 Cu concentrations in *S. patens* shoots of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences between treatments ($p > 0.05$) as determined by Duncan's Test.

No significant differences were obtained between treatments. Values during senescence after the first growing season, however, were much smaller than during vegetative growth and reproduction. Since no data for these plant phenological stages are available for the third growing season, clear statements on potential effects of long-term exposure on Cu uptake cannot be drawn without additional experimental proof. Cu concentrations above 20-30 mg kg^{-1} dry shoot weight represent the critical toxicity level for most crop species (Marschner 1995). This level is still 2-5 times higher than the concentration of Cu found in leaves of *S. patens* at any time during the short- and long-term studies. Cu in crop plants such as tomato is generally accumulated in roots with values similar to those found in *S. patens*, i.e. up to 250 mg kg^{-1} dry weight (Rahimi & Bussler 1974). Up to 60% of this Cu might be bound to the cell wall fraction and the cell wall-plasma membrane interface (Marschner 1995). Translocation into shoots is about 2 orders of magnitude lower, again similar to results in this study, and thus highly restricted. Concentrations of Cu in roots increase proportional to the availability in soil, and thus the concentration in roots and not that in shoots needs to be considered for toxicology assessments.

3.5.2.4 Lead (Pb). Pb principally occurs in the +2 oxidation state in soils. Under the oxidizing conditions, the Pb^{2+} ion becomes less soluble as soil pH is raised (McBride 1994). Complexation with organic matter, chemisorption on oxides and silicate clays, and precipitation as carbonate, hydroxide, or phosphate are all favored at higher pH. Soil Pb concentrations generally range from 10 to 84 ppm, with values for the US often found between 17 and 26 ppm (McBride 1994).

Similar to Cd and Cr, Pb concentrations in soil cores declined with depth ($F(2,115) = 115$, $p < 0.05$) (Figure 3.48), and were significantly lower after the third growing season (Figure 3.49). Ni amended cores usually displayed lower Pb concentrations in soil than controls with largest differences under anoxic conditions (i.e. at 5.0 and 7.5 cm depths of the senescence of the first growing season) with values about 50% lower than in the controls (Figure 3.49). Ni amendment resulted in higher Pb uptake into roots (Figure 3.50); however, these differences were often not statistically significant except for higher concentrations in roots at the depth of 7.5 cm during senescence after both growing seasons (Figure 3.51).

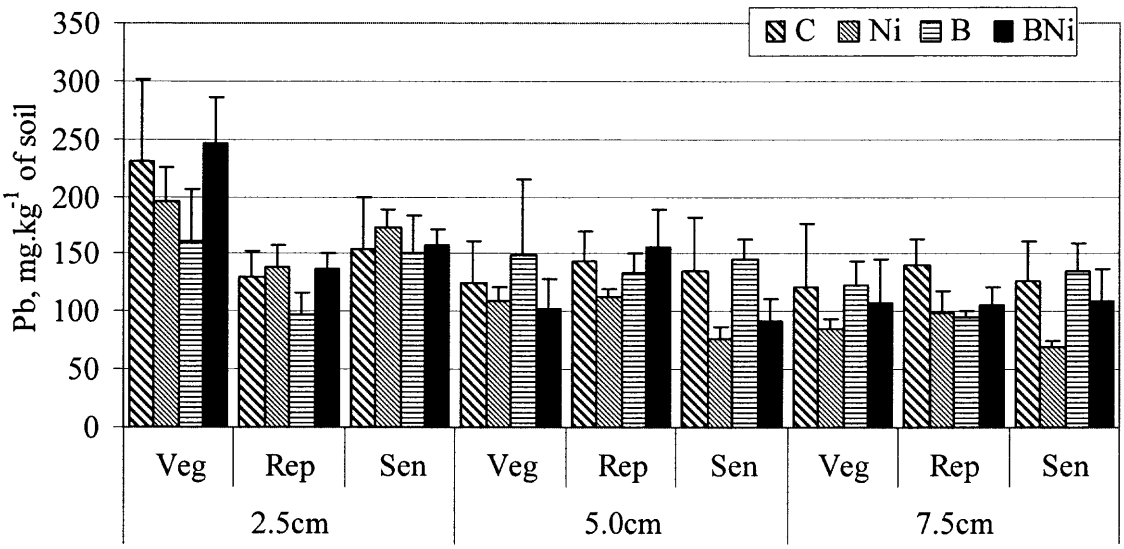

Figure 3.48 Pb concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

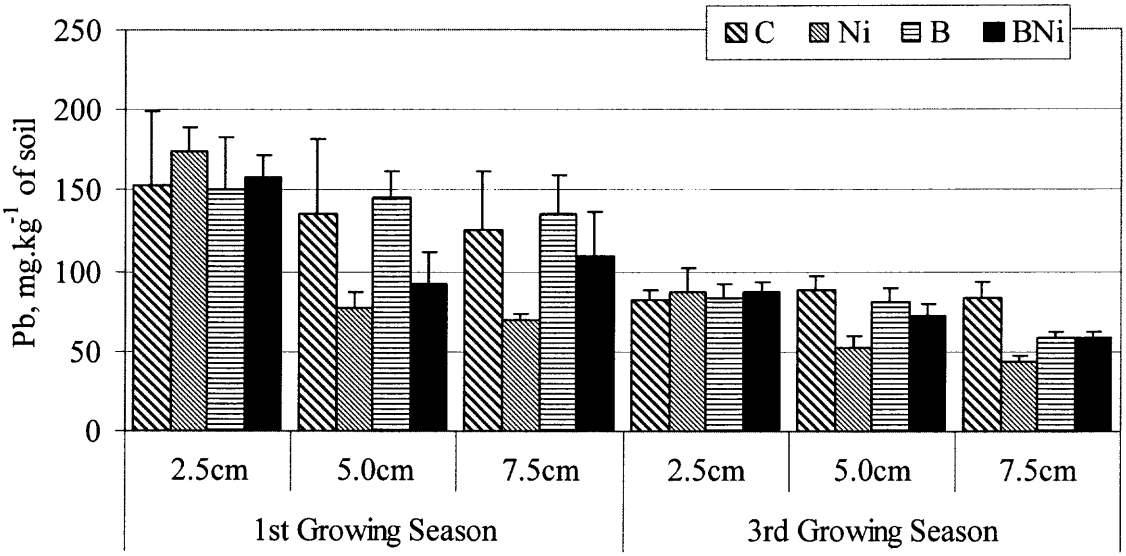

Figure 3.49 Pb concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

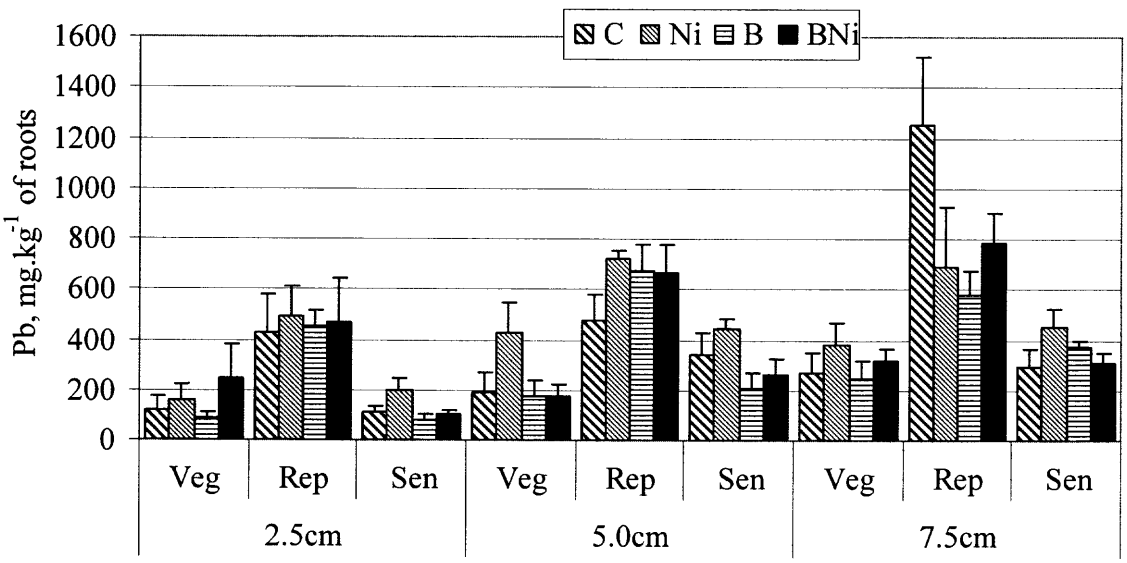

Figure 3.50 Pb concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

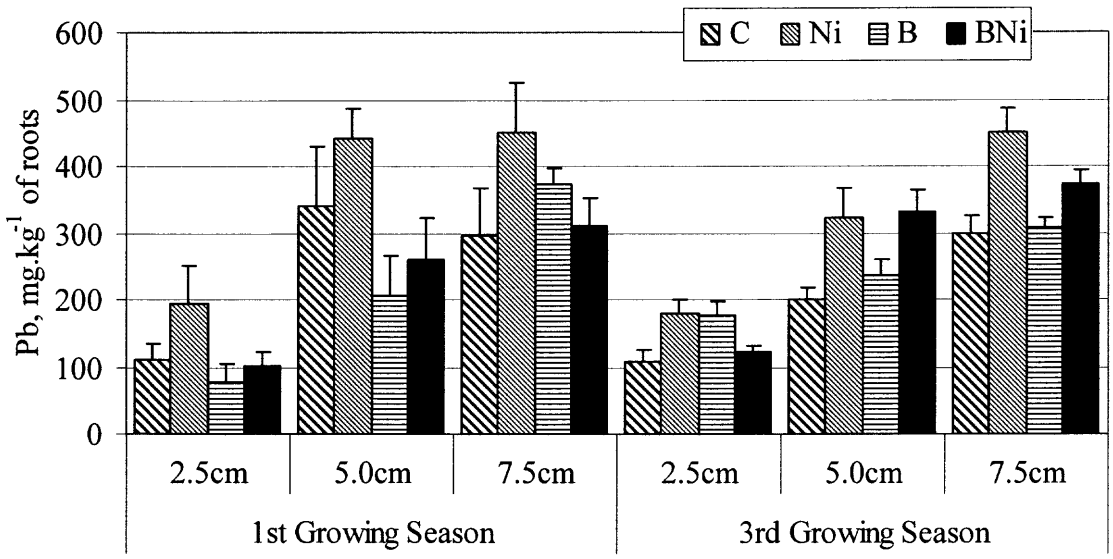

Figure 3.51 Pb concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

Generally, higher Pb uptake was obtained with depth even though concentrations had declined after the third growing season where concentrations in roots reached between equal and about 30% lower concentrations than in the first growing season. Differences were generally not statistically significant. Large differences between treatments were not found after the third growing season, suggesting no long-term effect of Ni amendment on Pb uptake and accumulation in the roots.

The availability of Pb in soil is meant to be very limited for plant uptake since pyromorphyte ($Pb_5(PO_4)_3Cl$) will be formed under oxic conditions as the most important Pb-containing mineral while galena (PbS) will be the most stable and common Pb-containing mineral under anoxic conditions (Nriagu 1984). Pb availability has therefore been enhanced by the addition of chelators such as EDTA leading to increased uptake and translocation (Blaylock et al. 1997, Piechalak et al. 2003). Translocation of lead into

shoots was not influenced by Ni amendment during the first ($t(8) = 1.45$, $p = 0.66$) and the third ($t(8) = -1.54$, $p = 0.10$) growing seasons (Figure 3.52).

The highest Pb concentrations were measured during vegetative growth confirming previous assumptions of impacts of growth stages and seasonal variation on heavy metal uptake (Fitzgerald et al. 2003). These values declined slightly during the first growing season, and stayed low with a reduction of about 60% after the third growing season (Figure 3.52).

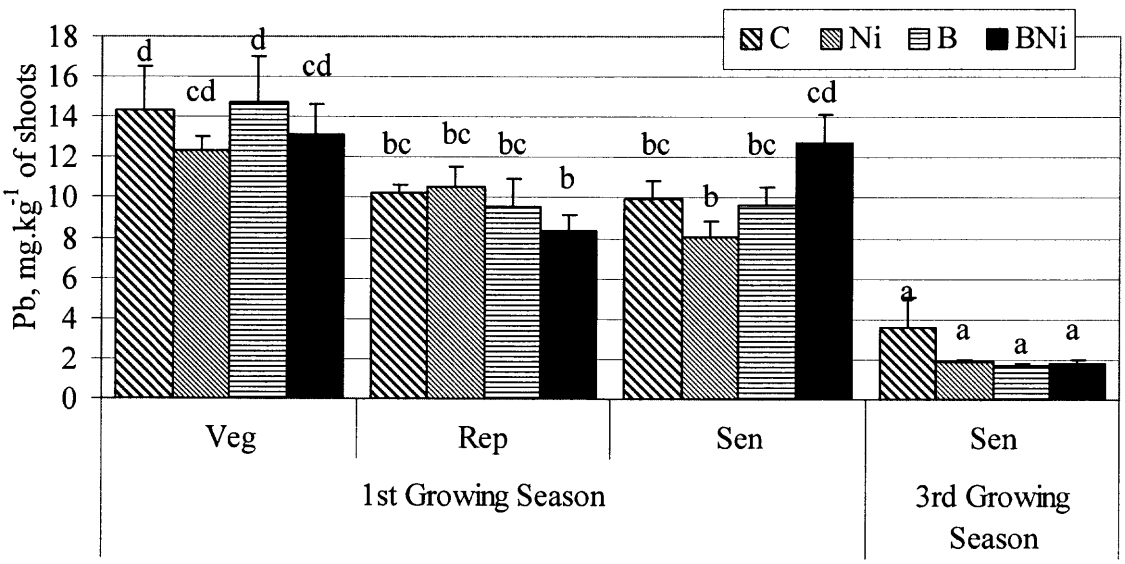

Figure 3.52 Pb concentrations in *S. patens* shoots of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences between treatments ($p > 0.05$) as determined by Duncan's Test.

Since Pb uptake into roots is comparable after the first and third growing season, the much lower translocation into shoots after the third season might be related to long-term adsorption of Pb to root cell walls rather than decreased translocation into shoots.

Translocation of Pb in plants is limited, with most Pb staying within the root cell walls (Mace et al. 1997). For salt marsh plants, a clear partitioning of Pb within the plant in the case of monocotyledons was found with much higher concentrations of Pb in the roots of *Agrostis stolonifera, S. tabernaemontani* and *Spartina* spp. (Fitzgerald et al. 2003). Only in *P. australis*, Pb and Cu were partitioned fairly evenly between roots and shoots while in the dicotyledon *P. maritima,* Pb accumulated mainly in the shoots confirming other studies that found higher accumulation of metals in the shoots of dicotyledons (Rozema et al. 1985, Otte et al. 1991).

Pb concentrations up to 600 mg kg^{-1} in *S. partens* roots and up to10 mg kg^{-1} in its shoots are far below values obtained for hyperaccumulators such as, for example, cabbage that might reach concentrations of up to 5,000 mg kg^{-1} in shoots (Shen et al. 2002). Generally, both values for roots and shoots are in the range of those encountered in other plants (Kovacheva et al. 2000).

3.5.2.5 Zinc (Zn). As a chalocophile, Zn is often found as the sulfide mineral sphalerite (ZnS) in rocks, which weathers to release the soluble Zn^{2+} ion under oxidizing conditions into soils. The +2 oxidation state is the only one possible in the soil (McBride 1994). Zn has a medium mobility, because Zn^{2+} is held exchangeable on clays and organic matter. At higher pH, chemisorption on oxides and aluminosilicates and complexation with humus, however, lower the solubility of Zn^{2+} significantly. Under reducing conditions, release of Zn^{2+} from dissolving Fe oxides may initially increase availability. Soil Zn concentrations generally range from 17 to 125 ppm, with values for the US between 34 and 84 ppm (McBride 1994).

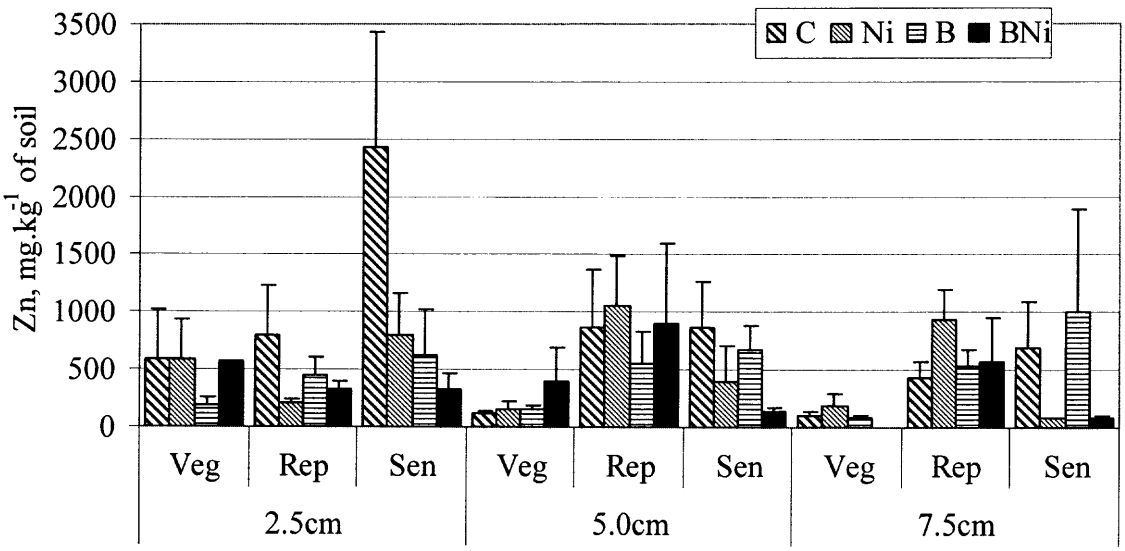

Figure 3.53 Zn concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

Zn concentrations in soil cores were not affected by phenological stages ($F(2,104) = 3.00$, $p > 0.05$), depths ($F(2, 104) = 1.08$, $p > 0.05$), or treatments ($F(3.104) = 1.53$, $p > 0.05$) during the first growing season (Figure 3.53). Ni amendment reduced Zn concentrations during senescence at all depths during the first season, but these differences were not displayed after the third growing season when concentrations, however, were much lower than during the first season (Figure 3.54). Zn concentrations in roots were not affected by Ni amendment, except for the lowest depth (7.5 cm) where Ni amendment resulted in higher Zn uptake both in the short- as well as the long-term experiment ($F(11,116) = 5.42$, $p < 0.05$) (Figure 3.55).

Uptake into roots was not significantly different between short- and long-term studies. However, Ni addition to cores resulted in higher Zn concentrations in roots at a

depth of 7.5 cm during senescence of the third growing season (Figure 3.56). In shoots, Zn concentrations were not significantly different between treatments during vegetative growth ($t(4) = 0.55$, $p = 0.61$), reproduction ($t(8) = 2.63$, $p = 0.14$) and senescence ($t(8) = -0.09$, $p = 0.93$) of the first growing season and senescence ($t(8) = 1.26$, $p = 0.24$) of the third growing season (Figure 3.57). Values for Zn concentrations during the first year, however, are extremely high, and exceed those of other studies by at least 2 orders of magnitude (Marschner 1995, Linnik 2003).

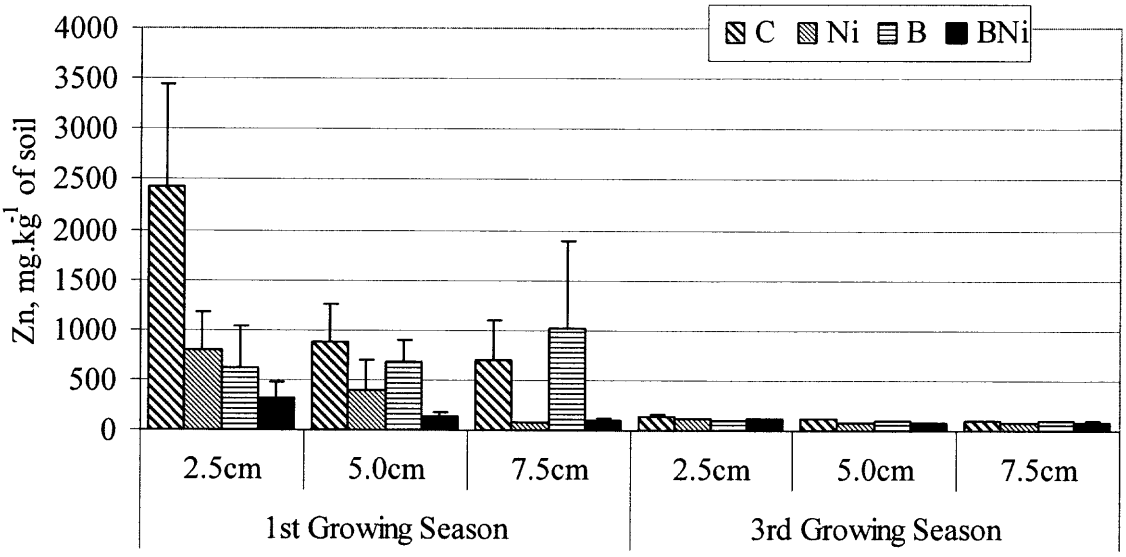

Figure 3.54 Zn concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

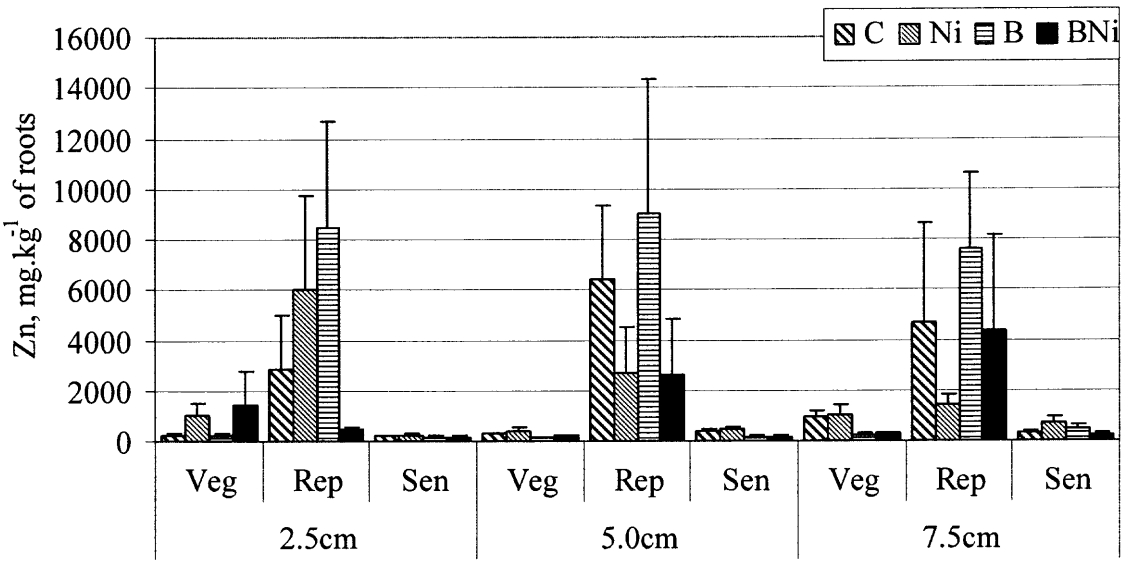

Figure 3.55 Zn concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during vegetative growth (Veg), reproduction (Rep), and senescence (Sen). Bars and error bars represent mean and standard error values.

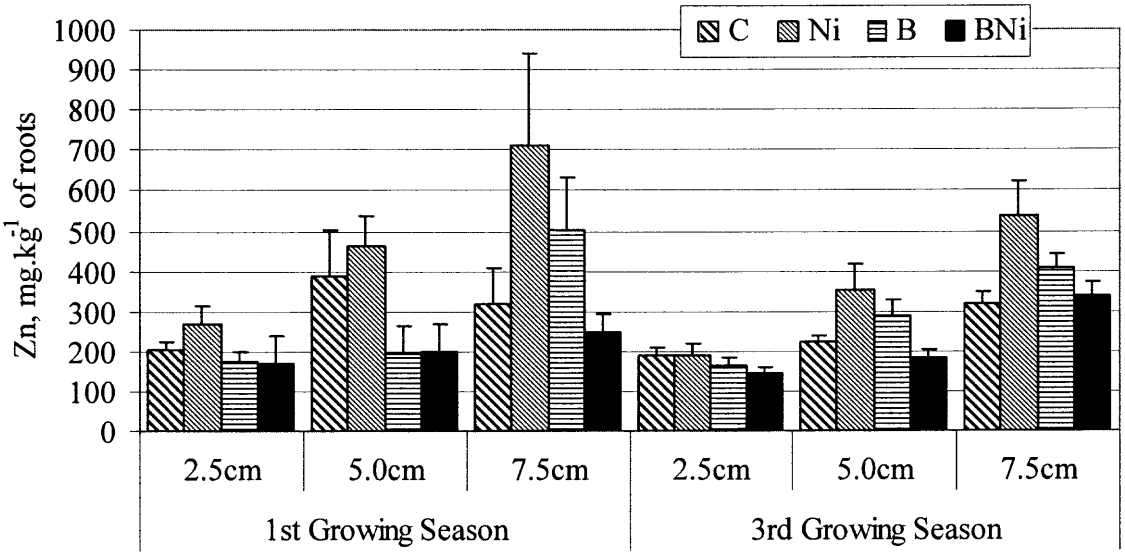

Figure 3.56 Zn concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values.

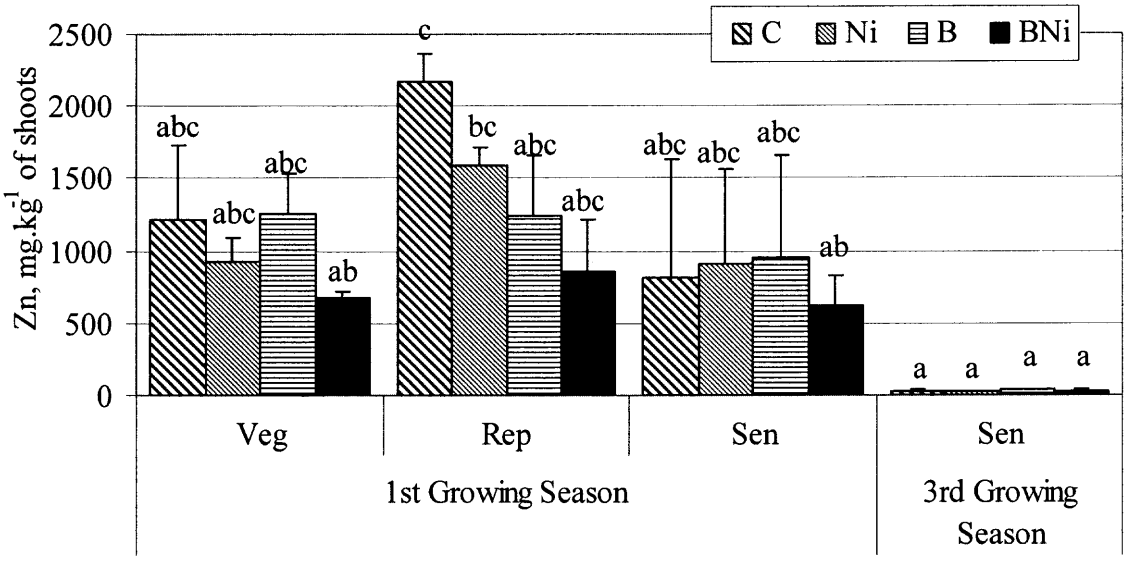

Figure 3.57 Zn concentrations in *S. patens* shoots of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), and Ni and benomyl addition (BNi) during senescence after one growing season and three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent insignificant differences between treatments ($p > 0.05$) determined by Duncan's Test.

Although repeated analyses of the same samples confirmed the validity of these results, the original, unprocessed core samples were not available for replicate analyses. Therefore, Zn values in shoots of the first growing season are considered to be methodological artifacts that cannot be included into any comparative analysis.

Zn is generally taken up as Zn^{2+} and can be accumulated in both roots and shoots after translocation (Marschner 1995). Values of about 250 mg kg^{-1} of root material have been obtained for beans, which is comparable to values for *S. patens* obtained in all treatments (Cakmak & Marschner 1988). In shoots, values of 30, 37 and 60 mg kg^{-1} have been reported for cabbage, beans and okra, respectively (Marschner 1995). These values resemble those found in *S. patens* after the third growing season, and thus again indicate

methodological problems for the analysis of Zn in shoots during the first season. In contrast to Cu, binding to cell walls is not considered to be the major mechanism excluding Zn from uptake and translocation (Vazquez et al. 1992). Tolerance to Zn is mainly achieved through sequestration in vacuoles where Zn is complexed with organic acids (Godbold et al. 1984).

3.5.2.6 Manganese (Mn). In soils, Mn occurs in three oxidation states, +2, +3, and +4 (McBride 1994). The most reduced form of Mn, the Mn^{2+} ion is the stable form in soil solution. Mn^{3+} is a powerful oxidant that either disproportionates to Mn^{2+} and Mn^{4+} or oxidizes water to liberate O_2. Both Mn^{3+} and Mn^{4+} are stable only in the solid phase of soils, where they form insoluble oxide and hydroxide minerals of variable structure and oxidation state (McBride 1994). The Mn^{2+} ion is released from these solids by spontaneous dissolution or cation exchange, especially under acidic or reducing conditions. Mn solubility is controlled by redox potential and the pH of the soil. Mn^{2+} is a very soluble species in water, forming hydroxide and carbonate precipitates only at a pH >7. However, despite being the most weakly complexing transition metal, Mn^{2+} binds to organic matter, oxides, and silicates and its solubility decreases as the pH is raised above 6. Low Eh favors the reduction of insoluble Mn oxides and increases the solubility of Mn^{2+}. Soil Mn concentrations range from 80 to 1300 ppm worldwide, with concentrations found in the US generally between 260 and 840 ppm (McBride 1994).

In the setup, Mn concentrations were affected by Ni amendment and generally not significantly different in depths (Figure 3.58), except for the upper 0.7 cm depth where concentrations up to 1 order of magnitude higher than at the lower depths were found (data not shown). Since highly oxic conditions as well as highest contents of organic

material were found at this depth, sparingly soluble Mn minerals and adsorbed species are supposed to dominate at this depth. Although Rhodochosite ($MnCO_3$) will be the first precipitate, it does not occur as a stable solid phase (Glasby & Schulz 1999). In the solid phase in the seawater, various MnO_2 phases and MnOOH appear to be more stable (Glasby & Schulz 1999). At lower depths, reduced redox potentials result in higher concentrations of mobile Mn^{2+} in the water phase that might leach out of the system and cause their decline with depth.

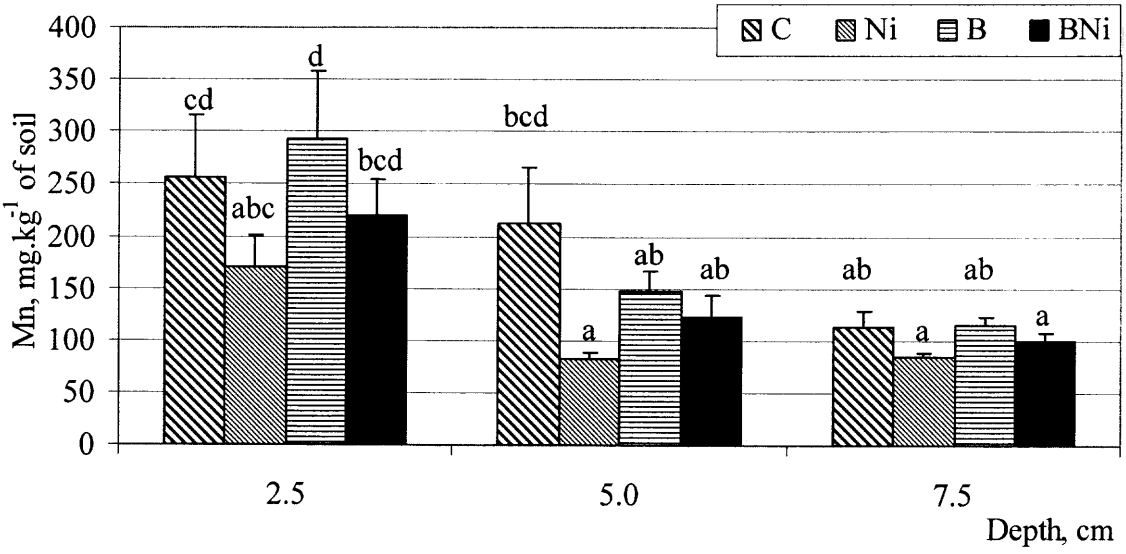

Figure 3.58 Mn concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

The same distribution pattern of Mn as for soil was obtained for Mn concentrations in roots (Figure 3.59). The highest, and the only significantly different values were obtained for the upper 0.7 cm (data not shown), while concentrations at the

remaining depths were about 5-times lower. Ni amendment did not result in significant difference in Mn concentrations in roots compared to the control ($t(8) = 0.93$, $p = 0.38$). Translocation into shoots was also not affected by Ni amendment (Figure 3.60). Concentrations in shoots are well below critical toxicity contents of Mn in shoots of other plants. These can vary depending on the plant species with low values of about 200 mg kg^{-1} dry weight for corn, and high values of 5300 mg kg^{-1} dry weight for sunflower (Marschner 1995).

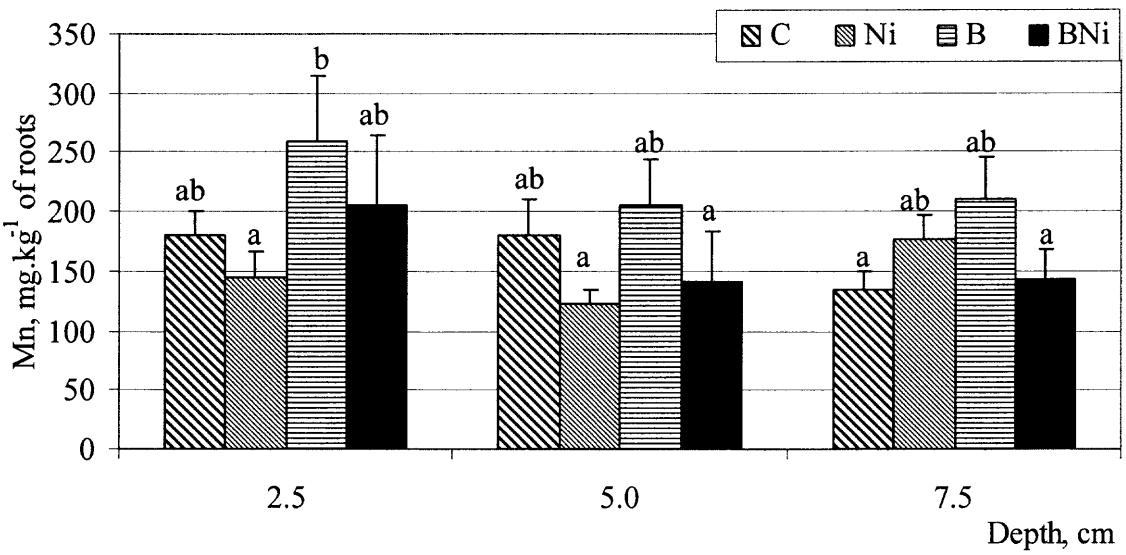

Figure 3.59 Mn concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

Mn^{2+} is by far the dominant form in plants, and thus easily taken up and translocated in the plant. Mn^{2+} can readily be oxidized to Mn^{3+} or Mn^{4+}, and thus plays a significant role in redox processes in the plant (Marschner 1995). Although Mn acts as a cofactor in more than 35 enzymes, at high concentrations it can replace Mg^{2+} in several critical complexes, for example, in the Mg.ATP energy-transmitting system. Normal functioning of this system as well as of other systems therefore relies on sequestration of Mn^{2+}, generally in vacuoles (Pfeffer et al. 1986).

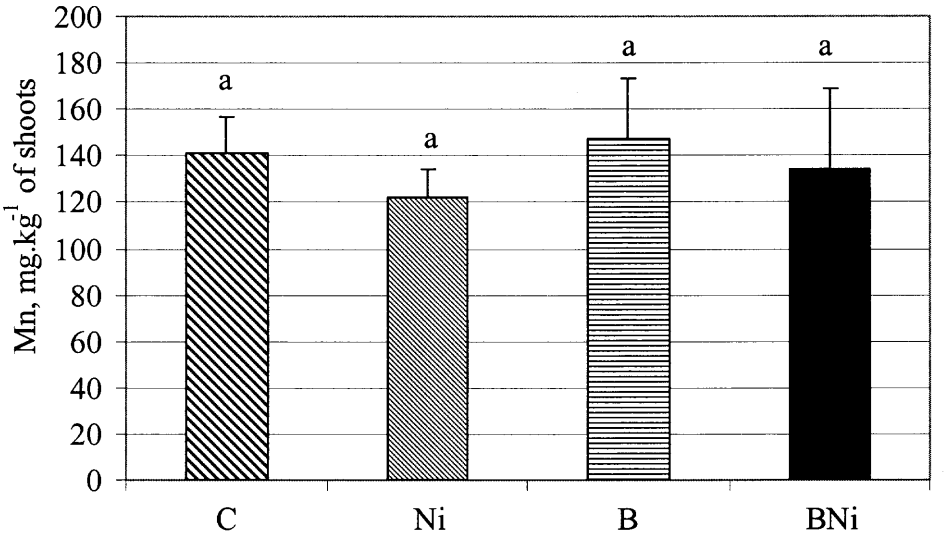

Figure 3.60 Mn concentrations in shoots taken from *S. patens* growing on soil cores without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) as determined by Duncan's Test.

3.5.2.7 Iron (Fe). In contrast to Mn, Fe concentrations in soil were relatively stable without depth or treatment effects (Figure 3.61), even though significantly higher concentrations of Fe were found for the control at a depth of 0.7 cm (data not shown). Fe uptake into roots was also not affected by Ni amendment, except at the depth of 7.5 cm

where highest Fe concentrations in roots were found with significantly higher values in Ni amended cores than in control cores ($F(7,119) = 15.70, p < 0.05$) (Figure 3.62).

Fe concentrations in roots increased with depth suggesting higher availability most likely following redox-dependent reduction of Fe^{3+} to Fe^{2+}. Concentrations in shoots were not different between treatments ($t(8) = 0.81, p = 0.44$) (Figure 3.63). Values in the shoots with about 200 mg kg^{-1} dry weight represent normal levels of Fe concentrations between critical deficiency contents that range for other plants between 50-150 mg kg^{-1} dry weight, and the critical toxicity content above 500 mg kg^{-1} dry weight (Marschner 1995). Fe toxicity is a well-known problem in waterlogged soils (e.g. rice paddies) that provide similar conditions with reduced zones in the depth profile as in the experimental setup.

Translocation, however, does obviously not result in excessive concentrations of Fe in shoots of *S. patens*. The extremely high Fe concentrations in roots and here especially under anoxic conditions at the depths of 5.0 and 7.5 cm might actually not be located in the root, but rather be present as plaque on their surface. Fe as well as Mn coatings on the epidermal cells have been observed on roots of a variety of wetland plants, such as *Oryza sativa* (Green & Etherington 1977), *S. alterniflora* (Mendelssohn & Postek 1982), *Thypha latifolia* (Taylor et al. 1984), and *P. australis* (Batty et al. 2000).

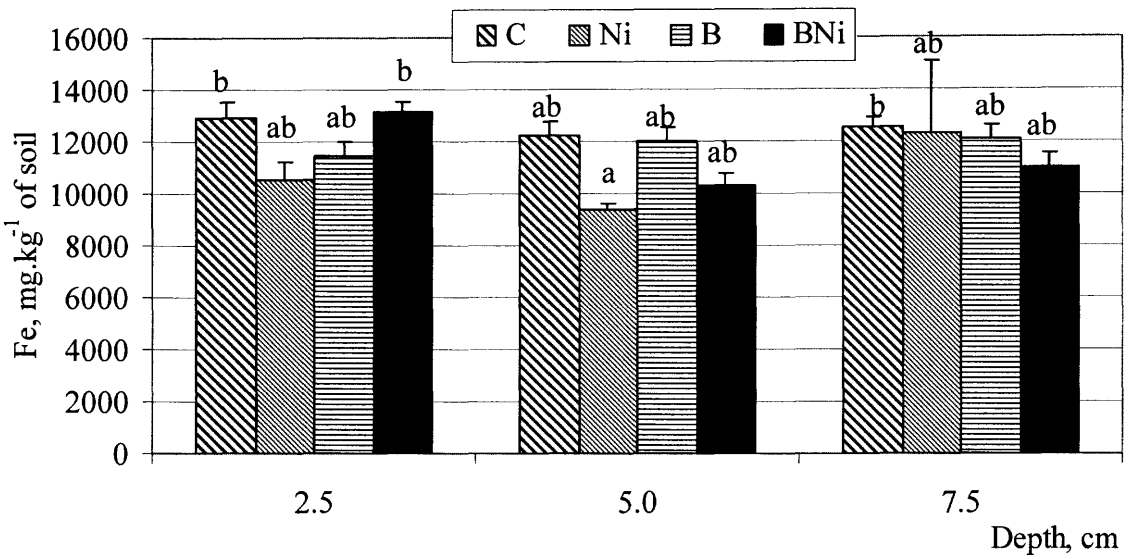

Figure 3.61 Fe concentrations in soil taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

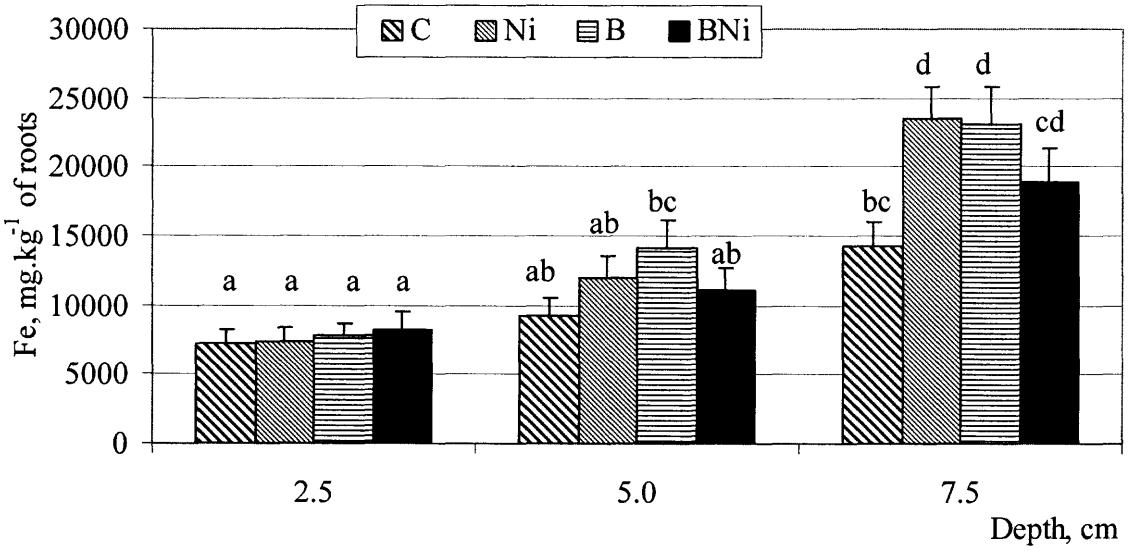

Figure 3.62 Fe concentrations in *S. patens* roots taken at different depths (2.5, 5.0 and 7.5 cm) of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

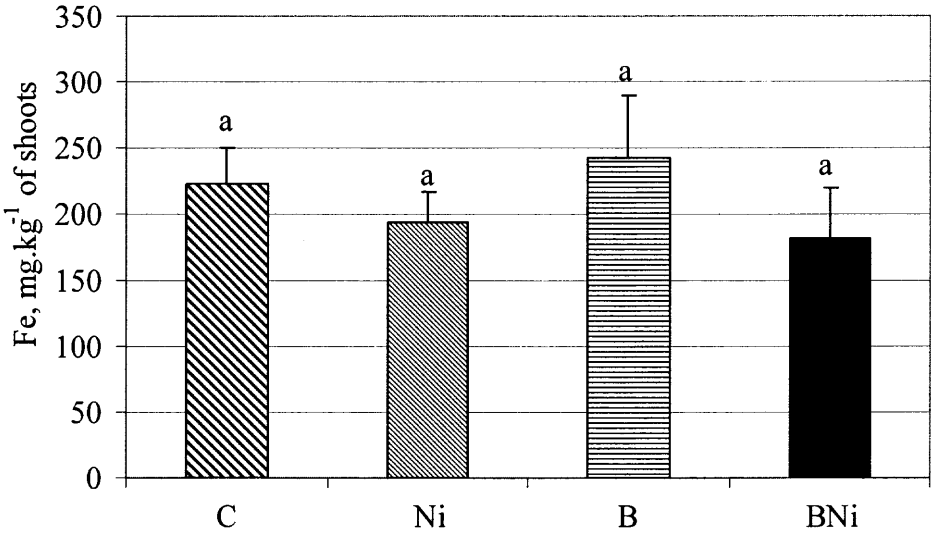

Figure 3.63 Fe concentrations in *S. patens* shoots of treatments; without treatment (C), Ni addition (Ni), benomyl addition (B), or Ni and benomyl addition (BNi) during senescence after three growing seasons. Bars and error bars represent mean and standard error values. Identical letters represent non-significant differences between treatments ($p > 0.05$) determined by Duncan's Test.

3.5.3 Analysis of Heavy Metals (Summary)

Monocotyledonous salt marsh plant species are assumed to concentrate metals primarily in roots (Otte et al. 1991), however, with a high degree of variability between metals and between various plant species (Fitzgerald et al. 2003). Other factors affecting uptake of heavy metals include differences in age and growth stages, seasonal variations, presence of iron plaques on the roots, levels of metal contamination in the locality, soil properties such as pH and redox potential, tidal inundation and salinity (Wright & Otte 1999). Of all heavy metals analyzed, Cu, Fe, Mn, Ni, and Zn are essential in plants, while no biological functions are currently known for Cd, Cr, and Pb (Davies et al. 2001). Uptake routes are either passive, only driven by the concentration gradient across the membrane, or inducible substrate-specific and energy-dependent (Nies 1999, Schuetzenduebel & Polle

2002). The results show that all metals analyzed can be taken up by plant roots and be translocated into shoots. Uptake and translocation were not affected by benomyl-treatment or increased Ni concentrations. Since benomyl application did not result in detectable suppression or reduction of AMF, conclusions cannot be drawn on effects of AMF on heavy metal uptake and translocation, both of which have been found enhanced in the presence of AMF (Rabie 2005)

None of the heavy metals displayed uptake or translocation values typical for hyperaccumulating plants, but rather values in the typical range found for many agricultural plants. Since all values in plant tissues were above the critical deficiency contents and below the critical toxicity contents, effects on plant growth performance should not be expected. Ni amendment did not increase uptake and translocation in most cases although effects of Ni amendment were displayed for root uptake of certain heavy metals (i.e. Cu, Pb, Zn and Fe) at the depth of 7.5 cm (Table 3.8). These effects could be a function of redox potential with amended Ni^{2+} being more soluble and thus available at much higher concentrations than under oxic and suboxic conditions. Ni^{2+}, in addition to redox dependent formation of Fe^{2+}, could compete with other metals for adsorption sites on organic material which is present in about 10-fold lower concentration at the depth of 7.5 cm compared to the 2.5 cm depth. Release from organic material could increase mobility and result in either enhanced leaching or in increased uptake by or adsorption to roots.

Table 3.8 Additional Effect of Ni Amendment on Uptake of Other Metals by *S. patens* ($p < 0.05$)

Metal	ShootS		Roots	
	1st growing season	3rd growing season	1st growing season	3rd growing season
Cd	None	None	None	None
Cr	None	None	None	None
Cu	None	None	None	at 7.5 cm
Pb	None	None	at 7.5 cm	at 7.5 cm
Zn	None	None	at 7.5 cm	at 7.5 cm
Fe	N.d.	None	N.d.	at 7.5 cm
Mn	N.d.	None	N.d.	None

n.d. not determined

Adsorption to roots might be enhanced by plaque formation, e.g. that of Fe^{3+} deposits. Fe oxidation occurs in the rhizosphere of many wetland plants based on the presence of Fe oxy-hydroxide precipitates that often coat root surfaces (Mendelssohn et al. 1995). Fe plaques on root surfaces may be caused by root-induced oxidation of precipitated and immobile sulfides thereby releasing metals followed by Fe precipitation around the roots as oxy-hyrodoxide (Wright & Otte 1999). Diffusion of oxygen into reduced, sulfate- and sulfide-rich environments as found in estuarine environments through plant roots could mobilize metals precipitated and co-precipitated as sulfides (Hsieh & Yang 1997). Co-precipitation of other heavy metals in oxy-hydroxide or adsorption to Fe or Mn plaque can result in higher concentrations of heavy metals on the

root surface. Fe and Mn plaque, for example, enhance Cu adsorption to roots and thus can act as Cu reservoir (Ye et al. 2001). The amount adsorbed depends on the amount of Fe or Mn on the roots and the amount of Cu in the environment (Ye et al. 2001). The concentration of Cu in or on the roots is also affected by the plant phenological stage. Studies on concentrations of dissolved forms of some heavy metals similar to the study such as Cd, Cr, Cu, Mn, Pb, and Zn and their species in the water of the Dnieper reservoirs and the Dnieper-Bug estuary in Ukraine, for example, show that seasonal dynamics of dissolved organic matter influenced the complexation of the metals and thus their availability (Linnik 2003).

CHAPTER 4

CONCLUSIONS AND FUTURE PERSPECTIVES

The general outcome of this study was that moderate heavy metal contamination found in sediments of Harrier Meadow did not affect growth performance of the common salt marsh plant *S. patens* and associated arbuscular mycorrhizal fungi colonizing its roots during key points of the growing season. Although considerable uptake of all heavy metals into or adsorption to roots was encountered, translocation from below- into above-ground parts of *S. patens* was not significant, and always below regulatory action limits (maximum limit levels for vegetative leaves of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn are 0.2, 2.30, 73.3, 425.50, 500, 67.90, 0.3 and 99.40 $mg.kg^{-1}$, respectively). Thus, the risks associated with the restoration of moderately contaminated salt marshes with *S. patens*, i.e., the potential of *S. patens* to act as a conduit for the movement of toxic metals into the food web, are minimal and thus of low public health concern.

Although these results answer the basic questions addressed in this thesis, several topics need more attention in future studies. These topics include the experimental setup, the assessment of short- and long-term environmental quality, the diversity of AMF, as well as the fate of heavy metals in the system.

The experimental setup in the greenhouse with soil cores containing *S. patens* from Harrier Meadow resulted in quite consistent environmental conditions in cores within and between treatments, with values for most physicochemical parameters being not significantly different at comparable depths. This was expected since the same setup had been used for studies on *S. patens*-AMF interactions before (Burke et al. 2002a, Burke et al. 2003). These studies, however, dealt with an organic matrix that was quite

homogeneous. This is in contrast to cores from Harrier Meadow where the mineral matrix is extremely heterogeneous and also contaminated with moderate concentrations of heavy metals. Both factors might affect the experimental setup on a small scale without being detected as variable environmental determinants. Since all cores from Harrier Meadow were contaminated, no real uncontaminated control was available that could be used to evaluate direct effects of Ni addition on AMF or plant growth performance. In all cases, adaptation of the individual organisms (i.e. AMF, plants) to moderate contamination and thus limited effects of additional Ni on these organisms cannot be excluded. Future research should try to include such an uncontaminated control, and manipulate uncontaminated cores in comparison to contaminated ones.

Long-term evaluation of environmental stability in all cores attempted by retrieving concentrations and spectra of total hydrolysable amino acids (THAA) from all cores resulted in similar pattern for all cores. These results were similar to those reported for different environments and environmental conditions despite substantial quantitative differences in concentrations of THAA (Friedel & Scheller 2002). Unfortunately, the focus on THAA analysis covers all amino acids present in an environment whether available for organismal nutrition or not. The absence of treatment effects between non-treated, Ni-, or Benomyl-treated cores, as well as the only small differences between these and Benomyl/Ni-amended cores in the long-term experiment might therefore reflect long-term stability of certain amino acids, potentially adsorbed to soil minerals or representing low-nutrient value resources both of which escape degradation by consumers (Dauwe et al. 1999). Very recent studies also indicated that THAA in soils are mineralized rapidly with half-life times for most amino acids far less than half a day

(Jones et al. 2005). Thus, although widely used to assess environmental quality (i.e. organic matter quality), the THAA analysis might not be appropriate for this purpose in long-term studies as attempted here.

Future studies should therefore include analysis of enzymatically hydrolysable amino acids (EHAA) in which the amino acid pool in the organic matter is enzymatically hydrolyzed mimicking digestion and thus determining the bioavailable fraction of this pool (Medernach et al. 2001). Changes in EHAA as well as in THAA and in their amino acid composition are indicative of degradation, with ratios between EHAA and THAA providing a more sensitive index on changes and thus lability of organic material (Medernach et al. 2001). Although in the long-term experiment no large changes in the amino acid concentrations and spectra were obtained between treatments, a more sensitive measure using EHAA/THAA ratios as well as studies on the kinetics of short- and long-term changes in the amino acid concentrations and spectra could probably provide a more adequate means to determine differences in soil quality in the cores as a function of treatments, and thus potential effects of treatments on organisms and related processes.

Measures for AMF were similar on plants from all treatment even though small, statistically significant differences were obtained for percent root length colonized, and arbuscular or vesicular colonization. Since none of the treatments eliminated AMF, these differences could not be related to treatment effects, but were suggested to be the result of potential shifts in AMF community structure. Since community structure analyses of AMF were not within the scope of this thesis, further investigations need to include studies on AMF diversity, both in soil and roots which would potentially allow us to

differentiate a higher diversity of dormant stages in soil, and a lower diversity of AMF actively infecting roots. A similar outcome, though with functional AMF belonging to different groups, could be obtained in comparative analyses of non-treated and Ni-treated cores. Plants on these cores did not show significant differences in root length colonized, and arbuscular or vesicular colonization. Thus, although no treatment effects (i.e. of Ni amendment) were detected, such effects could still be a function of different AMF. Future perspectives should therefore include studies on the diversity of AMF in relation to treatments and seasonality.

Although plant growth performance assessment based on the foliar N content, stable isotope ratios ($\delta^{13}C$), carboxylation efficiency (CE), CO_2 saturated photosynthetic capacity (A_{max}), estimations of optimal transfer efficiency of open photosynthesis II (PSII) reaction centers (F_v/F_m), sprout numbers, and shoot and root biomass generally showed only seasonal variation, some variation was displayed for parameters such as A_{max}, F_v/F_m, and shoot and root biomass where significant effects of Ni amendment were found during the reproduction. These effects were no longer significant at the end of the growing season, and were not obtained in Benomyl/Ni treatments. Since environmental characteristics such as temperature, pH, redox potential, NH_4^+, sulfide and TOC as well as the analysis of AMF do also not provide significant differences between treatments that could be specifically related to lower plant growth performance in Ni-amended cores during reproduction, additional evidence for potential effects of Ni amendment needs to be established in future studies. No such evidence was found in Ni uptake and translocation patterns in plants of all four treatments.

None of the heavy metals displayed uptake or translocation values typical for hyperaccumulating plants, but rather values in the typical range found for many agricultural plants. Since all values in plant tissues were above the critical deficiency contents and below the critical toxicity contents, effects on plant growth performance should not be expected. Ni amendment did not increase uptake and translocation in most cases although effects of Ni amendment were displayed for root uptake of certain heavy metals (i.e. Cu, Pb, Zn and Fe) at the depth of 7.5 cm. Since heavy metals dissolved or precipitated in water were not included into the analyses on heavy metal uptake and translocation, a mass balance cannot be presented. This fact could have serious implications on explanations of some of the results obtained in this study since reductions in concentration levels of several metals could be due to leaching into water that was subsequently discarded in the attempt to standardize seawater salinity. Leached metals, however, could also have adsorbed to filamentous algae that grew on the walls of the water buckets effectively immobilizing them during the growing season in a part of the system that was not analyzed. At the end of the growing season, mineralization of the algae could have resulted in a release of heavy metals and them entering the soil system again by diffusion which would explain increased heavy metal concentrations in the lower parts of the cores at senescence. These assumptions, however, are highly speculative and need additional experiments that include highly controlled conditions and analyses of all phases potentially involved in heavy metal adsorption.

APPENDIX

DIAGRAMS OF HEAVY METALS

A. Diagrams of MineQL+ calculations

A.1 Solubility Diagram of Hydroxylapatite in Seawater without Heavy Metals

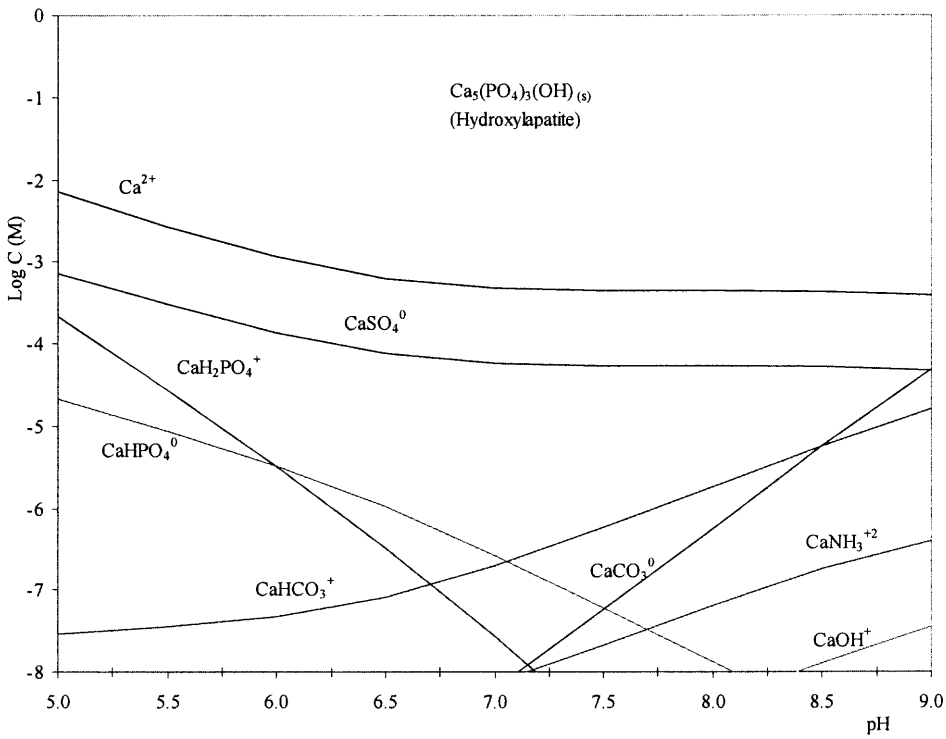

Figure A.1 Solubility diagram (log C-pH) of hydroxylapatite in simulated seawater without heavy metals.

A.2 Speciation of Heavy Metals in Water as a Function of Concentration and LogC pH under Suboxic Conditions

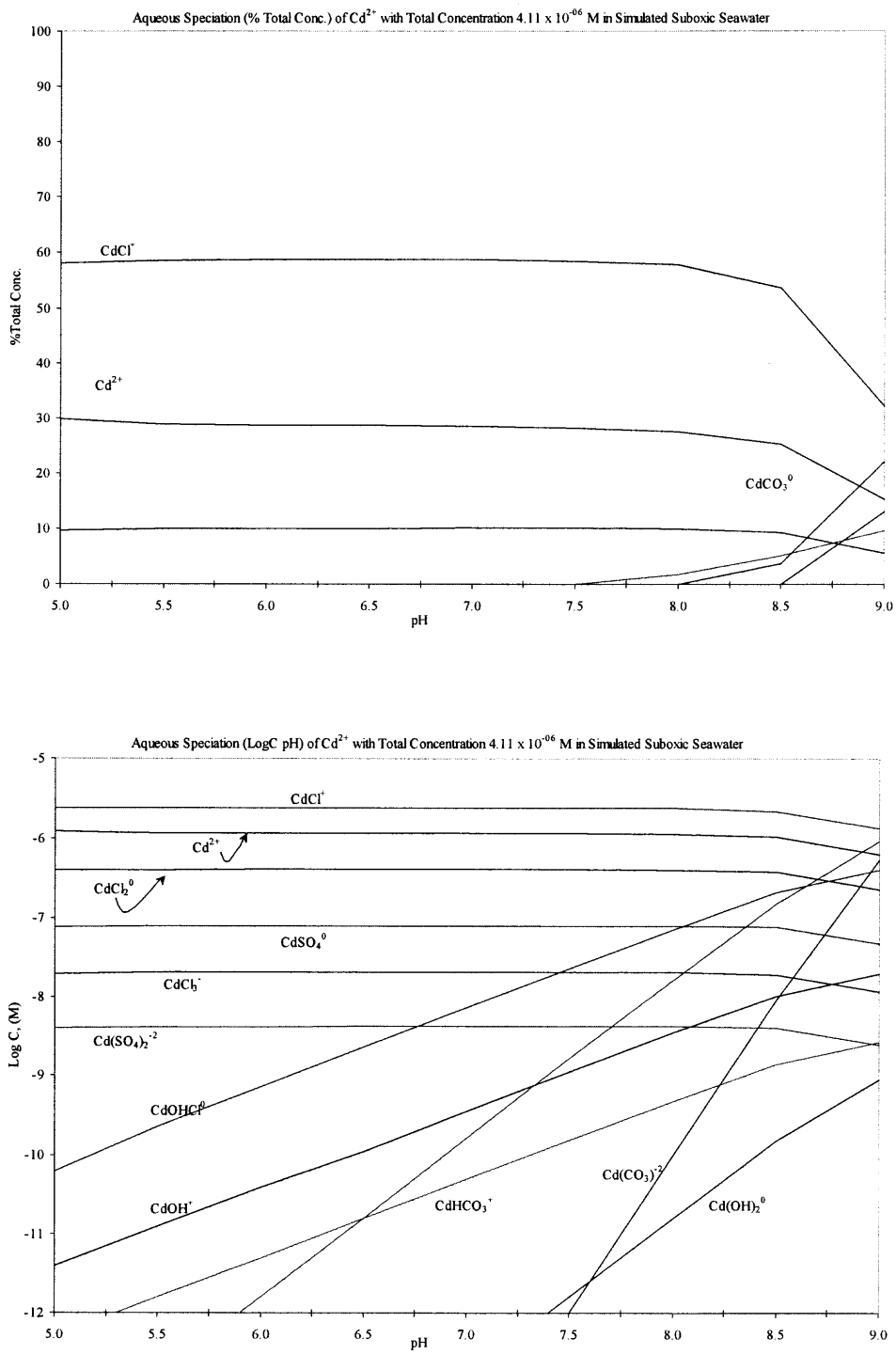

Figure A.2 Speciation of Cd in suboxic seawater.

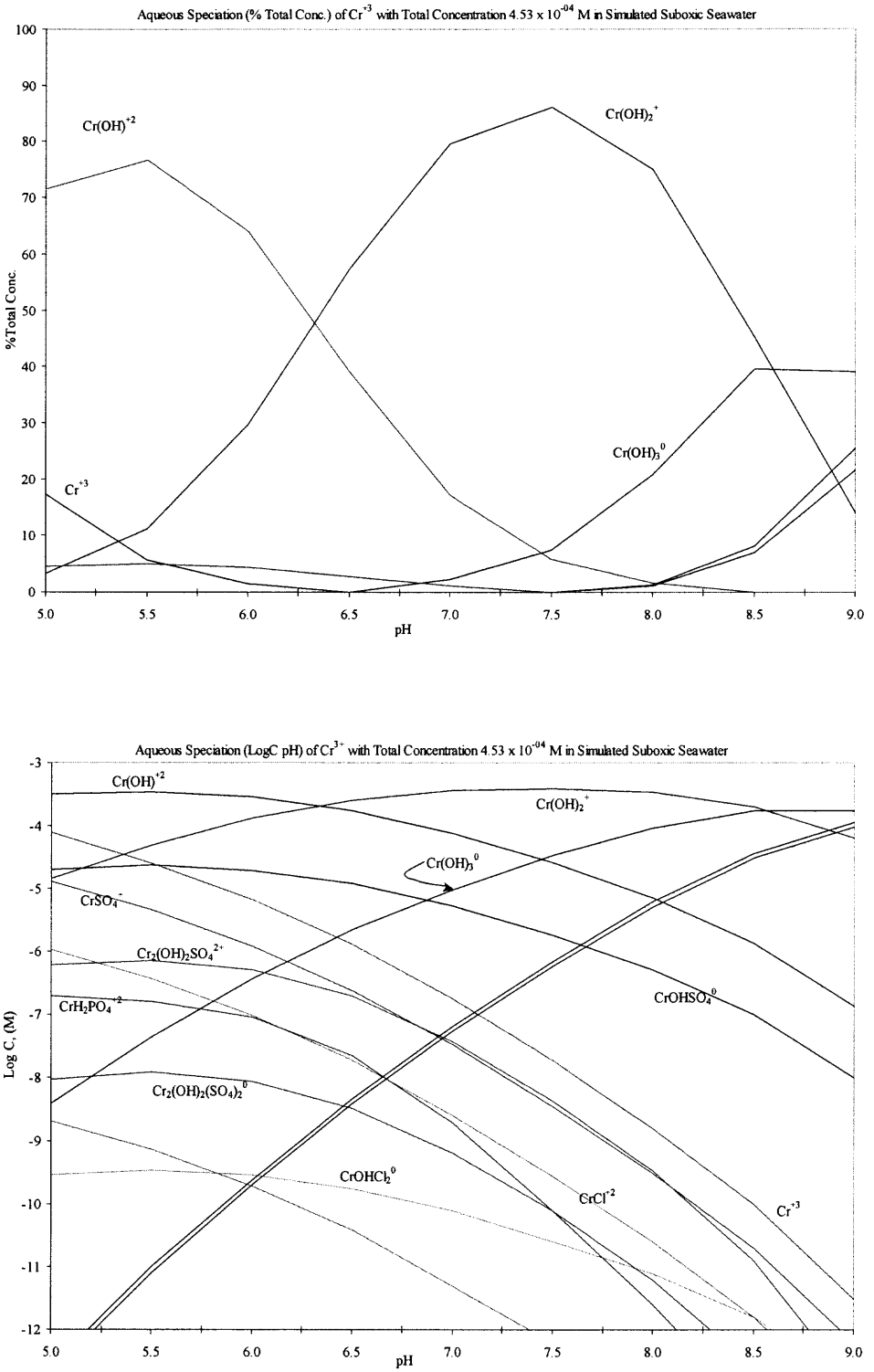

Figure A.3 Speciation of Cr in suboxic seawater.

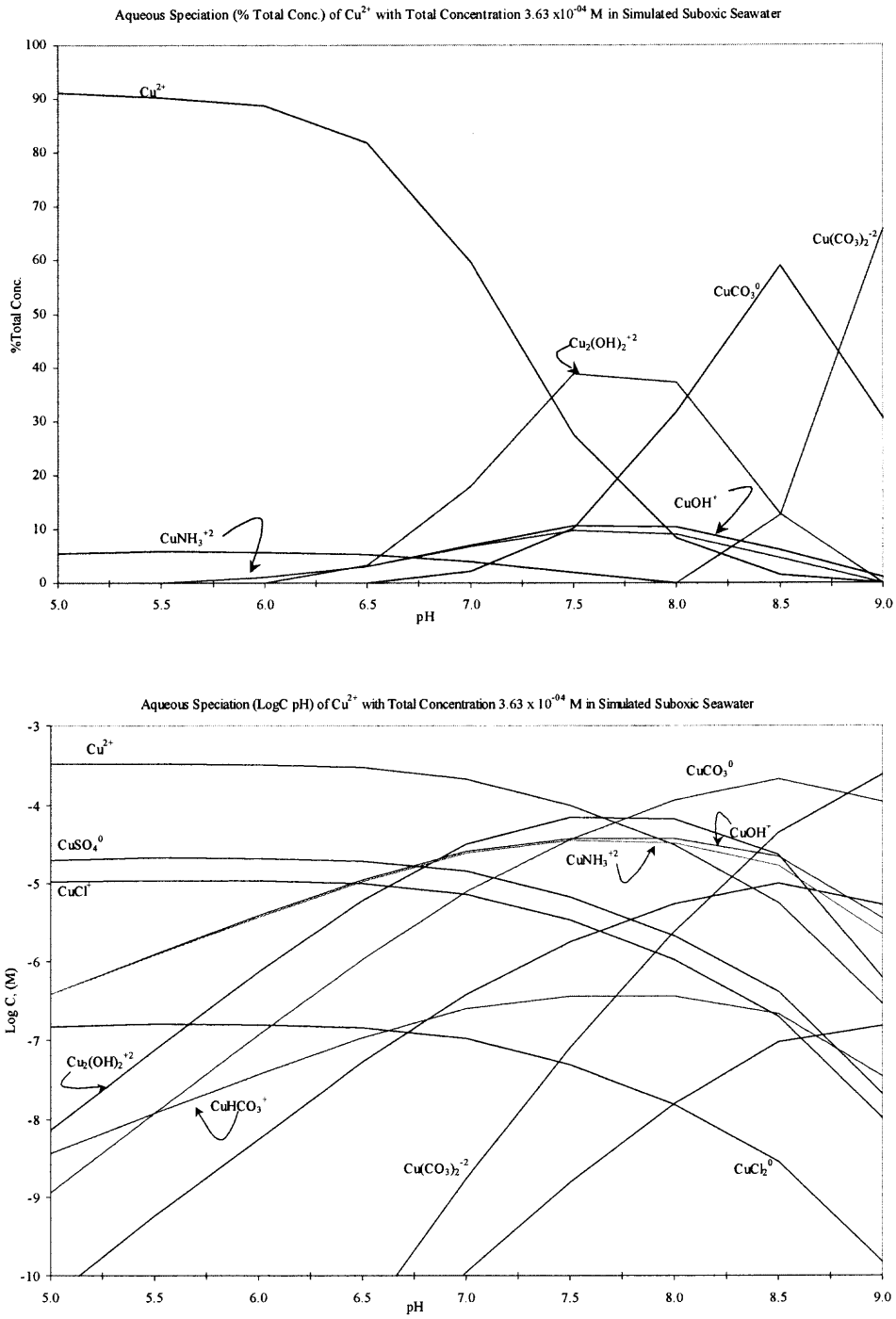

Figure A.4 Speciation of Cu in suboxic seawater.

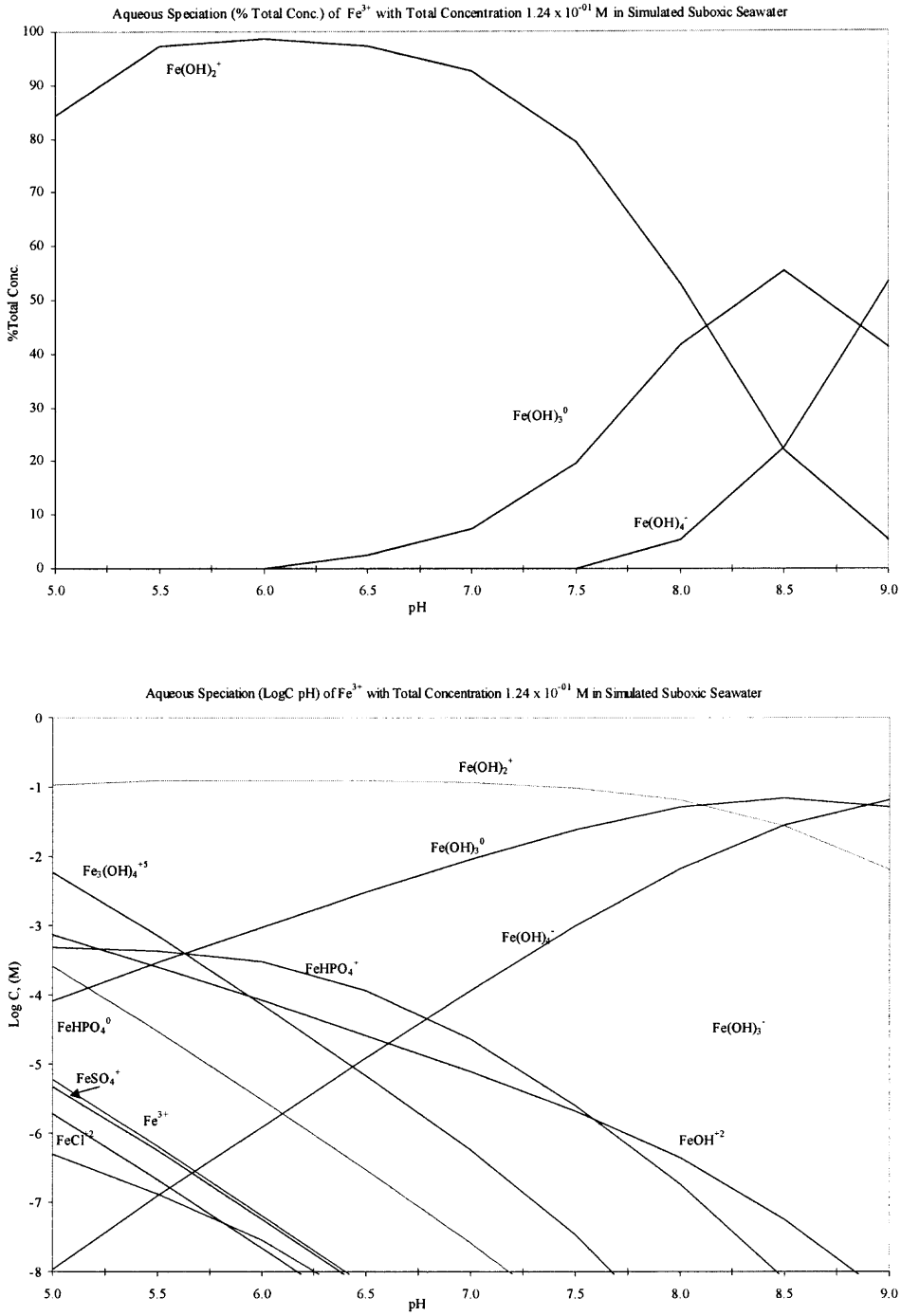

Figure A.5 Speciation of Fe (III) in suboxic seawater.

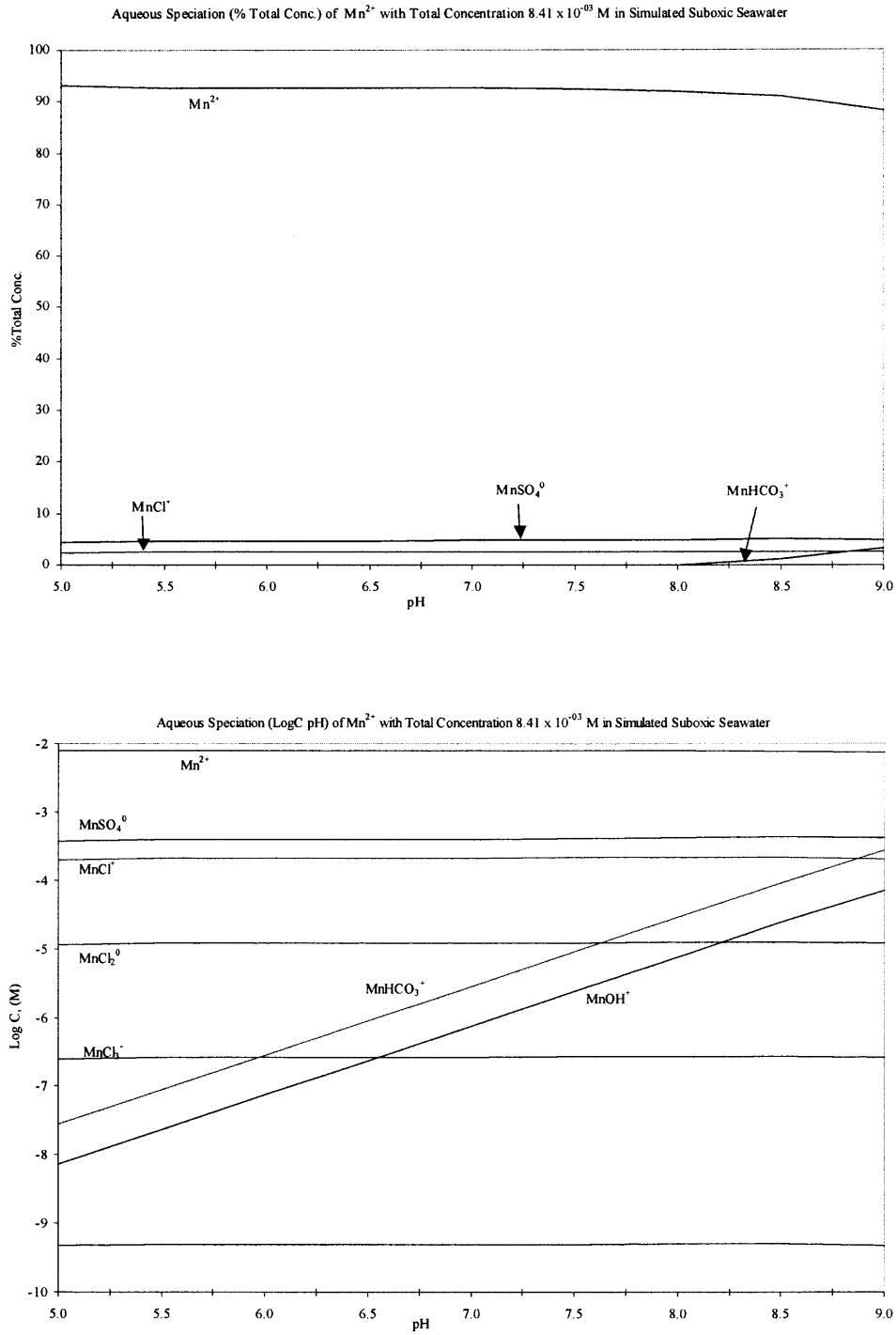

Figure A.6 Speciation of Mn in suboxic seawater.

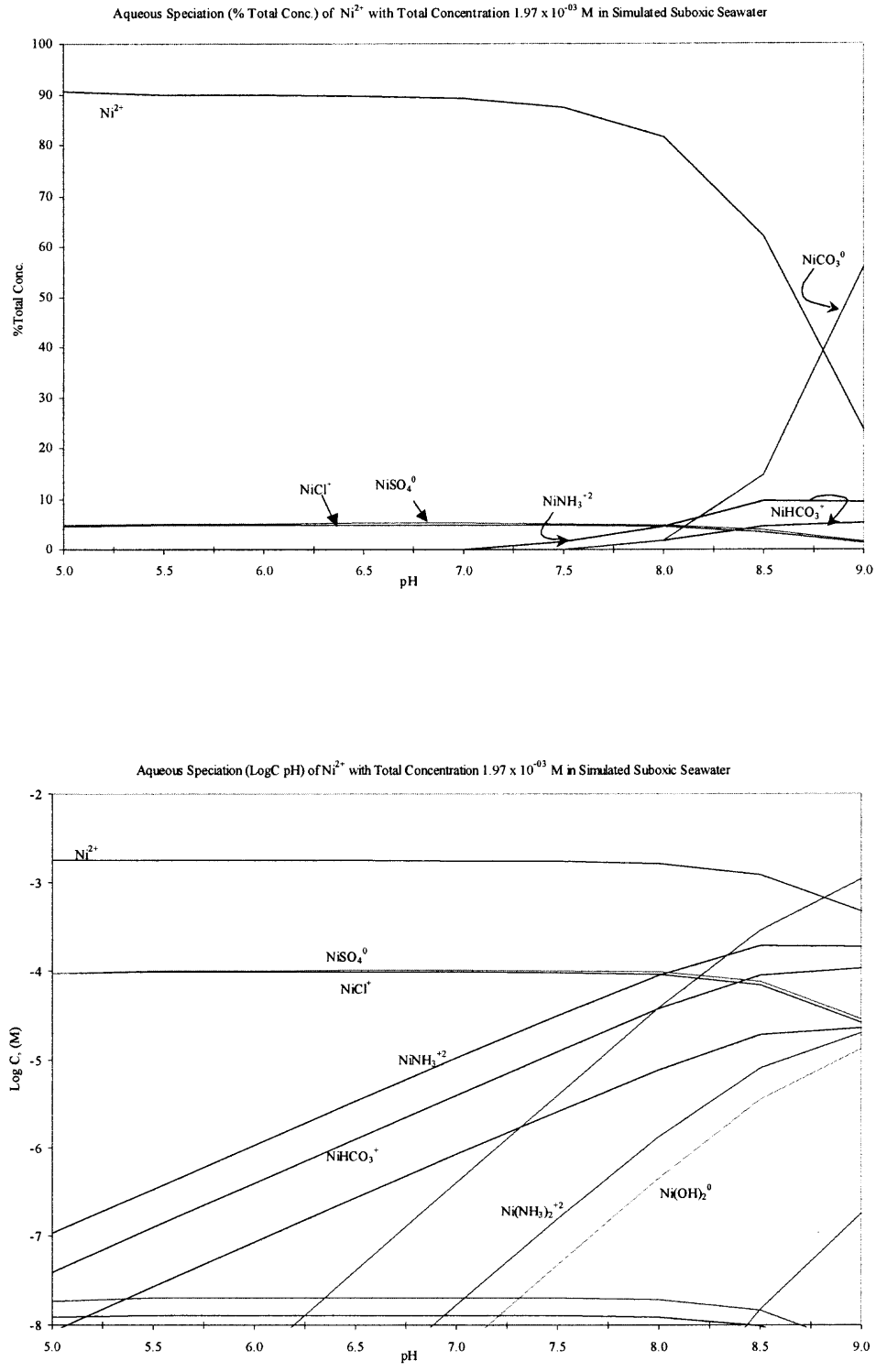

Figure A.7 Speciation of Ni in suboxic seawater.

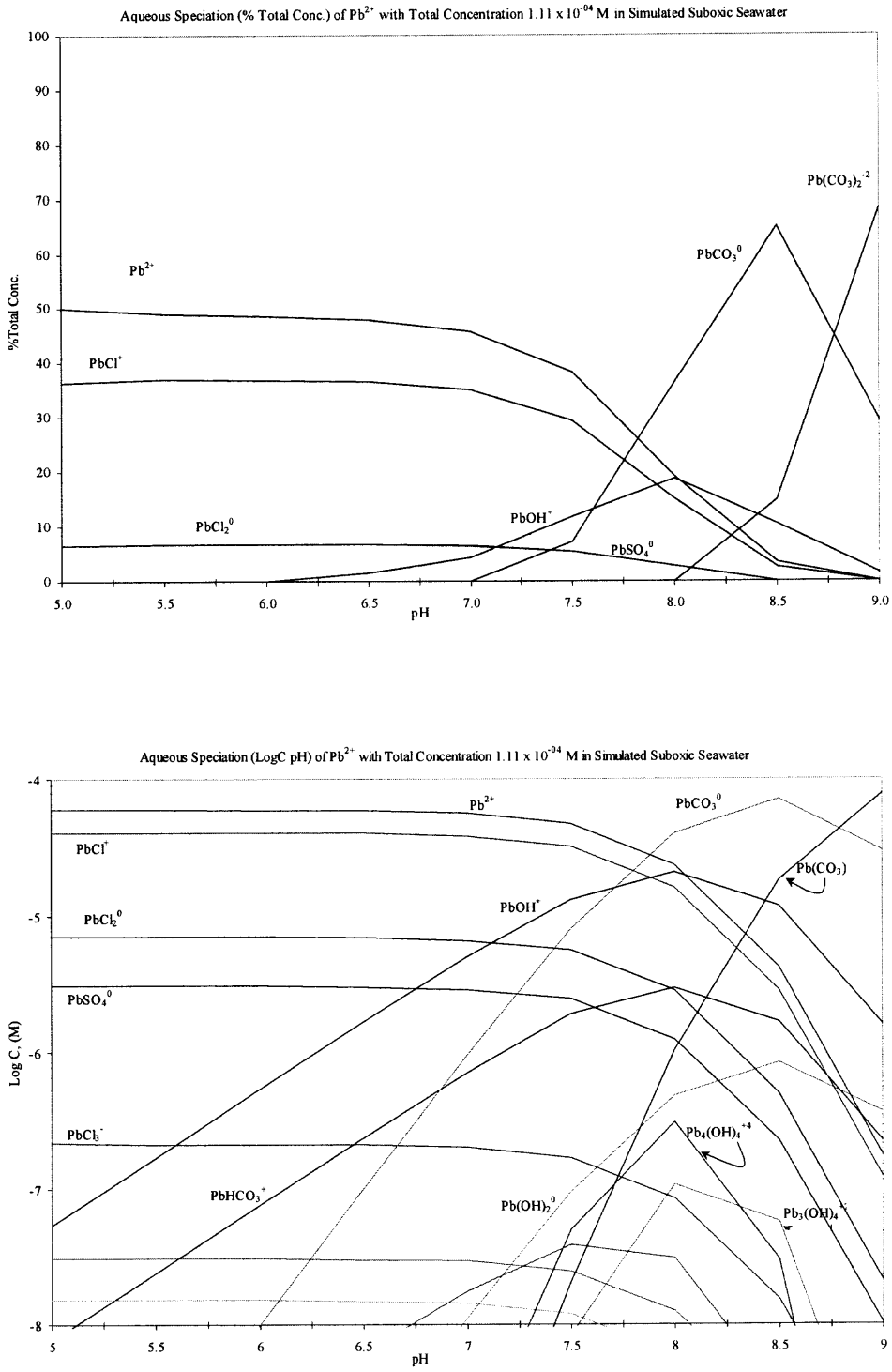

Figure A.8 Speciation of Pb in suboxic seawater.

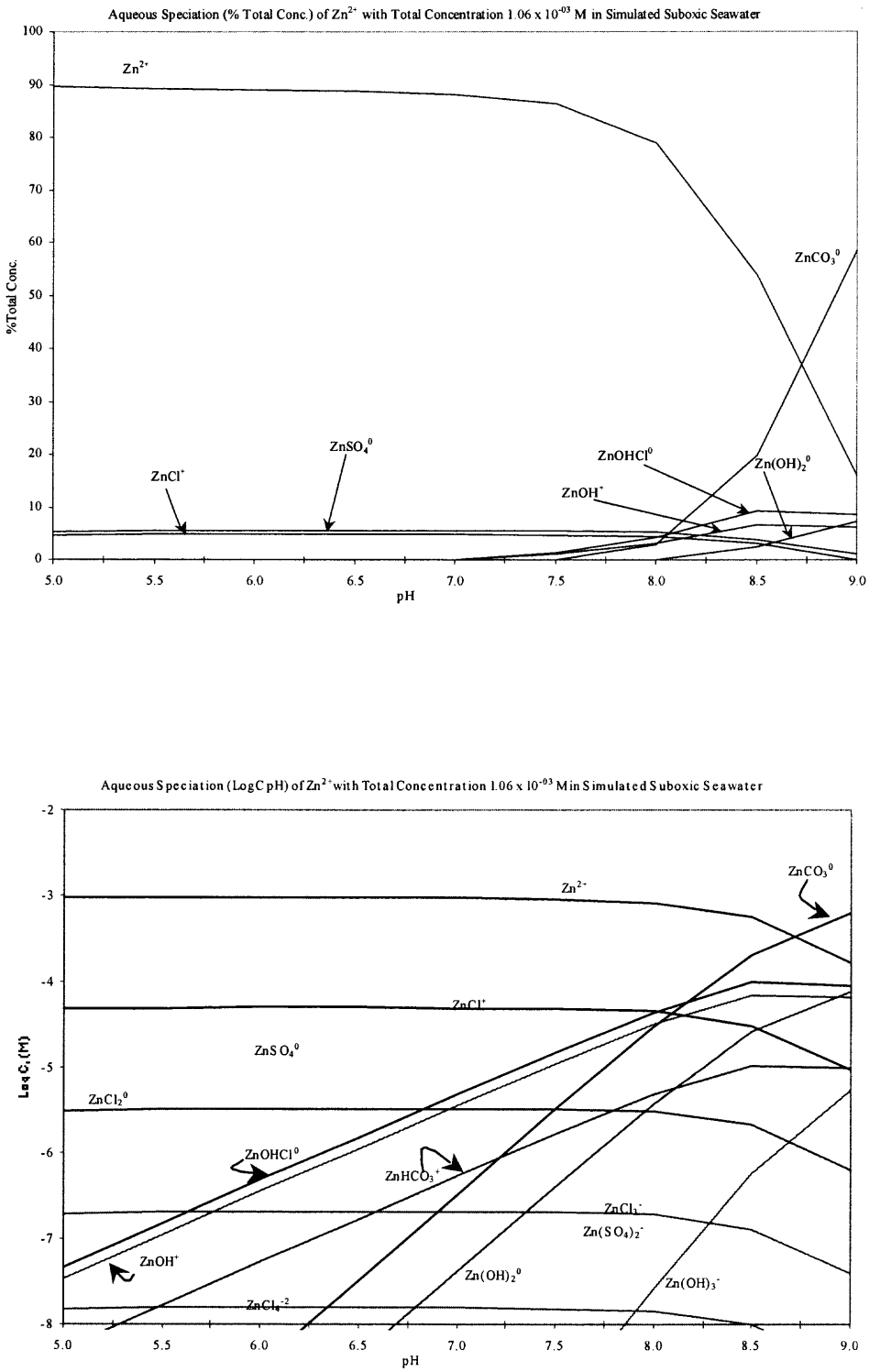

Figure A.9 Speciation of Zn in suboxic seawater.

A.3 Solubility Diagrams for Heavy Metal Minerals under Suboxic Conditions

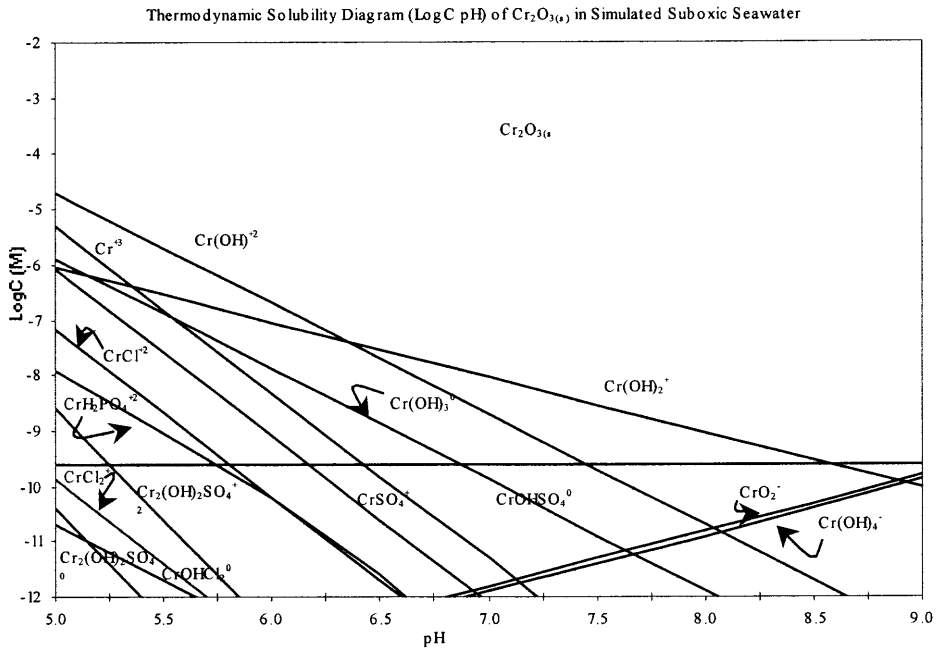

Figure A.10 Solubility diagram of Cr-minerals in suboxic seawater.

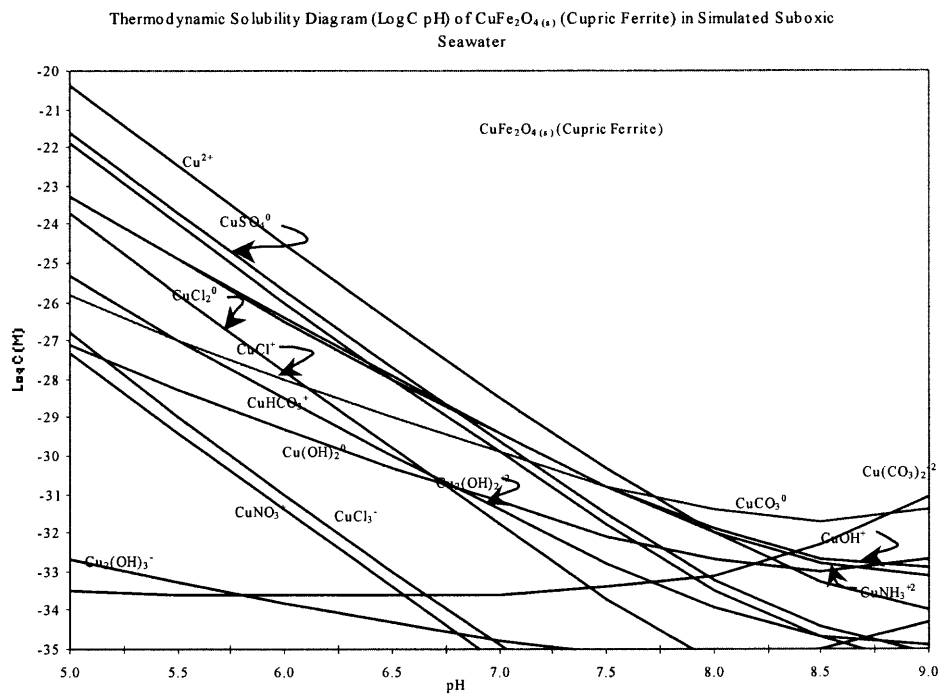

Figure A.11 Solubility diagram of Cu-minerals in suboxic seawater.

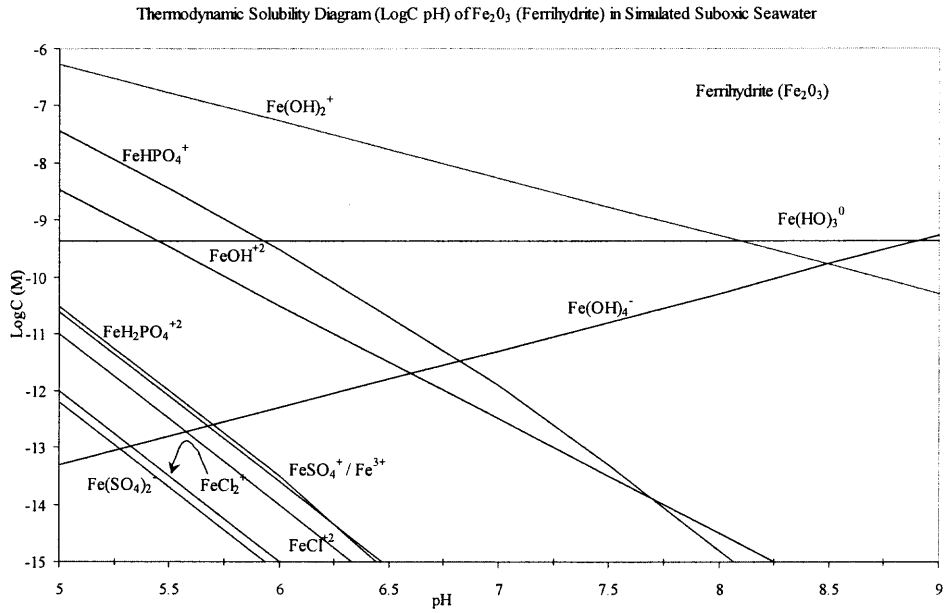

Figure A.12 Solubility diagram of Fe(III)-minerals in suboxic seawater.

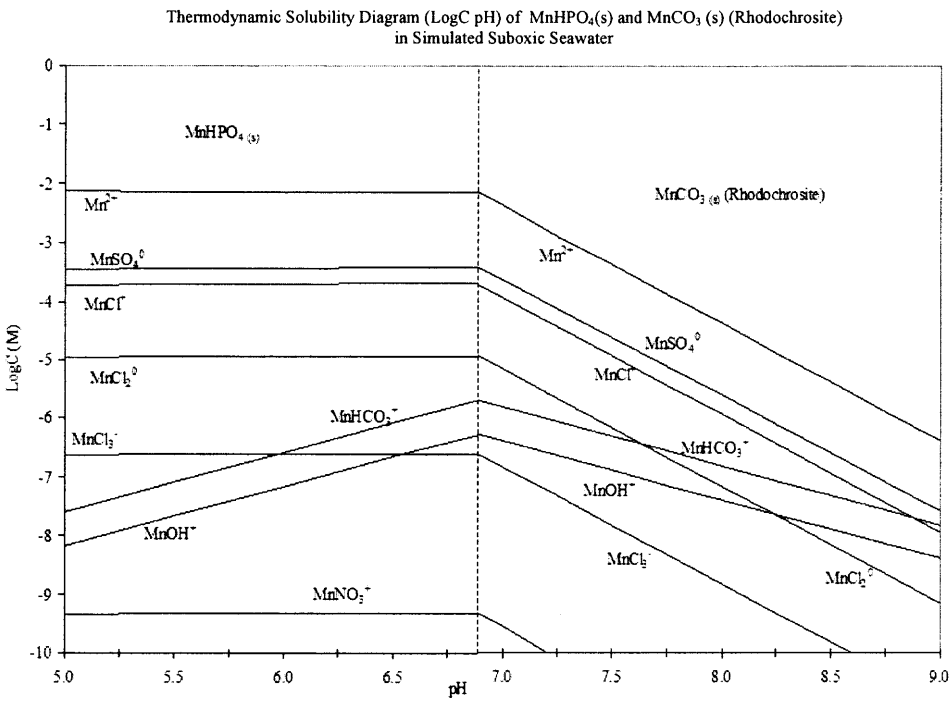

Figure A.13 Solubility diagram of Mn-minerals in suboxic seawater.

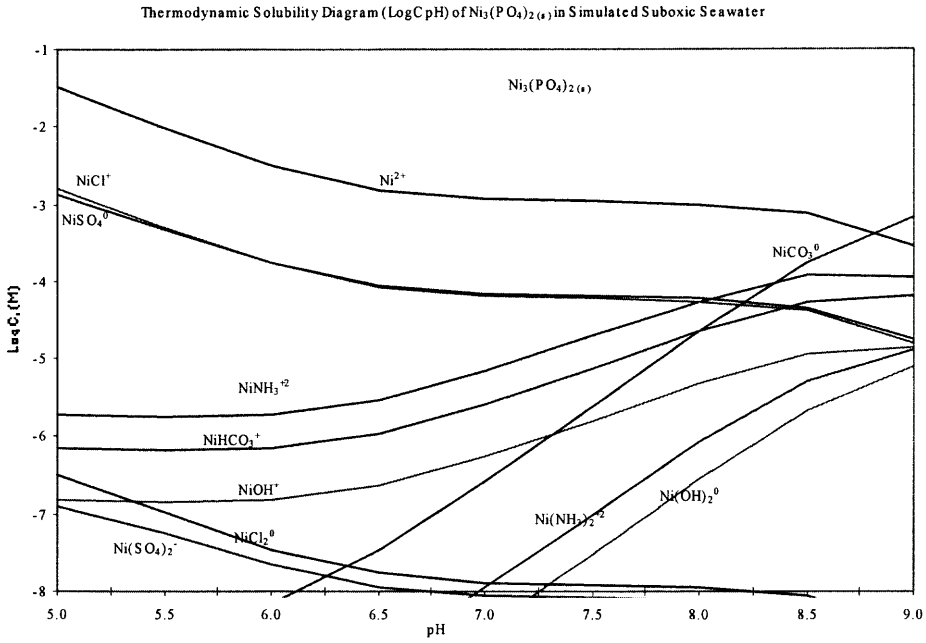

Figure A.14 Solubility diagram of Ni-minerals in suboxic seawater.

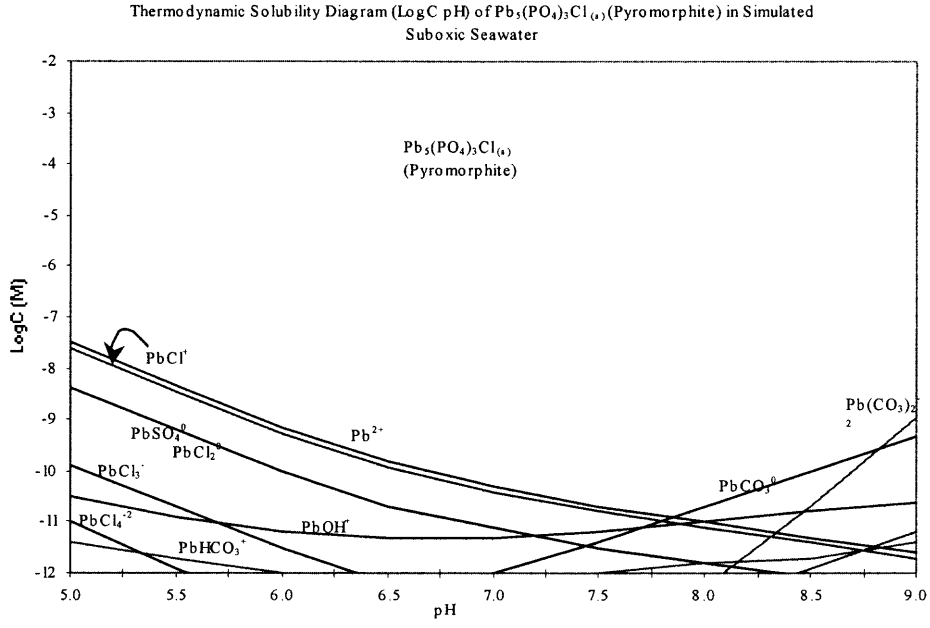

Figure A.15 Solubility diagram of Pb-minerals in suboxic seawater.

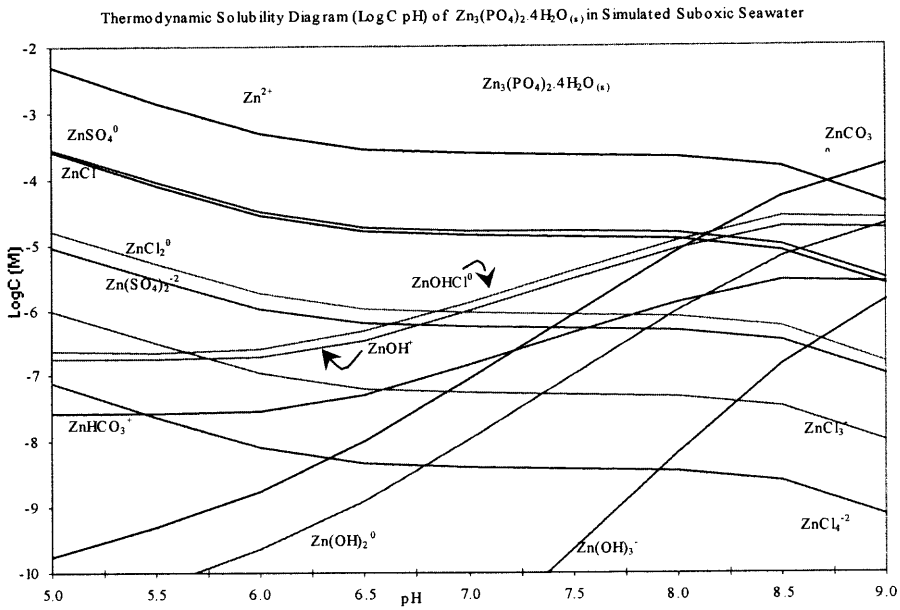

Figure A.16 Solubility diagram of Zn-minerals in suboxic seawater.

A.4 Speciation of Heavy Metals in Water as a Function of Concentration. and LogC pH under Anoxic Conditions

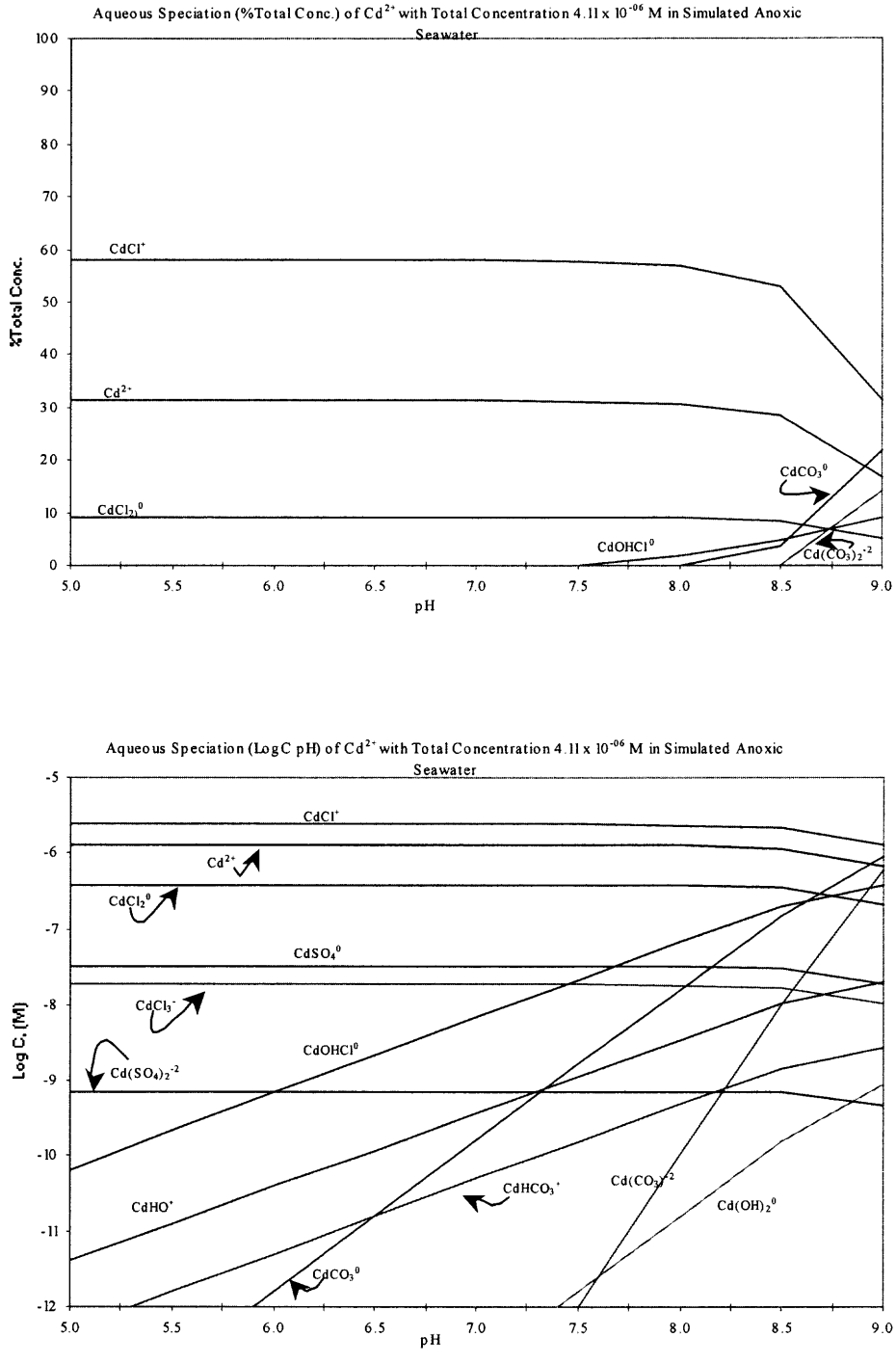

Figure A.17 Speciation of Cd in anoxic seawater.

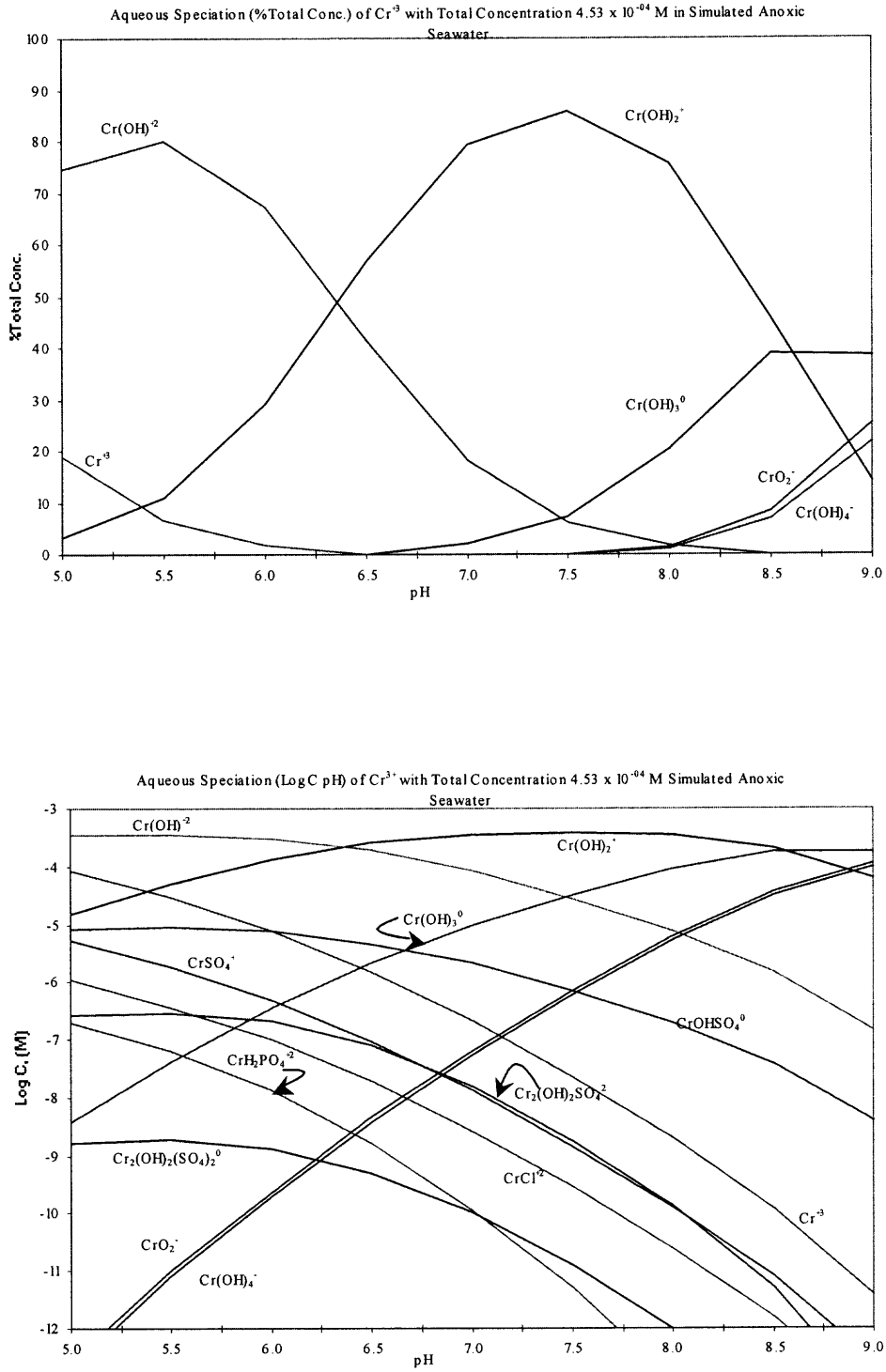

Figure A.18 Speciation of Cr in anoxic seawater.

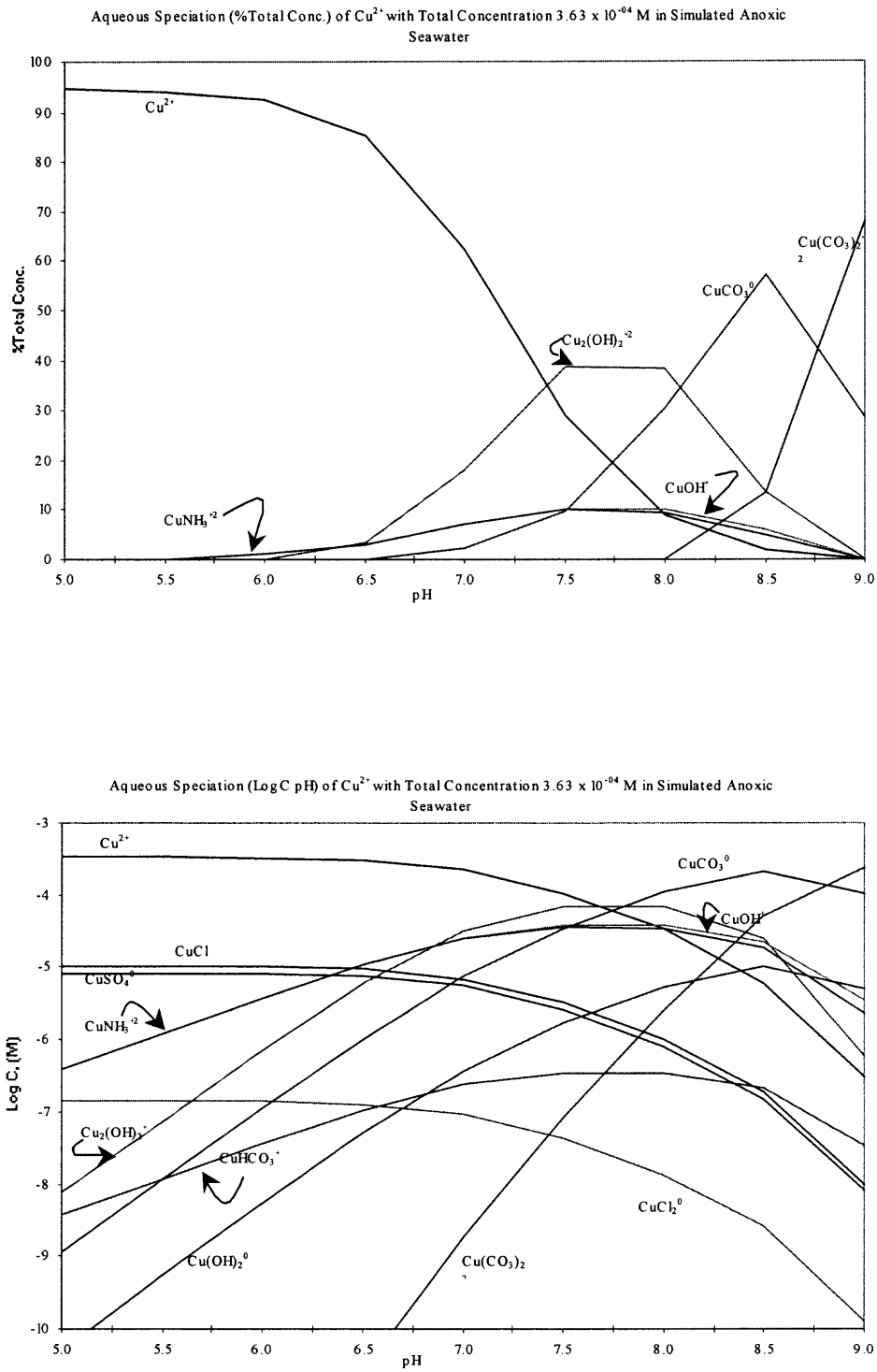

Figure A.19 Speciation of Cu in anoxic seawater.

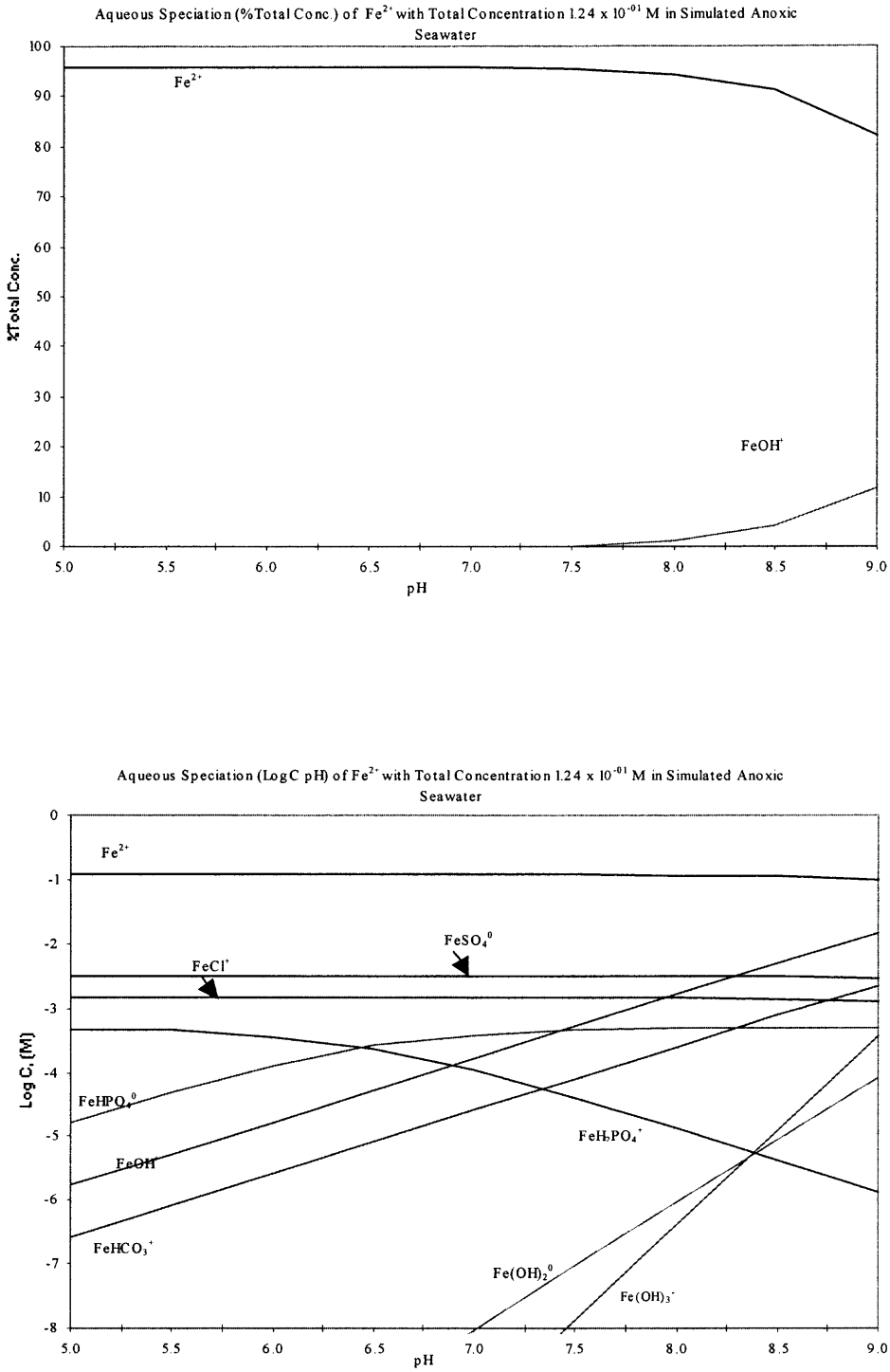

Figure A.20 Speciation of Fe (II) in anoxic seawater.

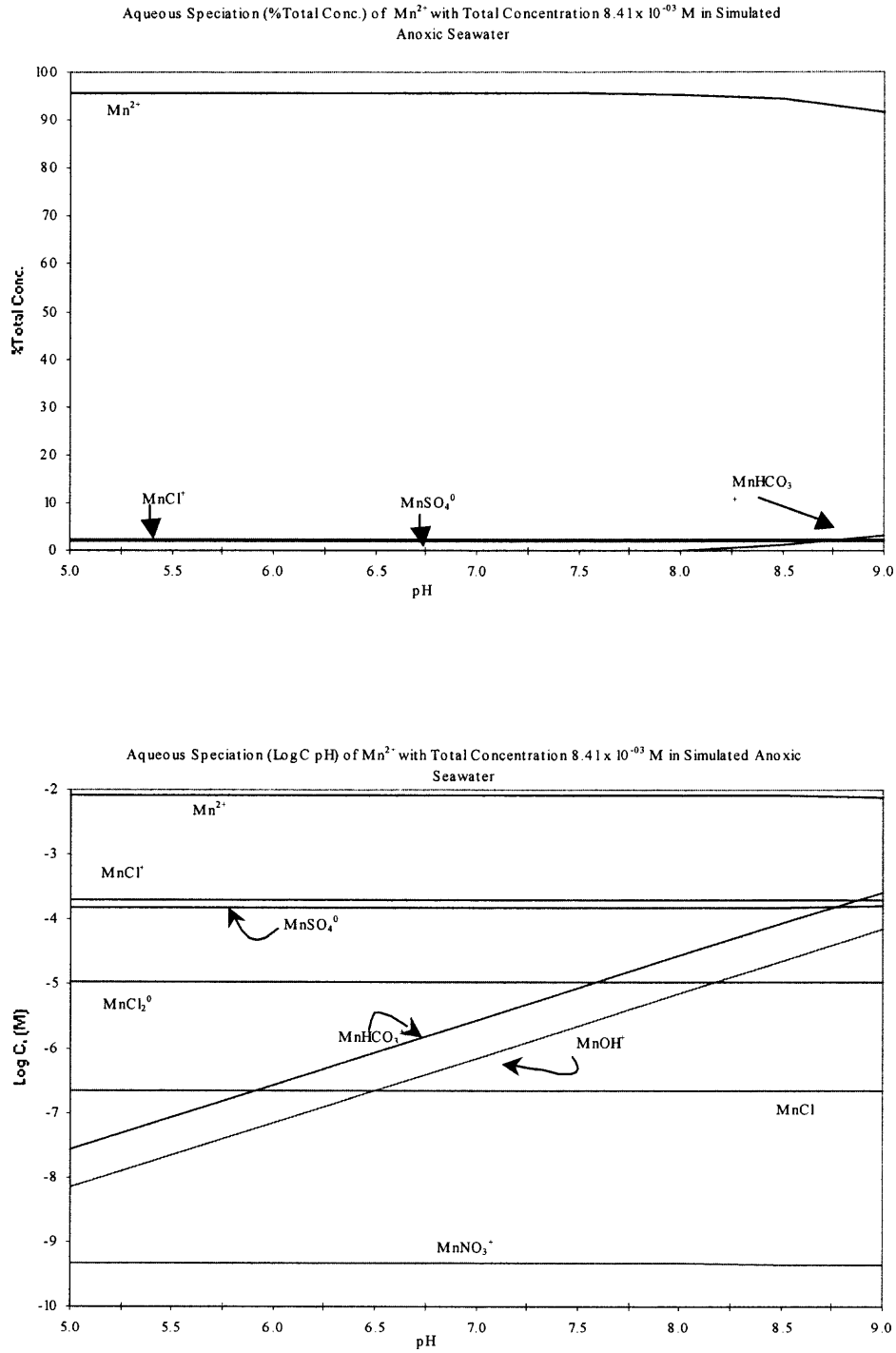

Figure A.21 Speciation of Mn in anoxic seawater.

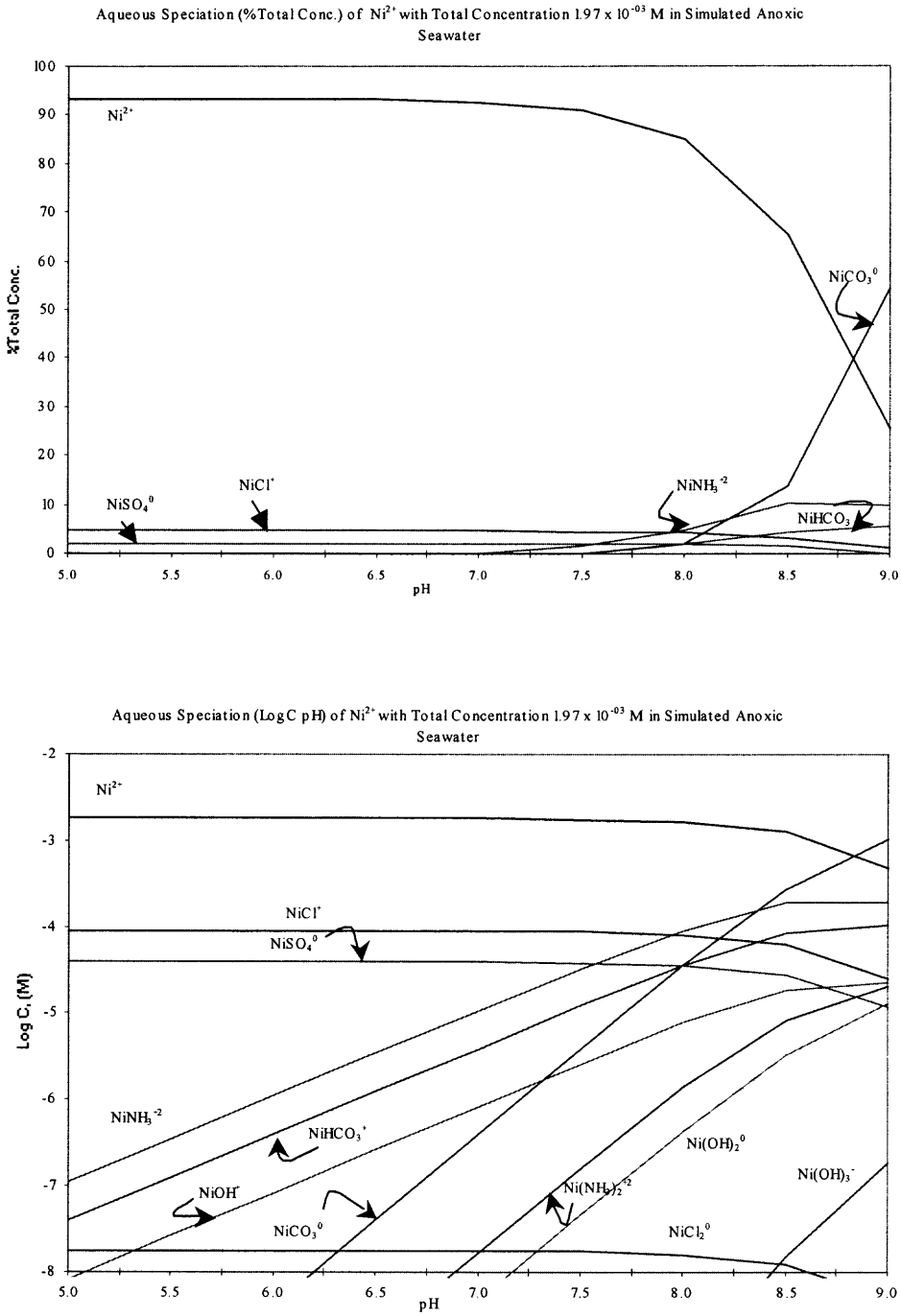

Figure A.22 Speciation of Ni in anoxic seawater.

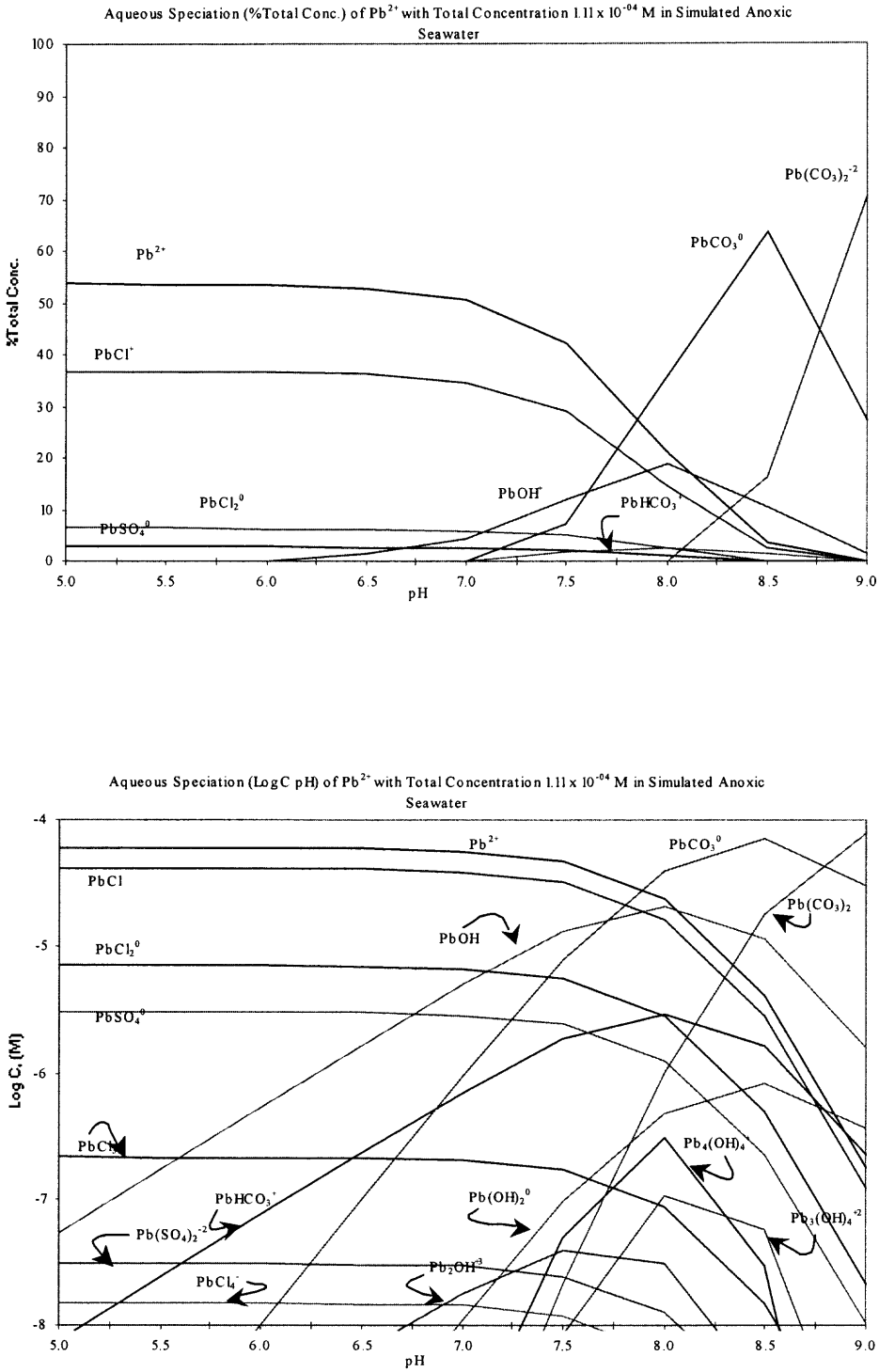

Figure A.23 Speciation of Pb in anoxic seawater.

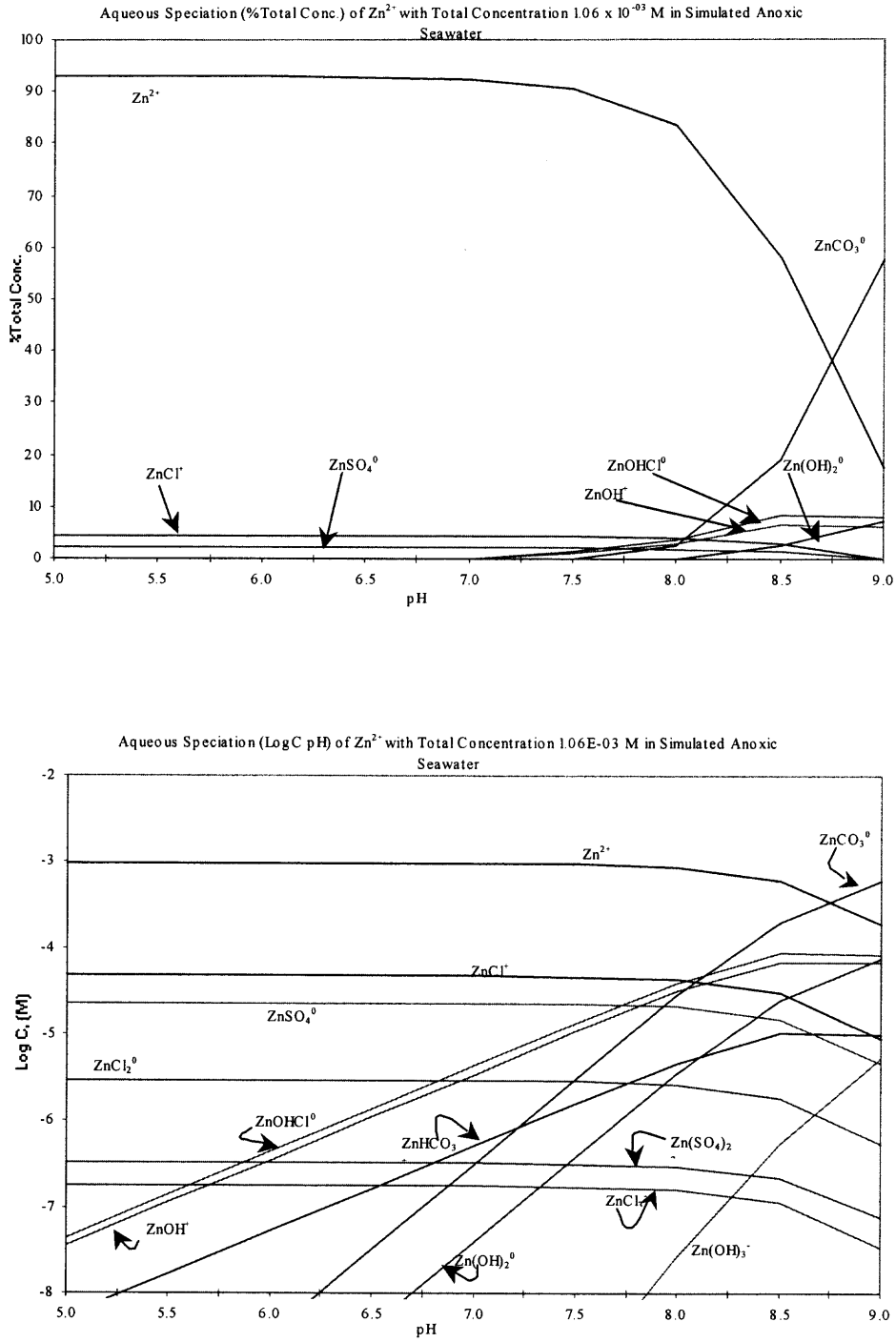

Figure A.24 Speciation of Zn in anoxic seawater.

A.5 Solubility Diagrams for Heavy Metal Minerals under Anoxic Condition

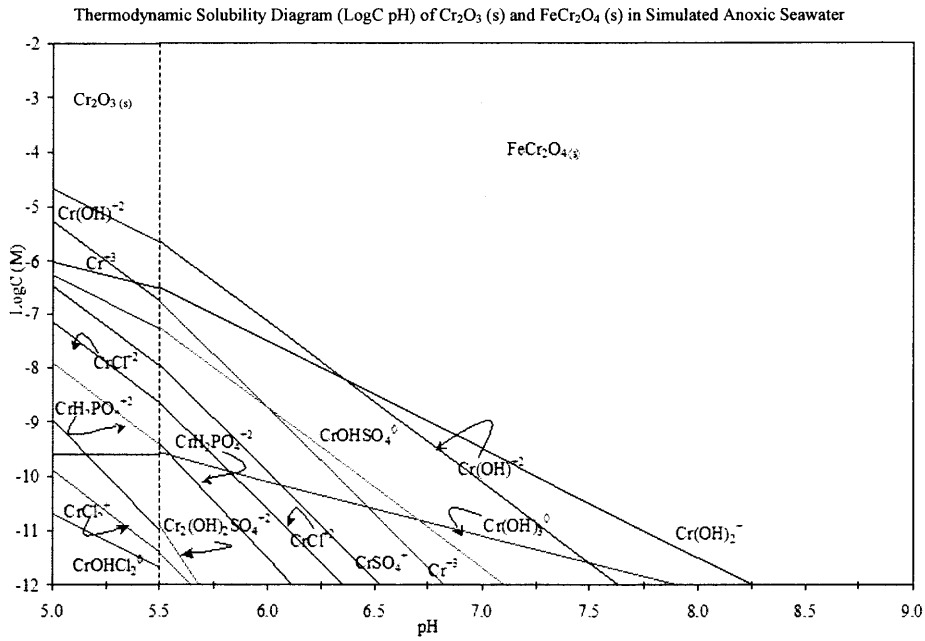

Figure A.25 Solubility diagram of Cr-minerals in anoxic seawater.

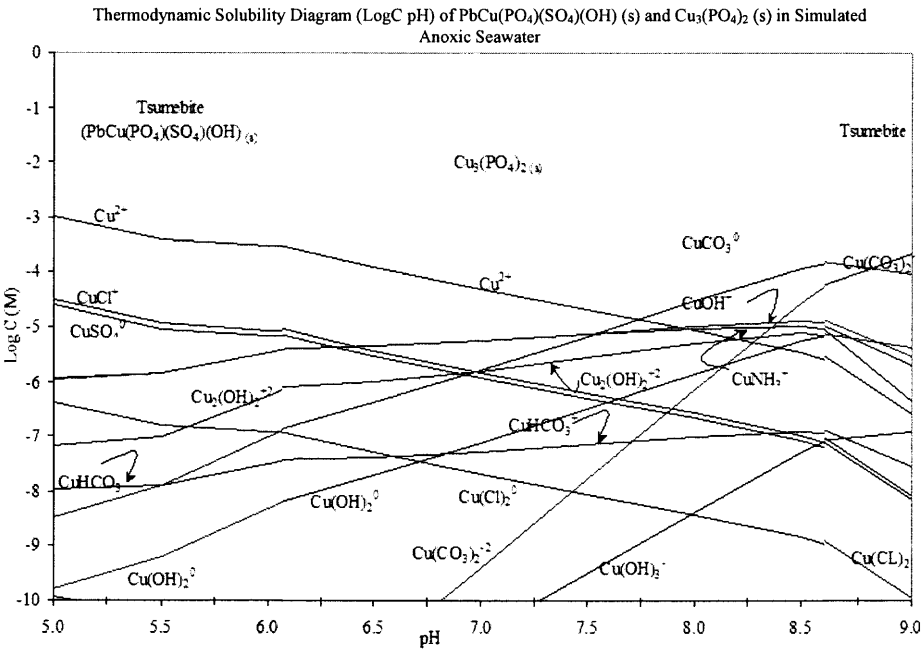

Figure A.26 Solubility diagram of Cu-minerals in anoxic seawater.

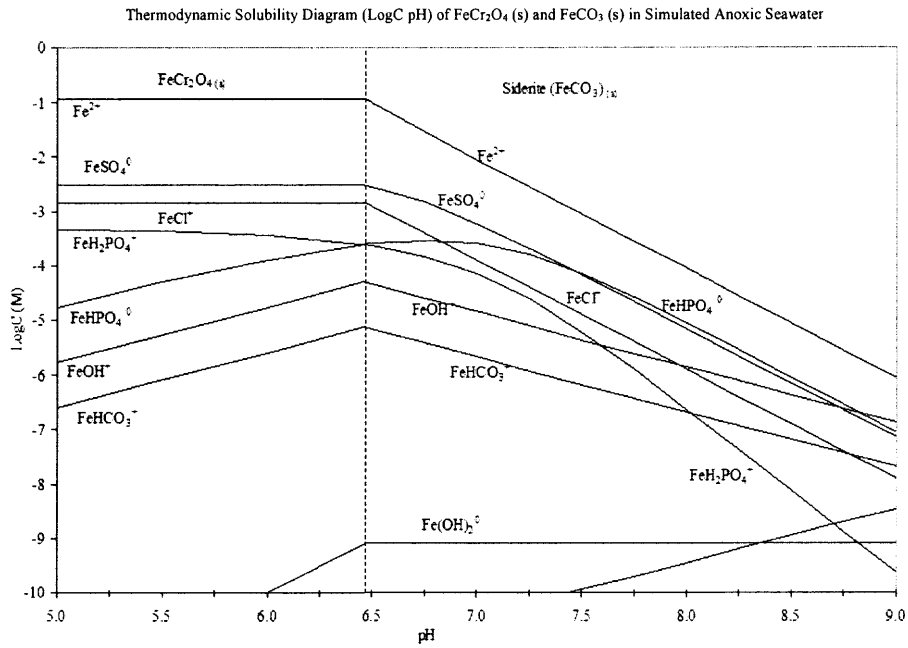

Figure A.27 Solubility diagram of Fe-minerals in anoxic seawater.

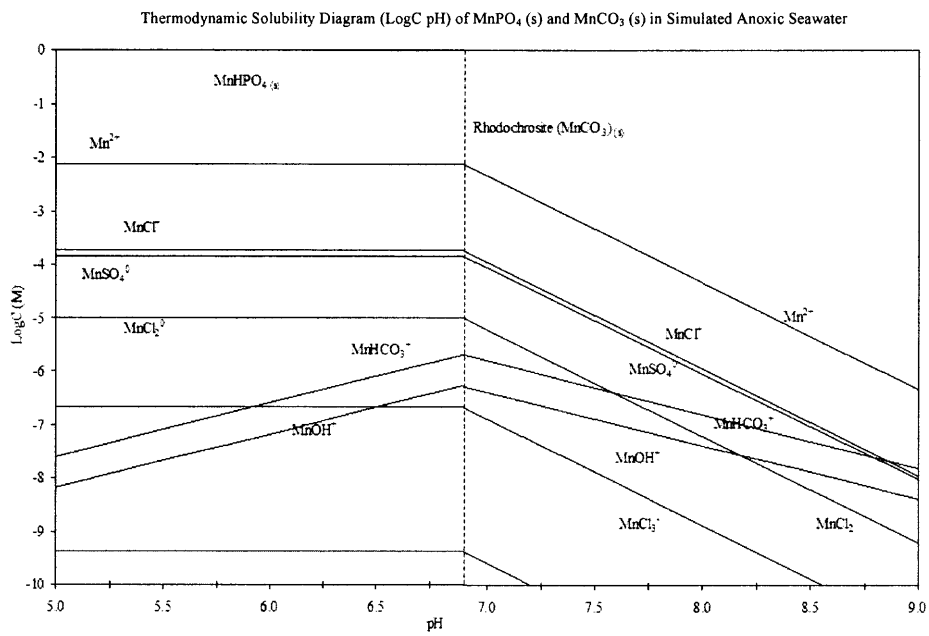

Figure A.28 Solubility diagram of Mn-minerals in anoxic seawater.

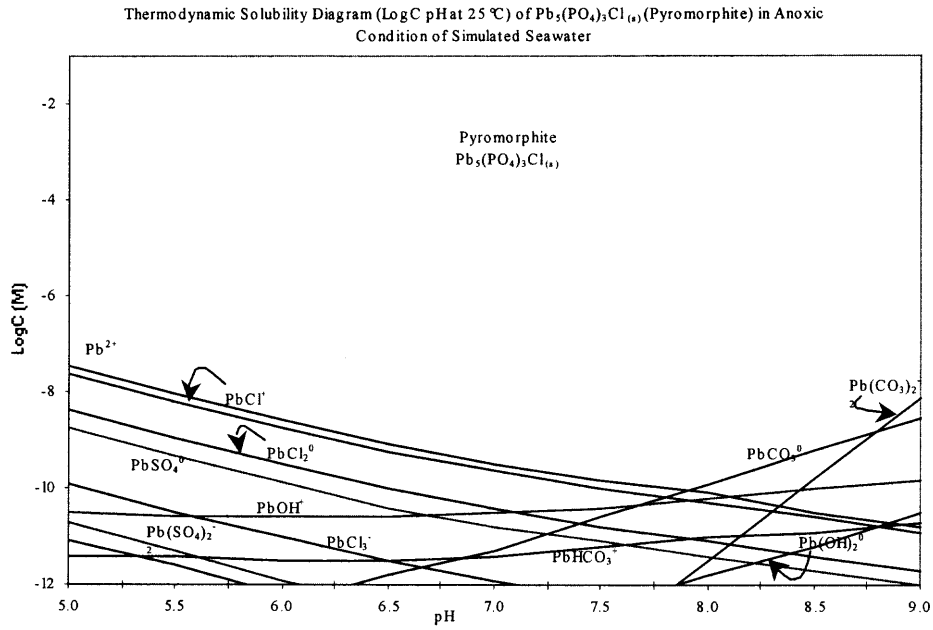

Figure A.29 Solubility diagram of Pb-minerals in anoxic seawater.

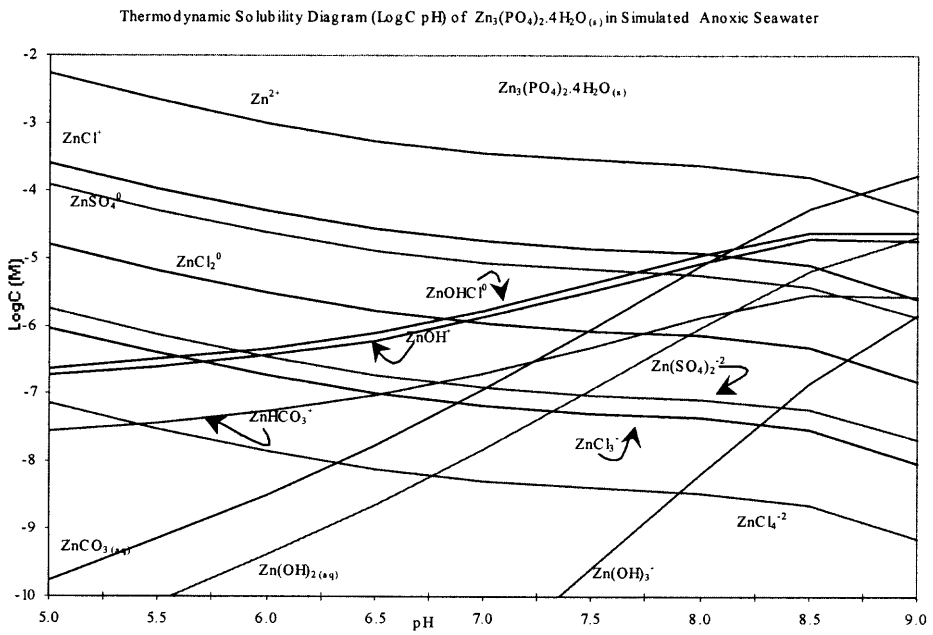

Figure A.30 Solubility diagram of Zn-minerals in anoxic seawater.

REFERENCES

Adam P (1990) Salt marsh ecology. Cambridge University Press, Cambridge, UK.

Allen HE, Fu G, Boothman W, DiToro DM, Mahony JD (1991) Determination of acid volatile sulfide and selected simultaneously extractable metals in sediment. US Environmental Protection Agency, Washington, DC.

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, Cambridge, UK.

Ames RN, Reid CPP, Porter LK, Cambardella C (1983) Hyphal uptake and transport of nitrogen from two N-labelled sources by *Glomus mosseae*, a vesicular-arbusuclar mycorrhizal fungus. New Phytol. 95:381-396.

Amsberry L, Baker MA, Ewanchuk PJ, Bertness MD (2000) Clonal integration and the expansion of *Phragmites australis*. Ecol. Appl. 10:1110-1118.

Anastasiou CJ, Brooks JR (2003) Effects of soil pH, redox potential, and elevation on survival of *Spatina patens* planted at a West Central Florida salt marsh restoration site. Wetlands 23:845-859.

Andersson E, Simoneit BRT, Holm NG (2000) Amino acid abundances and stereochemistry in hydrothermally altered sediments from the Juan de Fuca Ridge, northeastern Pacific Ocean. Appl. Geochem. 15:1169-1190

Atkinson MJ, Bingman C (1998) Elemental composition of commercial seasalts. J. Aquat. Sci. 8:39-43.

Bagwell CE, Lovell CR (2000) Microdiversity of culturable diazotrophs from the rhizoplanes of the salt marsh grasses *Spartina alterniflora* and *Juncus roemerianus*. Microb. Ecol. 39:128-136.

Bagwell CE, Piceno YM, Ashburne-Lucas A, Lovell CR (1998) Physiological diversity of the rhizosphere diazotroph assemblages of selected salt marsh grasses. Appl. Environ. Microbiol. 64:4276-4282.

Baker AJM, Reeves RD, Hajar ASM (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J.&C. Presl (Brassicaceae). New Phytol. 127:61-68.

Bandyopadhyay BK, Pezeshki SR, DeLaune RD, Lindau CW (1993) Influence of soil oxidation-reduction potential and salinity on nutrition, -15N uptake, and growth of *Spartina patens*. Wetlands 13:10-15.

Batty LC, Baker AJM, Wheeler BD, Curtis CD (2000) The effect of pH and plaque on the uptake of Cu and Mn in *Phragmites australis* (Cav.) Trin ex Steudel. Annals Bot. 86:647-653.

Baude FJ, Pease HL, Holt RF (1974) Fate of benomyl on field soil and turf. J. Agr. Food Chem. 22:413-418.

Behrends B, Liebezeit G (1999) Particulate amino acids in Wadden Sea waters - seasonal and tidal variations. J. Sea Res. 41:141-148.

Bertness MD (1991) Zonation of *Spartina patens* and *Spartina alterniflora* in a New England salt marsh. Ecology 72:138-148.

Bertness MD (1992) The ecology of a New England marsh. Am. Sci. 80:260-268.

Bertness MD, Callaway R (1994) Positive interactions in communities. Trend Ecol. Evol. 9:191-193.

Bijlmakers LL, de Swart EOAM (1995) Large-scale wetland-restoration of the Ronde Venen, The Netherlands. Water Sci. Technol. 31:197-205.

Blaylock M, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I (1997) Enhanced accumulation of Pb in Indian Mustard by soil-applied chelating agents. Environ. Sci. Technol. 31:860-865.

Boesch DF, Turnar RE (1984) Dependence of fishery species on salt marshes: the role of food and refuge. Estuaries 7:460-468.

Borman BT, Cromack JK, Russell WOI (1993) Influences of alder on soils and long-term ecosystem productivity. In: Tarrant RF (ed) Biology and Management of Alder. Oregon State University Press, Corvallis, OR, USA, pp. 47-56.

Boucher DH (1985) The biology of mutualism, ecology and evolution. Oxford University Press, New York, USA.

Brookins DG (1988) Eh, pH diagrams for geochemistry. Springer Verlag, New York, NY, USA.

Broome SW, Seneca ED, Woodhouse WW (1988) Tidal salt marsh restoration. Aquat. Bot. 32:1-22.

Brundrett MC, Melville L, Peterson RL (1994) Practical methods in mycorrhiza research. Mycologue Publications, Waterloo, Ontario, Canada.

Bryan GW, Langston WJ (1992) Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. Environ. Pollut. 76:89-131.

Buchmann N, Brooks JR, Rapp KD, Ehleringer JR (1996) Carbon isotope composition of C4 grasses is influenced by light and water supply. Plant, Cell Environ. 19:392-402.

Burke D, Hahn D (2000) The effect of mycorrhizae on nitrogen-fixing bacteria associated with the salt marsh grass *Spartina patens*. In: Nieder WC, Waldman JR (eds) Final Reports of the Tibor T. Polgar Fellowship Program 1999, section II. Hudson River Foundation, New York, p. 28.

Burke D, Hamerlynck E, Hahn D (2002a) Effect of arbuscular mycorrhizae on soil microbial populations and associated plant performance of the salt marsh grass *Spartina patens*. Plant Soil 239:141-154.

Burke D, Hamerlynck EP, Hahn D (2002b) Interactions among plant species and microorganisms in salt marsh sediments. Appl. Environ. Microbiol. 68:1157-1164.

Burke D, Hamerlynck EP, Hahn D (2003) Interactions between the salt marsh grass *Spartina patens*, arbuscular mycorrhizal fungi and sediment bacteria during the growing season. Soil Biol. Biochem. 35:501-511.

Burke D, Weis JS, Weis P (2000) Release of metals by the leaves of the salt marsh grasses *Spartina alternifolia* and *Phragmites australis*. Estuar. Coast. Shelf Sci. 51:153-159.

Cakmak I, Marschner H (1988) Increase in membrane permeability and exudation of roots of zinc deficient plants. J. Plant Physiol. 132:356-361.

Callaway RM (1997) Positive interactions in plant communities and the individualistic-continuum concept. Oecologia 112:143-149.

Cammack R, Fernandez VM, Schneider K (1988) Nickel in hydrogenases from sulfate-reducing, photosynthetic, and hydrogen-oxidizing bacteria. In: Lancaster JRj (ed) The bioorganic chemistry of nickel. Verlag Chemie, Weinheim, Germany, pp. 167-190.

Campbell GC, Tessier A (1996) Ecotoxicology. In: Jagoe CH (ed) Ecotoxicology of metals in the aquatic environment: geochemical aspects. Lewis Publishers, Boca Raton, FL.

Carbonell-Barrachina AA, Arabi MA, DeLaune RD, Gambrell RP, Patrick WHj (1998) The influence of arsenic chemical form and concentration on *Spartina patens* and *Spartina alterniflora* growth and tissue arsenic concentration. Plant Soil 217:33-43.

Caris C, Hördt W, Hawkins H-J, Römheld V, George E (1998) Studies of iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants. Mycorrhiza 8:35-39.

Cary EE, Allaway WH, Olson OE (1977) Control of chromium concentrations in food plants. 1. Absorption and translocation of chromium by plants. J. Agric. Food Chem. 25:300-304.

Chalmers AG (1979) The effects of fertilization on nitrogen distribution in a *Spartina alterniflora* salt marsh. Estuar. Coast. Shelf Sci. 36:105-131.

Chang WM (1985) Soil column leaching studies with [phenyl-14C(U)]benomyl., E.I. Du Pont de Nemours and Co., Inc., Agricultural Chemicals Department, Experimental Station (Unpublished report No. AMR 426-85). Wilmington, Delaware.

Chaudri AM, McGrath SP, Giller KE, Rietz E, Sauerbeck DR (1993) Enumeration of indigenous *Rhizobium leguminosarum* biovar *trifolii* in soils previously treated with metal-contaminated sewage sludge. Soil. Biol. Biochem. 25:301-309.

Chen J, Li Y, Yin K, Jin H (2004) Amino acids in the Pearl River estuary and adjacent waters: origins, transformations and degradation. Cont. Shelf Res. 24:1877-1894.

Cooke JC, Butler RH, Madole G (1993) Some observations on the vertical distribution of vesicular arbuscular mycorrhizae in roots of salt marsh grasses growing in saturated soils. Mycologia 84.:547-550.

Cooke JC, Lefor MW (1990) Comparison of vesicular-arbuscular mycorrhizae in plants from disturbed and adjacent undisturbed regions of a coastal salt marsh in Clinton Connecticut, USA. Environ. Manage. 14:131-137.

Cooper KM, Tinker PB (1981) Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. IV. Effect of environmental variables on movement of phosphorus. New Phytol. 88:327-339.

Cowie GL, Hedges JI (1992) Improved amino acid quantification in environmental samples: charge-matched recovery standards and reduced analysis time. Mar. Chem. 37:223-238.

Currin CA, Newell SY, Paerl HW (1995) The role of standing dead *Spartina alterniflora* and benthic micoralgae in salt marsh food webs; considerations based on multiple stable isotope analysis. Mar. Ecol-Prog. Ser. 121:99-116.

Cutler JM, Rains DW (1974) Characterization of cadmium uptake by plant tissue. Plant Physiol. 54:67-71.

Dai T, Wiegert RG (1997) A field study of photosynthetic capacity and its response to nitrogen fertilization in *Spartina alterniflora*. Estuar. Coast. Shelf Sci. 45:273-283.

Dame RF (1989) The importance of *Spartina alterniflora* to Atlantic Coast estuaries. Rev. Aquat. Sci. 1:639-660.

Damesin C, Rambal S, Joffre R (1998) Seasonal and annual changes in leaf 13C in two co-occurring Mediterranean oaks: relations to leaf growth and drought progression. Funct. Ecol. 12:778-785.

Daniell TJ, Husband R, Fitter AH, Young JPW (2001) Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. FEMS Microbiol. Ecol. 36:203-209.

Dauwe B, Middelburg J (1998) Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. Limnol. Oceanogr. 43:782-798.

Dauwe B, Middelburg JJ, Herman PMJ, Heip CHR (1999) Linking diagenetic alteration of amino acids and bulk organic matter reactivity. Limnol. Oceanogr. 44:1809-1814.

Davies FT, Puryear JD, Newton RJ, Egilla JN, Saraiva Grossi JA (2001) Mycorrhizal fungi enhance accumulation and tolerance of chromium in sunflower (*Helianthus annuus*). J. Plant Physiol. 158:777-786.

DeLaune RD, Feijtel TC, Patrick WH (1989) Nitrogen flows in a Louisiana gulf coast salt marsh: spatial considerations. Biogeochemistry 8:25-37.

DeLaune RD, Pezeshki SR (1991) Role of soil chemistry in vegetative ecology of wetlands. Trends in Soil Sci. 1:101-113.

Díaz G, Azcón-Aguilar C, Honrubia M (1996) Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. Plant Soil 180:241-249.

Dong S, Scagel CF, Cheng L, Fuchigami LH, Rygiewicz PT (201) Soil temperature and plant growth stage influence nitrogen uptake and amino acid concentration of apple during early spring growth. Tree Physiol. 21:541-547.

Doyle MO, Otte ML (1997) Organism-induced accumulation of iron, zinc and arsenic in wetland soils. Environ. Pollut. 96:1-11.

Duncan DB (1995) Multiple range and multiple F-tests. Biometrics 11:1-42.

Eaton AD, Clesceri LS, Greenberg AE (1995) Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC, USA.

Egerton-Warburton LM, Allen EB (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecol. Appl. 10:484-496.

Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Pearcy RW, Ehleringer J, Mooney HA, Rundel PW (eds) Plant physiological ecology: field methods and instrumentation. Chapman and Hall, New York, pp. 281-300.

Engels C, Marschner H (1992) Root to shoot translocation of macronutrients in relation to shoot demand in maize (*Zea mays* L.) grown at different root zone temperature. Z. Pflanz. Bodenkunde. 155:121-128.

EPA (1983) Metals (atomic absorption methods). In: Anonymous (ed) Methods for Chemical Analysis of Water and Wastes (EPA-600/4-79-020). US EPA, Cincinnati, Ohio, USA, pp. 55-72.

EPA (1986) Test methods for evaluating solid waste, physical/chemical methods 3rd ed - 4 vols: EPA 530/SW-846, U.S. Government Printing Office (GPO), Superintendent of Documents, Washington, DC.

Eskew DL, Welch RM, Norwell WA (1984) Nickel in higher plants. Further evidence for an essential role. Plant Physiol. 76:691-693.

Fitzgerald EJ, Caffrey JM, Nesaratnam ST, McLoughlin P (2003) Copper and lead concentrations in salt marsh plants on the Suir Estuary, Ireland. Environ. Pollut. 123:67-74.

Flemming CA, Trevors JT (1989) Copper toxicity and chemistry in the environment: a review. Water, Air, Soil Pollut. 44:143-158.

Fliessbach A, Martens R, Reber HH (1994) Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol. Biochem. 26:1201-1205.

Florijn PJ, Van Beusichem ML (1993) Uptake and distribution of cadmium in maize inbred lines. Plant Soil 150:25-32.

Förstner U, Wittmann GTW (1981) Metal pollution in the aquatic environment. Springer-Verlag, New York.

Fox MJ, York AT (1995) Draft Environmental Impact Statement on the Special Area Management Plan for the Hackensack Meadowlands District, NJ, U.S. Environmental Protection Agency-Region II and U.S. Army Corps of Engineers-New York District.

Francis R, Read DJ (1994) The contributions of mycorrhizal fungi to the determination of plant community structure. Plant Soil 159:11-25.

Friedel JK, Scheller E (2002) Composition of hydrolysable amino acids in soil organic matter and soil microbial biomass. Soil Biol. Biochem. 34:315-325.

Frostegård Å, Tunlid A, Bååth E (1993) Phospholipid fatty acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl. Environ. Microbiol. 59:3605-3617.

Galloway JN, Thornton JD, Norton SA, Volcho HL, McLean RA (1982) Trace metals in atmospheric deposition: A review and assessment. Atmos. Environ. 16:16-77.

Gambrell R (1994) Trace and toxic metals in wetlands - a review. J. Environ. Qual. 23:883-891.

Gambrell RP, Wiesepape JB, Patrick WHj, Duff MC (1991) The effects of pH, redox, and salinity on metal release from a contaminated sediment. Water, Air, and Soil Pollut. 57/58:359-367.

Geoffrey PG, Horst DS (1999) EH, pH diagrams for Mn, Fe, Co, Ni, Cu and As under seawater conditions: application of two new types of E_h, pH diagrams to the study of specific problems in marine geochemistry. Aquat. Geochem. 5:227-248.

Giddens J, Todd RL (1984) Rhizosphere microorganisms-overview. In: Kral DM, Hawkins SL (eds) Microbial-Plant Interactions, ASA special publication number 47. Soil Science Society of America, Madison, Wisconsin, pp. 51-68.

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem. 30:1389-1414.

Glasby GP, Schulz HD (1999) EH, pH diagrams for Mn, Fe, Co, Ni, Cu and As under seawater conditions: application of two new types of Eh, pH diagrams to the study of specific problems in marine geochemistry. Aquat. Geochem. 5:227–248.

Godbold DL, Horst WJ, Collins JC, Thurmann DA, Marschner H (1984) Accumulation of zinc annd organic acids in roots of zinc tolerant and non-tolerant ecotypes of *Deschampsia caespitosa*. J. Plant Physiol. 116:59-69.

Green MS, Etherington JR (1977) Oxidation of ferrous iron by rice (*Oryza sativa* L.) roots: a mechanism for waterlogging tolerance? J. Exp. Bot. 28:678-690.

Griffiths BS, DiazRavina M, Ritz K, McNicol JW, Ebblewhite N, Baath E (1997) Community DNA hybridisation and %G+C profiles of microbial communities from heavy metal polluted soils. FEMS Microbiology Ecology 24:103-112.

Haag-Kewer A, Schäfer HJ, Heiss S, Walter C, Rausch T (1999) Cadmium exposure in *Brassica juncea* causes a decline in transpiration rate and leaf expansion without effect on photosynthesis. J. Exp. Bot. 50:1827-1835.

Hamel C, Nesser C, Barrantes-Cartin U, Furlan V, Smith DL (1991) Endomycorrhizal fungal species mediate 15N transfer from soybean to maize in non-fumigated soils. Plant Soil 138:41-47.

Han FX, Sridhar MBB, Monts DL, Su Y (2004) Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea*. New Phytol. 162:489-499.

Harnett DC, Hetrick BAD, Willson BWT, Gibson DJ (1993) Mycorrhizal influence on intra-and interspecific neighbor interactions among co-occurring prairie grasses. Ecology 81:787-795.

Hartnett DC, Wilson GWT (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. Ecology 80:1187-1195.

Haugen J-E, Lichtentaler R (1999) Amino acid diagenesis, organic crbon and nitrogen mineralization in surface sediments from the inner Oslofjord, Norway. Geochim. Cosmochim. Acta 55:1649-1661.

Hayman DS (1986) Mycorrhizae of nitrogen-fixing legumes. Mircen J. Appl. Microb. 2:121-145.

Hershberger LW, Arce GT (1993) International programme on chemical safety: Environmental health criteria 148, Benomyl, World Health Organization, Geneva.

Hetrick BAD, Harnett DC, Wilson GWT, Gibson DJ (1994) Effects of mycorrhizae, phosphorous availability and plant density on yield relationships among competing tallgrass prairie grasses. Can. J. Bot. 72:168-176.

Hetrick BAD, Wilson GWT, Leslie JF (1991) Root architecture of warm- and cool-season grasses: Relationship to mycorrhizal dependence. Can. J. Bot. 69:112-118.

Hines ME, Evans RS, Sharak Genthner BR, Willis SG, Friedman S, Rooney-Varga JN, Devereux R (1999) Molecular phylogenetic and biogeochemical studies of sulfate-reducing bacteria in the rhizosphere of *Spartina alterniflora*. Appl. Environ. Microbiol. 65:2209-2216.

Hines ME, Knollmeyer SL, Tugel JB (1989) Sulfate reduction and other sedimentary biogeochemistry in a northern New England salt marsh. Limnol. Oceanogr. 34:578-590.

Hobbie JE, Fletcher MM (1988) The aquatic environment. In: Lynch JM, Hobbie JE (eds) Micro-organisms in action: Concepts and applications in microbial ecology. Blackwell Scientific Publications, Oxford, pp. 132-162.

Hodge A (2000) Microbial ecology of the arbuscular mycorrhiza. FEMS Microbiol. Ecol. 32:91-96.

Hoefnagels MH, Broome SW, Shafer SR (1993) Vesicular-arbuscular mycorrhizae in salt marshes in North Carolina. Estuaries 16:851-858.

Hogue EJ, Neilson GH (1986) Effect of root temperature and varying cation ratios on growth and leaf cation concentration of apple seedlings grown in nutrient solution. Can. J. Plant Sci. 66:637-645.

Howe JA, Loeppert RH, DeRose VJ, Hunter DB, Bertsch PM (2003) Localization and speciation of Chromium in subterranean clover using XRF, XANES, and EPR spectroscopy. Environ. Sci. Technol. 37:4091-4099.

Hsieh YP, Yang CH (1997) Pyrite accumulation and sulfate depletion as affected by root distribution in a *Juncus* (needle rush) salt marsh. Estauries 20:640-645.

http://www.ffp.csiro.au/research/mycorrhiza/vam.html (2004) .

http://www.meadowlands.state.nj.us/eip/wl-harrier.html (2004).

Huxman TE, Hamerlynck EP, Moore BD, Smith SD, Jordan DN, Zitzer SF, Nowak RS, Coleman JS, Seemann JR (1998) Photosynthetic down-regulation in *Larrea tridentata* exposed to elevated atmospheric CO_2: interaction with drought under glasshouse and field (FACE) exposure. Plant, Cell Environ. 21:1153-1161.

Jack McCormick and Association I (1977) Investigation of aquatic and terrestial mercury contamination in the vincinity of the former location of the Wood Ridge Chemical Corporation processing plant.

Jacob J, Greitner C, Drake DG (1995a) Acclimation of photosynthesis in relation to Rubisco and non-structural carbohydrate contents and *in situ* carboxylase activity in *Scirpus olneyi* grown in elevated CO_2. Plant Cell Environ. 18:875-884.

Jacob J, Greitner C, Drake DG (1995b) Acclimation of photosynthesis in relation to Rubisco and non-structural carbohydrate contents and *in situ* carboxylase activity in *Scirpus olneyi* grown in elevated CO_2. Plant, Cell Environ. 18:875-884.

Joner EJ, Leyval C (1997) Uptake of ^{109}Cd by roots and hyphae of *Glomus mosseae/Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. New Phytol. 135:253-260.

Jones DL, Kemmitt SJ, Wright DJ, Cuttle SP, Bol R, Edwards AC (2005) Rapid intrinsic rates of amino acid biodegradation in soils are unaffected by agricultural management strategy. Soil Biol. Biochem. 37:1267-1275.

Jones MD, Hutchinson TC (1988) Nickel toxicity in mycorrhizal birch seedlings infected with *Lactarius rufus* or *Scleroderma flavidum*. I. Effects on growth, photosynthesis, respiration and transporation. New Phytol. 108:451-459.

Jones NB, Gilligan JP (1983) *o*-phtaldialdehyed precolumn derivatization and reversed-phase HPLC of polypeptide and physiological fluids. J.Chromatogr. 266:471-482.

Kahiluoto H, Ketoja E, Vestberg M (2000) Creation of a non-mycorrhizal control for a bioassay of AM effectiveness. 1. Comparison of methods. Mycorrhiza 9:241-258.

Kahiluoto H, Vestberg M (2000) Creation of a non-mycorrhixal control for a bioassay of AM effectiveness. 2. Benomyl application and soil sampling time. Mycorrhiza 9:259-270.

Kelly DP, Wood AP (1998) Microbes of the sulfur cycle. In: Burlage RS, Atlas R, Stahl D, Geesey G, Sayler G (eds) Techniques in Microbial Ecology. Oxford University Press, New York, pp. 31-57.

Kelly JJ, Häggblom MM, Tate RL (2003) Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter as indicated by analysis of microbial community phospholipid fatty acid profiles. Biol. Fert. Soils 38:65-71.

Khan SU, Schnitzer M (1971) Sephadex gel filtration of fulvic acid: the identification of major components in two low molecular weight fractions. Soil Sci. 112:321-238.

Kilham K, Firestone MK (1983) Vesicular-arbuscular mycorrhizal meditation of grass response to acidic and heavy metal deposition. Plant Soil 72:39-48.

Kneib RT (1997) The role of tidal mrashes in the ecology of estuarine nekton. Oceanogr. Mar. Biol. Annu. Rev. 35:163-220.

Kochian LV (1991) Mechanisms of micronutrient uptake and translocation in plants. In: Mortvedt JJ (ed) Micronutrients in agriculture. Soil Sci. Soc. Am. Book Series No. 4, Madison, WI, USA, pp. 229-296.

Koomen I, McGrath SP, Giller KE (1990) Mycorrhizal infection of clover is delayed in soils contaminated with heavy metals from past sewage sludge applications. Soil Biol. Biochem. 22:871-873.

Kormanick PP, McGraw AC (1982) Quantification of vesicular-arbuscular mycorrhizas in plant roots. In: Schenck NC (ed) Methods and Principals of Mycorrhizal Research. The American Phytopathological Society, St Paul, Minnesota, pp. 37-45.

Kovacheva P, Djingova R, Kuleff I (2000) On the representative sampling of plants for multielement analysis. Phytol. Balcanica 6:91-102.

Krom MD, Davison P, Zhang H, Davison W (1994) High-resolution pore-water sampling with a gel sampler. Limnol. Oceanogr. 39:1967-1972.

Larcher W (1995) Physiological plant ecology. Springer Verlag, Berlin, Germany.

Larsson EH, Asp H, Bornman JF (2002) Influence of prior Cd^{2+} exposure on the uptake of Cd^{2+} and other elements in the phytochelatin-deficient mutant, cad1-3, of *Arabidopsis thaliana*. J. Exp. Bot. 53:447-453.

Lasat MM (2002) Phytoextraction of toxic metals: A review of biological mechanisms. Journal of Environmental Quality 31:109-120.

Levine JM, Brewer JS, Bertness MD (1998) Nutrients, competition and plant zonation in a New England salt marsh. J. Ecol. 86:285-292.

Linderman RG (1988) Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. Phytopathology 78:366-371.

Lindroth P, Mopper K (1979) HPLC determination of subpicomole amounts of amino acids by precolum fluorescence derivatization with *o*-pthaldialdehyde. Anal. Chem. 51:1667-1674.

Ling-lee M, Ashford AE, Chilvers GA (1977) A histochemical study of polysaccharide distribution in eucalypt mycorrhizas. New Phytol. 78:329-335.

Linnik PN (2003) Complexation as the most important factor in the fate and transport of heavy metals in the Dnieper water bodies. Anal. Bioanal. Chem. 376:405-412.

Losi ME, Amrhein C, Frankenberger WT (1994) Factors affecting chemical and biological reduction of Cr(VI) in soil. Environ. Toxicol. Chem. 13:1727-1735.

Luoma SN (1983) Bioavailability of trace metals to aquatic organisms--a review. Sci. Total Environ. 28:1-22.

Luther GWI, Church TM (1988) Seasonal cycling of sulfur and iron in porewaters of a Delaware salt marsh. Mar. Chem. 23:295-309.

Mace JE, Graham RC, Amrhein C (1997) Anthropogenic lead distribution in rodent-affected and undisturbed soils in Southern California. Soil Sci. 162:46-50.

Mansfield SD, Barlocher F (1993) Seasonal variation of fungal biomass in the sediment of a salt marsh in New Brunswick. Microbial Ecol. 26:37-45.

Mantoura RFC, Dickson A, Riley JP (1978) The complexation of metals with humic materials in natural waters. Estuar. Coast. Mar. Sci. 6:587-594.

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, San Diego, CA.

Marsh BH, Arthur MF (1989) Aerobic metabolism of [phenyl(U)-14C] benomyl in Keyport Silt Loam., Battelle Memorial Institute (Unpublished report No. AMR 1112-88, prepared for E.I. Du Pont de Nemours and Co., Inc.), Columbus, Ohio.

Mason RP, Sullivan KA (1998) Mercury and methylmercury transport through an urban watershed. Water Res. 32:321-330.

McBride M (1994) Environmental chemistry of soils. Oxford University Press, New York.

McCabe OM, Otte ML (2000) Revegetation of metal mine tailings under wetland conditions. In: Trouart J (ed) Proceedings of the 14th Annual National Meeting -- Vision 2000 and environmental commitment. American Society for Surface Mining and Reclamation, Austin, TX.

McClung CR, van Berkum P, Davis RE, Sloger C (1983) Enumeration and localization of N2-fixing bacteria associated with roots of *Spartina alterniflora* Loisel. Appl. Environ. Microbiol. 45:1914-1920.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115:495-501.

McMichael BL, Burke JJ (1998) Soil temperature and root growth. HortScience 33:947-951.

Medernach L, Grémare A, Amouroux JM, Colomines JC, Vétion G (2001) Temporal changes in the amino acid contents of particulate organic matter sedimenting in the Bay of Banyuls-sur-Mer (northwestern Mediterranean). Mar. Ecol. Prog. Ser. 214:55-65.

Mendelssohn IA, Kleiss BA, Wakeley JS (1995) Factors controlling the formation of oxidized root channels: a review. Wetlands 15:37-46.

Mendelssohn IA, Postek MT (1982) Elemental analysis of deposits on the roots of *Spartina alterniflora* Loisel. Am. J. Bot. 69:904-912.

Merharg AA (2003) The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations: review. Mycol. Res. 107:1253-1265.

Mitchell AK, Hinckley TM (1993) Effects of foliar nitrogen concentration on photosynthesis and water use efficiency in Douglas-fir. Tree Physiol. 12:403-410.

Mitsch WJ, Gosselink JG (1993) Wetlands. John Wiley & Sons, New York.

Moors JW, Ramamoorthy S (1984) Heavy metals in natural waters. Springer Verlag, New York.

Morgutti S, Sacchi GA, Cocucci SM (1984) Effects of Ni^{+2} on proton extrusion, dark CO_2 fixation and malate synthesis in maize roots. Physiol. Plantarum 60:70-74.

Moriarty DJW, Iverson RL, Pollard PC (1986) Exudation of organic carbon by the seagrass *Halodule wrightii* Aschers and its effect on bacterial growth in sediment. J. Exp. Mar. Biol. Ecol. 96:115-126.

Morrison GM, Wei CH (1991) Electroanalysis of metal speciation and its relevance to ecotoxicology. Anal. Proc. 28:70-71.

Mortimer RJG, Davey JT, Krom MD, Watson PG, Frickers PE, Clifton RJ (1999) The effect of macrofauna on porewater profiles and nutrient fluxes in the intertidal zone of the Humber estuary. Estuar. Coast. Shelf Sci. 48:683-699.

Mortimer RJG, Krom MD, Hall POJ, Hulth S, Ståhl H (1998) Use of gel probes for the determination of high resolution solute distributions in marine and estuarine pore waters. Mar. Chem. 63:119-129.

Mullins GL, Sommers LE (1986) Cadmium and zinc influx characteristics by intact corn (*Zea mays* L.) seedlings. Plant Soil 96:153-164.

Naidoo G, McKee KL, Mendelssohn IA (1992) Anatomical and metabolic responses to waterlogging and salinity in *Spartina alterniflora* and *S. patens* (Poaceae). Am. J. Bot. 79:765-770.

Nedeau EJ, Merritt RW, Kaufman MG (2003) The effect of industrial effluent on an urban stream benthic community: water quality vs. habitat quality. Environ. Pollut. 123:1-13.

Newsham KK, Watkinson AR, West HM, Fitter AH (1995) Symbiosis fungi determine plant commuinty structure: changes in a lichen-rich community induced by fungicide application. J. Ecol. 9:442-227.

Nies DH (1999) Microbial heavy metal resistance. Appl. Microbiol. Biotechnol. 51:730-750.

Nriagu JO (1984) Formation and stability of base metal phosphates in soil and sediments. In: Nriagu JO, Moore PB (eds) Phosphate Minerals. Springer-Verlag, New York, pp. 319-329.

O'Connor PJ, Smith SE, Smith FA (2002) Arbuscular mycorrhizas influence plant diversity and community structure in a semiarid herbland. New Phytol. 154:209-218.

Odum HT (2000) Heavy metals in the environment: using wetlands for their removal. Lewis Publishers, Boca Ration, FL, USA.

Otte ML, Bestebroer SJ, van der Linden JM, Rozema J, Broekman R (1991) A survey of zinc, copper and cadmium concentrations in salt marsh plants along the Dutch coast. Environ. Pollut. 72:175-189.

Otte ML, Kearns CC, Doyle MO (1995) Accumulation of arsenic and zinc in the rhizosphere of wetland plants. Bull. Environ. Contam. Toxicol. 55:154-161.

Pagencopf G (1993) Type of metal ions and its toxicity on water systems. In: H S (ed) Concepts on metal ion toxicity. Mir, Moscow, Russia, pp. 88-100.

Patriquin DG, Keddy C (1978) Nitrogenase activity (acetylene reduction) in a Nova Scotian salt marsh: its association with angiosperms and the influence of some edaphic factors. Aquat. Bot. 4:227-244.

Paul EA, Clark FE (1996) *Soil Microbiology and Biochemistry*. Academic Press, New York.

Pawlowska TE, Blaszkowski J, Ruehling A (1996) The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland. Mycorrhiza 6:499-505.

Pedesen CT, Sulvia DM (1997) Limitations to using benomyl in evaluating mycorrhizal functioning. Biol. Fert. Soils 25:163-168.

Penning de Vries FWT, Bruinsting AHM, van Laar HH (1974) Products, requirements and efficiency of biosynthesis: a quantitative approach. J. Theor. Biol. 45:339-377.

Peterson BJ, Howarth RW (1987) Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt marsh estuaries of Sapelo Island, Georgia. Limnol. Oceanogr. 32:1195-1213.

Peterson RL (1992) Adaptations of root structure in relation to biotic and abiotic factors. Can. J. Bot. 70:661-675.

Pezeshki SR, DeLaune RD (1996) Responses of *Spartina alterniflora* and *Spartina patens* to rhizosphere oxygen deficiency. Acta Oecol. 17:365-378.

Pezeshki SR, Matthews SW, DeLaune RD (1991) Root cortex structure and metabolic responses of *Spartina patens* to soil redox conditions. Environ. Experi. Bot. 31:91-97.

Pfeffer PE, Tu S-I, Gerasimovicz WV, Cavanaughk JR (1986) *In vivo* ^{31}PNMR studies of corn root tissue and its uptake of toxic metals. Plant Physiol. 80:77-84.

Piechalak A, Tomaszewska B, Baralkiewicz D (2003) Enhancing phytoremediative ability of *Pisum sativum* bt EDTA application. Phytochemistry 64:1239-1251.

Polle A, Schützendübel A (2003) 7 Heavy metal signalling in plants: linking cellular and organismic responses. In: Hirt H, Shinozaki K (eds) Topics in current genetics vol 4/2003: plant responses to abiotic stress, Vol 4. Springer-Verlag GmbH, Berlin, pp. 187-215.

Portnoy JW (1999) Salt marsh diking and restoration: Biogeochemical implications of altered wetland hydrology. Environ. Management 24:111-120.

Rabie GH (2005) Contribution of arbuscular mycorrhizal fungus to red kidney and wheat plants tolerance grown in heavy metal-polluted soil. Afr. J. Biotechnol. 4:332-345.

Rahimi A, Bussler W (1974) Kupfermangel bei höheren Pflanzen und sein histochemischer Nachweis. Landwirt. Forsch. 30:101-111.

Rauser WE (1995) Phytochelatins and related peptides. Plant Physiol. 109:1141-1149.

Reekie EG, Bazzaz FA (1987) Reproductive effort in plants. I carbon allocation to reproduction. Am. Nat. 129:876-896.

Reid CPP (1984) Mycorrhizae: A root soil interface in plant nutrition. In: Kral DM, Hawkins SL (eds) Microbial-plant interactions, ASA special publication number 47. Soil Science Society of America, Madison, Wisconsin, pp. 29-50.

Roman CT, Niering WA, Warren RS (1984) Salt marsh vegetation change in response to tidal restriction. Environ. Manage. 8:141-150.

Rooney-Varga JN, Devereux R, Evans RS, Hines ME (1997) Seasonal changes in relative abundance of uncultivated sulfate-reducing bacteria in a salt marsh sediment and the rhizosphere of *Spartina alterniflora*. Appl. Environ. Microbiol. 63:3895-3901.

Rozas LP, McIvor CC, Odum WE (1988) Intertidal rivulets and creek banks: corridors between tidal creeks and marshes. Mar. Ecol. Prog. Ser. 47:303-307.

Rozema J, Arp W, van Diggelen J, van Esbroek M, Broekman R, Punte H (1986) Occurrence and ecological significance of vesicular arbuscular mycorrhiza in the salt marsh environment. Acta Bot. Neerl. 35:457-467.

Rozema J, Otte ML, Broekman RA, Punte H (1985) Accumulation of heavy metals in estuarine salt marsh sediment and uptake of heavy metals by salt marsh halophytes. In: Lekkas T (ed) Heavy Metals in the Environment, Vol. 1. CEP Consultants, Edinburgh, pp. 545–547.

Rublee PA (1982a) Bacteria and microbial distribution in estuarine sediments. In: Kennedy VS (ed) Estuarine Comparisons. Academic Press, New York, pp. 159-182.

Rublee PA (1982b) Seasonal distribution of bacteria in salt marsh sediments in North Carolina, USA. Estuar. Coast. Shelf Sci. 15:67-74.

Ryan DL (1989) Soil column leaching of [phenyl(U)-14C]benomyl in a rice paddy soil, E.I. Du Pont de Nemours and Co., Inc. (Unpublished report No. AMR 1512-89), Wilmington, Delaware.

Ryan MH, Angus JF (2003) Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. Plant Soil 250:225-239.

Sadiq M (1992) Toxic metal chemisty in marine environments. Mercel Dekker, Inc., New York.

Sage R, F., Pearcy RW (1987) The nitrogen use efficiency of C3 and C4 plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopdium album* (L.) and *Amaranthus retroflexus* (L.). Plant Physiol. 84:954-963.

Salt DE, Prince RC, Pickering IJ, Raskin I (1995) Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol. 109:1427-1433.

Schreiber U, Bilger W, Hormann H, Neubauer C (1998) Chlorophyll fluorescence as a diagnostic tool: Basics and some aspects of practical relevance. In: Raghavendra AS (ed) Photosynthesis, a comprehensive treatise. Cambridge University Press, Cambridge, UK, pp. 320-336.

Schuetzenduebel A, Polle A (2002) Plant responses to abiotic stresses: Heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53:1351-1365.

Scott-Fordsmand JJ (1997) Toxicity of nickel ot soil organisms in Denmark. Rev. Environ. Contam. T. 148:1-34.

Shahandeh H, Hossner LR (2000) Plant screening for chromium phytoremediation. Int. J. Phytoremediat. 2:31-51.

Shen Z-G, Li X-D, Wang C-C, Chen H-M, Chua H (2002) Lead phytoextraction from contaminated soil with high-biomass plant species. J. Environ. Qual. 31:1893-1900.

Sinicrope TL, Hine PG, Warren RS, Niering WA (1990) Restoration of an impounded salt marsh in New England. Estuaries 13:25-30.

Smart RM, Barko JW (1980) Nitrogen nutrition and salinity tolerance of *Distichlis spicata* and *Spartina alterniflora*. Ecology 61:630-638.

Smeyers-Verbeke J, De Graeve M, Francois M, De Jaegere R, Massart DL (1978) Cd uptake by intact wheat plants. Plant, Cell Environ. 1:291-296.

Smith MD, Hartnett DC, Rice CW (2000) Effects of long-term fungicide applications on microbial properties in tallgrass prairie soil. Soil Biol. Biochem. 32:935-946.

Smith MD, Hartnett DC, Wilson GWT (1999) Interacting influence of mycorrhizal symbiosis and competition on plant diversity in tallgrass prairie. Oecologia 121:574-582.

Smith SE, Read DJ (1997) Mycorrhizal Symbiosis. Academic Press, San Diego.

Sparks DL (1995) Environmental soil chemistry. Academic Press, San Diego, CA, USA.

Steever EZ, Warren RS, Niering WA (1976) Tidal energy subsidy and standing crop production of *Spartina alterniflora*. Estuar. Coast. Mar. Sci. 4:473-478.

Stoltz E, Greger M (2002) Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environ. Exp. Bot. 47:271-280.

Sun Y-P, Unestam T, Lucas SD, Johanson KJ, Kenne L, Finlay R (1999) Exudation-reabsorption in a mycorrhizal fungus, the dynamic interface for interaction with soil and soil microorganisms. Mycorrhiza 9:137-144.

Sundby B, Vale C, Cacador I, Caterino F, Madureira MJ, Caetano M (1998) Metal-rich concretions on the roots of salt marsh plants: mechanisms and rate of formation. Limnol. Oceanogr. 43:245-252.

Tagliavini M, Hogue EJ, Neilson GH (1991) Influence of phosphorus nutrition and root zone temperature on growth and mineral uptake of peach seedlings. J. Plant Nutr. 14:1267-1276.

Tan KH (2003) Humic matter in soil and the environment: principles and controversies. Marcel Dekker, Inc., New York.

Taylor GJ, Crowder AA, Rodden R (1984) Formation and morphology of an iron plaque on the roots of *Thypha latifolia* L. grown in solution culture. Am. J. Bot. 71:666-675.

Teal JM, Weinstein MP (2002) Ecological engineering, design, and construction considerations for marsh restorations in Delaware Bay, USA. Ecol. Engineering 18:607-618.

Thurman EM (1986) Organic geochemistry of natural waters. Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht, The Netherlands.

Tonin C, Vandenkoornhuyse P, Joner EJ, Straczek J, Leyval C (2001) Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. Mycorrhiza 10:161-168.

Toselli M, Flore JA, Maragoni B, Masia A (1999) Effects of root-zone temperature on nitrogen accumulation by non-bearing apple trees. J. Hortic. Sci. Biotech. 74:118-124.

Valiela I, Teal JM (1974) Nutrient limitation of salt marsh vegetation. In: Reimold RJ, Queen WH (eds) Ecology of Halophytes. Academic Press, New York, pp. 547-563.

Valiela I, Teal JM (1979) The nitrogen budget of a salt marsh ecosystem. Nature 280:652-656.

van Assche F, Clijsters H (1985) Inhibition of photosynthesis by heavy metals. Photosynth. Res. 7:31-40.

Van Cleve K, Viereck LA, Schlentner RL (1971) Accumulations of nitrogen in alder (*Alnus*) ecosystems near Fairbanks, Alaska. Arctic Alpine Res. 3:101-114.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72.

Van Duin WE, Rozema J, Ernst WHO (1989) Seasonal and spatial variation in the occurrence of vesicular-arbuscular mycorrhizae in salt marsh plants. Agric. Ecosyst. Environ. 29:107-110.

Vazquez MD, Barcelo J, Poschenrieder C, Madico J, Hatton P, Baker AJM, Cope GH (1992) Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can hyperaccumulate both metals. J. Plant. Physiol. 140:350-355.

Von Cammerere S, Farquhar GD (1981) Some relationships between the biochemistry and of photosynthesis and gas exchange of leaves. Planta 153:367-387.

Walker LR, Chapin FS (1987) Interactions among processes controlling successional change. Oikos 50:131-135.

Wattt RK, Ludden PW (1999) Nickel-binding proteins. CMLS Cellular Molecular Life Sciences 56:604-625.

Weis JS, Weis P (2004) Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. Environ. Int. 30:685-700.

Weissenhorn I, Leyval C, Belgy G, Berthelin J (1995a) Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. Mycorrhiza 5:245-251.

Weissenhorn I, Mench M, Leyval C (1995b) Bioavailability of heavy metals and arbuscular mycorrhiza in a sewage-sludge-amended sandy soil. Soil Biol. Biochem. 27:287-296.

Welsh DT, Bourgues S, de Wit E, Herbert RA (1996) Seasonal variations in nitrogen-fixation (acetylene reduction) and sulfate-reduction rates in the rhizosphere of *Zostera noltii*: nitrogen fixation by a sulfate-reducing bacteria. Mar. Biol. 125:619-628.

West HM, Fitter AH, Watkinson AR (1993) The influence of three biocides on the fungal associates of the roots of *Vulpia ciliata* ssp. *ambigua* under natural conditions. J. Ecol. 81:345-350.

Whitaker G, Terrell CR (1992) Federal programs for wetland restoration and use of wetlands for nonpoint source pollution control. Ecological Eng. 1:157-170.

White DS, Howes BL (1994) Translocation, remineralization, and turnover of nitrogen in the roots and rhizomes of *Spartina alterniflora*. Am. J. Bot. 81:1225-1234.

Whiting GJ, Gandy EL, Yoch DC (1986) Tight coupling of root-associated nitrogen fixation and plant photosynthesis in the salt marsh grass *Spartina alterniflora* and carbon dioxide enhancement of nitrogenase activity. Appl. Environ. Microbiol. 52:108-113.

Wilson GWT, Hartnett DC, Smith MD, Kobbeman K (2001) Effects of mycorrhizae on growth and demography of tallgrass prairie forbs. Am. J. Bot. 88:1452-1457.

Wright DJ, Otte ML (1999) Wetland plant effects on the biogeochemistry of metals beyond the rhizosphere. Biol. Environ. 99B:3-10.

Wright SF, Millner PD (1994) Dynamic processes of vesicular-arbuscular mycorrhizae: a mycorrhizosystem within the agroecosystem. In: Stewart BA (ed) Advances in soil science, soil biology: effects on soil quality. Lewis Publishers, Boca Raton, pp. 29-59.

Yamashita Y, Tanoue E (2003) Distribution and alteration of amino acids in bulk DOM along a transect from bay to oceanic waters. Mar. Chem. 82:145-160.

Ye ZH, Baker AJM, Wong MH, Willis AJ (2003) Copper tolerance, uptake and accumulation by *Phragmites australis*. Chemosphere 50:795–800.

Ye ZH, Cheung KC, Wong MH (2001) Copper uptake in Typha latifolia as affected by iron and manganese plaque on the root surface. Can. J. Bot. 79:314-320.

Zhang Y-J, Bryan ND, Livens FR (1996) Complexing of metal ions by humic substances. In: Clark SB (ed) ACS symposium series 651. Humic and fulvic acids: isolation, structure and environmental role. American Chemical Society, Washington, DC, pp. 194-206.