# ผลของการเติมฟอสเฟตต่อการตรึงแคดเมียมในดิน การดูดซึมแคดเมียมสู่พืช รูปเคมีของแคดเมียม และการเคลื่อนย้ายแคดเมียมในต้นข้าว

นางสาวนีน่า ซีเบียส



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาโท

สาขาวิชาการจัดการสิ่งแวดล้อม (สหสาขาวิชา)

# EFFECTS OF PHOSPHATE ADDITION ON IMMOBILIZATION AND PHYTOAVAILABILITY OF Cd IN SOIL AND THE CHEMICAL FORM AND TRANSLOCATION OF Cd WITHIN RICE PLANTS

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**A Thesis Submitted in Partial Fulfillment of the Requirements**
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| By | Miss Nina Siebers |
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นางสาวนีน่า ซีเบียส : ผลของการเติมฟอสเฟตต่อการตรึงแคดเมียมในดิน การดูดซึมแคดเมียมสู่พืช รูปเคมีของแคดเมียม และการเคลื่อนย้ายแคดเมียมในต้นข้าว (EFFECTS OF PHOSPHATE ADDITION ON IMMOBILIZATION AND PHYTOAVAILABILITY OF Cd IN SOIL AND THE CHEMICAL FORM AND TRANSLOCATION OF Cd WITHIN RICE PLANTS)
อ.ที่ปรึกษาวิทยานิพนธ์ : อ.ดร.จันทรา ทองคำเภา, 158 หน้า.

งานวิจัยนี้ได้ศึกษาผลของฟอสเฟตต่อพีเอชของดินและต่อความเข้มข้นของแคดเมียมในรูปที่พืชดูดซึมได้ รวมถึงการกระจายตัวของแคดเมียมในต้นข้าว โดยทำการทดลองทั้งในกระถาง และในพื้นที่ปนเปื้อนจริง ทั้งนี้การทดลองในกระถางใช้ดินที่ปนเปื้อนแคดเมียมสูง (> 60 มิลลิกรัมต่อกิโลกรัมดิน) ส่วนการทดลองในพื้นที่จริงทดลองทั้งในดินที่มีการปนเปื้อนแคดเมียมต่ำ (< 0.5 มิลลิกรัมต่อกิโลกรัมดิน) และสูง โดยดินที่ศึกษาได้เติมปุ๋ยฟอสเฟตสูตร 0-52-34 ซึ่งประกอบด้วย $P_2O_5$ 52% และ $K_2O$ 34% ในระดับที่แตกต่างกันสี่ช่วงดังนี้ 0 (ชุดควบคุม), 50, 200 และ1000 มิลลิกรัมต่อกิโลกรัมดิน การตรึงแคดเมียมในดินประเมินจากผลทดลอง ผังลำดับส่วนองค์ประกอบทางเคมี ทั้งในการทดลองแบบกระถาง และในพื้นที่จริง นอกจากนี้ยังได้ศึกษาผลของการเติมฟอสเฟตที่มีผลต่อรูปทางเคมี และลำดับส่วนองค์ประกอบย่อยส่วนต่างๆ ภายในเซลล์ของแคดเมียมในต้นข้าว

ในการเติมฟอสเฟตพบว่ามีผลทำให้พีเอชของดินเพิ่มขึ้น และการดูดซับแคดเมียมในดินเพิ่มขึ้น ซึ่งมีผลทำให้ความเข้มข้นของแคดเมียมในรูปที่ละลายได้ และรูปที่แลกเปลี่ยนไอออนได้ลดลง อีกทั้งยังเพิ่มผลผลิตมวลแห้งในการทดลองในกระถาง และพื้นที่จริง เมื่อเติมฟอสเฟตมากขึ้น ผลของความเข้มข้นของแคดเมียมที่พบในพืชที่ได้รับจากการทดลองในกระถางแตกต่างจากผลการศึกษาที่ได้ในพื้นที่จริง เนื่องจากในดินที่ปลูกในกระถางมีความเข้มข้นสูงและมีผลให้พืชแสดงอาการความเป็นพิษที่รุนแรงของแคดเมียม อย่างไรก็ตามพบว่าการดูดซึมแคดเมียมเข้าสู่ต้นข้าวทั้งต้นในการทดลองในกระถางไม่เปลี่ยนแปลงแม้ว่าการเติมฟอสเฟตมากขึ้น ซึ่งแตกต่างจากการทดลองในพื้นที่โดยความเข้มข้นของแคดเมียมลดลงเมื่อเติมฟอสเฟตเพิ่มขึ้น ในระยะที่พืชเจริญเติบโตมากขึ้นพบว่าความเข้มข้นแคดเมียมจะลดลง และลดลงมากขึ้นเมื่อเติมฟอสเฟตมากขึ้น โดยเป็นผลจากผลการเจือจางแคดเมียมเนื่องจากมวลของข้าวเพิ่มมากขึ้น รูปของแคดเมียมมีการเปลี่ยนแปลงเมื่อเติมฟอสเฟตและทำให้การดูดซึมเข้าสู่พืชลดลง การทดลอง ลำดับส่วนองค์ประกอบย่อยส่วนต่างๆ ภายในเซลล์แสดงถึงการกระจายตัวของแคดเมียมในพืชในรูปที่ไม่เคลื่อนที่ในผนังเซลล์เมื่อเติมฟอสเฟตเพิ่มขึ้น

สาขาวิชาการจัดการสิ่งแวดล้อม ..... ลายมือชื่อนิสิต .....
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NINA SIEBERS: EFFECTS OF PHOSPHATE ADDITION ON IMMOBILIZATION AND PHYTOAVAILABILITY OF Cd IN SOIL AND THE CHEMICAL FORM AND TRANSLOCATION OF Cd WITHIN RICE PLANTS, THESIS ADVISOR: CHANTRA TONGCUMPOU, Ph.D., 158 pp.

In this work the effects of phosphate (P) on the pH, Cd phytoavailability, and Cd distribution in rice plants was examined in a pot experiment and field experiments using soil exhibiting a high (> 60 mg Cd/kg soil) Cd concentration for the pot experiment and high (> 60 mg Cd/kg soil) and low (< 0.5 mg/kg) Cd concentration for the field experiment. The soil was treated with four P levels, 0 (control), 50, 200, and 1000 mg P/kg soil in the form of a commercial P-fertilizer (0-52-34; 52% $P_2O_5$ and 34% $K_2O$). Cd immobilization in soil was evaluated by a chemical fractionation scheme for both the pot and the field experiments. Additionally, the effects of various P concentrations (0-1000 mg/L) on the chemical forms and subcellular fractionation of Cd within rice plants were examined.

The addition of P increased the pH and Cd adsorption by the soil and decreased the Cd concentration in the soluble and exchangeable Cd fraction. There was an increase in dry matter yield with increasing P addition for the pot as well as for the field experiment. Results obtained for plant uptake for the pot experiment were different compared to the field experiment. Due to high Cd concentrations present in soil, plants showed severe Cd toxicity symptoms. Cd uptake by the whole rice plants was not altered with increasing P addition. This was different for the field experiment as with increasing P application the Cd concentration in plants decreased. With increasing growth stage the Cd concentration in plants was decreased being more pronounced for higher levels of P application ascribed to a reduced Cd uptake, translocation, and as a result of the dilution effect of the plant's Cd with increasing biomass. The chemical forms of Cd within the rice plants were altered after P addition and the uptake was decreased. The subcellular fractionation showed a redistribution of the plant's Cd to the immobile cell wall fraction with higher levels of P addition.



Field of Study: Environmental Management Student's Signature.....

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# ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| ATP | Adenosine Triphosphate |
| CEC | Cation Exchange Capacity |
| DOA | Department of Agriculture |
| EFSA | European Food Safety Authority |
| F-AAS | Flame Atomic Absorption Spectrometer |
| GF-AAS | Graphite Furnace Atomic Absorption Spectrometer |
| GIS | Geographic Information System |
| GSH | Glutathione |
| HCS | High Concentration Site |
| JCEFA | Joint FAO/WHO Expert Committee on Food Additives |
| LCS | Low Concentration Site |
| MOA | Ministry of Agriculture |
| NCE-EHWM | National Center of Excellence for Environmental and Hazardous Waste Management |
| PTWI | Provisional Tolerable Weekly Intake |
| SD | Standard Deviation |
| SOM | Soil Organic Matter |
| SM&T | Standard, Measurement and Testing Programme of the European Union |
| TEM | Transmission Electron Microscopy |
| USEPA | United States Environmental Protection Agency |

# CHAPTER I

# INTRODUCTION

## 1.1 General Statement

Cadmium (Cd) is a natural occurring heavy metal, which is also referred to as a transition metal. Human activities have raised its natural concentrations because of the mining, smelting, and processing of ores. Cd is a by-product of the zinc industry; therefore, Cd can be found in increasing amounts in soils around those industries. The most important role of soil for humans is its fertility or agricultural productivity, which helps to ensure the survival of humans (Adriano, 2001). Cd is one of the most ecotoxic metals and has severe effects on biological soil activities, plant metabolism, and the health of humans (Kabata-Pendias, 2001). Therefore, conserving soil's productivity is of crucial concern for humankind (Kabata-Pendias, 2001; Adriano, 2001). After the evaluation of Cd in food by the European Food Safety Authority (EFSA) the provisional tolerable weekly intake (PTWI established by the joint FAO/WHO Exert Committee on Food Additives and endorsed by the Scientific Committee for Food) of 7 μg/kg body weight per week was lowered to 2.5 μg/kg body weight per week, resulting in a need of action to assure safe crops.

In recent years, there has been an increasing interest in mining in the area containing the Phatat Pha Daeng and Mae Tao Mai Sub-districts of Mae Sot District, Tak Province, Thailand. The area was classified as a zinc-rich area, and mining activities have been ongoing since 1977. The Department of Agriculture (DOA) under the Ministry of Agriculture (MOA) conducted a six-year study and found that the region is contaminated with Cd, a metal that coexists naturally with zinc (Zn) (Simmons et al., 2003). Due to the fact that Cd is bioavailable for soil organisms as well as for plants (*phytoavailable*) and constitutes a risk to human health, there has been increased interest in the development of technologies to remediate contaminated sites.

Many methods to immobilize Cd in soil and, by this, reduce its phytoavailability have already been studied. Liu et al. (2008) studied the influence of iron plaque (Fe(III) in the rhizosphere) on the uptake and accumulation of Cd by rice

seedlings and found that the addition of Fe tends to diminish the negative effects of Cd on the physiological indices of rice seedlings to some extent. However, a major problem with this kind of application is that a high Fe concentration also induces phytotoxicity and consequently reduces plant growth (Batty and Younger, 2003). Haghiri (1974) also reported a reduced uptake of Cd by soybean shoots after the enhancement of another heavy metal, namely Zn, due to competition between them. Li et al. (2008) found that pH plays an important role for Cd uptake by rice plants. They used limestone to increase the pH and obtained a 12.5 to 16.5 fold increase in the grain yield and a 23.0-50.4% decrease in the Cd concentration. Bolan et al. (2003a) found comparable results for reduced Cd uptake by plants after the addition of lime up to a certain concentration. However, at higher liming rates, the uptake of Cd increased due to the $Ca^{2+}$ of the lime that increased the concentration of $Cd^{2+}$ in the soil solution due to competition for adsorption sites. Another promising approach of Bolan et al. (2003b) is the addition of phosphate as $KH_2PO_4$, which increases the pH, negative surface charge, and Cd adsorption of the soil, resulting in a decreased uptake of Cd by mustard plants.

This study focused on investigating the Cd contamination in rice-based agricultural soil within Phatat Pha Daeng and Mae Tao Mai Sub-districts, Mae Sot District, Tak Province, Thailand, where rice, soybeans, and garlic have been cultivated for at least three generations. In this study, the effects of phosphate addition as commercial P-fertilizer mainly consisting out of $KH_2PO_4$ on the Cd uptake of rice plants in Mae Sot, Tak district, will be studied. There are two primary aims of this study: The first is to determine the effects of phosphate addition on soil properties, including the soil's Cd distribution as well as its phytoavailability. The second aim is to ascertain the requirements for a safe cultivation of rice in this area. The hypotheses that will be tested are that the phosphate content in the soil affects the immobilization of Cd and with that the phytoavailability of Cd in rice plants.

## 1.2 Objectives

There are three main objectives of this study:

1. To analyze the effects of phosphate addition on the properties of rice paddy soil and the bioavailable soil's Cd fraction.
2. To analyze the effects of phosphate addition on the immobilization and

phytoavailability of Cd by rice plants.

3. To study the effects of phosphate addition on the translocation and chemical form of Cd present in the rice plant.

## 1.3 Hypothesis

Three hypotheses were made about the effects of the phosphate content in rice paddy soil:

1. The phosphate content in soil affects the soil's properties and with that the bioavailable fraction of Cd present in the rice paddy soil.
2. The phosphate content in soil affects the immobilization of Cd and with that the phytoavailability of Cd in rice plants.
3. The phosphate content in soil affects the translocation and chemical form of Cd within the rice plant.

## 1.4 Scope of the Study

The scope of this research is as follows:

1. One pot experiment with Jasmine rice 105 was performed using a high Cd contamination (> 60 mg Cd/kg soil).
2. The soil used in the pot experiment was obtained from a contaminated site from the Mae Sot District.
3. The bioavailable fractions 1 and 2 of Cd and Zn in soil were determined using the BCR method based upon the Standards, Measurements and Testing Programme of the European Union (SM&T).
4. The total Cd and Zn concentrations in soil were determined using microwave assisted digestion.
5. The soil characteristics like pH, redox potential, soil texture, and organic matter content of soil from Mae Sot District in the Tak Province was determined using different methods.
6. The effect of phosphate addition as a commercial P-fertilizer mainly consisting out of $KH_2PO_4$ on soil properties like pH and redox potential was studied.
7. A field study with Jasmine rice 105 was performed selecting an area of low

Cd contamination (< 0.5 mg Cd/kg soil) and high Cd contamination (> 60 mg Cd/kg soil) to determine the effects of phosphate addition to the same parameters as studied in the pot experiment.

8. The effect of phosphate addition on the subcellular distribution and chemical form of Cd in rice plants was studied.

# CHAPTER II

# THEORETICAL BACKGROUND AND LITERATURE REVIEW

## 2.1 Behavior of Heavy Metals in Soil and Plants

### 2.1.1 Cadmium

Cd is one of the most ecotoxic compounds (Mengel, 1991; Sillanpää and Jansson, 1992; Kabata-Pendias, 2001). It is one of the most mobile heavy metals in soil. (Amberger, 1996). The main sources for the introduction of Cd into the environment are traffic, industrial activities, sewage sludge applications, and mineral fertilization. Phosphate fertilizers, in particular, contain Cd concentrations of up to 300 mg/kg (Amberger, 1996; Alloway and Steinnes, 1999).

The availability of Cd increases as pH decreases. Hornburg and Brümmer (1993) as well as Amberger (1996) state that a pH of 6.5 is suitable for starting Cd mobilization. In soil, Cd has a high affinity to bind to Fe- and Mn-oxides and organic substances as well as clay particles.

High concentrations of Cd inhibit photosynthesis, disturb $CO_2$ fixation and the water content, and change the permeability of the cell membrane within the plant (Alloway, 1999; Kabata-Pendias, 2001). Symptoms of Cd toxicity are stunned growth and unspecific chlorosis and necroses. The chlorosis is mainly due to the deficiency of Fe. The excess Cd may interact directly or indirectly with the foliar Fe content; thus, the concentration of foliar is decreased when soil contain high Cd concentrations (Das et al., 1997). The Cd taken up by a plant are mostly found as free ion ($Cd^{2+}$), inorganic complexes ($CdCl^{+}$, $CdCl_2$, and $CdSO_4$), and organic complexes (McLaughlin and Singh, 1999). Whether Cd is needed by plants is as of yet unknown.

### 2.1.2 Zinc

In soil, Zn is one of the most mobile heavy metals (Alloway, 1999; Kabata-Pendias, 2001), making it ubiquitous in its occurrence. Typical source activities leading to the discharge of Zn include the smelting of ores that contain Zn, industrial applications of products containing Zn (e.g., colors and protective coatings), the fertilization of agricultural land (especially when pig slurry is used), as well as the use

of Zn-containing pesticides (Kiekens, 1999; Schilling, 2000; Kabata-Pendias, 2001). At a pH value of < 6.0 the phytoavailability of Zn increases (Hornburg and Brümmer, 1993). Zn mainly binds to clay minerals, Fe- and Mn-(hydr)oxides, and organic substances (Zeien, 1995). Plants mainly uptake $Zn^{2+}$ ions; though, chelated forms are also absorbed. Within the plant, Zn is relatively mobile; however, during conditions of deficiency, mobilization can be limited. No Zn, for example, would be mobilized from older leaves. As a part of enzymes, Zn is involved in catabolic reactions and in the RNS-/DNA-transcription and translation. Furthermore, Zn influences the permeability of the membrane and the plant's resistance to droughts (Kiekens, 1999; Schilling, 2000; Kabata-Pendias, 2001). A plant's Zn deficiency occurs below a Zn concentration of 30 mg/kg dry weight of the plant material (Amberger, 1996). This deficiency is accompanied by symptoms like stunted growth, the deformation of stems and leaves, and the discoloration of leaves. When toxic concentrations are present (about 500 mg Zn/kg dry weight), unspecific chlorosis as well as a disturbance of growth occurs (Amberger, 1996).

## 2.2 The Bioavailability of Metals

There are different operationally defined geochemical fractions heavy metals can partition into, namely (i) exchangeable (F1), (ii) bound to carbonate or weakly specifically adsorbed (F2), (iii) bound to iron-manganese oxides (F3), (iv) bound to organic matter (F4), and (v) the residual fraction (F5). Fractions 1 and 2 are known to be the most bioavailable fractions; thus, the focus of preventing Cd uptake by plants should lay in decreasing the amounts of Cd present in these fractions. To achieve this, soil properties have to be changed, for the distribution of Cd in these fractions is greatly affected by soil properties such as the pH, redox potential, cation exchange capacity (CEC), organic matter, and clay content (Tu et al., 2000).

Plants can only take up the heavy metals present in soil solution; therefore, the following three parameters are of crucial importance: the quantity of heavy metals in the soil (i.e., the storage of heavy metals), the intensity of the heavy metals (i.e., the amounts of heavy metals in the soil solution), and the kinetic reactions that occur (i.e., the rates of their subsequent delivery out of storage) (Brümmer, 1986). The most important parameter determining the mobility and phytoavailability of heavy metals

in soil is their adsorption onto the solid phase. During adsorption, the unspecific bonding through ion exchange and the specific adsorption is differentiated. The ion exchange is reversible and stoichiometrical, whereas the specific adsorption is based on the attachment of the heavy metal cations onto the ligands of the soil surface under the partial formation of covalent bonds (Alloway, 1999). It has to be taken into account that heavy metals with high affinities for forming hydroxycomplexes also show high specific adsorption. The equilibrium constant p*K* of the reaction $M^{2+} + H_2O$ □ $MOH^+ + H^+$ is responsible for the adsorption behavior of the heavy metal. Brümmer (1986) give the following p*K* values for the affinity of the specific adsorption of Cd and Zn: $Zn^{2+}$(p*K*=9.0) > $Cd^{2+}$(p*K*=10.1) meaning that $Cd^{2+}$ is more mobile than $Zn^{2+}$. Other important processes for the immobilization of heavy metals are the coprecipitation and coagulation of slightly soluble compounds (often with phosphate).

Besides the type of heavy metal binding, which is mainly characterized through the pH, $E_h$, amounts of heavy metals present, and amounts and quality of the exchangers, phytoavailability is mainly influenced by the speciation of the heavy metal in the soil solution. The main species present is dependent on the metal, the availability of organic and inorganic ligands, and the soil pH (see Table 2.1).

**Table 2.1.** The main species of Cd and Zn present in soil solutions of acidic and basic soil (Sposito, 1998).

| Heavy Metal | Acidic Soils | Basic Soils |
|---|---|---|
| Cd | $Cd^{2+}$, $CdSO_4^0$, $CdCl^+$, org. complexes | $Cd^{2+}$, $CdSO_4^0$, $CdCl^+$, $CdHCO_3$, $CdCO_3^0$, $CdHPO_4^0$, $Cd(OH)^+$, org. complexes |
| Zn | $Zn^{2+}$, $ZnSO_4^0$, org. complexes | $Zn^{2+}$, org. complexes, $ZnOH^+$, $Zn(OH)_2^0$, $ZnCO_3^0$, $ZnHCO_3^+$, $ZnPO_4^+$, $ZnHPO_4^0$, $ZnSO_4^0$, $ZnB(OH)_4^+$ |

## 2.3 Phosphorus

Phosphorous is crucial for plants, for insufficient phosphorous could lead to delayed crop maturity, reduced flower development, low seed quality, and decreased crop yield. This is because it is an essential component of adenosine triphosphate (ATP), which is necessary for most biochemical processes and for DNA formation or

cell development. It is very important to manage the phosphorous content of soil, as not only is less phosphorous harmful for crops, but too much phosphorous in soil possesses a threat for the environment. The higher the phosphorous content is in the soil, the more it can leach out and be transported into other systems like lakes--where it can lead to eutrophication. Additionally, applying phosphorous at levels above what a crop needs does not have any positive effects on crop yield or growth; it only costs more money and can harm the environment.

### 2.3.1 Phosphorus Cycle

The phosphorous cycle is dynamic and involves soil, plants, and microorganisms. The different forms of phosphorous that are present in soil are grouped into the following categories: (i) plant available inorganic phosphorous, (ii) organic phosphorous, (iii) adsorbed phosphorous, and (iv) primary mineral phosphorous. The major phosphorous transformation processes include the uptake of phosphorous by plants and its recycling through the return of plant and animal residues, weathering and precipitation, mineralization and immobilization, and adsorption and desorption. In natural systems, the phosphorous taken up by plants is returned to the soil by plant residues and animal residues, but for crops the situation is different. A portion of the phosphorous present in the soil is removed with the crop after being taken up by the plants; because of this, after a certain time, the application of a phosphorous fertilizer will be necessary. In Figure 2.1, a simplified version of the terrestrial phosphorous cycle is shown.

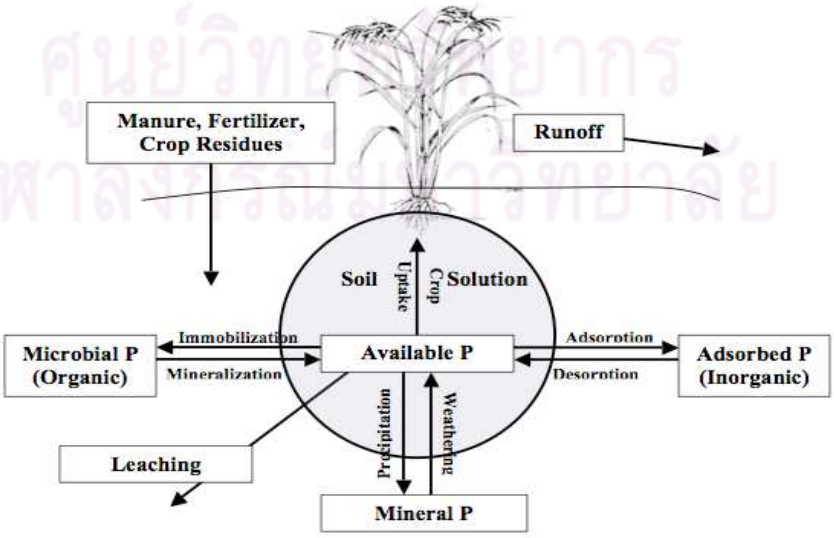



**Figure 2.1.** Simplified version of the terrestrial phosphorous cycle (after Hyland et al., 2005).

*Weathering and Precipitation.* Plant-available phosphorous is increased by the weathering of phosphorous rich minerals into plant available forms. On the other hand, when inorganic phosphorous reacts with dissolved iron, manganese, or aluminium in acidic soils or calcium in alkaline soils, insoluble phosphate minerals are formed, which are not accessible by plants.

*Immobilization and Mineralization.* Microorganisms in soil are able to immobilize plant-available phosphorous by consuming it and transform it into organic forms of phosphorous that are not available to plants. As the microorganisms die, the organic phosphorous is slowly released, making it available again.

The organic phosphorous present in soil is converted by microorganisms into plant-available orthophosphates, and with this, it increases the plant-available phosphorous pool.

*Adsorption and Desorption.* Plant available phosphorous is also able to adsorb to soil surfaces or iron and aluminum, decreasing the available pool again. Every soil has a maximum amount of phosphorous that it can adsorb. Phosphorous-saturated soil will lose its excess phosphorous by runoff or leaching to the environment.

Adsorption is a dynamic process, meaning the adsorbed phosphorous can easily desorb into the soil solution again. The most important parameter for adsorption-desorption processes is the soil's pH. In Figure 2.2, the impact of soil pH on plant-available phosphate is presented. At a pH range of 6.0-7.5 the plant available phosphorous fraction is highest.

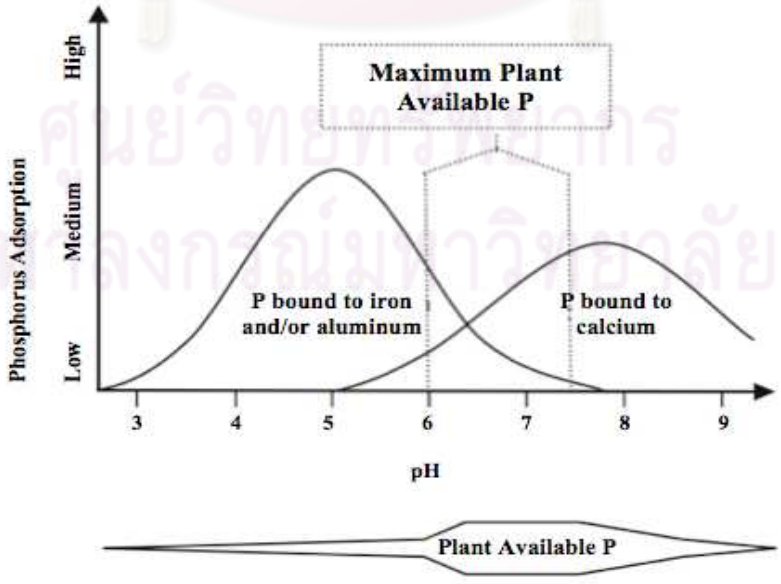



**Figure 2.2.** Impact of soil pH on plant available phosphorous (Hyland et al., 2005).

*Runoff.* The major cause of phosphorous loss in fields is runoff because the water carries away phosphorous bound to particulates as well as solution phosphorus from manure and fertilizers. To prevent runoff from fields, erosion control practices have to be applied (Stevenson and Cole, 1999; Hyland et al., 2005).

### 2.3.2 Behavior of native phosphorus in soil during rice propagation

For an effective P-fertilizer application to rice paddy soil in ecological and economic terms, a good understanding about the dynamics of the available phosphorus during rice propagation is essential. The fate of native phosphorus in loamy soil, which is predominantly found in the Mae Sot District, was studied by Uwasawa et al. (1988). In loamy rice paddy soil the main pool contributing to the bioavailable pool is the organic phosphorus pool (Org-P), followed by the inorganic phosphorus pool (Inorg-P).

They found that the Inorg-P contributing to the available phosphorus pool, mainly consisted of up to 57% of reductant soluble phosphorus (Oc-P) (covered by coatings of Fe and Al oxides or hydroxides), followed by iron phosphorus (Fe-P), which made up a fraction of the 29% of the total Inorg-P. Minor fractions were aluminum and calcium phosphorus (Al-P and Ca-P), representing 7% of the total Inorg-P each.

During submergence and subsequent rice cultivation the fractions contributing to the available phosphorus pool changed significantly. The Al-P decreased significantly, whereas the Fe-P fraction decreased during submergence and rice cultivation. The Ca-P fraction decreased to nearly nil under anaerobic conditions. The Org-P fraction was transformed under submerged conditions into Inorg-P, which was utilized by the plants. The Inorg-P fraction that was not uptaken was transformed into Oc-P under desiccation, resulting in an increase of this fraction. Uwasawa et al. (1988) found that the available phosphorus aside from Org-P consisted of Al-P and Fe-P.

## 2.4 Fertilizer

PK-fertilizer 52-34, the fertilizer used in this study for both the pot and field study, consists of 52% phosphoruspentoxide ($P_2O_5$) and 34% potassiumoxide. The

phosphorus in this fertilizer is completely water soluble and when in contact with soil particles, moisture from the soil dissolves the fertilizer particles. When dissolved in the soil solution, the phosphorus in the fertilizer exists as orthophosphates ($H_2PO_4^-$ and $HPO_4^{2-}$) and is readily available for plant uptake (Halvin et al., 1999). The pool of soluble phosphorus that is available for plants is increased after P fertilizer application, but with time, the soluble phosphorus is transformed into other forms that are less soluble and less bioavailable.

### 2.4.1 Behavior of added phosphorus to soil during rice propagation

Added phosphorus in the form of a P-Fertilizer is distributed at the beginning of the treatment into the Al-P, Fe-P, and Oc-P fractions. During the first week of soil submergence, the Org-P fraction decreased significantly, more than in the soil without P-fertilizer application. Uwasawa et al. (1988) found that the Al-P decreased up to 50% from the initial value during rice cultivation. It was also observable that under anaerobic conditions the Fe-P content constantly increased.

Uwasawa et al. concluded that most of the applied phosphorus accumulated in the Al-P and Fe-P fractions; furthermore, the bioavailability of phosphorus in the Al-P fraction was higher than it was in the Fe-P fraction. One part of the accumulated phosphorus came from the uptake by the rice plants, whereas the other part was immobilized and fixed as Oc-P during soil desiccation.

## 2.5 Rice

### 2.5.1 Growth stages of rice plants

This study focuses on the rice cultivar Jasmine Rice 105; in Thailand, it is also known under the official name Thai Hom Mali 105. For Jasmine Rice 105, the vegetative growth period is about 55 days long, the reproduction process takes 35 days, and the time it takes to reach maturity is another 30 days, so altogether the total growth process takes 120 days. The morphological development of rice can be divided into three phases, namely the seedling, vegetative, and reproduction stages (Counce et al., 2000). The late vegetative phase and the early reproductive phase occur simultaneously.

*Seedling development*

The seedling development period includes the time the plant starts off as a dry, unimbibed seed to a seed where a prophyll from a coleoptile has emerged. The germination of a dry rice seedling must imbibe water, and either a coleoptile (i.e., the leaf that generally emerges first with no collar and no blade, only a leaf sheath) or a radicle can emerge first, but in a normal sequence the coleoptile emerges before the radicle (Figure 2.3).

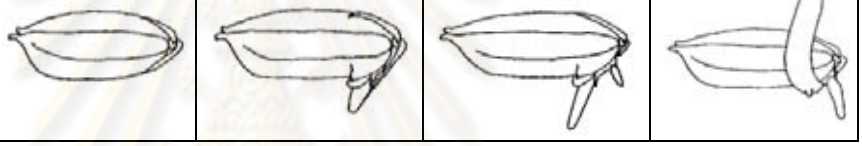
| **Growth Stage** | **S0** | **S1** | **S2** | **S3** |
|---|---|---|---|---|
| **Morphological Criteria** | Dry unimbibed seed | Emergence of coleoptiles | Emergence of a radicle | Emergence of a prophyll from a coleoptile |
| **Illustration** |  | | | |

**Figure 2.3.** Normal sequence of morphological rice development during the seedling phase; S=seedling stage (after Counce et al., 2000).

*Vegetative phase*

The vegetative phase is divided into stages: V1, V2, V3, up until the V*N* stage, where *N* is the final number of leaves with collars on the main stem. A phyllochron is measured by taking the time between the formation of two leaf collars on successive leaves. In this system, the vegetative growth stages are measured by each successive leaf collar formation as demonstrated in Figure 2.4. In this figure a rice cultivar with 13 true leaves on the main stem is illustrated, but the number of stages at this phase varies with each cultivar.

| **Growth Stage** | **V1** | **V2** | **V3** | **V4** | **V5** |
|---|---|---|---|---|---|
| **Morphological Marker** | Collar formation on the first complete leaf (Leaf 1) of the main stem | Collar formation on Leaf 2 of the main stem | Collar formation on Leaf 3 of the main stem | Collar formation on Leaf 4 of the main stem | Collar formation on Leaf 5 of the main stem |
| **Illustration** |  |  |  |  |  |
| **Growth Stage** | **V6** | **V7** | **V8** | **V9 ($V_{F-4}$)** | **V10 ($V_{F-3}$)** |
| **Morphological Marker** | Collar formation on Leaf 6 of the main stem | Collar formation on Leaf 7 of the main stem | Collar formation on Leaf 8 of the main stem | Collar formation on Leaf 9 of the main stem | Collar formation on Leaf 10 of the main stem |
| **Illustration** |  |  |  |  |  |
| **Growth Stage** | **V11 ($V_{F-2}$)** | **V12 ($V_{F-1}$)** | **V13 ($V_F$)** | | |
| **Morphological Marker** | Collar formation on Leaf 11 of the main stem | Collar formation on Leaf 12 of the main stem | Collar formation on Leaf 13 (the flag leaf) of the main stem | | |
| **Illustration** |  |  |  | | |

**Figure 2.4.** Sequence of morphological rice development during the vegetative phase. $V_F$ denotes the flag leaf and $V_{f-1}$ denotes the nth node before the flag leaf (V9-V13) (after Counce et al., 2000).

*Reproductive phase*

With the initiation of panicle structures from the shoot apex, the reproductive phase starts, and it contains different stages (Figure 2.5).

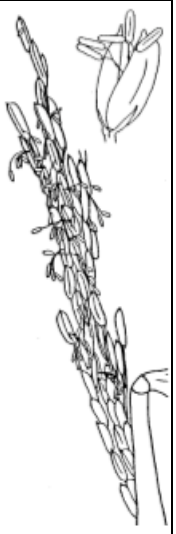
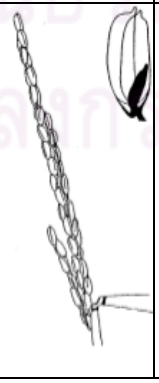
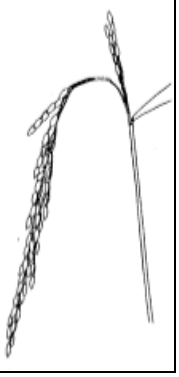
| **Growth stage** | **R0** | **R1** | **R2** | **R3** | **R4** |
|---|---|---|---|---|---|
| **Morphological Marker** | Panicle development has been initiated | Panicle branches have formed | Flag leaf collar formation | Panicle exertion from the boot, tip of panicle is above the collar or flag leaf | One or more florets on the main stem panicle has reached anthesis |
| **Illustration** |  |  |  |  |  |
| **Growth stage** | **R5** | **R6** | **R7** | **R8** | **R9** |
| **Morphological Marker** | At least one caryopsis on the main stem panicle has elongated to the end of the hull | At least one caryopsis on the main stem panicle has elongated to end of the hull | At least one grain on the main stem panicle has a yellow hull | At least one grain on the main stem panicle has a brown hull | All grains which have reached R6 have brown hulls |
| **Illustration** |  |  |  |  |  |

**Figure 2.5.** Sequence of morphological rice development during the reproductive phase (after Counce et al., 2000).

The stages are denoted as: panicle initiation (R0), panicle differentiation (R1), flag leaf collar formation (R2), panicle exertion (R3), anthesis (R4), grain length and width expansion (R5), grain depth expansion (R6), grain dry down (R7), single grain maturity (R8), and complete panicle maturity (R9). A panicle is able to fully develop within the stages R0 to R3. After harvesting, the grain continues to develop, which leads to chemical and physical changes in the endosperm.

For a better understanding of what is meant by the different parts of the plants mentioned above, the external morphological structure of a rice plant is illustrated in Figure 2.6.

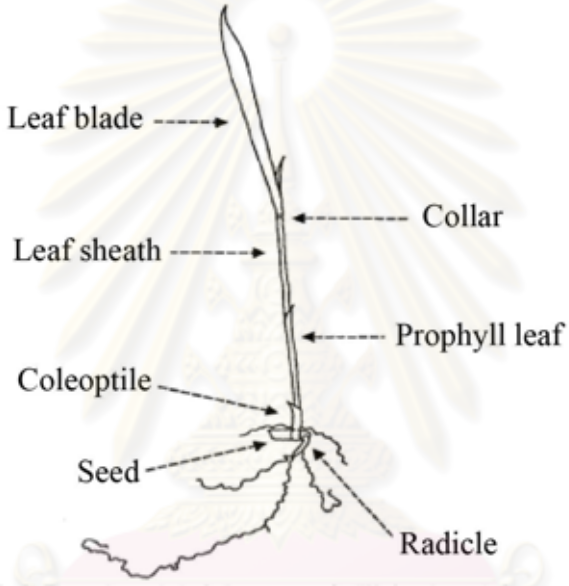



**Figure 2.6.** External morphological structure of a rice plant (after Counce et al., 2000).

## 2.6 Uptake, Translocation, and Sequestration Mechanisms of Cd by Plants

The mechanisms of Cd uptake and translocation by plants are not completely understood (Jarvis et al., 1976; Homma and Hirata, 1984; Herren and Feller, 1997; Hart et al., 1998; Tanaka et al., 2003; He et al., 2007; He et al., 2008). Without proper knowledge about the uptake and translocation mechanisms of Cd by plants, it is not possible to prevent the uptake of Cd by food crops and with that by humans, which can lead to severe health effects.

### 2.6.1 Root uptake

There are different mechanisms among Cd uptake by roots, but the main mechanism is the absorption and transport of aqueous ionic $Cd^{2+}$, which has been the focus of most related studies. Jarvis et al. (1976) compared the short term uptake of Cd by living ryegrass roots with and without the presence of other divalent cations like $Ca^{2+}$, $Mn^{2+}$, and $Zn^{2+}$ and found that the uptake was depressed in the presence of other divalent cations. This observation is due to the chemical similarity of some divalent ions that are essential for plant nutrition with Cd; therefore, Cd is able to use selective transport mechanisms in plant cell membranes, normally used by specific essential nutrients like $Ca^{2+}$ and $Zn^{2+}$ (Jarvis et al., 1976; Kim et al., 2002). There have been much less studies on inorgaic and organic complexes even though they contribute as well to the solubility of Cd, and some forms of complexes are also directly taken up by roots (McLaughlin and Singh, 1999). Another proposed mechanism is the penetration of Cd through the cortical tissue of the root. The uptake of Cd does not require a specific $Cd^{2+}$ transporter system because the activity of the $H^{+}$-translocating ATPase within the plasma membrane creates a negative electrical potential at the exterior surface of the plasma membrane. At the cytosol, on the other hand, there is very little $Cd^{2+}$ present. The $Cd^{2+}$ taken up by the roots is driven by this large negative membrane potential, and $Cd^{2+}$ is adsorbed across the plasma membrane of the root cells (McLaughlin and Singh, 1999). The $Cd^{2+}$ absorption kinetics shows biphasic characteristics with a saturable component when the $Cd^{2+}$ activities in the adsorption solution are low, and a linear component when these $Cd^{2+}$ activities are high. Linear-uptake kinetic components represent the binding of Cd to the apoplastic components that remain after desorption and the nonspecific $Cd^{2+}$ influx via a cation channel (McLaughlin and Singh, 1999), whereas the saturable component is the result of carrier-mediated transport across the root-cell plasma membrane (He et al., 2007). As for now, it is uncertain whether the uptake is through a Cd specific carrier or opportunistic via a carrier for another divalent cation.

### 2.6.2 Translocation within the rice plant

The translocation of Cd within the rice plant was studied by Kashiwagi et al. (2009). They found that during the five different growth stages (i.e., the vegetative stage, the pre-heading stage, the heading stage, the maturity stage, and the fully-ripe

stage), Cd was translocated from lower leaves to upper leaves, and then from upper leaves to culms and ears. They found that the lower leaves are the primary Cd storage organs because the Cd concentration was highest during the early stages but decreased to nearly nil during the later growth stages. They also found that the Cd uptake from soil is highest during the pre-heading stage, after which it continuously decreases. During the heading stage, the uptake of Cd can be negative due to leaves falling to the ground. Kashiwagi et al. (2009) concluded that the Cd concentration in grain is determined by the Cd already accumulated in the leaves and stems before the heading stage.

The Cd translocation within the plant is likely to be controlled by the general translocation properties of the leaves, stems, and roots via the xylem and phloem. To enter the xylem, solutes have to be taken up into root cells for passage through the root endodermis. There is some evidence for a second, wholly apoplastic pathway for the entry of water and possibly cations into the xylem in certain regions of the root. This was the focus of a study by Tanaka et al. (2003), who determined the Cd concentrations in the xylem exudates and the phloem sap. Cd was detected in the xylem exudates as well as in the phloem sap obtained through the cut ends of stylets of the brown planthopper. These results provide direct evidence for the translocation of Cd in rice plants via the xylem and phloem. Once Cd enters the xylem, it complexes to several ligand as organic acids and, if present, phytochelatins (Sanità di Toppi and Gabrielli, 1999). Most likely, some degree of cycling of metal cations occurs from the shoots back to the roots via the phloem (Welch, 1995).

### 2.6.3 Sequestration of Cd within the rice plant

The activity of Cd must be kept low in the cytosol because Cd preferentially binds to sulphhydryl ligands, and with this, competes with essential metals like Zn, Ni, and Cu for active functional binding sites in essential cytosolic metabolites. To regulate the Cd activity in the cytosol, the plants use several response mechanisms; the most important response mechanisms of rice plants are (i) immobilization, (ii) the synthesis of phytochelatins, the synthesis of metallothioneins (iii), and (iv) compartmentalization.

The barrier Cd has to overcome in order to enter the plant are the roots. It is there where a big portion of the Cd that is present in the rice plant is immobilized by means of the cell wall (Sanità di Toppi and Gabrielli, 1999). Kim et al. (2002) found

that only a little portion of total Cd present in the plant is translocated to the shoot and that about 82% is retained in the roots of rice seedlings.

Another response of plants to Cd stress is the activation of a system related to its sulphur metabolism. This system is able to produce low molecular weight cysteine rich polypeptides, which are important complexing agents and termed *phytochelatins*. Phytochelatins are used to bind Cd(II) with its thiolic groups of cysteine, which are then transported into vacuoles, where they possibly transfer their Cd to higher molecular weight metallothionein-type proteins for storage (Sanità di Toppi and Gabrielli, 1999). It was suggested that this mechanism is only important at high Cd exposures (> 5µM Cd), and that for lower Cd exposures reduced glutathione (GSH) may be the most important cytosolic complexer of $Cd^{2+}$ (McLaughlin and Singh, 1999).

The compartmentalization of Cd into the vacuoles is an important mechanism used to detoxify the Cd in the plant and to prevent its free circulation in the cytosol. As mentioned before, the Cd is transported to the vacuoles by the $S^{2-}$ rich low molecular weight phytochelatins, which transfer the complexed Cd to the metallothioneins through a membrane transport protein that is located in the vacuolar membrane. Another way for $Cd^{2+}$ ions to enter the vacuoles is a $Cd^{2+}/2H^{+}$ antiporter (Salt and Wagner, 1993). In the vacuoles, an acidic pH dominates, which lets the complex between the acid-labile sulfides and Cd dissociate again. Then, Cd is complexed by vacuolar organic acids (e.g., citrate, oxalate, malate) (Sanità di Toppi and Gabrielli, 1999).

### 2.6.4 Bioavailability and toxicity of Cd in plant foods

The Cd transfer from soil to grain is unique for rice, as Cd is transported to the grain without the accompanying presence of Zn at very high concentrations in areas of Zn mining. The grain Cd concentration is increased up to 100-fold the background levels, whereas Zn concentration in grain is not increased even at very high Zn concentrations in soil (5000 mg/kg) (Chancy et al., 2001). This phenomenon could only be observed for rice plants; other food crops of the western world that grown under aerobic conditions show an increase of Cd as well as Zn under elevated Zn concentrations in soil. Due to the fact that Zn normally is 100-times the Cd concentration in contamination soils, the Zn uptake is also increased greatly by crops

grown under aerobic conditions. Thus, prior to an accumulation of high Cd concentrations in grains that would exceed existing Cd standards in food crops, Zn-phytotoxicity would occur first. This mechanism protects humans and livestock from elevated Cd concentrations in western food crops under a normal geochemical Cd:Zn ratio (Chaney, 1993).

Another important factor to focus on is the chemical form of Cd present in the plant. The two most toxic forms for the plant is inorganic Cd (mainly associated with nitrate/nitrite, chloride, and aminophenol) and water soluble Cd, which combines with organic acids and $M(PO_4)_2$. These forms are very mobile, able to penetrate into the symplasm, and present in the soluble fraction, which enables the Cd to be translocated to every plant part. Thus, these Cd forms are able to reach and accumulate in plant parts normally used for human consumption like rice grains (Wu et al., 2005). Cd that is integrated into pectates and proteins is neither as mobile as inorganic nor soluble Cd, yet since it mainly binds to proteins, it disturbs enzyme activity, resulting in plant toxicity symptoms (He et al., 2008). Another form of Cd that can be found in plants are undissolved Cd phosphates ($CdHPO_4$ and $Cd_3(PO_4)_2$) and cadmium oxalics, which possess low toxicity and mobility.

## 2.7 Literature Review

### 2.7.1 Phosphate addition and its effects on the bioavailable fractions of Cd in soil

Kirkham (2006) stated that the bioavailable fractions (F1 and F2) are strongly affected by the pH and surface charge. The pH and bioavailable fractions are affected by the addition of different amendments like phosphorus, silicon, or zinc. The results of Brown et al. (2004), as reviewed by Kirkham (2006), show that phosphate addition reduces Cd availability. Different forms of phosphate were added to soil contaminated with Cd near a Zn and Pb smelter, namely 1% P-$H_3PO_4$, a +P-triple superphosphate (TSP), and 1% P-phosphate rock. They found that the Cd taken up by tall fescue (*Festuca arundinaceae)* decreased the most after the addition of 3.2% P-TSP. The addition of 1% P- $H_3PO_4$ and 1% P-TSP also resulted in decreased Cd plant uptake (compared to the control), while the addition of phosphate rock showed no effect. These results suggest that the form of phosphate applied is of crucial importance for

decreasing the Cd uptake in plants.

Bolan et al. (2003b) applied phosphate in the form of $KH_2PO_4$ to seven different Cd contaminated soils and studied the effects of phosphate addition on the immobilization and phytoavailability of *Brassica juncea* (L.) mustard. They found that the pH and surface charge of the slightly acidic soils can be increased by the addition of phosphate. The increase in pH is due to the ligand-exchange (i.e., $OH^-$ ions) phosphate adsorption reactions. The increased amount of $OH^-$ ions causes the formation of the $Cd(OH)_2$ complex, which precipitates and thus immobilizes the Cd. The formation of insoluble $Cd_3(PO_4)_2$ complexes is also enhanced and thus decreasing Cd uptake by plants. Li et al. (2008) also studied the effects of chemical fertilizers containing $KH_2PO_4$ as the phosphate source on Cd uptake by rice. They found that the pH increased about 0.66 units to 1.49 units when more Ca-Mg-P fertilizer was applied (5-15 g fertilizer on 7.5 kg soil), resulting in the decrease of the rice's phytoavailability. This was reflected in the decreasing concentrations of Cd in the rice grain and straw as the additions of Ca-Mg-P fertilizer increased. The concentration decreased from 0.264 mg/kg Cd in the grain (control) to 0.193 mg/kg and 0.187 mg/kg for lower and higher fertilizer concentration, respectively. In the straw, concentrations of 1.17 mg Cd/kg and 0.88 mg Cd/kg of rice straw could be found for lower and higher fertilizer concentration, respectively, which were lower than the Cd concentration of 3.99 mg/kg from the control.

### 2.7.2 Zn and its effect on the bioavailable fraction of Cd in soil

Cd's uptake behavior mimics that of Zn, and they are known to compete with each other for binding sites. In the literature, it has been reported that Zn applications can increase or decrease Cd accumulation in crops.

Grant and Bailey (1996) studied the uptake of Cd by flaxseed in the presence of Zn at different sites. They found that not only did the simple interactions between Cd and Zn reduce the Cd uptake, but interactive interactions between Zn, P, and Cd under specific conditions also had an influence on the Cd uptake.

The Cd concentration in the seed negatively correlated with the Zn concentration in the soil. This is attributed to the competition between Zn and Cd for binding sites and translocation by the plant. Homma and Hirata (1984) found that Cd uptake rates by rice seedlings were as high as the uptake rates of Zn when the

concentrations of both ion species were equal to or below 1.0 mMol/$m^3$. When both ion species were at higher concentrations, the Zn uptake rate was more than 2.5 fold higher than the uptake rate of Cd. The increase in the uptake rate of Zn compared to that of Cd at higher concentrations can be seen in the linear uptake of Zn. As the Zn concentration increases, the Cd absorption rate scarcely increased as the Cd concentration increased. Jarvis et al. (1976) observed that the Cd uptake by ryegrass was suppressed when Zn was added to the uptake solution. After 240 min the ryegrass took 91% of the Cd present in solution, whereas only 80% of the Cd was taken up after the addition of Zn. McKenna et al. (1993) observed that Zn decreased the accumulation of Cd in the young leaves of lettuce and spinach but not in the old leaves when plants were grown in a solution culture. The accumulation of Cd in the roots and old leaves did not change significantly after the addition of Zn. The retention of Cd in the roots was favored after the addition of moderate concentrations of Zn; thus, Zn seems to interfere with the translocation of Cd from roots to young leaves. At higher Zn concentrations, Zn might have interfered with the Cd uptake by roots. The authors report that the strong antagonistic Zn effect on Cd accumulation in young lettuce and spinach leaves at low solution Cd was not observable at higher Cd concentrations. They, therefore, conclude that the Cd concentration in the edible parts of lettuce and spinach grown on Zn-Cd contaminated soil might not be lowered when they have higher Zn concentrations, which may lower the plant's bioavailability to Cd. Moraghan (1993) also observed that Zn has an antagonistic effect on Cd in flaxseed when Cd is present at a low Cd concentration. Additionally, he found that high levels of phosphate reduces the Zn concentrations in the tissues and increases the occurrence of Zn deficiencies, resulting in increased Cd uptake.

### 2.7.3 Zn and phosphate and their effect on the bioavailable fraction of Cd in soil

In a study, the addition of phosphate enhanced the immobilization of Cd and made it less bioavailable for mustard (Bolan et al., 2003b). However, in another study, the addition of phosphate was also shown to reduce the phytoavailability of Zn and, therefore, increase the amount of Cd taken up by flaxseed. A reason for this observation was found by Grant and Bailey (1996). They indicate that the Zn accumulation may be restricted by Zn availability, for the accumulation of Zn is

unrelated to seed yield. Most of the Zn uptaken by plants is in the form of $Zn^{2+}$, whereas phosphate is taken up as an anionic form of $PO_4^{3-}$ by plants. Due to the electrostatic attraction between the divalent Zn cation and the phosphate anion a bond is formed, which cannot readily be broken; thus, excess phosphorus can bind a great amount of Zn and make it unavailable for plants, resulting in a Zn deficiency (Hopkins and Ellsworth, 2003). Another explanation given for the increased Cd uptake after phosphate addition in the presence of Zn may be the dilution of the plant's Zn. When only a limited Zn concentration is available, the plant's Zn becomes diluted as the plant yield increases, thus, causing less competition between Cd and Zn for binding sites.

Taking these findings into account, this proposed study would like to determine the effects the high Zn concentrations in the soil at Mae Sot have on the application of phosphate to reduce the Cd taken up by rice plants.

### 2.7.4 The uptake and distribution mechanisms of Cd within the rice plant and the effects of phosphate addition

Liu et al. (2007) found that the Cd concentration and quantity accumulation in different parts of the rice plant were as follows: root > stem > leave > grain; over 98% of the Cd taken up was found in the roots and stems.

Li et al. (2009) determined the effects of phosphate and other amendment additions on the distribution and accumulation of Cd in rice. They found that regardless of the type of amendment, the order of Cd accumulation in the different parts of the rice plant was as follows: root > straw > grain. It was proposed that the translocation of Cd from the root to the straw and grain was restrained after phosphate addition, but the mechanisms involved were not apparent.

There is only little information available about the transport, intracellular distribution, and binding of Cd within a rice plant (Makoto et al., 1986; He et al., 2008; Zhang et al., 2009) Since cellular sequestration of Cd can greatly affect the level of free Cd in the cell and thus potentially influence the movement of Cd throughout the plant, it is important to study the subcellular distribution of Cd within plants.

Subcellular fractionation is a powerful tool to study the sequestration of Cd within a plant as it allows to separate different organelles present in plant tissue. The

fractionation of plant material is possible due to the fact that different organelles possess different physical properties as size, density, charge, and other attributes. To be able to separate the organelles, two steps are necessary, namely (1) homogenization (disruption of the cellular organization), and (2) separation of the different populations of organelles by fractionation of the homogenate. The homogenate is used to partition the fractions by means of differential centrifugation. The fractions resolved can mainly be classified into four groups, namely (1) cell wall fraction containing cytosceletal networks, (2) chloroplast-shoot/trophoplast-root, (3) membranes and organelles, and the (4) soluble fraction containing the cytosol (Weigel and Jäger, 1980; Pasquali et al. 1999).

Another interesting approach is to study whether the chemical form of Cd within the plant changes with increasing phosphate concentration or not (Wu et al., 2005), as the formation of compounds with low biological activity reduces Cd toxicity.

In this proposed study subcellular fractionation experiments are carried out in order to obtain a better understanding on how phosphate affects the uptake and behavior of Cd within a rice plant. Consequently, sequestration and chemical form of Cd within the rice plant will be determined, and how the sequestration and chemical form is affected by the addition of phosphate to the soil.

# CHAPTER III

# METHODOLOGY

## 3.1 Materials

### 3.1.1 Chemicals

Chemicals used in this study are listed in Table 3.1.

**Table 3.1.** Chemical list.

| Chemicals | Supplier / Grade |
|---|---|
| Acetic Acid | Merck / anhydrous GR for analysis |
| Ammonium fluoride | Merck / GR for analysis |
| Ammonium molybdate | Univar / analytical reagent |
| Bromine | Merck / ACS, ISO, Reag. Ph Eur |
| Cadmium nitrate | Carlo Erba / for analysis |
| DL-Dithiothreitol | Bio Basic Inc. / ultra pure |
| Ethanol | Merck / for synthesis |
| Formic acid 99% | Carlo Erba / ACS, for analysis |
| Hydroxylamine hydrochloride | Carlo Erba / ACS, for analysis |
| Hydrochloric acid fuming 37% | Merck / for analysis |
| Hydrogen peroxide | Merck / (stabilized) for analysis |
| L-Ascorbic acid | Unilab / laboratory reagent |
| P-fertilizer | Haffa Chemicals Norhern Europe |
| Potassium antimonyl tartrate | Unilab / laboratory reagent |
| Potassium dihydrogen orthophosphate | Riedel-de Haën / extra pure |
| Nitric acid 65% | Merck / for analysis |
| Sodium chloride | Carlo Erba / AnalytiCals |
| Sodium hydroxide | Merck / for synthesis |
| Sodium thiosulphate 5-hydrate | BDH / AnalaR® |
| Sucrose | Merck / for microbiology |
| Sulphuric acid 95-97% | Merck / for analysis |
| Triethanolamine | Carlo Erba / BP-FU |
| Tris-hydrochloric acid | Bio Basic Inc. / reagent |

### 3.1.2 Glassware

The used pipettes and glassware are listed in Table 3.2.

**Table 3.2.** Pipettes and glassware used in this study.

| Pipettes and glassware | Supplier |
|---|---|
| Beaker (10, 100, 500, and 1000 mL) | SCHOTT AG, Mainz, Germany |
| DURAN® Volumetric flask: 25 mL (±0.04 mL) and 50 mL (±0.06 mL) with stopper from PE | SCHOTT AG, Mainz, Germany |
| Eppendorf Research® (10-100, 100-1000, and 500-5000 µL) with epTIPS pipette tips | Eppendorf AG, Hamburg, Germany |
| Graduated cylinder: 25 mL (± 0.40 mL), scaling 25:0.5 | SCHOTT AG, Mainz, Germany |
| Oak Ridge Centrifuge Tubes (50 mL) | NALGENE Labware, |
| Pipette: 5, 10 mL (± 0.05 mL) scaling 5:0.1; 10:0.1 | Precicolor HBG W.- Germany |
| Pyrex® fluted funnel, short stem | Pyrex, Germany |
| Volumetric Flask: 25 mL (± 0.04 mL), 50 mL (± 0.06 mL), 100 mL (± 0.10 mL), 500 mL (± 0.25 mL), 1000 mL (± 0.40 mL) with stopper from PE | SCHOTT AG, Mainz, Germany |
| Watch glass | SCHOTT AG, Mainz, Germany |
| Whatman filter paper No. 40 (Ø 110 mm.) | Whatman, USA |

### 3.1.3 Instruments

*Balance*

An analytical balance model TE214S purchased from Satorius (Elk Grove, IL, USA) was used in this study.

*Centrifuge*

A Heraeus Sorvall Biofuge Stratos Refidgerated High Speed Centrifuge (Hanau, Germany) was used to separate the soil/plant parts from the extraction solvent.

*DI-water*

Deionized water was obtained from a PURELAB ultra (18.2 MΩ-cm) purchased from ELGA Veolia Water STI (St. Maurice Cedex, France).

*Flame Atomic Adsorption Spectometer (Flame-AAS)*

For Cd and Zn analysis a Flame-AAS model ZEEnit 700 Tech was used.

*Graphite Furnace Atomic Adsorption Spectrometer (GF-AAS)*

For Cd analysis a GF-AAS model AAnalyst 800, Perkin Elmer, was used.

*Hot plate and stirrer*

A hot plate with integrated stirring function model Cimarec, Barnstedd/Thermolyne was used for the experiments.

Microwave Digester

For microwave assisted digestion a microwave digester model ETHOS SEL, MILESTONE was used.

*Oven*

Soil and plant samples were dried in a model FD 115 drying oven purchased from BINDER GmbH (Tuttlingen, Germany).

*pH and Conductivity meter*

A pH and conductivity meter model sensION 1 portable pH meter with gel-filled pH electrode from HACH LANGE (Düsseldorf, Germany) was used for pH and redox potential measurements.

*Shaker*

A mechanical shaker model Sseriker II PNP (Bangkok, Thailand) was used for shaking extraction batches.

*UV/VIS-Spectrophotometer*

For total and available phosphorus analysis a UV/VIS-spectrophotometer model Heyios α, Thermo Electron Corporation was used.

## 3.2 Methods

This research was divided into four parts (Figure 3.1).

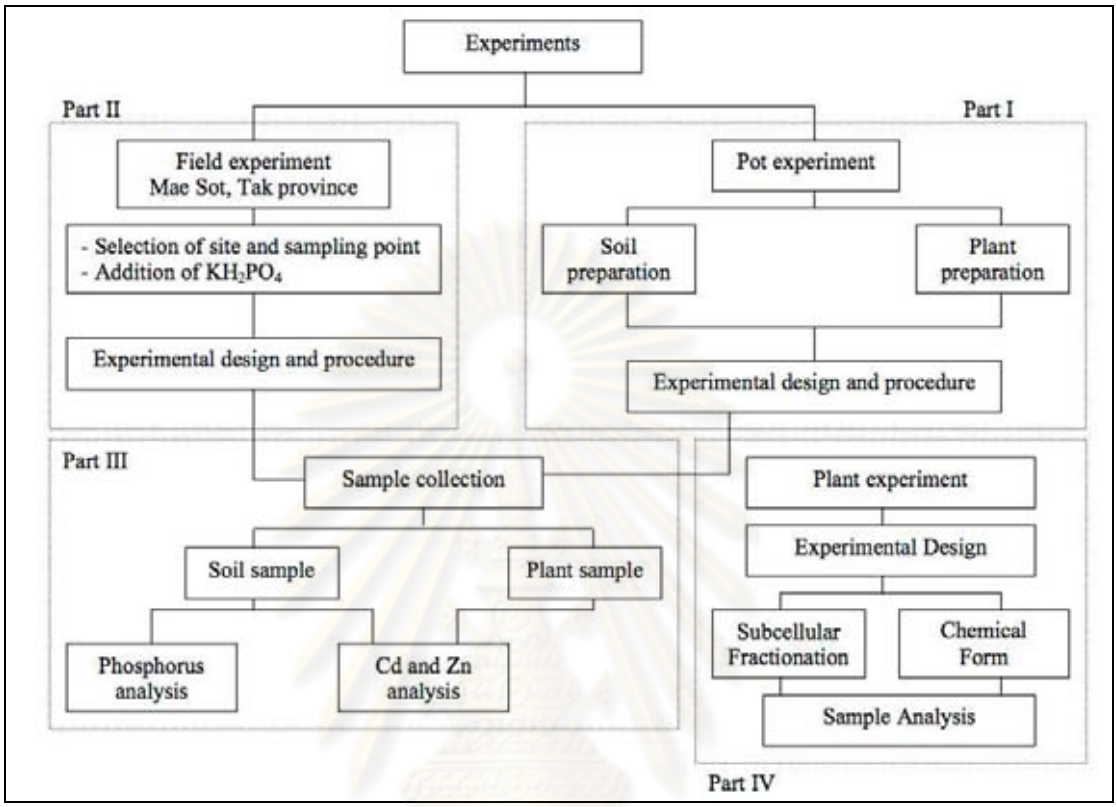



**Figure 3.1.** Schematic diagram of experiments.

Part I focused on the pot experiment, which involved the necessary soil and plant preparations. The results obtained from the pot experiment were used to select the correct treatment for Part II, the field experiment, which incorporated the site selection and soil preparation for the field experiment. Part III included the sample collection from the pot and field experiments and the analysis in respect to the total and available phosphorus, Cd and Zn in the soil, as well as the analyses of Cd and Zn in plants. To obtain a better understanding for the findings from Part I and II, an additional plant experiment was conducted, which was the fourth part of the experimental framework. In this part the subcellular distribution and the chemical forms of Cd within the rice plant were determined.

### 3.2.1 Pot experiment

#### 1) Soil preparation for the pot experiment

##### 1.1) Soil sample

A soil sample with high Cd contamination was collected at a depth of 0 to 30 cm (root zone) one time as a bulk for the pot experiment from the Mae Sot District and was further prepared.

##### 1.2) Preparation of pots

The soil for the pot experiment obtained from the Mae Sot District exhibited a high Cd contamination of >60 mg Cd/kg soil. The oven-dried soil was crushed using a grinder at 24000 rpm and sieved (mesh 2-3 mm). Every pot was filled with 5 kg dried and crushed soil and then submerged with water at a height of 2-5 cm above soil level.

##### 1.3) Phosphorus added

For the pot experiment four phosphate concentrations were applied, namely, 0 (control), 50, 200, and 1000 mg P/kg soil. To obtain those concentrations commercial fertilizer (0-52-34) containing 52% $P_2O_5$ and 34% $K_2O$ was used. One kilogram commercial fertilizer contains 227.1 g P so that the following amounts of the P-fertilizer were used for each concentration (Table 3.3):

**Table 3.3.** Phosphate concentration in pots and amount of fertilizer added to pots.

| No.-Set | P concentration (mg/kg) | Amount P in 5 kg pot (g) | Fertilizer added per pot (g) |
|---|---|---|---|
| P-0 | 0 | 0.00 | 0.00 |
| P-1 | 50 | 0.25 | 1.10 |
| P-2 | 200 | 1.00 | 4.40 |
| P-3 | 1000 | 5.00 | 22.02 |

After addition of the P-fertilizer the pots stood for 2 weeks to reach equilibrium before further treatment.

#### 2) Plant preparation

Seeds of the Jasmine rice 105 cultivar (Thai Hom Mali 105) were surfaced

sterilized and germinated in DI-water for 3 days. After germination the seedlings were transplanted into the pots with a density of three plantlets per pot and the strongest plant was selected after 2 weeks so that a density of one plantlet per pot was obtained.

**3) Experimental Design**

The pot experiment was performed outside and protected from rain and pests at an experimental plot at the Kasetsart University, Kamphaengsaen Campus. Four concentrations (control included) and five replicates per treatment were done for three rice growth stages (after 30, 90, and 120 days). The P-fertilizer was added to the pots and mixed thoroughly. Each concentration had three pots, one of them for sampling of plants and soil solution after 30 days (vegetative stage), 90 days (panicle formation), and 120 days (grain filling stage).

### 3.2.2 Field experiment

**1) Site selection**

Two different fields used for rice propagation were selected in the area of the Mae Sot District for the field experiment. Each field had a different level of Cd contamination namely <0.5 mg Cd/kg soil and >60 mg Cd/kg soil, respectively. The Geographic Information System (GIS) was used for the site and sampling point selection.

**2) Soil preparation for the field experiments**

At the beginning the soil in the fields was roughly ploughed. After one week, the soil was again ploughed into regular furrows for one to two times. After another 1-2 weeks the soil was harrowed and saturated with water to a height of about 5 to 10 cm. Then, the soil was ready for transplanting and the growth of weed was inhibited as well.

**2.1) Addition of phosphorus to soil**

137.4 kg fertilizer (phosphate content of 227.1 g P/kg fertilizer) per rai were added to the soil to a depth of 30 cm to obtain a phosphate concentration of 50 mg/kg soil.

**3) Experimental design**

Two field sites were selected to conduct the field study; one site possessed a low Cd contamination of <0.5 mg Cd/kg soil and the other site a high Cd contamination of >60 mg Cd/kg soil, respectively. To each field a P-fertilizer was added to a depth of 30 cm to obtain a phosphate concentration of 50 mg P/kg soil. Soil and plant samples were taken at five different sampling points for each field and samples were taken at five different stages, namely before amendment addition and transplantation of rice seedlings (background), shortly after P-fertilizer application (background-P), after 60 days of plant growth (vegetative stage), 90 days of plant growth (panicle formation), and 120 days of plant growth (grain filling stage).

### 3.2.3 Sample collection and analysis

**1) Total and available phosphorus analysis of the soil sample**

**1.1) Total phosphorus analysis**

The total phosphorus was determined using the NaOBr method proposed by Dick and Tabatabai (1977). In this method, the following reagents and methods were used.

Reagents:

(1) The sodium hypobromite solution (NaOBr-NaOH) is prepared by slowly adding 3 mL of bromine (0.5 mL/min) to 100 mL of 2 M NaOH under constant stirring. This reagent is prepared every day freshly.

(2) 90% formic acid

(3) 2.5 M $H_2SO_4$

(4) The ammonium molybdate-antimony potassium tartrate solution is prepared by dissolving 12 g of ammonium molybdate A.R. [$(NH_4)_6Mo_7O_{24}.4H_2O$)] in 250 mL DI-water. After that 0.2908 g of antimony potassium tartrate A.R. ($KSbO.C_4H_4O_6$) are dissolved in 100 mL of DI-water. Both solutions are added to 1 L of 2.5 M sulfuric acid, and the volume is diluted to 2 L with DI-water.

(5) The ascorbic acid solution is prepared by dissolving 1.056 g of ascorbic acid ($C_6H_8O_6$) in 200 mL of reagent (4).

(6) The standard phosphorus solution (P=50 mg/L) is prepared by dissolving 0.2195 g of potassium dihydrogen phosphate ($KH_2PO_4$) in DI-water. The solution is

diluted to 1 L with DI-water.

A sub-sample of 100-200 mg soil is oxidized with addition of 3 mL of an alkaline hypobromite solution (NaOBr-NaOH). The sample is swirled and heated in a sand bath at 260 to 280 °C. The content is evaporated to dryness and additional 30 min. Then, the flasks are removed from the sand bath and allowed to cool down for 5 min. After this, 4 mL of DI-water and 1 mL of formic acid are added and mixed before 25 mL of 0.5 M $H_2SO_4$ are added as well. The stoppered flasks are manually shaken and the sample is transferred to a centrifuged tube and the content is centrifuged at 12000 rpm for 1 min.

1 to 2 mL of the sample are placed into a 25 mL volumetric flask and 4 mL ascorbic reagent are added. The solution is mixed and bulked to volume. The absorbance of the solution is measured after 30 min at a wavelength of 720 nm. The concentration of total phosphorus will be calculated using the following equation:

$$\text{Total P, mg/kg} = \left[\text{Concentration of P in initial formic acid/}H_2SO_4\text{ solution, mg/L}\right] \cdot \left[0.03\text{ L/mass of soil, kg}\right]$$

**1.2) Available phosphorus analysis**

The available phosphorus was determined using the Bray II method (Bray and Kurts, 1945). In this method, the following reagents and methods were used.

Reagents:

(1) The Bray extracting solution is prepared by dissolving 2.22 g ammonium fluoride A.R. ($NH_4F$) in DI-water and transferred to a volumetric flask. 17 mL concentrated hydrochloric acid are added and the solution is bulked to volume with DI-water.

(2) The Reagent A 17.14 g ammonium molybdate A.R. [$(NH_4)_6Mo_7O_{24}.4H_2O$)] are dissolvd in 200 mL warm DI-water. 0.392 g potassium antimonyl tartrate A.R. ($KSbO.C_4H_4O_6$) are separately dissolved in 150 mL DI-water. 500 mL DI-water are placed in a 2 L volumetric flask, and 200 mL concentrated sulphuric acid are added slowly with mixing. When cooled, the cooled molybdate and tartrate solutions are added, mixed, and bulked to volume with DI-water.

(3) For Reagent B 0.53 g L-ascorbic acid ($C_6H_8O_6$) are dissolved in DI-water

and transferred to a 500 mL volumetrrc flaks. Then, 70 mL of Reagent A are added and bulked to volume with DI-water. The solution is prepared every day freshly.

(4) The standard phosphorus solution (P=50 mg/L) is prepared by dissolving 0.2195 g potassium dihydrogen orthophosphate A.R. ($KH_2PO_4$) in 100 mL DI-water, and the solution is transferred to a 1 L volumetric flask.

A sub-sample of 5 g soil is placed in an erlenmeyer flask and 50 mL Bray II extraction solution (soil:extraction solution ratio is 1:10) are added. The flask is shaken vigorously for 1 min and the solution is then filtered using a Whatman filter No. 40 (Ø 110 mm); then, 0.5 mL of sample solution are placed in a colourimeter tube and 2 mL ascorbic acid reagent are added. After that, the solution has to stand for 30 min and then the absorbance is measured at a wavelength of 882 nm. The concentration of available phosphorus will be calculated using the following equation:

$$\text{AvailablePhosphorus,mg/kg} = \frac{[\text{P conc measured, mg/2.5 mL}] \cdot [\text{Dilution factor}]}{[\text{Sample weight, g}]}$$

**2) Analysis of total Cd and Zn in soil samples**

The soil was digested by using acid in a closed vessel for heating in a microwave. 0.5 g of soil was placed in a vessel and 9 mL of HCl (37%) and 3 mL of $HNO_3$ (65%) were added and gently swirled to homogenize the solution. After that, the vessels were closed and placed according to the instructions of the microwave (USEPA, 1996).

**3) Analysis of the fraction of Cd and Zn in soil samples**

The phytoavailable fractions of Cd and Zn were extracted by applying the BCR methods I and II. In these methods, the soil is suspended in acetic acid (0.11 Mol/L) (the soil-to-solution ratio is 1:40) to release the exchangeable, water, and acid soluble Cd ions that are bound to carbonates fractions (F1). The soil solution is shaken for 16 h at 350 rpm at room temperature and then centrifuged for 20 min at 4800 rpm. The supernatant is analyzed directly for Cd and Zn using graphite furnace atomic adsorption spectrometer (GF-AAS) and 20 mL DI-water is added to the residues. After washing the residues by manual shaking for 5 min, they are

centrifuged for 15 min at 4800 rpm to get the residues for Fraction two (F2).

In BCR II, the residues of BCR I are suspended in hydroxylammonium chloride (0.1 Mol/L) (the soil-to-solution ratio is 1:40) at a pH of 2 to release the Cd ions that are bound to iron/manganese oxides and represent the reducible fraction; the pH is adjusted with 2 Mol/L $HNO_3$. The following procedure was performed as stated in the method to determine the BCR I fraction (Tokalioglu et al., 2003).

**5) Analysis of Cd and Zn in plant samples**

The plants were harvested according to growth stage. For the pot and the field experiment the plants were taken out carefully to avoid damage of the roots. After that, the plants were washed thoroughly with DI-water to remove soil. The plant samples were separated into roots, upper plant parts (stems and leaves), and panicles/grains. Fresh weight and dry weight were taken after drying the samples at 105 °C for 48 h.

*Shoots*

The dried and grinded shoots were digested on a hot plate (80 °C) by adding 8 mL concentrated $HNO_3$. The samples were heated until the copious $NO_2$ fume evolution subsides. After this, the samples were cooled down and 1-2 mL of concentrated reagent-grade $H_2O_2$ was added. They were returned to the hot plate again to maintain a solution temperature of 180 °C to 200 °C and covered with a watch glass to prevent loss of the sample. The samples were digested until the denseness of the white fumes had dissipated and only wisps of white vapors were visible. After cooling the samples down, they were filtered using a Whatman filter No. 41 and the volumes were adjusted to the final volumes by adding DI-water. The Cd and Zn concentrations were measured by a GF-AAS and F-AAS, respectively.

*Roots*

The dried and grinded roots samples were digested on a hot plate (80 °C) by adding aqua regia ($HCl:HNO_3$; 3:1) and heated until the copious $NO_2$ fume evolution subsides. The beakers were covered with a watch glass to prevent loss of sample. After cooling down the samples were filtered and analyzed as described for the shoot samples.

### 3.2.4 Subcellular fractionation and determination of chemical forms of Cd

To obtain a better understanding about the effect of phosphate on the Cd distribution and chemical form within the rice plant, the subcellular distribution and chemical form of Cd within the rice plant were studied. This experiment was separately set up as a hydroponic pot experiment. The phosphate concentrations selected for this study were 0 (control), 50, 200, and 1000 mgP/L nutrient solution. Details for each experimental step are given below.

**1) Plant culture.**

Rice seeds (Jasmine 105) were germinated and cultivated in soil until they were 4 weeks old. After that, they were transplanted into a basic nutrient solution. For each treatment three replicates were conducted and the plants were grown at a plant density of three plants per pot. The initial pH was set to be 5.0-5.1. After 2 weeks, $CdNO_3$ at a concentration of 0.3 mg $Cd^{2+}$/L was added to the nutrient solution as well as different phosphate concentrations (0, 50, 200, and 1000 mg/L) and the plants were treated with this solution for 2 weeks. The pH was adjusted to a value of 5.0-5.1 every third day. After a total time period of 8 weeks, the plants were harvested and divided into roots and shoots and immediately frozen at -80 °C (Makoto et al., 1986).

**2) Subcellular fractionation**

**2.1) Homogenization**

The roots and upper plant parts were homogenized separately using a pestle and a motar. The plant samples were grinded at 4 °C in a medium containing 0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), and 1 mM DL-dithiothreitol.

**2.2) Fraction I**

The homogenized plant material was filtrated using a nylon cloth with a mesh size of 240 μm obtaining a liquid and residues. The residues were washed twice with grinding medium and then pooled with the first filtrate. The pooled washes were then centrifuged at 300 x g for 30 s. The obtained pellet was combined with the residues from the nylon cloth filtration and mainly contained cell walls and cell wall debris and was appointed to be the cell wall fraction (I).

**2.3) *Fraction II***

The filtrate from the previous step was centrifuged at 1500 x g for 10 min (root sample 2500 x g for 20 min) and the pellet obtained was the chloroplast-shoot/trophoplast root containing fraction (II).

**2.4) Fraction III**

The supernatant was then centrifuged at 20000 x g for 45 min to sediment the cell organelles. The pellet obtained was designated to the organelle fraction (III). The supernatant obtained was considered as soluble fraction (F IV).

**2.5) Measurement**

Fraction I was measured by drying it at 70 °C to constant weight; then, the samples were digested according to being roots and shoots as described in chapter 3.2.3, 5) and measured using a GF-AAS. The re-suspended fraction II and fraction II, as well as the soluble fraction were first digested on a hot plate by adding $HNO_3$ and $H_2O_2$ as described in chapter 3.2.3, 5) and then measured using a GF-AAS.

**3) Determining the chemical forms of Cd**

In the method proposed by Wu et al. (2005) Cd in different chemical forms was extracted in the order of the extraction solutions listed below. In the method, frozen shoot and root tissues are homogenized in the extraction solution using a motar and pestle. The resulting mash is diluted at the ratio of 1:100 (w/v), and shaken for 22 h at 25 °C. The homogenate is then centrifuged at 5000 x g for 10 min, obtaining the first supernatant solution. The pellet was re-suspended twice in extraction solution and shaken for 2 h at 25 °C, centrifuged at 5000 x g for 10 min, and then the supernatant of the three suspending and centrifuge steps is pooled for each of the five extraction solutions. Each of the pooled supernatant solution is then evaporated in an oven at 70 °C to constant weight. The samples were digested as described in chapter 3.2.3, 5).

(1) 80% ethanol, extracting inorganic Cd giving priority to nitrate/nitrite, chloride, and aminophenol Cd.

(2) DI-water, extracting water-soluble Cd of organic acid, and $M(PO_4)_2$.

(3) 1 M NaCl, extracting Pectates and protein integrated Cd.

(4) 2% HAC, extracting undissolved Cd phosphates including $CdHPO_4$ and $Cd_3(PO_4)_2$.

(5) 0.6 M HCL, extracting cadmium oxalic.

### 3.2.5 Soil characterization

Soil characterization was performed at the Soil Plant and Agricultural Material Testing and Research Unit at the Kasetsart University, Kamphaengsaen Campus. Soil organic matter was determined using the Walkley and Black method, the cation exchange capacity using $NH_4OAc$ at pH equal to 7.0, and soil texture using the pipette method.

### 3.2.6 Statistical Analysis

All the data were statistically analyzed using one-way ANOVA at a significance level of $P < 0.05$ with Microsoft Excel. Mean separations were compared by using ANOVA ($P < 0.05$) to test the difference between control and treatment. Duncan's New Multiple Range Test (DMRT) was used to obtain groupings of the mean values.

Additionally, Pearson's correlation with adjacent t-test was performed to test a linear relationship between the level of P addition and the available Cd and Zn concentration in soil samples.

The LOD and LOQ for the GF-AAS was found to be 1.64 and 4.18 μg/L and samples were corrected if below, respectively.

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 Pot Experiment

### 4.1.1 Physical and chemical properties of soil

The soil used for this pot experiment exhibited the basic characteristics presented in Table 4.1.

**Table 4.1.** Basic characteristics of the soil used in this experiment.

| pH | SOM[a)] | CEC[b)] | Particle Size composition [%] | | | Total P | Total Cd | Total Zn |
|---|---|---|---|---|---|---|---|---|
| 1:1 $H_2O$ | [%] | [meq/100g] | Clay | Silt | Sand | [mg/kg] | [mg/kg] | [mg/kg] |
| 7.51 | 2.22 | 18.66 | 13.63 | 47.90 | 38.47 | 437 | 82 | 2339 |

[a)]SOM: Soil organic matter; [b)]CEC: Cation exchange capacity.

The pH value of the submerged soil (negative redox potential) was slightly alkaline, which is slightly outside the optimum pH for submerged soil of 6.5-7.0 for rice propagation (Brink and Belay, 2006). The soil organic matter content was moderate according to the Soil Survey and Land Classification Center (1997), suggesting that the clay contributed most to the cation exchange capacity (CEC). The CEC is the property of soils to exchange one cation with another, which is the total amount of cations adsorbed on the soil, expressed in miliequivalents (meq) per hundred grams of dry clay (Hunter, 1982). The CEC was typical for a loam soil. High CEC values favor the sorption of heavy metals in soil; thus, reducing the plant availability because the CEC of a soil is a measure of the negative charge density of a soil as function of the soil's ability to adsorb positively charged cations. Elevated Cd and Zn concentration were found due to intense mining activities in this area. The Cd:Zn ratio of the soil differed compared to the natural ratio of 1:100 (compare section 2.6.4) and was reduced to about 1:30. This reduction of the natural Cd:Zn ratio found in contaminated soil should not have had an adverse effect on Cd uptake as rice plants are the only food crop taking up Cd unaffected by the soil's Zn concentration (compare section 2.6.4). Normally, at high Zn concentrations in soil and a natural Cd:Zn ratio, the plant shows Zn toxicity symptoms before accumulating high

amounts of Cd. With a reduced Cd:Zn ratio the plant might not show Zn toxicity symptoms before accumulating high Cd concentrations resulting in a threat to human health.

### 4.1.2 Fertilizer analysis

The commercial fertilizer used in this study was of a 0-52-34 formulation containing 52% $P_2O_5$ and 34% $K_2O$. A main source for Cd introduction into soils besides mining activities is the application of P-fertilizers because they are often manufactured from Cd-enriched raw phosphates and can contain up to 300 mg Cd/kg fertilizer (Amberger, 1996; Alloway und Steinnes, 1999).

To avoid further Cd pollution by using Cd-enriched P-fertilizers, in this study a fertilizer exhibiting a low Cd concentration was used (see Table 4.2), meaning that for a highest fertilizer application 12.50 μg Cd and 0.21 mg Zn were added to each pot containing 5 kg soil, which does not alter the initial metal concentration. The Zn concentration was around 17 fold the Cd concentration

**Table 4.2.** Cd and Zn content of the fertilizer used in this study.

| Total Cd [mg/kg fertilizer] | Total Zn [mg/kg fertilizer] |
|---|---|
| 0.568 ± 0.027 | 9.750 ± 1.532 |

Mean ± SD, n=2.

### 4.1.3 pH and redox potential of soil samples

After P addition a significant increase in soil pH was observed for the highest level of P addition ranging between 0.11 and 0.41 pH units for different growth stages (see Table 4.3).

**Table 4.3.** pH values for different growth stages and levels of P addition.

| Level of P Addition [mg/kg] | pH values for different growth stages | | | | SDV[a)] |
|---|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage | |
| 0 | 7.51 ± 0.17[a] | 7.28 ± 0.27[a] | 7.13 ± 0.23[a] | 7.41 ± 0.13[a] | |
| 50 | 7.61 ± 0.25[a,b] | 7.23 ± 0.41[a] | 7.15 ± 0.13[a] | 7.44 ± 0.30[a] | NS[b)] |
| 200 | 7.63 ± 0.11[a,b] | 7.21 ± 0.12[a] | 7.39 ± 0.21[a] | 7.40 ± 0.11[a] | NS |
| 1000 | 7.63 ± 0.05[b] | 7.55 ± 0.27[b] | 7.54 ± 0.08[b] | 7.52 ± 0.08[b] | 0.038 |

Mean ± SD, n=5; [a)]SDV: Significantly different values of the corresponding pH between P treatments and the control at $P < 0.05$; [b)]NS: No significance. Numbers followed by the same letter in each column are not significantly different at $P < 0.05$ by DMRT.

It is generally accepted that the pH of a soil is one of the most important factor

controlling the uptake of heavy metals by plants (Seuntjens et al., 2004; Amini et al., 2005; Basta et al., 2005). The increase in pH after P addition was due to ligand exchange phosphate adsorption reactions (Bolan et al., 2003b). During this mechanism surface hydroxyl groups coordinated with a metal cation (e.g., Fe and Al) of the solid phase are replaced by phosphate ions. This exchange mechanism mostly involves Fe- and Al-oxyhydroxides as solid phase. The mechanisms are presented in Figure 4.1. Due to the released $OH^-$ ions the pH of the soil increases with increasing P addition.

$$\left[\equiv AlOH_2^+\right]OH + H_2PO_4^- = \left[\equiv AlOH_2^+\right]H_2PO_4 + OH^-$$

$$Al\begin{matrix}OH\\OH\\OH\end{matrix} + H_2PO_4^- = Al\begin{matrix}OH\\OH\\H_2PO_4\end{matrix} + OH^-$$

**Figure 4.1.** Chemical reactions showing reactions with hydrous oxides or ligand exchange and fixation of phosphate by silicate clays (Reddy and DeLaune, 2008).

The slight decrease of the initial pH for every growth stage compared to the initial soil could be explained by the redox condition of the soil during the measurement (Table 4.4). Before rice growth, the soil exhibited a positive redox potential meaning that aerobe conditions were dominant, whereas for rice propagation the soil has to be submerged. Under submerged conditions the entrained oxygen present in soil is quickly consumed and lack of free oxygen causes a reducing environment of the soil.

**Table 4.4.** Redox potential for different growth stages and levels of P addition.

| Level of P Addition [mg/kg] | Redox potential for different growth stages [mV] | | | | SDV[a)] |
|---|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage | |
| 0 | 129.5 ± 34.47 | -85.82 ± 83.99 | -172.5 ± 24.61 | -128.5 ± 118.2 | |
| 50 | 53.54 ± 103.6 | -146.3 ± 52.76 | -184.7 ± 62.16 | -6.760 ± 43.42 | NS[b)] |
| 200 | 23.88 ± 119.7 | -177.0 ± 54.12 | -230.1 ± 8.721 | -52.36 ± 130.2 | NS |
| 1000 | 107.1 ± 60.50 | -234.2 ± 17.53 | -278.5 ± 5.816 | -254.8 ± 49.14 | NS |

Mean ± SD, n=5; [a)]SDV: Significantly different values of the corresponding redox potential between P treatments and the control at $P < 0.05$; [b)]NS: No significance.

This process influences the pH resulting in an increase in pH of acid soils and a decrease in pH of alkali soils to a range of 6.7-7.2 (Ponnamperuma, 1972). Due to

an accumulation of carbon dioxide in the soil under flooded conditions, the alkalinity of the loam soil present in Mae Sot was reduced and the pH decreased because of the formation of carbonic acid (Sahrawat, 2005). This could be observed for the control treatment between the initial stage and the vegetative stage; at initial conditions the soil was drained, whereas for the vegetative stage the soil was submerged decreasing the initial slightly alkaline pH of the loam soil.

### 4.1.4 Total phosphorus in soil samples

The total P content in the soil was around 437 mg/kg. With increasing level of P addition a significant increase of total P was observable (Table 4.5).

**Table 4.5.** Total P concentration for different growth stages and levels of P addition.

| Level of P Addition [mg/kg] | Total P concentration for different growth stages [mg/kg] | | | | |
|---|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage | SDV[a)] |
| 0 | 436.9±73.19[a] | 315.8±54.96[a] | 301.5±57.37[a] | 281.6±36.77[a] | |
| 50 | 448.1±129.9[b] | 485.8±73.72[b] | 417.6±79.31[b] | 459.8±43.41[b] | 0.023 |
| 200 | 581.3±154.8[c] | 628.3±116.3[c] | 596.0±104.3[c] | 608.3±80.58[c] | $3.1 \cdot 10^{-4}$ |
| 1000 | 1462±347.6[d] | 1361±30.45[d] | 1369±164.7[d] | 1405±119.8[d] | $2.4 \cdot 10^{-7}$ |

Mean ± SD, n=5; [a)]SDV: Significantly different values of the corresponding total P concentration between P treatments and the control at $P < 0.05$; Numbers followed by the same letter in each column are not significantly different at $P < 0.05$ by DMRT.

No clear trend for the reduction of the total P amount in soil over time was visible; thus, a reduction of total P over time was insignificant ($P < 0.05$). This is due to the small amount of total P removed from soil by plant uptake compared to the total amount present. It is well accepted that about 0.2% of a plant's dry weight is made up by P (Schachtman et al., 1998). When taking the dry weight of the rice plants into account (see section 4.1.8), an average rice plant of this study needed a total of about 330 mg P during its complete growth. Additionally, the standard deviations were partly very high, which also might disguise a trend for a depleting P concentration with increasing time. The high standard deviations could be a result of incomplete homogenization during the crushing process of the original soil obtained from the Mae Sot District so that slightly different initial P concentrations were present in the five pots constituting a sample variant.

### 4.1.5 Available phosphorus in soil samples

The initially available P content in soil before rice propagation was around 2 mg/kg. According to Mallarino et al. (2000), an available P content of 11-14 mg/kg insures optimal plant growth. The available P found in the soil used for the pot experiment was below the optimal range, making the addition of a P-fertilizer for propagation necessary. In this study 4 levels of P-fertilizer were added, namely 0 (control), 50, 200, and 1000 mg/kg. After the addition of 200 and 1000 mg P/kg a significant increase of the available P concentration in soil from 1.78 mg/kg to 10.73 and 225.40 mg/kg for the initial stage was observed, respectively, whereas no significance for an addition of 50 mg P/kg was detectable (see Table 4.6). The significant increase was due to the very high water solubility of the P-fertilizer, which readily dissolved and increased the concentration in soil solution (Havlin et al., 1999).

**Table 4.6.** Available P concentration for different growth stages and levels of P addition.

| Level of P Addition [mg/kg] | Available P concentration for different growth stages [mg/kg] | | | | |
|---|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage | SDV[a)] |
| 0 | 1.777±0.493[a] | 3.995±0.935[a] | 3.647±1.075[a] | 2.191±1.381[a] | |
| 50 | 2.965±0.861[a] | 4.169±1.390[a] | 3.856±0.880[a] | 2.515±0.288[a] | NS[b)] |
| 200 | 10.73±4.014[b] | 15.11±2.449[b] | 14.36±1.975[b] | 6.631±0.800[b] | $3.6 \cdot 10^{-3}$ |
| 1000 | 225.4±8.663[c] | 243.8±19.53[c] | 233.9±18.45[c] | 228.1±13.76[c] | $2.6 \cdot 10^{-9}$ |

Mean ± SD, n=5; [a)]SDV: Significantly different values of the corresponding available P concentration between P treatments and the control at $P < 0.05$; [b)]NS: No significance; Numbers followed by the same letter in each column are not significantly different at $P < 0.05$ by DMRT.

After the addition of 200 mg/kg, the available P concentration was in the optimum range for plant growth. A slight increase of the available P content for all levels of P addition (including control) was observed during the vegetative stage of the rice plants, suggesting that bound P was released due to the change of the redox potential from being positive during the initial stage to be negative for the vegetative stage after submergence, as was found by Uwasawe et al. (1988) (compare section 2.3.2). As mentioned in section 4.2.2, under anaerobe conditions ferric ions are reduced to ferrous ions thus releasing its bound P (Boström et al., 1982). During the single plant growth stages the available P content was continuously depleted. This effect is due to the uptake by rice plants. For the first and second level of P addition

with 0 and 50 mg/kg, respectively, the concentration was below the optimum range for all growth stages, whereas for an addition of 200 mg/kg the concentration decreased below the optimum only for the maturity stage. The insignificant increase in available P after an addition of 50 mg/kg P into the soil can be explained by the fast fixation of available P by soil. Ochwoh et al. (2005) found that up to 60% of the added P from a fertilizer was transformed into less labile forms within 1 d. After P addition, the soil was aged for around 2 weeks so that the soil fixed most of the available P added. Due to this observation, it could be concluded that a repeated addition of the P-fertilizer is necessary to keep the available P concentration constantly in a range for optimum plant growth. This is only true for a P level of 200 mg/kg because after the addition of 1000 mg/kg, the available P concentration was about 22 fold the optimum range for every growth stage. This raises the question if a repeated addition of P-fertilizer at lower concentrations or a one-time addition at a higher concentration would be beneficial. The economical as well as ecological examination of this question would have exceeded the scope of this project.

### 4.1.6 Total Cd and Zn concentrations in soil samples

The total Cd concentration in soil was not affected by the amount of applied fertilizer (Table 4.7). No significance was observable for all levels of P addition ($P < 0.05$). Due to the heterogeneous nature of soil, the initial Cd concentrations in the single pots were slightly different; however, they are in an acceptable range. The soil used in this study was obtained from the Mae Sot District. In this study, every experimental pot was sample sacrificed for each sampling stage; thus, the concentration in latter stages was found to be higher than for the initial stage for each treatment. Nonetheless, the data was shown to be insignificantly different.

**Table 4.7.** Total Cd concentration for different growth stages and levels of P addition.

| Level of P Addition [mg/kg] | Total Cd concentration in soil for different growth stages [mg/kg] | | | | |
|---|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage | SDV[a)] |
| 0 | 79.29 ± 6.721 | 82.90 ± 3.853 | 85.53 ± 5.004 | 79.36 ± 6.680 | |
| 50 | 72.30 ± 3.812 | 79.41 ± 1.553 | 83.36 ± 4.404 | 82.45 ± 2.741 | NS[b)] |
| 200 | 71.36 ± 0.573 | 82.62 ± 4.346 | 82.75 ± 3.058 | 83.14 ± 1.730 | NS |
| 1000 | 71.95 ± 1.959 | 81.68 ± 3.699 | 79.52 ± 1.726 | 82.61 ± 1.909 | NS |

Mean ± SD, n=5; [a)]SDV: Significantly different values of the corresponding Cd concentration between P treatments and the control at $P < 0.05$; [b)]NS: No significance.

Similar results were obtained for the total Zn concentration showing an insignificant difference for every level of P addition, suggesting that Zn was not affected by the different rates of fertilizer application (Table 4.8).

**Table 4.8.** Total Zn concentration for different growth stages and levels of P addition.

| Level of P Addition [mg/kg] | Zn concentration for different growth stages [mg/kg] | | | | |
|---|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage | SDV[a)] |
| 0 | 2414 ± 153.2 | 2254 ± 73.63 | 2365 ± 87.77 | 2323 ± 103.3 | |
| 50 | 2423 ± 100.0 | 2242 ± 108.6 | 2333 ± 76.61 | 2123 ± 172.1 | NS[b)] |
| 200 | 2352 ± 78.33 | 2311 ± 18.09 | 2285 ± 41.02 | 2316 ± 50.49 | NS |
| 1000 | 2395 ± 97.40 | 2363 ± 85.94 | 2280 ± 51.80 | 2357 ± 31.54 | NS |

Mean ± SD, n=5; [a)]SDV: Significantly different values of the corresponding Zn concentration between P treatments and the control at $P < 0.05$; [b)]NS: No significance.

### 4.1.7 Cd and Zn fractions in soil samples

In the sequential extraction procedure the extractable Cd and Zn from soil media was measured using acetic acid (denoted as F1) and hydroxylammonium chloride (BCR II), which are exchangeable, reducible and oxidizable metals (denoted as F2), respectively. The results for every extraction step are presented in Figure 4.2.

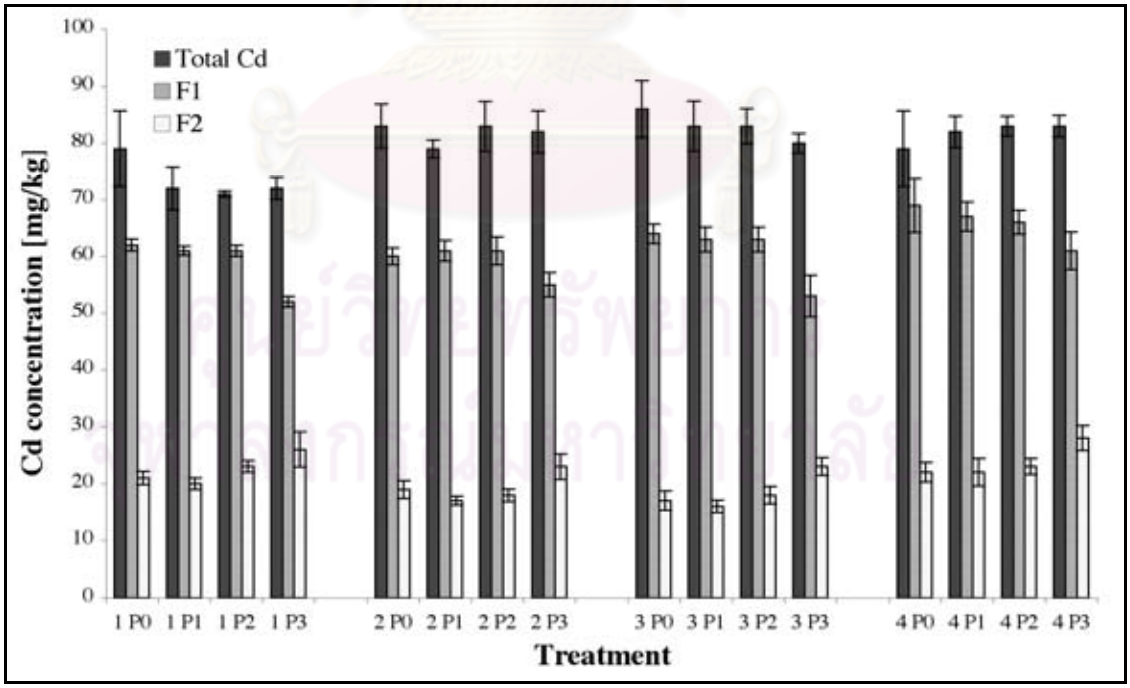



**Figure 4.2.** Cd concentrations of the different fractions for the 4 growth stages with respect to the total amount in the studied soil. Total Cd, concentration of the total Cd in the soil; F1, exchangeable, water, and acid soluble Cd fraction; F2, iron-manganese oxides associated Cd fraction; P0, P1, P2, and P3 level of P addition of 0, 50, 200, and 1000 mg/kg, respectively.

For a P addition of 50-1000 mg/kg, a decrease of F1 of 1.6-16.1%, 0.0-8.3%, 1.6-17.2%, and 2.9-11.6% for soil sampling during initial, vegetative-, panicle formation-, and maturity-stage conditions was observed, respectively. The decrease of Cd was only significant ($P < 0.05$) for the highest level of P addition compared to the control treatment. According to a decrease of the F1 fraction, an increase in the less mobile F2 fraction of 0.0-19.2%, 0.0-17.4%, 0.0-26.1%, and 0.0-21.4% for initial, vegetative-, panicle formation-, and maturity-stage conditions was visible, respectively, which was only significant for the highest level of P addition. To test a linear relationship between the addition of P and the Cd concentration measured in F1, Pearson's correlation with adjacent t-test was performed. Before analysis the data were graphed to ensure that the data show a linear relationship (Figure 4.3).

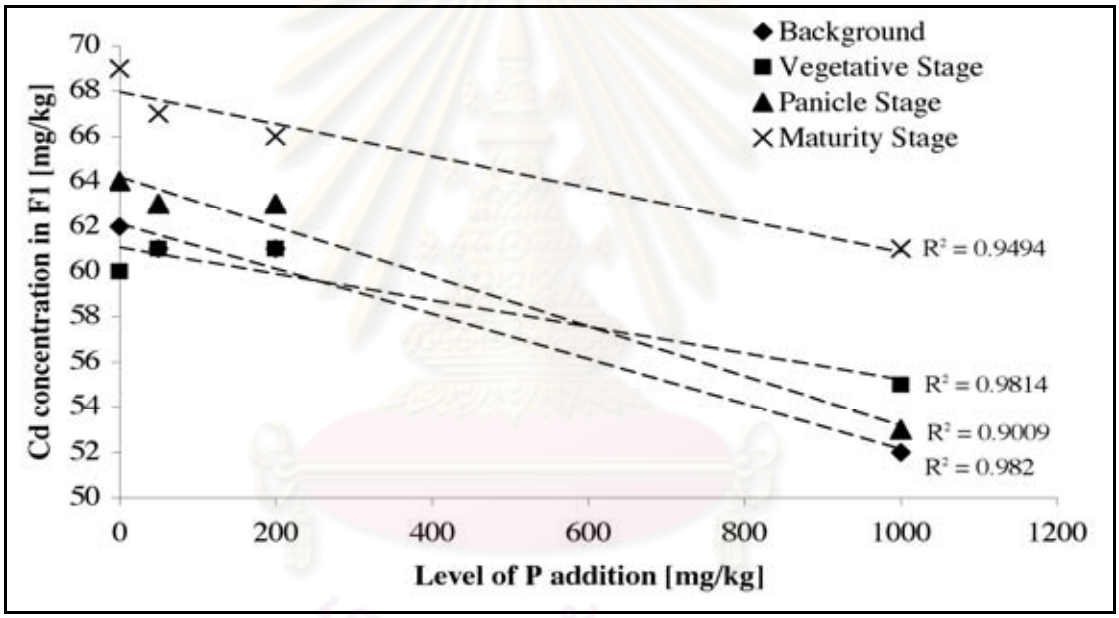



**Figure 4.3.** Correlation curves for the available Cd fraction (F1) for different growth stages.

From Figure 4.3 a linear relationship was demonstrated and significant ($P < 0.05$) negative correlation coefficients of $r = -0.9891$, $r = -0.9492$, $r = -0.9907$, and $r = -0.9744$ for soil sampling during initial-, vegetative stage-, panicle stage-, and maturity stage-conditions were obtained, respectively. The correlation coefficients imply that with increasing P application the Cd concentration in F1 decreased.

It is an overall accepted fact that Cd represents one of the most mobile heavy metals in soil. Tokalioglu et al. (2003) found that Cd is the most mobilized element among Cu, Pb, Ni, Fe, Co, Mn, Zn, and Cr; thus, most of the Cd present in the solid

phase of the soil can be found in the easily mobile forms. In this study, approximately 75% of the total Cd concentration was measured in the first extraction stage (F1), whereas only 25% was found in the second extraction stage (F2). The sum of F1 and F2 exceeded the total Cd concentration for some samples (see Figure 4.3). The total Cd concentration was obtained by microwave-assisted digestion with aqua regia as reagent. Ahnstrom and Parker (1999) revealed that only 74-86% of the total Cd for different soils was extracted with that procedure. To overcome this problem they tried to double-digest the samples, resulting in a slight increase of the recovery to 86-91%, but it still was not possible to extract some refractory Cd. They also compared several sequential extraction procedures and found that for some soil the Cd concentration in the oxidizable fraction was above 75% of the total Cd concentration. Tokalioglu et al. (2003) found comparable results and claimed that about 75% of the soil's Cd can be found in the first two fractions, making Cd such an ecotoxic heavy metal. Based on the high percentage found in F1 and the low extraction efficiency of the microwave digestion procedure the sum of F1 and F2 sometimes exceeded the total Cd amount.

The influence of $KH_2PO_4$ on Cd fractionation in soil contaminated with Cd was also studied by Hettiarachchi et al. (2000). It was found that with increasing P level (0, 1100, and 2200 mg/kg) the Cd concentration in the exchangeable fraction was significantly decreased, whereas the Cd concentration in the carbonate (II) and oxide (III) fraction increased with increasing level of P addition. As reviewed in chapter 2.7.1, comparable results were found by many other researchers.

The mechanisms responsible for the decrease of F1 and a concomitant increase in F2 after the addition of water-soluble phosphate compounds are widely discussed in literature. They can be described as phosphate induced adsorption (Pierzynski and Schwab, 1993; Naidu et al., 1994; Bolan et al., 1999) due to an increase in pH (Levi-Minzi and Petruzelli, 1984) and precipitation as $Cd_3(PO_4)_2$ (McGowen et al., 2001).

As described in section 4.1.3, the pH of the soil is increased after addition of water-soluble $KH_2PO_4$ due to ligand exchange and fixation reactions. With an increase in pH, the negative surface charge of soil with a high clay and/or organic matter content increases as well. Clay and organic matter possess a high amount of active surface sites, which will be, dependent on the pH, protonated or deprotonated exhibiting a positive or negative charge, respectively. In this study the soil pH was increased after P addition; increasing the negative surface charge of the functional sites of the clay present in the soil; thus, the positively charged Cd-cations were

adsorbed by the negative surface charge of the soil and with this immobilized (Bolan et al., 2003b). Another mechanism for the reduction of the Cd activity is surface complex formations after the addition of phosphate. In the reaction proposed in Figure 4.1, phosphate anions form a complex with the soil surface featuring the adsorption of Cd-cations onto the adsorbed anions. Precipitation as metal phosphates and hydroxides has also been proposed to be a main mechanism for the immobilization of metals. However, precipitation of Cd as hydroxide compounds ($Cd(OH)_2$) of low solubility was unlikely to occur because of the low pH (precipitation starts at a pH range of 8.0-9.5). Additionally, the solubility of the insoluble $Cd_3(PO_4)_2$ was too high to control the activity of Cd in soil solution (Xiong, 1995; Bolan et al., 2003b).

In this study the addition of $KH_2PO_4$ increased the soil pH and thus increased the negative surface charge of the variable charged components of the clay present in the soil. It is assumed that this lead to an enhanced Cd immobilization as shown by the redistribution of Cd to a less mobile fraction (F2).

Unlike Cd, Zn exhibited a different trend for the fractions F1 and F2 (see Figure 4.4).

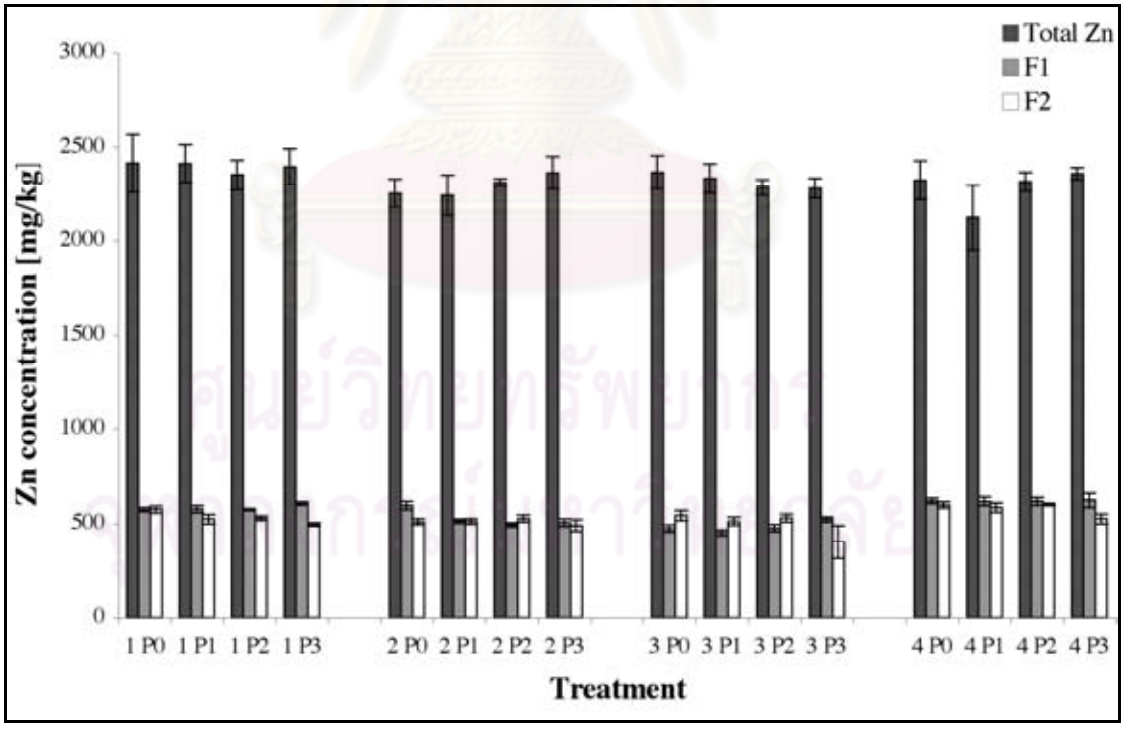



**Figure 4.4.** Zn concentrations of the different fractions for the 4 growth stages with respect to the total amount in the studied soil. Total Zn, concentration of the total Zn in the soil; F1, exchangeable, water, and acid soluble Zn fraction; F2, iron-manganese oxides associated Zn fraction; P0, P1, P2, and P3 level of P addition of 0, 50, 200, and 1000 mg/kg, respectively.

After P addition of 50-1000 mg/kg, an increase in F1 of 0.1-1.5%, 0.0%, and 0.0-0.9% for soil sampling was observed during initial-, vegetative-, panicle formation-, and maturity-stage conditions, respectively. Accordingly, F2 seemed to decrease 2.1-3.1%, 0.0-1.9%, and 1.1-5.4% for soil sampling during background, vegetative-, panicle formation-, and maturity-stage conditions, respectively. However, the only significant decrease in F2 was observed for the highest level of P addition compared to the control, whereas no significance for an increase in F1 was detectable.

The correlation coefficients for the Zn concentration in F1 with increasing level of P addition are $r$ = 0.9750, $r$ = -0.4541, $r$ = 0.9454, and $r$ = 0.8866 for soil sampling during initial-, vegetative-, panicle formation-, and maturity-stage conditions, respectively. Except for the vegetative stage, all correlation coefficients were positive meaning that with increasing P the Zn in F1 increased as well (Figure 4.5). Significant correlations were obtained for every growth stage except for the vegetative stage. The positive correlation and low linearity obtained for the vegetative stage could be an effect of the plant uptake. Kashiwagi et al (2009) found that the main Cd uptake was during the pre-heading stage, which represents the end of the vegetative stage. As Cd and Zn are chemically related to each other it can be assumed that the main Zn uptake also occurred during that stage. Similarly, Ramanathan and Krishnamoorthy (1973) found that the main uptake of minerals took place between tillering and flowering. Due to the high uptake by the rice plants during that stage (about 200-700 mg Zn/kg dry weight of the rice plants, see Table 4.12), the Zn present in F1 was depleted faster than redistributed from the other fractions. During the panicle and maturity stage, the rice plants accumulated only minor amounts of Zn; thus, the equilibrium between the single Zn fractions in soil was reached again and an increase of Zn in F1 with increasing P was observed.

Zn is an essential micronutrient for plants and Zn deficiency can lead to toxicity symptoms. Due to the increased Cd and Zn concentrations in soil from the Mae Sot District one can find a very high available Zn fraction (F1). Normally, for other crops at such high available Zn levels danger of Zn phytotoxicity would exists, but for rice plants this is no problem, as only necessary Zn will be accumulated (compare section 2.6.4).

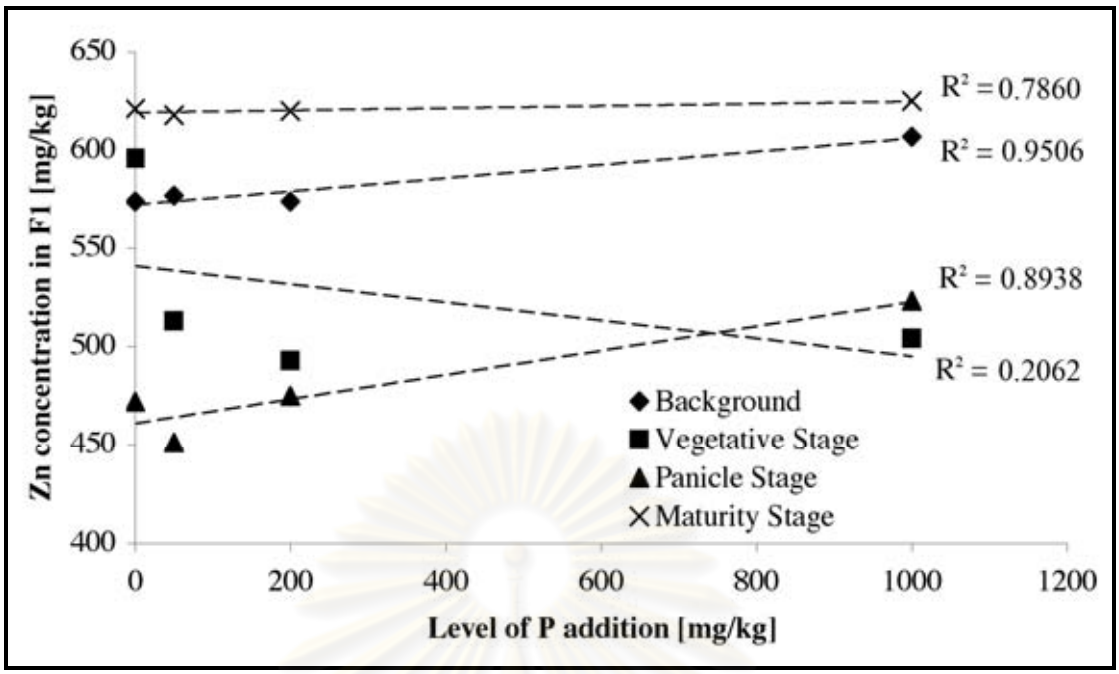



**Figure 4.5.** Correlation curves for the available Zn fraction (F1) for different growth stages.

With increasing level of P addition there was no great change in the Zn distribution between F1 and F2. However, the seemingly slight increase in F1 was also found by Ahumada et al. (1997) who studied the Zn speciation in phosphate-affected soils. They could observe this effect on andosol soil showing low pH (5.3), high SOM (7.21%) and a very high CEC (102.2 cMol/kg), making a comparison between the soil used in this study difficult. They ascribed this effect to the high abundance of Zn in the exchangeable and organically bound fraction, making Zn more available for the plant.

As Cd and Zn are chemically related, they are showing similar behavior in the soil-plant system. It can be assumed that, as a result of an increased adsorption of Cd by functional surface sites of the soil, there was competition between Cd and Zn for binding sites with Cd showing a higher affinity (Homma and Hirata, 1984). Hence, more sites were adsorbed with Cd, whereas Zn was released resulting in an increase of the mobile fraction. However, there is no proof to evoke this mechanism.

In conclusion, the increase in F1 and the decrease in F2 were not significant (except for F2 at the highest level of P addition compared to the control) and altogether the change in F1 and F2 was only minor, suggesting only a marginal, if any, effect of the rate of P application on the Zn distribution in F1 and F2 at such high soil Zn concentrations.

### 4.1.8 Dry mater yield of plant samples

The effect of P application at different rates on the dry matter yield of the different plant parts was measured for different growth stages and the results are presented in Table 4.9. There was no significance detectable for the dry matter yield of the whole rice plants between the different levels of P application. However, an increase of the dry matter yield with increasing addition of P fertilizer was observed (see Table 4.10).

**Table 4.9.** Dry matter yield of different rice plant parts for different levels of P addition and growth stages.

| Growth Stage | Dry matter yield of different plant parts [g/pot] | | | |
|---|---|---|---|---|
| | 0 [mg/kg] | 50 [mg/kg] | 200 [mg/kg] | 1000 [mg/kg] |
| **Vegetative Stage** | | | | |
| Roots | 0.410 ± 0.475 | 1.000 ± 1.124 | 0.870 ± 1.751 | 1.950 ± 2.297 |
| Stems + Leaves | 1.790 ± 2.303 | 3.240 ± 3.038 | 2.690 ± 5.764 | 5.170 ± 6.074 |
| **Panicle Stage** | | | | |
| Roots | 2.280 ± 1.272 | 3.150 ± 1.622 | 5.825 ± 4.184 | 7.600 ± 5.041 |
| Stems + Leaves | 11.54 ± 4.796 | 16.73 ± 8.195 | 20.65 ± 11.99 | 26.50 ± 13.77 |
| Panicles | 2.300 ± 0.964 | 3.750 ± 2.278 | 8.733 ± 4.508 | 5.933 ± 4.869 |
| **Maturity Stage** | | | | |
| Roots | 1.960 ± 0.868 | 4.000 ± 1.423 | 9.375 ± 1.879 | - [c)] |
| Stems + Leaves | 12.36 ± 7.617 | 22.30 ± 9.263 | 33.83 ± 3.634 | - |
| Grains | 4.660 ± 2.687 | 12.50 ± 6.687 | 12.28 ± 5.022 | - |

Mean ± SD, n=5; [c)]no samples were obtained due to Cd toxicity.

For the panicle and the maturity stage some rice plants died due to Cd toxicity so that replicates between three to five were obtained, except for the maturity stage for a P application of 1000 mg/kg, where no plants survived due to Cd toxicity (compare section 2.1.1).

Identical observations were made by Chien et al. (2003) who studied the effects of phosphorus on the rice grain yield. The rice grain yield was increased by 95.5% after the application of a PK-fertilizer. Similarly, Mortvedt (1987) reported the enhanced growth of plants after the addition of phosphorus.

The enhanced plant growth is due to the fact, that the available phosphorus naturally present in the soil is below the range of 11-14 mg/kg needed for optimum plant growth (compare section 4.1.5). Available phosphorus contents are often depleted in agricultural used soil making the application of fertilizers necessary. After the addition of the P-fertilizer, the available phosphorus content increased for an

application rate of 200 mg/kg to the optimum range; at the highest level of P addition the available P content was about 22 fold the optimum available P range, resulting in a restoration of the soil with respect to the available P content, and the plant growth resumed at a normal rate.

**Table 4.10.** Dry matter yield of the whole rice plant for different levels of P addition and growth stages.

| Level of P Addition [mg/kg] | Dry matter yield of the rice plants [g/pot] | | | |
|---|---|---|---|---|
| | Vegetative Stage | Panicle Stage | Maturity Stage | SDV[a)] |
| 0 | 2.200 ± 0.976 | 16.12 ± 5.340 | 18.98 ± 5.397 | |
| 50 | 4.240 ± 1.584 | 25.66 ± 7.199 | 39.80 ± 9.152 | NS[b)] |
| 200 | 3.560 ± 1.287 | 33.88 ± 8.141 | 55.49 ± 13.36 | NS |
| 1000 | 7.170 ± 2.277 | 40.73 ± 11.20 | -[c)] | NS |

Mean ± SD, n=5; [a)]SDV: Significantly different values of the corresponding dry matter yield between P treatments and the control at $P < 0.05$; [b)]NS: No significance; [c)]no samples were obtained due to Cd toxicity.

### 4.1.9 Cd and Zn concentrations in plant samples

#### 1) Cd and Zn in the whole rice plants

The Cd concentration in the rice plants was affected by the addition of different levels of P-fertilizers, as presented in Table 4.11. Even though there was no significant effect on the Cd content in the plants for all stages, the results showed that the Cd concentration was reduced for the panicle and maturity stage compared to the vegetative for every level of P addition. The partly high SDs were a result of the distinctive Cd toxicity symptoms as some of the plants showed nearly no symptoms, while others showed stunned growth or discoloration being a hint for the damage of the chlorophyll having an effect on nutrient uptake from soil and thus on the uptake of Cd (compare section 2.1.1). Additionally, some replicates constituting a sample variant did not survive also being responsible for an increase in the SDs.

It could be observed that the Cd was accumulated during the vegetative stage, which is consistent with the findings of Kashiwagi et al. (2009), who found that almost all Cd was accumulated during pre-heading, which is the end phase of the vegetative stage (compare section 2.6.2).

**Table 4.11.** Cd concentration for the whole rice plant (dry weight) for different levels of P addition and growth stages.

| Level of P Addition [mg/kg] | Cd concentration in the whole rice plant [mg/kg] and for different levels of P addition | | | |
|---|---|---|---|---|
| | Vegetative Stage | Panicle Stage | Maturity Stage | SDV[a)] |
| 0 | 0.859 ± 0.253 | 0.661 ± 0.231 | 0.561 ± 0.413 | |
| 50 | 0.791 ± 0.362 | 0.569 ± 0.281 | 0.271 ± 0.079 | NS[b)] |
| 200 | 1.634 ± 0.526 | 0.961 ± 0.245 | 0.343 ± 0.180 | NS |
| 1000 | 1.085 ± 0.062 | 1.036 | -[c)] | NS |

Mean ± SD, n=5; [a)]SDV: Significantly different values of the corresponding Cd concentration between P treatments and the control at $P < 0.05$; [b)]NS: No significance; [c)]no samples were obtained due to Cd toxicity.

The Cd concentration in the plant samples seemed to increase with higher levels of P application for the vegetative and panicle stage. This finding is a result of augmented complex formations at the root cell wall with increasing anion concentration as will be discussed in section 4.1.9, 2). Due to this the Cd concentration in the roots was increased (compare Table 4.13).

The Cd content of the plant continuously decreased for all treatments with increasing time. The Cd concentration for the maturity stage for a P application of 50 and 200 mg P/kg was slightly lower compared to the control. From this it can be inferred that the translocation of the Cd from the roots to the shoots was restrained after P addition like found by Li et al. (2009). Additionally, this effect can also be explained by the dilution of the plant's Cd as with increasing P application the biomass also increased. As the main Cd uptake takes place during the vegetative stage, less Cd is detectable per kg dry mass; similar results were also found by Williams and David (1977) and Prochnow et al. (2001).

Nevertheless, the decreased Cd concentration in the rice plants for the maturity stage for a P application of 50 and 200 mg/kg is insignificant and seemed to be due to an increased biomass as an effect of the fertilizer application. This assumption is also supported when taking the high available Cd concentration of the soil into account, as the available Cd concentration was indeed reduced after P application, but it was still high (compare section 4.1.7).

The plant's Zn concentrations are presented in Table 4.12. The effect of different levels of P application did not have a significant effect on the Zn concentration in the plants for different growth stages ($P < 0.05$). For all levels of P addition, the Zn concentration in the panicle and maturity stage decreased as the main

stage for nutrient uptake by plants is the vegetative stage (Ramanathan and Krishnamoorthy, 1973). During the panicle and maturity stage only minor amounts of Zn were accumulated resulting in a dilution of the plant's Zn with increasing biomass during the panicle and maturity stage.

For the three levels of P added to the soil (50, 200, and 1000 mg/kg) no effect of the initial Zn uptake during the vegetative stage compared to the control was observed. Even though different levels of P addition did not affect the Cd concentration, the Zn concentration for the panicle and maturity stage is slightly augmented for an addition of 50, 200, and 1000 mg P/kg compared to the control.

**Table 4.12.** Zn concentration for the whole rice plant (dry weight) for different levels of P addition and growth stages.

| **Level of P Addition** | **Zn concentration in the whole rice plant [mg/kg] and for different levels of P addition** | | | |
|---|---|---|---|---|
| **[mg/kg]** | **Vegetative Stage** | **Panicle Stage** | **Maturity Stage** | **SDV[a)]** |
| 0 | 404.4 ± 257.2 | 190.3 ± 44.83 | 177.2 ± 32.65 | |
| 50 | 262.8 ± 173.3 | 171.4 ± 38.23 | 184.2 ± 23.91 | NS[b)] |
| 200 | 719.5 ± 398.5 | 211.2 ± 35.73 | 265.8 ± 18.48 | NS |
| 1000 | 545.6 ± 478.2 | 224.0 | -[c)] | NS |

Mean ± SD, n=5; [a)]SDV: Significantly different values of the corresponding Zn concentration between P-treatments and the control at $P < 0.05$; [b)]NS: No significance; [c)]no samples were obtained due to Cd toxicity.

It can also be concluded that the high Zn concentration in soil did not have a negative effect on the Cd accumulation by plants after P application as found by many researchers (Grant and Bailey, 1996; Hopkins and Ellswoth, 2003). The increased Cd uptake was proposed to be a result of limited Zn availability. With low Zn concentrations in soil and excess P application the Zn and phosphate may form a bond that cannot be readily broken and thus decreasing the Zn uptake. With a decreased Zn uptake there is less competition between Cd and Zn resulting in an increase of the Cd uptake (compare section 2.7.3). This is not the case in this study, as the available Zn concentrations were not depleted with increasing P application, for very high Zn concentrations were present in the soil. Another explanation for the increased Cd uptake after phosphate addition in the presence of Zn may be the dilution of the plant's Zn. When only a limited Zn concentration is available, the plant's Zn becomes diluted as the plant yield increases, thus, causing less competition between Cd and Zn for binding sites. A decreased Zn concentration in the whole rice plant was not found.

**2) Cd and Zn in different parts of the rice plants**

Normally the Cd distribution in different rice plant parts follows the order root > straw > panicle/grain (Liu et al., 2007). For some stages and treatments this order was found to be slightly differing (see Table 4.13). Normally, the roots are the first barrier for Cd to penetrate the plant's interior. As a mean of detoxification, plants immobilize Cd at their roots cell wall through adsorption (compare section 2.6.3). It was also found, that the translocation of Cd from the roots to the straw and grain was restrained after phosphate addition (Li et al., 2009). This is why the Cd concentration in the roots is higher compared to the straw and panicle/grain content. Cd translocated from the roots to the straw is also immobilized by sequestration into the vacuoles (Sanità di Toppi and Gabrielli, 1999). Only a minor part is translocated to the panicle/grain; thus, the Cd content in the panicle/grain is lower compared to the straw and the roots.

**Table 4.13.** Cd concentration in different rice plant parts (dry weight) for different levels of P addition and growth stages.

| Growth Stage | Cd concentration in different plant parts [mg/kg] and for different levels of P addition | | | |
|---|---|---|---|---|
| | **0 [mg/kg]** | **50 [mg/kg]** | **200 [mg/kg]** | **1000 [mg/kg]** |
| **Vegetative Stage** | | | | |
| Roots | 0.317 ± 0.238 | 1.453 ± 0.649 | 0.601 ± 0.706 | 1.317 ± 0.419 |
| Straw | 0.980 ± 0.242 | 0.621 ± 0.426 | 2.465 ± 1.484 | 1.033 ± 0.169 |
| **Panicle Stage** | | | | |
| Roots | 0.083 ± 0.054 | 1.401 ± 0.123 | 1.548 ± 0.291 | 0.904 ± 0.325 |
| Straw | 0.728 ± 0.200 | 0.606 ± 0.156 | 0.857 ± 0.237 | 0.738 ± 0.539 |
| Panicles | 0.555 ± 0.443 | 0.469 ± 0.367 | 0.722 ± 0.360 | 0.577 |
| **Maturity Stage** | | | | |
| Roots | 1.270 ± 0.107 | 1.036 ± 0.255 | 0.682 ± 0.122 | - [a)] |
| Straw | 0.319 ± 0.478 | 0.013 ± 0.018 | 0.023 ± 0.010 | - |
| Grains | 0.252 ± 0.084 | 0.369 ± 0.148 | 1.030 ± 0.716 | - |

Mean ± SD, n=5; [a)]no samples were obtained due to Cd toxicity.

A reason for the altered Cd distribution within the single parts of the rice plant might be the high SDs caused by the distinctive Cd toxicity symptoms of the plants, as described in the previous chapter. With increasing P addition the Cd content in the roots seemed to increase for the vegetative and panicle stage. This effect can be explained by augmented Cd complex formations between $Cd^{2+}$, $HPO_4^{2-}$and $H_2PO_4^-$, respectively. As anions like $HPO_4^{2-}$, $H_2PO_4^-$, $Cl^-$, and $SO_4^{2-}$ exist at the outer part of

the root cell wall, $Cd^{2+}$ is attracted by them and adsorbs onto the cell wall, resulting in an increased Cd concentration found in the roots with increasing anion concentration (Jiang et al., 2007).

A trend for the straw and panicle/grain was not clearly distinguishable for an increasing level of P addition. However, the P-fertilizer failed to reduce the Cd concentration in the rice grains to the mean background for Thai rice Cd concentrations in grains of 0.043 ± 0.019 mg/kg rice (Simmons et al., 2005). The Joint FAO/WHO Expert Committee on Food Additives (JCEFA) (WHO, 2004) has proposed a maximum level of 0.2 mg/kg Cadmium in rice grains, which was also exceeded in this study.

For the amount of Zn in plant parts the distribution order of roots > straw > panicle/grain is more obvious compared to Cd (see Table 4.14).

**Table 4.14.** Zn concentration in different rice plant parts (dry weight) for different levels of P addition and growth stages.

| Growth Stage | Zn concentration in different plant parts [mg/kg] and for different levels of P addition | | | |
|---|---|---|---|---|
| | **0 [mg/kg]** | **50 [mg/kg]** | **200 [mg/kg]** | **1000 [mg/kg]** |
| **Vegetative Stage** | | | | |
| Roots | 2127 ± 116.8 | 951.7 ± 26.71 | 1833 ± 108.2 | 1358 ± 754.0 |
| Straw | 87.08 ± 30.38 | 103.0 ± 74.36 | 127.0 ± 76.12 | 61.35± 11.85 |
| **Panicle Stage** | | | | |
| Roots | 887.4 ± 103.1 | 892.1 ± 86.35 | 842.0 ± 190.1 | 886.6± 97.57 |
| Straw | 55.89 ± 9.938 | 46.22 ± 5.930 | 41.63 ± 7.691 | 53.91± 41.58 |
| Panicles | 55.35 ± 17.85 | 94.97 ± 38.64 | 53.16 ± 22.28 | 31.71 |
| **Maturity Stage** | | | | |
| Roots | 716.2 ± 138.0 | 968.1 ± 21.90 | 943.1 ± 64.63 | - [a)] |
| Straw | 66.37 ± 20.02 | 91.76 ± 47.36 | 143.6 ± 27.94 | - |
| Grains | 41.98 ± 19.27 | 34.91 ± 5.786 | 97.69 ± 38.68 | - |

Mean ± SD, n=5; [a)]no samples were obtained due to Cd toxicity.

The Zn concentration in the straw and the panicle/grain seemed to be unaffected with increasing P addition. This could be a result of the high available Zn concentration present. Even though the Zn concentration in the roots was decreased with increasing P concentration, the concentrations were still high that no reduction of the Zn amount translocated to the shoots was visible. The Zn concentrations found in the straw and panicle/grain for every growth stage were low compared to the Zn present in soil, which also confirms the finding of Chancy et al. (2001) that the Zn uptake by rice plants is unaffected by the soil's Zn concentration.

Conclusively it can be said that the P application did not have a negative effect on the plant's Zn concentration as the concentration was always higher than the established deficiency content of Zn in plants of 10-20 mg/kg dry weight (Kabata-Pendias, 2001).

## 4.2 Field Experiment

### 4.2.1 Physical and chemical properties of soil

The soil properties of the experimental sites are presented in Table 4.15. The soil properties of the high concentration sites (HCS) were similar compared to the soil properties of the soil used for the pot experiment, as the soil used for the pot experiment was obtained from this area. This is true for every parameter except for the Cd concentration of the high concentration site used for a P fertilizer addition of 50 mg/kg (HCS-P), which exhibited an initial total Cd and Zn concentration of 29 and 1342 mg/kg, respectively. This concentration difference for the two sites located directly next to each other can be explained by their location related to the Mae Tao Creek. The control site (HCS-Ctrl) borders directly on that creek with the mine located upstream being responsible for the high Cd and Zn concentrations due to leachate. The HCS-P laid inbound not adjacent to the Mae Tao Creek and thus exhibiting much lower Cd and Zn concentrations.

**Table 4.15.** Basic characteristics of the soil at the experimental sites.

| Parameters | Field Sites | | | |
|---|---|---|---|---|
| | LCS[c]-Ctrl | LCS-P | HCS[d]-Ctrl | HCS-P |
| **pH (1:1 $H_20$)** | 6.23 | 6.43 | 7.63 | 7.52 |
| **SOM[a] [%]** | 1.86 | 1.86 | 2.56 | 2.56 |
| **CEC[b] [meq/100g]** | -[e] | - | 18.66 | 18.66 |
| **Clay [%]** | - | - | 13.63 | 13.63 |
| **Silt [%]** | - | - | 47.90 | 47.90 |
| **Sand [%]** | - | - | 38.47 | 38.47 |
| **Total P [mg/kg]** | 259 | 245 | 434 | 451 |
| **Total Cd [mg/kg]** | 0.282 | 0.248 | 75 | 29 |
| **Total Zn [mg/kg]** | 111 | 70 | 3292 | 1342 |
| **Soil Texture** | - | - | Loam | Loam |

[a]SOM: Soil organic matter; [b]CEC: Cation exchange capacity; [c]LCS: Low concentration site; [d]HCS: High concentration site; [e]properties were not measured.

The low concentrations sites (LCS) showed different soil characteristics with respect to soil pH, total P, Cd, and total Zn compared to the HCS. The soil pH was more acidic with 6.23 and 6.43 for the low concentration site used as control (LCS-Ctrl) and the low concentration site used for a P-fertilizer addition of 50 mg/kg (LCS-P), respectively. These pH values were slightly outside the optimum pH range of 6.5-

7 for rice propagation (Brink and Belay, 2006). The total Cd and Zn concentrations were low with the LCS-Ctrl showing slightly higher Cd and Zn concentrations. The natural Cd:Zn ratio of 1:100 (compare section 2.6.4) was altered for every site with the LCS showing an increased ratio of about 1:350 and the HCS showing a reduced ratio of about 1:45. The total P content was also about 1.5 fold lower compared to the HCS. The soil characteristics of the LCS were not measured, but paddy field soil in the Mae Sot District was generally classified as sandy loam or loam soil, and characteristics of the LCS should be comparable to the HCS (Unhalekhaka and Kositanont, 2009).

### 4.2.2 pH and ORP of the soil samples

The pH values of the different sites are listed in Table 4.16. For the LCS, an increase of pH between the background condition (before amendment addition) and the background after the addition of 50 mg/kg (background-P) for the LCS-P was observable. The increase in pH of the LCS-Ctrl can be explained by the redox potential showing a positive value during this stage (see Table 4.17). As discussed in chapter 4.1.3, the pH of an acidic soil increases under negative redox conditions (submergence), which were present for the background-P. The pH of acidic soils increases under anaerobic conditions, for ferric ions are used as an electron-acceptor for oxidizing organic matter using protons (Sahrawat, 2005) as shown in Equation (1):

$$Fe_2O_3 + \frac{1}{2}CH_2O + 4H^+ = 2Fe^{2+} + \frac{5}{2}H_2O + \frac{1}{2}CO_2 \qquad (1)$$

The increase in pH of the LCS-P can be explained by the addition of P due to ligand exchange and fixation reactions (compare Figure 4.1) as the redox conditions were negative during all growth stages except for the maturity stage (Table 4.17).

**Table 4.16.** pH values for different growth stages and levels of P addition.

| Growth Stage | pH for different field sites | | | |
|---|---|---|---|---|
| | LCS[c)]-Ctrl | LCS-P | HCS[d)]-Ctrl | HCS-P |
| **Background[a)]** | 6.23 ± 0.28 | 6.43 ± 0.27 | 7.63 ± 0.12 | 7.52 ± 0.20 |
| **Background-P[b)]** | 6.62 ± 0.12 | 6.76 ± 0.14 | 7.22 ± 0.08 | 7.40 ± 0.04 |
| **Vegetative Stage** | 6.53 ± 0.12 | 6.65 ± 0.08 | 6.98 ± 0.07 | 6.97 ± 0.08 |
| **Panicle Stage** | 6.20 ± 0.33 | 6.16 ± 0.43 | 7.16 ± 0.09 | 7.11 ± 0.07 |
| **Maturity Stage** | 6.10 ± 0.38 | 5.95 ± 0.34 | 7.44 ± 0.13 | 6.96 ± 0.26 |

Mean ± SD, n=5; [a)]Background: Soil before treatment; [b)]Background-P: Soil after P addition before rice propagation; [c)]LCS: Low concentration site; [d)]HCS: High concentration site.

After the initial increase in pH, a continuously decrease for the LCS for the vegetative, panicle, and maturity stage could be observed. This decrease resulted from leachate of the mobile cations in soil solution under submerged conditions. As described by Campbell and Wansbrough (2005), the functional surface sites are balanced by the exchangeable bases ($Ca^{2+}$, $Mg^{2+}$, $K^{+}$, and $Na^{+}$), which are in equilibrium with the cations present in the soil solution. Under submerged conditions the cations in soil solution are washed out and replaced by $H_3O^{+}$ and $Al^{3+}$ (aq) ions. Due to this the soil pH decreases under submerged conditions with increasing time.

**Table 4.17.** Redox potential for different growth stages and levels of P addition.

| Growth Stage | Redox Potential for different field sites [mV] | | | |
|---|---|---|---|---|
| | LCS[c)]-Ctrl | LCS-P | HCS[d)]-Ctrl | HCS-P |
| **Background[a)]** | 55.44 ± 204.5 | -117.5 ± 60.75 | -37.76 ± 75.29 | -4.457 ±71.29 |
| **Background-P[b)]** | -195.5 ±52.45 | -197.6 ± 37.73 | -156.0 ± 31.45 | -214.8 ±28.92 |
| **Vegetative Stage** | -146.6 ±70.73 | -197.6 ± 37.73 | -21.20 ± 69.50 | -73.14 ±142.5 |
| **Panicle Stage** | 34.52 ± 158.8 | -60.10 ± 176.0 | -103.3 ± 94.62 | -282.4 ±88.74 |
| **Maturity Stage** | 265.7 ± 131.4 | 159.1 ± 147.0 | 175.6 ± 44.26 | 148.3 ± 29.15 |

Mean ± SD, n=5; [a)]Background: Soil before treatment; [b)]Background-P: Soil after P addition before rice propagation; [c)]LCS: Low concentration site; [d)]HCS: High concentration site.

The pH values of the HCS showed a different trend for the background and the background-P stage. The pH was slightly alkaline; therefore, it was decreasing after submergence (compare section 4.1.3). Even though the mean redox potential for the background stage was negative, some replicates showed a positive value, suggesting that not all areas of the experimental fields possessed the same conditions at this stage. Hence, an initial decrease in pH was observed after the submergence of the soil as all sample replicates of the background-P soil exhibited a negative redox potential. For the subsequent growth stages a decline of pH was observable due to leachate of the exchangeable bases from soil solution. An exception to this is the pH for the maturity stage for the HCS-Ctrl soil, as it rose again. An explanation for this could be the positive redox potential reversing Equation (1) resulting in a depletion of protons. For the HCS-P no such trend was observable, but the SD was quite high which may have disguised this trend.

### 4.2.3 Total phosphorus in soil samples

The initial total P concentrations are presented in Table 4.18. The initial total P concentration of the LCS was lower compared to the HCS, whereas LCS-Ctrl showed

a higher concentration compared to LCS-P. The total P concentrations seemed to increase up to the vegetative stage, but the SDs might adulterate this. From the vegetative stage to the maturity stage the total P concentration was continuously decreased. This effect might have been a result of P accumulation by rice plants during the growth stages. Additionally, this could explain the constant P content during background and background-P condition as there was no plant growth during those stages.

**Table 4.18.** Total phosphorus concentration for different growth stages and field sites.

| Growth Stage | Total P for different field sites [mg/kg] | | | |
|---|---|---|---|---|
| | LCS[c)]-Ctrl | LCS-P | HCS[d)]-Ctrl | HCS-P |
| **Background[a)]** | 279.5 ± 7.305 | 244.8 ± 24.99 | 496.9 ± 144.6 | 450.8 ± 96.74 |
| **Background-P[b)]** | 314.3 ± 56.33 | 250.2 ± 26.27 | 505.9 ± 43.30 | 488.2 ± 4.264 |
| **Vegetative Stage** | 295.8 ± 32.85 | 384.0 ± 49.33 | 450.9 ± 15.75 | 448.2 ± 4.527 |
| **Panicle Stage** | 273.3 ± 7.485 | 236.9 ± 4.341 | 427.9 ± 21.42 | 428.7 ± 35.10 |
| **Maturity Stage** | 267.5 ± 7.385 | 222.0 ± 25.48 | 421.9 ± 24.33 | 423.4 ± 17.05 |

Mean ± SD, n=5; [a)]Background: Soil before treatment; [b)]Background-P: Soil after P addition before rice propagation; [c)]LCS: Low concentration site; [d)]HCS: High concentration site.

Alike results were obtained for the LCS-P site showing a slight increase of the total P concentration between the background and background-P stage, for the P fertilizer was added at the background-P stage (see Figure 4.6). The concentration at the vegetative stage seemed to be an outlier as it was much higher compared to the other values, but the concentrations for panicle and maturity stage showed the same decline as LCS-Ctrl, caused by plant uptake.

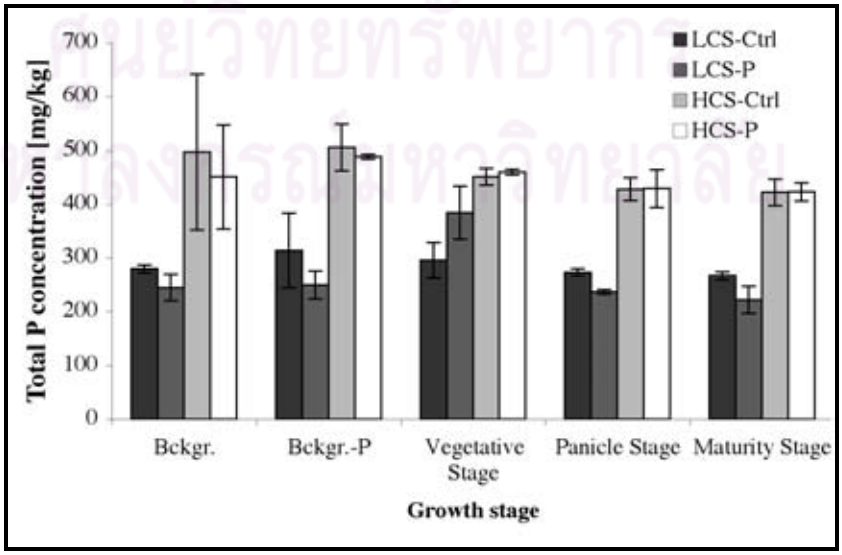



**Figure 4.6.** Total phosphorus concentration for different growth stages and field sites.

The trend for the HCS was well defined, as the total P concentration was constant for the background and background-P stage, and declining for the other stages due to the same reasons as for the LCS. The HCS-P showed an increase in total P between the background and background-P stage caused by the addition of 50 mg/kg P fertilizer between those stages, followed by a decrease in total P resulted by plant uptake.

### 4.2.4 Available phosphorus in soil samples

The trend for the available P concentration was like expected except for the LCS-P. For the other sites an increase between the background and the background-P stage was observable due to changing redox conditions as described in 4.1.5, which was more pronounced for the sites used for P addition (Table 4.19). The available P concentration decreased with increasing time during the vegetative, panicle, and maturity stage. An exception to this was the LCS-P showing an increased available P concentration.

**Table 4.19.** Available phosphorus concentration for different growth stages and field sites.

| Growth Stage | Available P for different field sites [mg/kg] | | | |
|---|---|---|---|---|
| | LCS[c)]-Ctrl | LCS-P | HCS[d)]-Ctrl | HCS-P |
| **Background[a)]** | 10.06 ± 3.027 | 25.23 ± 4.077 | 2.869 ± 1.212 | 13.21 ± 0.000 |
| **Background-P[b)]** | 12.84 ± 1.253 | 36.87 ± 6.705 | 5.090 ± 1.855 | 29.12 ± 4.175 |
| **Vegetative Stage** | 11.99 ± 1.885 | 42.84 ± 3.110 | 3.383 ± 1.734 | 11.66 ± 0.449 |
| **Panicle Stage** | 12.28 ± 2.524 | 40.96 ± 2.903 | 1.635 ± 0.318 | 12.12 ± 2.467 |
| **Maturity Stage** | 10.99 ± 3.692 | 44.53 ± 5.357 | 1.935 ± 0.792 | 12.56 ± 2.909 |

Mean ± SD, n=5; [a)]Background: Soil before treatment; [b)]Background-P: Soil after P addition before rice propagation; [c)]LCS: Low concentration site; [d)]HCS: High concentration site.

The decline of the available P content for LCS-Ctrl, HCS-Ctrl, and HCS-P was a result of the uptake by the rice plants (compare section 4.1.5). The initial concentrations for LCS-Ctrl, LCS-P, and HCS-P were already in the optimum range for plant growth, not falling below the limit value of 11 mg/kg (Mallarino et al., 2000). This is not true for the HCS-Ctrl possessing an initial available P concentration of 2.869 mg/kg with a subsequent decline during the vegetative, panicle, and maturity stage. The concentrations obtained for the LCS-P did not follow a trend for the vegetative, panicle, and maturity stage (see Figure 4.7). This may be due to the already high initial available P concentrations present in soil.

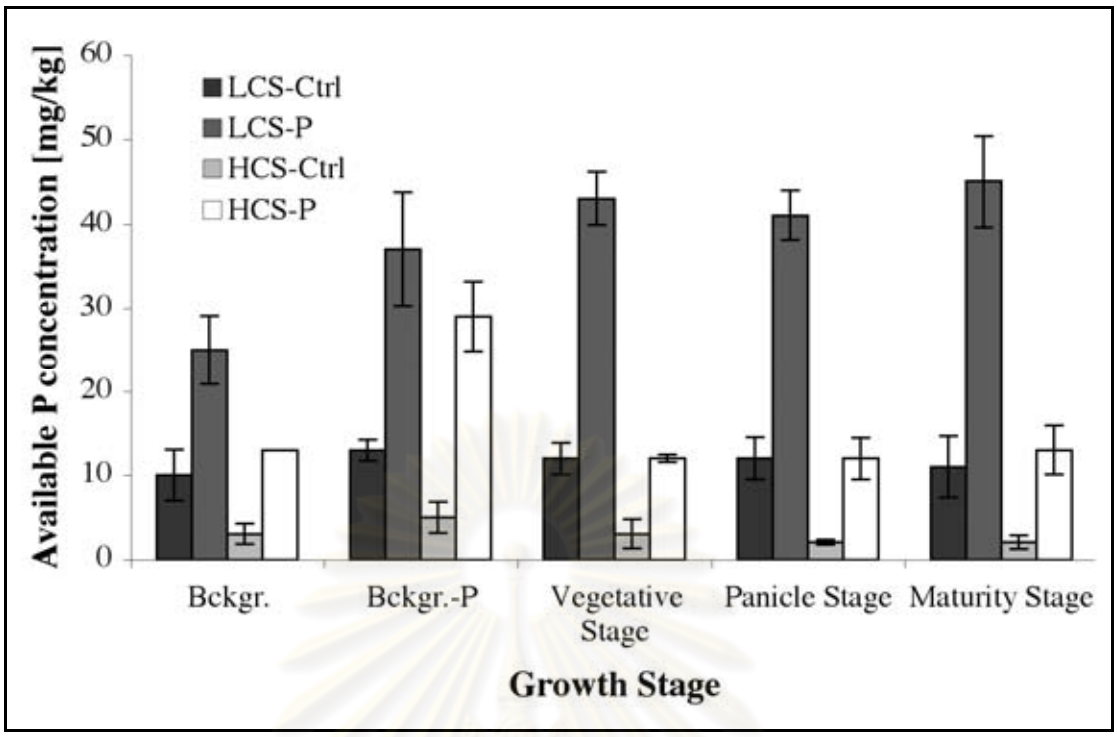



**Figure 4.7.** Available phosphorus concentration for different growth stages and field sites.

### 4.2.5 Total Cd and Zn concentrations in soil samples

The total Cd concentration in soil for the LCS and HCS are presented in Table 4.20. P addition did not alter the total Cd concentration in soil for the LCS.

**Table 4.20.** Total Cd concentration for different growth stages and field sites.

| Growth Stage | Total Cd concentration for different field sites [mg/kg] | | | |
|---|---|---|---|---|
| | LCS[c]-Ctrl | LCS-P | HCS[d]-Ctrl | HCS-P |
| **Background[a]** | 0.357 ± 0.088 | 0.282 ± 0.021 | 74.90 ± 4.030 | 28.77 ± 1.531 |
| **Background-P[b]** | 0.339 ± 0.047 | 0.254 ± 0.014 | 66.93 ± 9.776 | 24.91 ± 7.163 |
| **Vegetative Stage** | 0.410 ± 0.068 | 0.267 ± 0.026 | 68.34 ± 5.734 | 28.86 ± 6.423 |
| **Panicle Stage** | 0.418 ± 0.120 | 0.343 ± 0.027 | 65.16 ± 8.644 | 36.13 ± 4.829 |
| **Maturity Stage** | 0.400 ± 0.130 | 0.312 ± 0.040 | 69.76 ± 6.297 | 31.77 ± 7.013 |

Mean ± SD, n=5; [a]Background: Soil before treatment; [b]Background: Soil after P addition before rice propagation; [c]LCS: Low concentration site; [d]HCS: High concentration site.

The HCS-Ctrl featured a Cd concentration of about 70 mg/kg, while the HCS-P hold a total Cd concentration of approximately 30 mg/kg. The total Cd concentration for both HCS did not alter with augmented P addition, as reported in section 4.1.6.

For Zn similar results were gained as displayed in Table 4.21. The P addition

did not affect the total Zn concentration of the experimental sites.

**Table 4.21.** Total Zn concentration for different growth stages and field sites.

| Growth Stage | Total Zn concentration for different field sites [mg/kg] | | | |
|---|---|---|---|---|
| | LCS[c]-Ctrl | LCS-P | HCS[d]-Ctrl | HCS-P |
| **Background[a]** | 110.5 ± 22.45 | 70.13 ± 6.580 | 3389 ± 129.4 | 1342 ± 92.80 |
| **Background-P[b]** | 91.47 ± 9.812 | 68.30 ± 11.19 | 2854 ± 276.0 | 1376 ± 52.76 |
| **Vegetative Stage** | 84.60 ± 4.898 | 58.15 ± 7.421 | 3030 ± 208.0 | 1238 ± 291.4 |
| **Panicle Stage** | 127.1 ± 5.095 | 95.14 ± 7.731 | 2569 ± 277.0 | 1441 ± 32.10 |
| **Maturity Stage** | 100.4 ± 7.126 | 78.42 ± 3.174 | 2210 ± 249.1 | 1315 ± 194.0 |

Mean ± SD, n=5; [a]Background: Soil before treatment; [b]Background: Soil after P addition before rice propagation; [c]LCS: Low concentration site; [d]HCS: High concentration site.

### 4.2.6 Cd and Zn fractions in soil samples

Figure 4.8 displays the Cd concentrations in F1 and F2 with respect to the total Cd concentration in soil for the LCS.

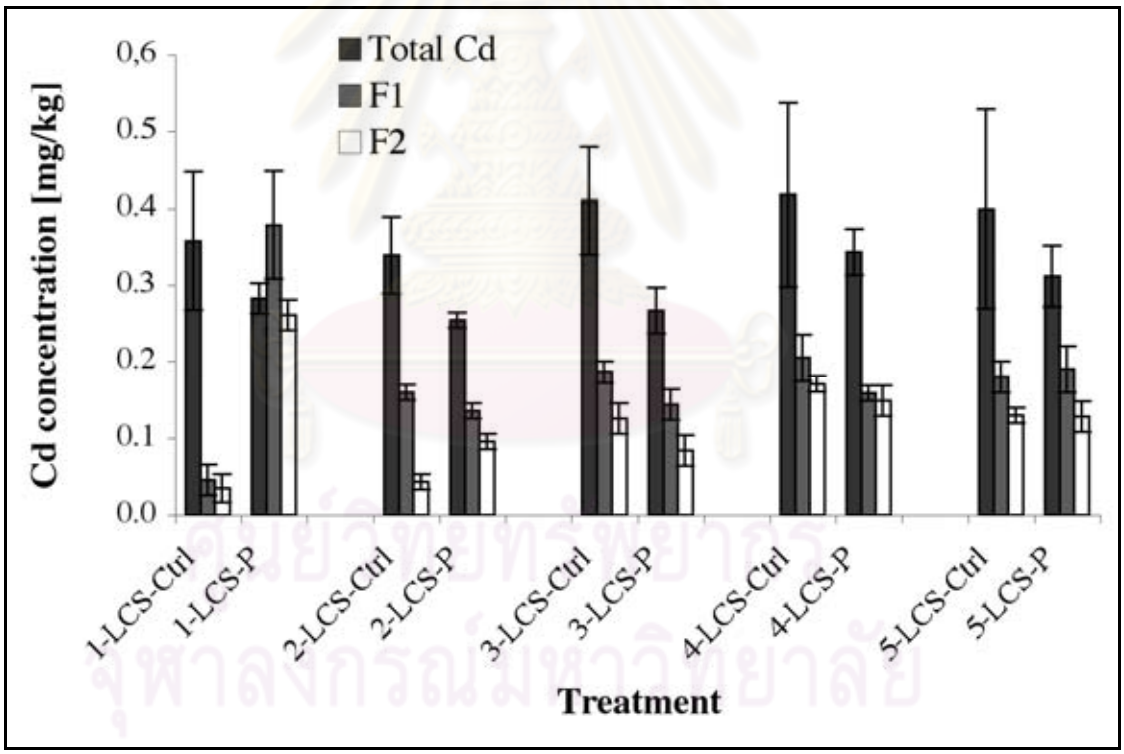



**Figure 4.8.** Cd concentrations of the different fractions for 5 different growth stages with respect to the total amount in the studied soil. Total Cd, concentration of the total Cd in the soil; F1, exchangeable, water, and acid soluble Cd fraction; F2, iron-manganese oxides associated Cd fraction; LCS-Ctrl, low concentration site control; LCS-P, low concentration site with 50 mg/kg P addition; 1-, 2-, 3-, 4-, and 5- represent the growth stages background, background after P addition, vegetative stage, panicle stage, and maturity stage, respectively.

The values for the LCS-P at the background stage were treated as outlier as the sum of the fractions are more than twice of the total Cd concentration present in soil. Like for the pot experiment, the Cd concentration in F1 was higher than the Cd concentration in F2 for every site and growth stage. At the LCS the P application did not have an effect on the Cd fractions in soil. The percentages of F1 with respect to the total Cd concentration in soil for the LCS-Ctrl are 12.9, 47.2, 45.4, 49.1, and 45.1%, while the F1 for the LCS-P made up 134.0, 53.5, 53.9, 46.4, and 61.0% of the total Cd concentration. F2 is comparable with F1 making up 9.9, 12.7, 30.7, 41.0, and 32.5 % for LCS-Ctrl and 92.5, 37.7, 31.5, 43.4, and 41.1% for the LCS-P (see Figure 4.9). It can be concluded that the P application did not affect the Cd distribution between the single Cd fractions in soil. This finding is surprising as a redistribution to less mobile fractions as found in the pot experiment and also for the HCS was expected, and should have been more pronounced for lower Cd concentrations.

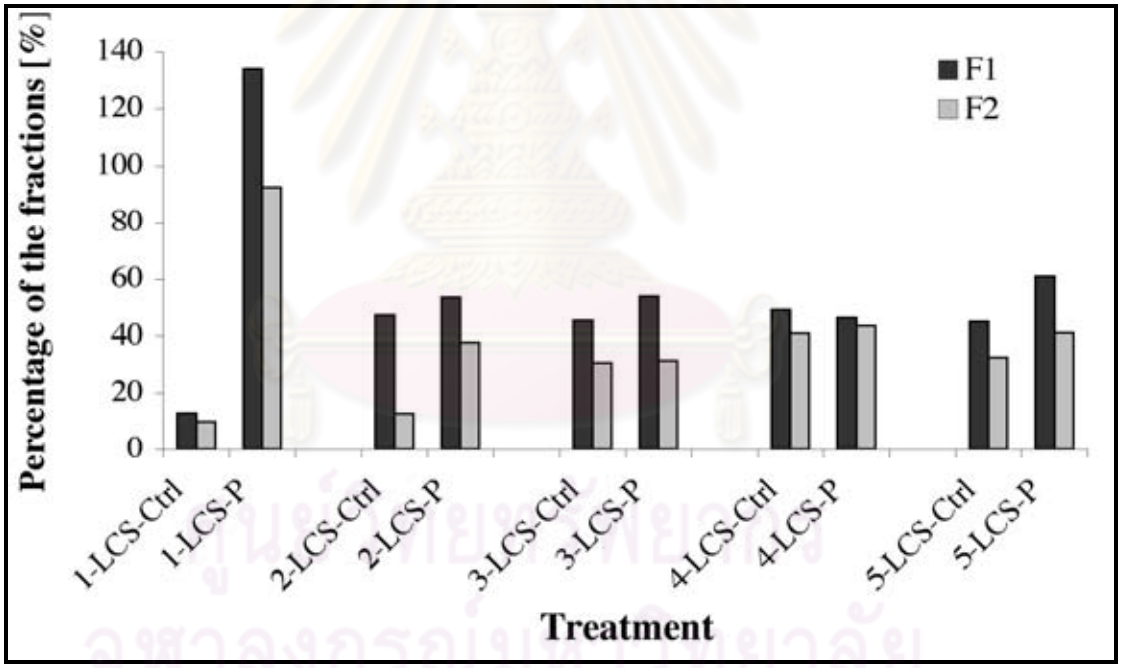



**Figure 4.9.** Percentages of Cd fractions with respect to the total Cd concentration in soil. F1, exchangeable, water, and acid soluble Cd fraction; F2, iron-manganese oxides associated Cd fraction; LCS-Ctrl, low concentration site control; LCS-P, low concentration site with 50 mg/kg P addition; 1-, 2-, 3-, 4-, and 5- represent the growth stages background, background after P addition, vegetative stage, panicle stage, and maturity stage, respectively.

The HCS-Ctrl displayed a similar Cd distribution between F1 and F2 compared to the Cd distribution in the pot experiment (see section 4.1.7). The HCS-P

exhibited a lower total Cd concentration than the HCS-Ctrl, making a direct comparison difficult (see Figure 4.10). When calculating the percentage of F1 and F2 with respect to the total Cd concentration (F1) present, it could be observed that F1 for the HCS-P is lower than F1 for the HCS-Ctrl. F1 for HCS-P was 62.1, 60.0, 51.7, 50.0, and 46.9% of the total Cd concentration, whereas F1 for the HCS-Ctrl was 72.0, 62.7, 76.5, 60.0, and 70.0% of the total Cd concentration for the background, background-P, vegetative, panicle, and maturity stage, respectively. F2 of the HCS-P was higher compared to F2 of the HCS-Ctrl with 41.4, 48.0, 37.9, 33.3, and 34.4% compared to 34.7, 31.3, 39.7, 30.8, and 34.4% of the total Cd content in soil for the five stages mentioned before, respectively (compare Figure 4.11).

These findings indicated a reduction of F1 with a concomitant increase in F2 with P application due to the same reasons as discussed previously in section 4.1.7.

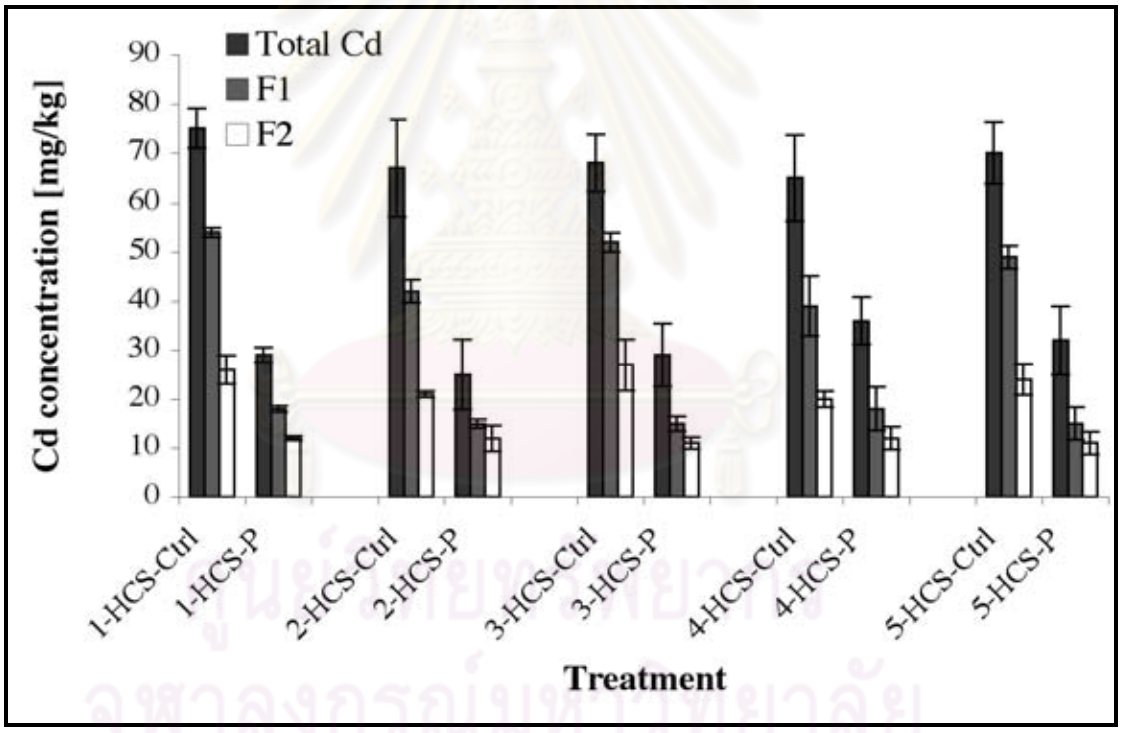



**Figure 4.10.** Cd concentrations of the different fractions for 5 different growth stages with respect to the total amount in the studied soil. Total Cd, concentration of the total Cd in the soil; F1, exchangeable, water, and acid soluble Cd fraction; F2, iron-manganese oxides associated Cd fraction; HCS-Ctrl, high concentration site control; HCS-P, high concentration site with 50 mg/kg P addition; 1-, 2-, 3-, 4-, and 5- represent the growth stages background, background after P addition, vegetative stage, panicle stage, and maturity stage, respectively.

Like for the pot experiment the sum of F1 and F2 sometimes slightly exceed the total Cd concentration found. This could be ascribed to the fact that the total Cd

was determined using microwave assisted digestion with aqua regia as reagent resulting in an incomplete digestion like found by Ahnstrom and Parker (1999). As a result of the high mobility of Cd in soil (about 75% of the Cd in soil were present in the oxidizable, exchangeable, and iron-manganese oxide fraction), the estimated total Cd in soil was below the sum of F1 and F2 (see section 4.1.7).

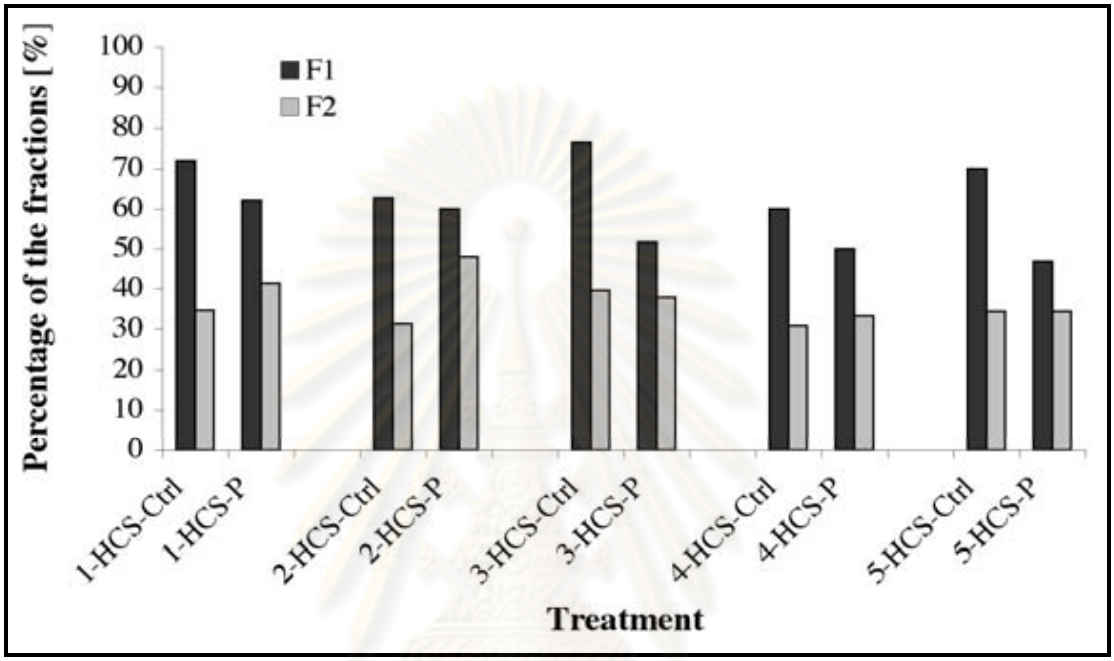



**Figure 4.11.** Percentages of Cd fractions with respect to the total Cd concentration in soil. F1, exchangeable, water, and acid soluble Cd fraction; F2, iron-manganese oxides associated Cd fraction; HCS-Ctrl, high concentration site control; HCS-P, high concentration site with 50 mg/kg P addition; 1-, 2-, 3-, 4-, and 5- represent the growth stages background, background after P addition, vegetative stage, panicle stage, and maturity stage, respectively.

The results of the single Zn fractions for the LCS are presented in Figure 4.12. For the LCS, a similar trend for the single Zn fraction was obtained due to the same reasons as discussed in the pot experiment (see section 4.1.7). When calculating the percentage of F1 with respect to total Zn present in soil, the LCS-P exhibited higher Zn in F1 compared to LCS-Ctrl, with 10.0, 10.3, 13.8, 8.4, and 10.3% compared to 7.2, 7.7, 10.6, 6.3, and 8.3%, for the five growth stages, respectively. For F2 the trend differed compared to the pot experiment as the Zn concentration in LCS-P was slightly higher than for the LCS-Ctrl, making up 11.4, 11.8, 13.8, 9.5, and 11.5% of the total Zn concentration in comparison to 9.0, 9.9, 11.8, 7.1, and 9.4% for the LCS-Ctrl for the five studied stages, respectively. For F2 no effect of the P-fertilizer was monitored (compare Figure 4.13).

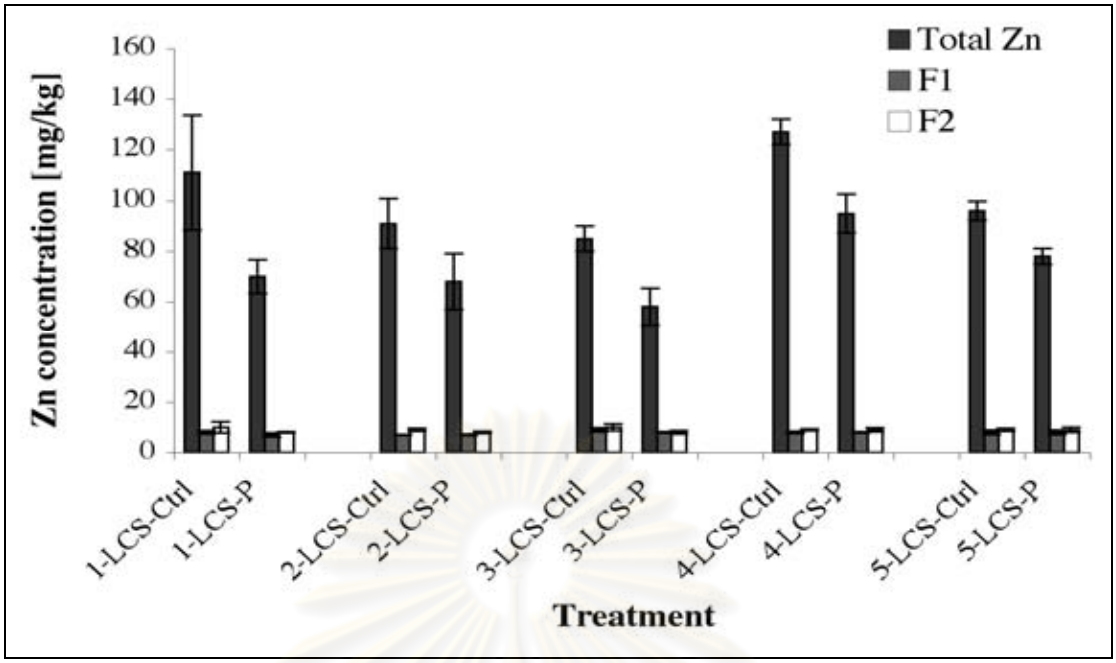



**Figure 4.12.** Zn concentrations of the different fractions for 5 different growth stages with respect to the total amount in the studied soil. Total Zn, concentration of the total Zn in the soil; F1, exchangeable, water, and acid soluble Zn fraction; F2, iron-manganese oxides associated Zn fraction; LCS-Ctrl, low concentration site control; LCS-P, low concentration site with 50 mg/kg P addition; 1-, 2-, 3-, 4-, and 5- represent the growth stages background, background after P addition, vegetative stage, panicle stage, and maturity stage, respectively.

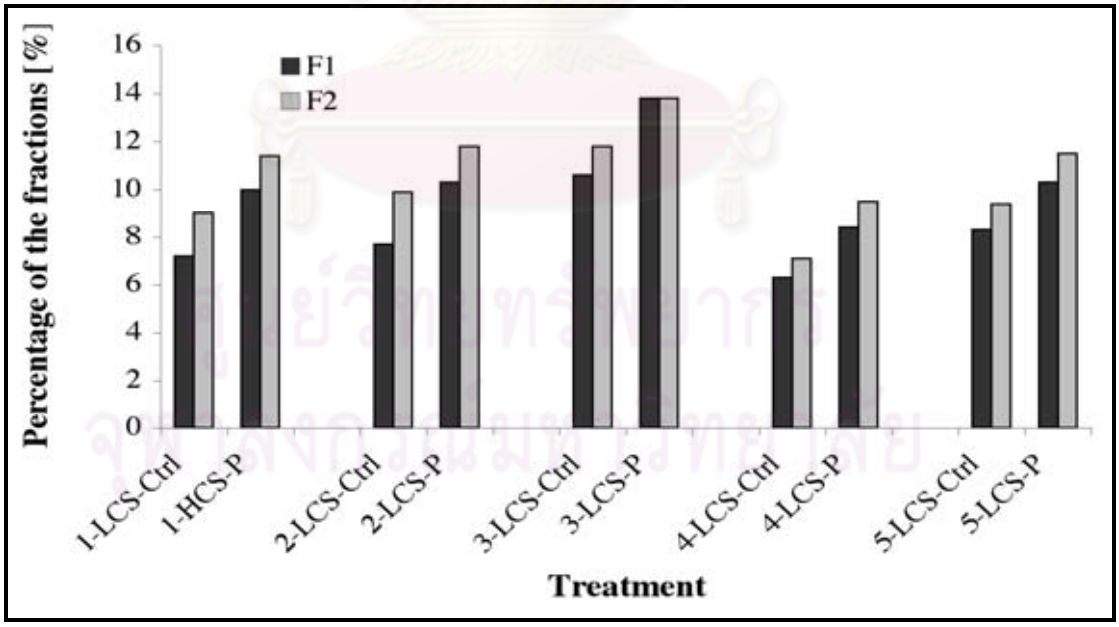



**Figure 4.13.** Percentages of Zn fractions with respect to the total Zn concentration in soil. F1, exchangeable, water, and acid soluble Cd fraction; F2, iron-manganese oxides associated Cd fraction; LCS-Ctrl, low concentration site control; LCS-P, low concentration site with 50 mg/kg P addition; 1-, 2-, 3-, 4-, and 5- represent the growth stages background, background after P addition, vegetative stage, panicle stage, and maturity stage, respectively.

The Zn concentrations for the HCS are shown in Figure 4.14.

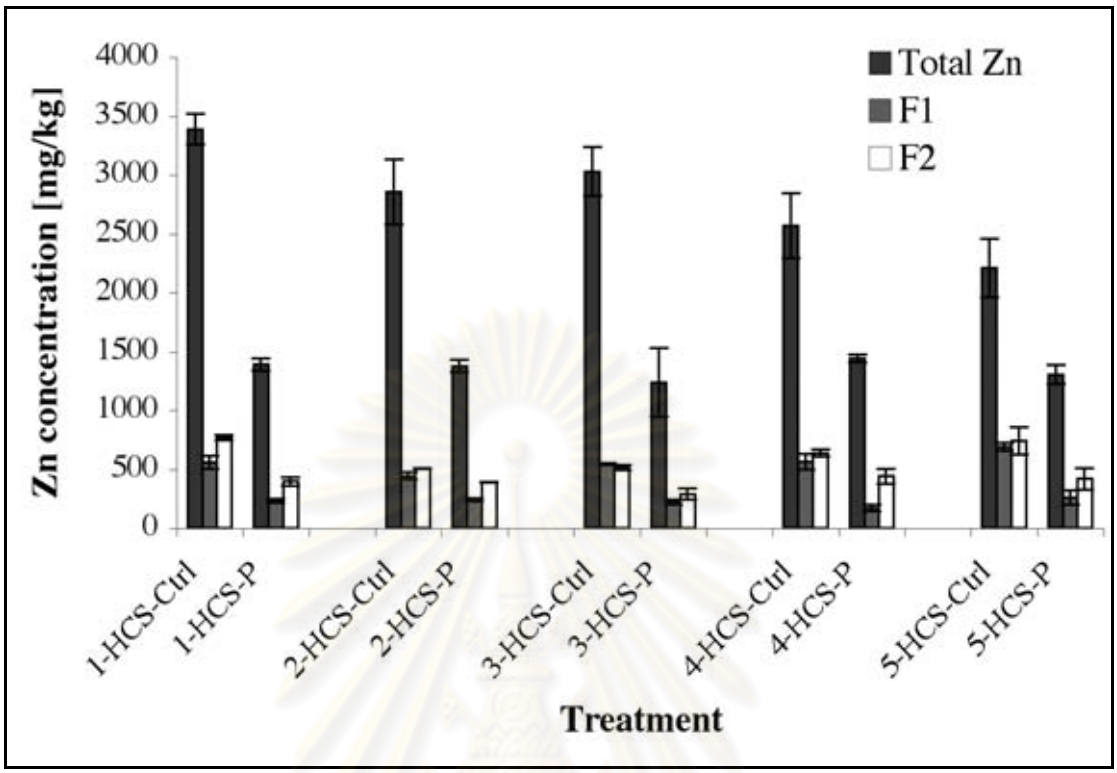



**Figure 4.14.** Zn concentrations of the different fractions for 5 different growth stages with respect to the total amount in the studied soil. Total Zn, concentration of the total Zn in the soil; F1, exchangeable, water, and acid soluble Zn fraction; F2, iron-manganese oxides associated Zn fraction; HCS-Ctrl, high concentration site control; HCS-P, high concentration site with 50 mg/kg P addition; 1-, 2-, 3-, 4-, and 5- represent the growth stages background, background after P addition, vegetative stage, panicle stage, and maturity stage, respectively.

The concentration of the HCS-Ctrl was about twice the HCS-P. Again a higher F2 compared to F1 was obtained. If calculating the percentages of F1 and F2 with respect to the total Zn concentration present in soil no effect of the P addition was visible as for the HCS-Ctrl percentages of 16.5, 15.6, 18.0, 22.0, and 31.3 for F1 and 22.6, 17.6, 16.9, 24.8, and 35.5% for F2 were determined for the five growth stages mentioned above. F1 for HCS-P represented 16.6, 17.5, 17.9, 11.9, and 19.8% of the total Zn concentration for the background, background-P, vegetative-, panicle-, and maturity-stage, respectively, whereas F2 amounted for 28.5, 28.3, 23.3, 30.5, and 32.1% of the total soil's Zn concentration for the five stages, respectively (compare Figure 4.15).

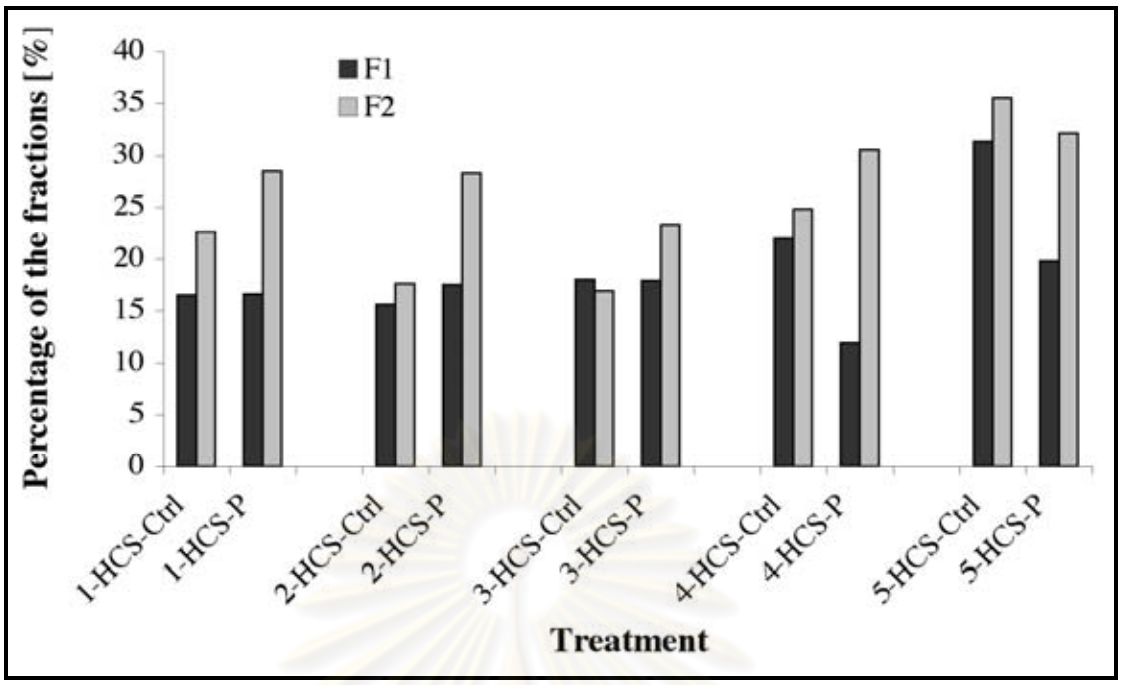



**Figure 4.15.** Percentages of Zn fractions with respect to the total Zn concentration in soil. F1, exchangeable, water, and acid soluble Cd fraction; F2, iron-manganese oxides associated Cd fraction; HCS-Ctrl, high concentration site control; HCS-P, high concentration site with 50 mg/kg P addition; 1-, 2-, 3-, 4-, and 5- represent the growth stages background, background after P addition, vegetative stage, panicle stage, and maturity stage, respectively.

### 4.2.7 Dry matter yield of plant samples

The dry matter yield increased with an application of 50 mg/kg of the P-fertilizer compared to the control site (compare Table 4.22). This effect was due to the same reasons as stated in section 4.1.8, where the same observation was made. The increase in dry matter yield was more pronounced for the HCS-P compared to the LCS-P (see Table 4.23) showing an increase of 31.1, 21.4, and 4.1% compared to 0.0, 6.3, and 21.4% for the vegetative-, panicle formation-, and maturity-stage, respectively. This effect could be explained by the available P concentrations initially found in soil.

The initial available P content during the background stage of the LCS-Ctrl was already in the range for optimum plant growth, whereas the available P in the HCS-Ctrl was around 3 mg/kg. With a P application of 50 mg/kg the available P content was increased to the optimum range (compare Table 4.19), promoting plant growth and thus the dry matter yield of the whole rice plant (see Table 4.23).

**Table 4.22.** Dry matter yield of the different parts of the rice plant for different field sites and growth stages.

| Growth Stage | Dry matter yield of the rice plants [g/plant] | | | |
|---|---|---|---|---|
| | LCS[a)]-Ctrl | LCS-P | HCS[b)]-Ctrl | HCS-P |
| **Vegetative Stage** | | | | |
| Roots | 7.104 ± 1.414 | 8.002 ± 1.389 | 4.940 ± 1.483 | 6.882 ± 1.514 |
| Straw | 26.94 ± 7.700 | 26.55 ± 7.540 | 26.10 ± 4.556 | 38.18 ± 9.237 |
| **Panicle Stage** | | | | |
| Roots | 11.73 ± 1.491 | 12.78 ± 4.368 | 15.54 ± 3.754 | 26.08 ± 7.177 |
| Straw | 109.3 ±16.28 | 115.9 ± 24.95 | 175.8 ± 9.911 | 222.2 ± 70.30 |
| Panicles | 15.43 ± 3.254 | 16.98 ± 2.519 | 23.10 ± 2.768 | 24.46 ± 8.741 |
| **Maturity Stage** | | | | |
| Roots | 18.28 ± 1.234 | 20.34 ± 1.141 | 17.62 ± 1.722 | 19.70 ± 2.112 |
| Straw | 86.78 ± 23.99 | 115.3 ± 21.19 | 127.6 ± 13.31 | 121.5 ± 26.98 |
| Grains | 59.98 ± 15.62 | 74.24 ± 11.52 | 63.42 ± 7.399 | 76.24 ± 11.32 |

Mean ± SD, n=5; [a)]LCS: Low concentration site; [b)]HCS: High concentration site.

**Table 4.23.** Dry matter yield of the whole rice plant for field sites and growth stages.

| Growth Stage | Dry matter yield of the rice plants [g/plant] | | | |
|---|---|---|---|---|
| | LCS[a)]-Ctrl | LCS-P | HCS[b)]-Ctrl | HCS-P |
| Vegetative Stage | 34.04 ± 14.03 | 34.55 ± 13.12 | 31.04 ± 14.96 | 45.06 ± 22.13 |
| Panicle Stage | 136.4 ± 55.28 | 145.6 ± 58.35 | 214.4 ± 90.42 | 272.8 ± 113.7 |
| Maturity Stage | 165.0 ± 34.52 | 209.9 ± 47.62 | 208.6 ± 55.23 | 217.4 ± 51.00 |

Mean ± SD, n=5; Mean ± SD, n=5; [a)]LCS: Low concentration site; [b)]HCS: High concentration site.

### 4.2.8 Cd and Zn concentrations in plant samples

#### 1) Cd and Zn concentrations in the whole rice plant

Table 4.24 presents the amount of Cd accumulated by the rice plants for different field sites and growth stages. For both HCS sites, the Cd concentrations in the rice plants decreased over time due to the Cd dilution effect with increasing biomass comparable to the pot experiment, but this effect was insignificant when comparing the treatment site with the control site. The initial Cd concentrations in soil of the LCS were slightly different, but it is assumed that a direct comparison is possible. It was found that for the panicle and maturity stage the LCS-P displayed a lower Cd concentration compared to the LCS-Ctrl. Similar findings were made for the HCS. A comparison of the HCS-Ctrl to the HCS-P is difficult, as the two sites exhibited different initial Cd concentrations with the control site featuring 2-3 fold the

Cd concentration of the treatment site (HCS-P). However, in literature the uptake kinetics of Cd by rice plants were widely studied (Cutler and Rains, 1974; Homma and Hitara, 1984; He et al., 2007) and it was found that the uptake of Cd increased steeply at low concentrations, whereas it increased moderately at higher concentrations. Up to a concentration of Cd in the absorption solution of 5 mg/L the uptake by rice roots was linear, whereas at concentration >5 mg/L the uptake slowed down and became non-linear (Cutler and Rains, 1974). The uptake was still increasing up to a solution's Cd concentration of 20 mg/L and did not stay constant. Similar results were obtained by Homma and Hitara (1984) up to a Cd solution's concentration of 8.90 μM. These findings allow the assumption that at such high Cd concentrations the uptake is not the same for the both sites, but they might not differ very much. If assuming that the uptake is as far as possible comparable for the both sites, a reduction of the plant's Cd concentration can be observed for the HCS-P. This may be attributed to different factors. Firstly, after the addition of P-fertilizer the bioavailable Cd concentration present in F1 was reduced. Secondly, the subcellular fractionation experiment revealed that with increasing P addition the uptake of Cd by the plants was reduced, which will be intensively discussed in section 4.4. Briefly, with increasing phosphate anions in solution more $Cd^{2+}$ were complexed and, thus, reducing the plant's uptake, as mainly free $Cd^{2+}$ is uptaken.

**Table 4.24.** Cd concentration for the whole rice plant (dry weight) for different field sites and growth stages.

| Growth Stage | Cd concentration for the whole rice plants [mg/kg] and for different field sites | | | |
|---|---|---|---|---|
| | LCS[a)]-Ctrl | LCS-P | HCS[b)]-Ctrl | HCS-P |
| Vegetative Stage | 0.106 ± 0.046 | 0.123 ± 0.068 | 13.27 ± 6.224 | 8.995 ± 2.109 |
| Panicle Stage | 0.557 ± 0.358 | 0.208 ± 0.220 | 7.730 ± 3.499 | 4.622 ± 1.939 |
| Maturity Stage | 0.183 ± 0.099 | 0.123 ± 0.084 | 4.135 ± 0.655 | 1.660 ± 0.440 |

Mean ± SD, n=5; [a)]LCS: Low concentration site; [b)]HCS: High concentration site.

For Zn the assumption of comparable uptake for different concentrations at a high level is not valid. As found by Homma and Hitara (1984) the Zn uptake into fresh roots is linear; thus, with higher Zn amounts present more Zn will be accumulated by the plant's roots. As displayed in Table 4.25, after the application of 50 mg P/kg to the LCS-P the plants seemed to take up less Zn compared to the control site, being distinct for the vegetative stage. This result indicated that the P application

decreased the Zn plant uptake. Many researchers have stated that high levels of P-fertilizer are known to reduce the Zn concentration in plant tissue (Lindsay, 1972; Racz and Haluschak, 1974, Moraghan, 1984; Loneragan and Webb, 1993). A reason for that is the bond formed between phosphate and Zn ions, which cannot readily be broken by plants and thus reduces the Zn uptake (compare section 2.7.3). For the HCS such a comparison is hard to make as the Zn uptake by roots was found to be linear; thus, the HCS-P took up less Zn compared to the HCS-Ctrl as the Zn concentration was around one-third of the HCS-Ctrl Zn concentration. However, it can be assumed that the same mechanisms are true for the HCS compared to the LCS, and that the reduced Zn concentration found in rice plants is partly caused by the P addition.

**Table 4.25.** Zn concentration for the whole rice plant (dry weight) for different field sites and growth stages.

| Growth Stage | Zn concentration for the whole rice plants [mg/kg] and for different field sites | | | |
|---|---|---|---|---|
| | LCS[a)]-Ctrl | LCS-P | HCS[b)]-Ctrl | HCS-P |
| Vegetative Stage | 44.45 ± 23.60 | 27.97 ± 2.679 | 155.3 ± 50.10 | 99.48 ± 8.111 |
| Panicle Stage | 48.72 ± 16.31 | 44.33 ± 8.964 | 127.9 ± 26.30 | 89.86 ± 18.14 |
| Maturity Stage | 46.85 ± 7.803 | 44.70 ± 14.63 | 124.6 ± 13.47 | 73.20 ± 5.625 |

Mean ± SD, n=5; [a)]LCS: Low concentration site; [b)]HCS: High concentration site.

**2) Cd and Zn in different parts of the rice plants**

The results for Cd concentration measured in different plant parts and for different growth stages are presented in Table 4.26. The distribution of Cd between the single plant parts was according to the literature (root > straw > panicle/grain, Li et al., 2009), except for three treatments of the LCS where it was slightly altered. The alteration was an effect of the high SDs obtained for the LCS. The high SDs were a result of the low initial Cd concentration present in soil. This was affirmed by the results obtained for the HCS as the initial concentrations were much higher, and more acceptable SDs were obtained. Additionally, all samples of the HCS showed a Cd distribution of the order roots > straw > panicle/grain.

If assuming similar uptake patterns of the rice plants between control and treatment sites, the uptake from the HCS was reduced for every plant part after the addition of 50 mg P/kg. The concentration in the rice grain for the treatment site was even half of the concentration in the grain of the control site. A similar trend was

found for the LCS.

**Table 4.26.** Cd concentration in different rice plant parts (dry weight) for different field sites and growth stages.

| Growth Stage | Cd concentration in different plant parts [mg/kg] and for different field sites | | | |
|---|---|---|---|---|
| | LCS[a)]-Ctrl | LCS-P | HCS[b)]-Ctrl | HCS-P |
| **Vegetative Stage** | | | | |
| Roots | 0.384 ± 0.231 | 0.431 ± 0.083 | 12.92 ± 0.875 | 14.70 ± 3.182 |
| Straw | 0.027 ± 0.059 | 0.040 ± 0.059 | 8.395 ± 5.502 | 7.951 ± 2.427 |
| **Panicle Stage** | | | | |
| Roots | 0.072 ± 0.020 | 0.051 ± 0.000 | 79.48 ± 36.74 | 45.57 ± 22.07 |
| Straw | 0.378 ± 0.408 | 0.252 ± 0.272 | 1.541 ± 0.626 | 0.364 ± 0.333 |
| Panicles | 0.033 ± 0.000 | 0.033 ± 0.047 | 0.491 ± 0.374 | 0.053 ± 0.030 |
| **Maturity Stage** | | | | |
| Roots | 0.280 ± 0.105 | 0.156 ± 0.104 | 18.59 ± 5.364 | 8.849 ± 2.656 |
| Straw | 0.081 ± 0.000 | 0.018 ± 0.021 | 3.158 ± 1.085 | 1.018 ± 0.553 |
| Grains | 0.096 ± 0.000 | 0.038 ± 0.037 | 0.706 ± 0.169 | 0.317 ± 0.166 |

Mean ± SD, n=5; [a)]LCS: Low concentration site; [b)]HCS: High concentration site.

The Zn concentrations in the different plant parts and for different growth stages are presented in Table 4.27. The Zn concentration in the roots was decreased for the LCS-P compared to the LCS-Ctrl for the vegetative stage.

**Table 4.27.** Zn concentration in different rice plant parts (dry weight) for different field sites and growth stages.

| Growth Stage | Zn concentration in different plant parts [mg/kg] and for different field sites | | | |
|---|---|---|---|---|
| | LCS[a)]-Ctrl | LCS-P | HCS[b)]-Ctrl | HCS-P |
| **Vegetative Stage** | | | | |
| Roots | 62.86 ± 23.35 | 39.83 ± 6.858 | 470.7 ± 29.24 | 280.8 ± 49.23 |
| Straw | 26.74 ± 2.780 | 24.04 ± 2.682 | 96.23 ± 41.67 | 67.01 ± 7.339 |
| **Panicle Stage** | | | | |
| Roots | 77.62 ± 12.15 | 70.43 ± 6.248 | 515.6 ± 160.9 | 536.2 ± 167.2 |
| Straw | 48.57 ± 18.44 | 44.21 ± 9.795 | 77.27 ± 18.69 | 42.76 ± 7.099 |
| Panicles | 25.91 ± 3.646 | 26.12 ± 3.501 | 37.98 ± 8.101 | 26.78 ± 6.115 |
| **Maturity Stage** | | | | |
| Roots | 80.39 ± 15.98 | 76.15 ± 19.48 | 623.5 ± 84.43 | 321.3 ± 38.73 |
| Straw | 61.07 ± 9.306 | 59.72 ± 24.31 | 111.4 ± 20.99 | 71.38 ± 7.428 |
| Grains | 15.29 ± 1.473 | 14.22 ± 1.033 | 14.83 ± 1.308 | 14.34 ± 1.237 |

Mean ± SD, n=5; [a)]LCS: Low concentration site; [b)]HCS: High concentration site.

This finding is caused by the different available Zn concentrations present in soil. The LCS-Ctrl featured a higher Zn concentration in F1, resulting in higher

adsorption by the root cell wall than for the LCS-P. The Zn concentrations in the single plant parts of the LCS-Ctrl were similar to the LCS-P for every growth stage (compare Table 4.27), suggesting no effect of the P-fertilizer on Zn plant uptake and translocation within the rice plant. The finding for the HCS was consistent with literature stating that only as much Zn is translocated by rice plants to the shoots as necessary (see section 2.6.4). The Zn concentration in root was elevated due to adsorption on the outer root cell wall, whereas the straw, panicle, and grain concentrations were comparable to those for the LCS exhibiting a much lower Zn concentration (compare Table 4.15). This finding allows the conclusion that only as much Zn is translocated to the shoots as necessary for the plant. The application of P did not affect the Zn distribution in the single plant parts.

## 4.3 Application in the Mae Sot District

In order to investigate a simple, low in costs, and effective way to remediate the paddy soil in the Mae Sot District, which is in some areas highly contaminated with Cd as a result of nearby Zn mines, a *in situ* treatment with a mineral P-fertilizer was studied. The addition of the P-fertilizer is known to reduce the Cd mobility in soil and additionally has a plant growth enhancing effect (see chapter 2.7). To estimate an effective level of P addition, pot experiments were conducted followed by a field site study. It was found that with increasing P addition the mobile Cd fraction in soil was reduced, but as a result of the high Cd concentration present the rice plants showed intense Cd toxicity symptoms resulting in stunned growth and death of some replicates; therefore, no assure data for a reduction of the Cd content in plants with higher P application were obtained. It is believed that the reduction of the Cd content in plants with increasing growth stage is probably mainly caused by the dilution effect with increasing biomass yield caused by P fertilization; however, additional research is necessary to prove this.

To test the efficiency of the P-fertilizer under field conditions, two sites exhibiting low (< 0.5 mg/kg) and high (> 60 mg/kg) Cd concentrations were selected and divided into control and treatment (addition of 50 mg P/kg) site; a concentration of 50 mg P/kg soil was selected as the pot study revealed a reduction of bioavailable Cd even for the lowest level of P addition. Additionally, other factors for the selection of 50 mg P/kg soil were costs and environmental aspects. The results from the field study revealed comparable results for the field site possessing high Cd concentration, as the bioavailable Cd's fraction was reduced after P addition, whereas no such trend was observed for the field site exhibiting a low Cd concentration. The Cd concentration in the rice plant seemed to be affected by P addition for the site high in Cd. This result is not ensured as the control and the treatment sites featured different Cd concentrations making a comparison difficult. Even though Cd concentrations were similar for pot experiment and the high concentration site (HCS), no Cd toxicity symptoms were observed for the field experiment. For the low concentration site (LCS), a reduction of the plant's Cd was detected, but for some sample replicates the Cd concentrations in rice grains still exceeded the Cd concentration in grains endorsed by the FAO/WHO of 0.2 mg/kg for rice grains (compare Appendix, Table

A-37).

To estimate the risk for the human health, not the total Cd content in rice grains is important, but the weekly intake that was set not to increase a value of 2.5 μg/kg body weight. In countries with rice as staple food this limit is rapidly exceeded.

Taking these finding into account, the application of a P-fertilizer at a rate of 50 mg/kg as *in situ* remediation for Cd contaminated sites with low and high contamination is solely not sufficient to reduce the Cd concentration in the edible parts of the rice plant to safe limits.

## 4.4 Subcellular fractionation

To obtain a better understanding about the effect of phosphate on the Cd distribution and chemical form within the rice plant, the subcellular distribution and chemical form of Cd within the rice plant were studied. This experiment was separately set up as a hydroponic pot experiment. The phosphate concentrations selected for this study were 0 (control), 50, 200, and 1000 mgP/L nutrient solution. The results of this experiment are discussed in the following chapters.

### 4.4.1 Roots

The effect of phosphate on the subcellular fractionation of Cd in the roots of rice plants was studied in this section. As phosphate source the P-fertilizer described in section 4.1.2 was used at different concentrations. The effect of the P-fertilizer on the subcellular fractions (F I: cell wall fraction, F II: trophoplasts, F III: membranes and organelles, and F IV soluble fraction) are presented in Table 4.28.

**Table 4.28** Subcellular fractions of Cd in the roots (fresh weight) of the rice plants grown with 0.3 mg/L $Cd^{2+}$ in nutrient solution.

| **Fraction**[a)] | **Level of P addition [mg/L]** | | | |
|---|---|---|---|---|
| **[µg/kg]** | **0** | **50** | **200** | **1000** |
| F I | 267.2 ± 2.826 (22.6%)[b)] | 261.8 ± 17.49* (22.5%) | 266.6 ± 5.129 (28.0%) | 240.3 ± 35.06* (29.6%) |
| F II | 391.2 ± 13.63 (33.2%) | 383.4 ± 140.8 (32.9%) | 265.9 ± 26.52* (27.9%) | 145.1 ± 6.191* (17.9%) |
| F III | 187.9 ± 18.94 (15.9%) | 167.1 ± 41.72* (14.3%) | 157.7 ± 21.87* (16.6%) | 61.09 ± 0.753* (6.3%) |
| F IV | 333.7 ± 26.19 (28.3%) | 353.8 ± 59.55* (30.3%) | 261.6 ± 3.276* (27.5%) | 376.4 ± 17.57 (46.3%) |
| Total | 1180 (100%) | 1166* (100%) | 951.7* (100%) | 812.8* (100%) |

Mean ± SD, n=3; [a)]F I: cell wall fraction; F II: trophoplast fraction; F III: membranes and organelles; F IV: soluble fraction; total: pseudototal Cd concentration (sum of all fractions); [b)]the numbers in the parentheses indicate the mean percentage of each Cd fraction in total Cd of roots.. *P < 0.05 (treatment vs. control)

After the addition of P the Cd concentration (with respect to the pseudototal Cd concentration) in F I and F IV was increased from 0.0-6.9% and from 0.0-18.0% for the highest level of P addition, respectively, whereas the Cd content in F II and F III were reduced from 0.3-15.3% and from 1.6-9.6% with increasing P addition,

respectively.

In literature only one study could be found studying the effect of phosphorus on the subcellular fractionation of Cd in corn and wheat plants (Yang et al., 1999). They found that with increasing P application the Cd content in the cell wall fraction was increased, whereas the other subcellular fractions decreased. They ascribed this effect to the involvement of phosphorus in sequestration of Cd ionic activity in the cell wall and vacuoles (organelles) by forming insoluble Cd phosphates. Same results were found in this study except the Cd concentration in F IV at a P application rate of 1000 mg/L. They also found that with increasing P the P content in the cell wall, vacuoles, and the cytoplasm of corn and wheat also increased with a P distribution of cell wall > vacuoles > cytoplasm.

The high Cd content in cytoplasm might be an outlier as for a P addition of 50 and 200 mg/L the Cd concentration decreases (compare Table 4.28). A decreasing trend of the Cd concentration in the cytoplasm (F IV) was also found for the shoot for every level of P addition, which will be discussed in section 4.4.2.

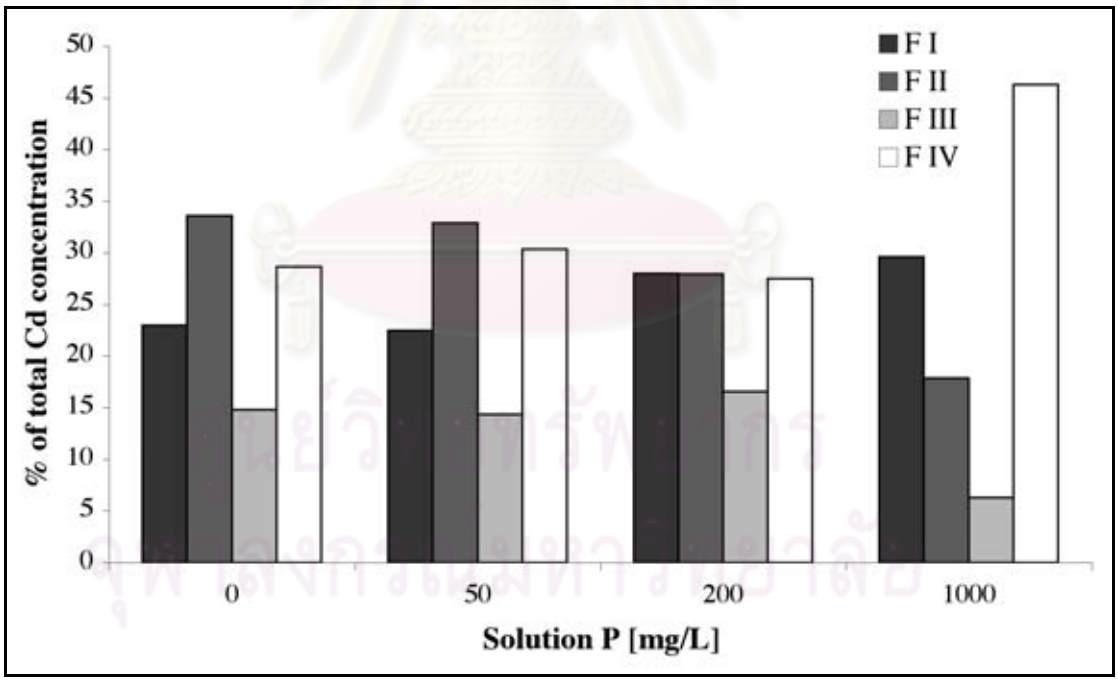



**Figure 4.16.** Percentage of different subcellular fractions in roots; F I: cell wall fraction; F II: chloroplast fraction; F III: membranes and organelles; F IV: soluble fraction.

Furthermore, a reduction of the intracellular Cd accumulation in rice plants with increasing P application was observed (see Table 4.28). This finding is analog to

the finding made by Yang et al. (1999) who also observed a reduction of the uptaken Cd by corn and wheat plants with increasing P. A reason for the reduced uptake of Cd by rice plants with high level of solution P can be the formation of Cd-phosphates. The pH of the nutrient solution was constantly kept at 5.0-5.1. At this pH the main P-species present is $H_2PO_4^-$ ($pK_2$=7.2) assisting the formation of $Cd(H_2PO_4)_2$ complexes reducing the free $Cd^{2+}$ activity in the nutrient solution. As stated in chapter 2.6.2, the uptake of Cd by the roots is dominated by diffusion of free $Cd^{2+}$. In a study conducted by Panfili et al. (2009) it was suggested that there is no uptake of non-dissociated Cd-complexes. Due to this reason the uptake of Cd with increasing level of P addition was reduced.

The increase in F I fraction can be a results of depositions on the cell wall. As the roots are the first barrier for Cd between the soil and the entrance to the plant cells, most of the Cd in the roots accumulated is immobilized by depositions. As anions like $HPO_4^{2-}$, $H_2PO_4^-$, $Cl^-$, and $SO_4^{2-}$ exists at the outer part of the root cell wall, $Cd^{2+}$ is attracted by them and adsorbs onto the cell wall, resulting in an increase of the Cd's root cell wall fraction (Jiang et al., 2007). The reduced Cd concentration in F II, F III, and F IV is due to the increased adsorption of Cd onto the cell wall, whereas the translocation from the cell wall to the interior plant parts did not increase, resulting in a fixation of the Cd (Youngdhal, 1977). The existence of these deposits, which prevent the translocation of Cd from the roots to the shoots, was directly proven by Jiang et al. (2007) by using transmission electron microscopy (TEM).

### 4.4.2 Shoots

The trends for the Cd content in the single fractions were consistent with the trends of the root fractions, except for F IV (compare Table 4.29). The F IV fraction initially slightly increased about 1.6-3.8% for an addition of 50 and 200 mg P/L, respectively, but for the highest level of P addition there was a slight reduction of 0.7% observable. The reduction of F IV is consistent with literature as Jiang et al. (2007) found comparable results. This trend is also favorable for rice propagation as the Cd found in the cytoplasm is highly mobile and thus able to reach the rice grains. Differently from the distribution in the roots, the initial Cd distribution between the four fractions was not relatively equal, yet F I amounts for the highest subcellular Cd, followed by F IV, and the F II and FIII fractions constituted the lowest Cd concentrations.

**Table 4.29.** Subcellular fractions of Cd in the shoots (fresh weight) of the rice plants grown with 0.3 mg/L $Cd^{2+}$ in nutrient solution.

| Fraction[a)] | Level of P addition [mg/L] | | | |
|---|---|---|---|---|
| [µg/kg] | 0 | 50 | 200 | 1000 |
| F I | 265.0 ± 9.667 (47.9%)[b)] | 194.9 ± 32.02* (44.0%) | 213.2 ± 48.43* (48.9%) | 97.15 ± 13.44* (58.7%) |
| F II | 72.95 ± 6.4 (13.2%) | 63.43 ± 2.586* (14.3%) | 49.12 ± 31.52* (11.3%) | 15.82 ± 0.69* (9.6%) |
| F III | 60.96 ± 31.95 (11.0%) | 53.79 ± 5.384* (12.2%) | 35.84 ± 31.97* (8.2%) | 7.642 ± 2.135* (4.6%) |
| F IV | 154.3 ± 33.96 (27.9%) | 130.6 ± 26.91* (29.5%) | 138.1 ± 75.85* (31.6%) | 45.01 ± 5.186* (27.2%) |
| Total | 553.2 (100%) | 442.7* (100%) | 436.3* (100%) | 165.6* (100%) |

Mean ± SD, n=3; [a)]F I: cell wall fraction; F II: chloroplast fraction; F III: membranes and organelles; F IV: soluble fraction; total: pseudototal Cd concentration (sum of all fractions); ); [b)]the numbers in the parentheses indicate the mean percentage of each Cd fraction in total Cd of shoots.. *P < 0.05 (treatment vs. control).

This distribution was also intensified with increasing P addition, as the Cd content in F I increased from 0.0-10.8%, whereas the F II and F III fractions were reduced from 0.0-3.6% and 0.0-6.4%, respectively (compare Figure 4.17).

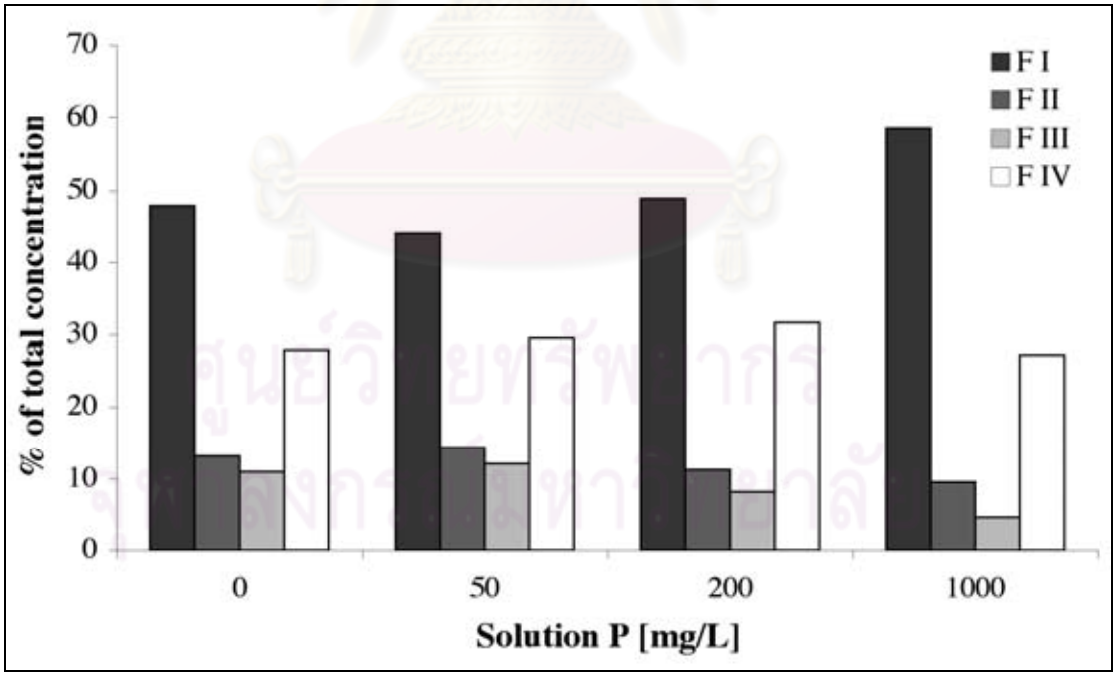



**Figure 4.17.** Percentage of different subcellular fractions in shoots; F I: cell wall fraction; F II: chloroplast fraction; F III: membranes and organelles; F IV: soluble fraction.

The total accumulated subcellular Cd concentration in shoots also decreased with increasing P content. It was found that about 46.9, 38.0, 45.8, and 20.4% of the

pseudotoal Cd concentration found in the roots for different levels of P application was present in the shoots. Additionally, the reduction of Cd in shoots was about 70.1%, whereas for the roots it only was 31.1%. Due to this it can be predicted that P has an effect on the translocation from the roots to shoots beside Cd deposition on the root cell wall, but the mechanisms behind that observation are not apparent.

The increase in the cell wall Cd can be explained by immobilization as detoxification mechanisms of plants responding to high Cd concentrations (see section 2.6.3). With increase of F I there is a concomitant decrease in F II, F III, and F IV observable, reducing the Cd toxicity within the rice plants.

The results imply that phosphate seems to affect the subcellular distribution and also the translocation of Cd from roots to shoots, but further experiments are necessary to validate this assumption.

## 4.5 Chemical Form

### 4.5.1 Roots

The different chemical forms of Cd present in plant tissue were characterized using different extraction solvents. Ethanol-extractable Cd ($Cd_{EtOH}$), deionized water-extractable Cd ($Cd_{DI}$), sodium chloride-extractable Cd ($Cd_{NaCl}$), acetic acid-extractable Cd ($Cd_{HAc}$), hydrochloric acid-extractable Cd ($Cd_{HC}$), and residual Cd ($Cd_{Res}$) were the main forms present in the plant tissue and the results for Cd extraction in roots are presented in Table 4.30. The pseudototal Cd concentrations for roots tissue was estimated summing up all fractions obtained except the Cd concentration for $Cd_{NaCl}$. Due to a high salt matrix in the sample solution instrumental problems of the GF-AAS occurred making a reliable measurement of the samples impossible.

**Table 4.30.** Chemical forms of Cd in the roots (fresh weight) of the rice plants grown with 0.3 mg/L $Cd^{2+}$ in nutrient solution.

| **Fraction**[a)] | **Level of P addition [mg/L]** | | | |
|---|---|---|---|---|
| **[µg/kg]** | **0** | **50** | **200** | **1000** |
| $Cd_{EtOH}$ | 94.25 ± 1.573 (33.0%)[b)] | 87.84 ± 0.095 (33.0%) | 86.18 ± 1.230* (38.1%) | 72.47 ± 0.173* (35.2%) |
| $Cd_{DI}$ | 89.73 ± 0.953 (31.4%) | 92.96 ± 0.072 (34.9%) | 87.66 ± 1.971 (38.8%) | 111.3 ± 4.448* (54.2%) |
| $Cd_{NaCl}$ | 0.000 ± 0.000 (-) | 611.6 ± 22.34 (-) | 570.6 ± 227.9 (-) | 43.39 ± 2.710 (-) |
| $Cd_{HAc}$ | 95.49 ± 5.407 (33.4%) | 81.53 ± 4.862 (30.7%) | 49.87 ± 3.329* (22.0%) | 19.91 ± 1.176* (9.7%) |
| $Cd_{HCl}$ | 5.897 ± 0.313 (2.1%) | 3.198 ± 0.406* (1.2%) | 2.000 ± 0.056* (0.9%) | 1.633 ± 0.014* (0.8%) |
| $Cd_{Res}$ | 0.202 ± 0.095 (0.1%) | 0.584 ± 0.020 (0.2%) | 0.377 ± 0.011 (0.2%) | 0.204 ± 0.011 (0.1%) |
| $Cd_{Total}$ | 285.6 (100%) | 266.1 (100%) | 226.1* (100%) | 205.5* (100%) |

Mean ± SD, n=2; [a)]$Cd_{EtOH}$: ethanol-extractable Cd, $Cd_{DI}$: deionized water-extractable Cd, $Cd_{NaCl}$: sodium chloride-extractable Cd, $Cd_{HAc}$: acetic acid-extractable Cd, $Cd_{HCl}$: hydrocloric acid-extractable Cd, $Cd_{Res}$: residual Cd, $Cd_{Total}$: pseudototal Cd concentration (sum of all fractions except $Cd_{NaCl}$); [b)]the values in blankets represent the percentage of each fraction at the given P application; *P < 0.05 (treatment vs. control).

In the roots without P addition to the nutrient solution, $Cd_{HAc}$ and $Cd_{EtOH}$ were most abundant constituting 33.4 and 33.0% of the pseudototal Cd concentration,

closely followed by the $Cd_{DI}$ form, representing 31.4%. From literature it can be assumed that the $Cd_{NaCl}$ would also constitute one of the main chemical forms (the implied concentrations are very high, see Table 4.30). He et al. 2008 found a comparable chemical form distribution in plant tissue of wild type and mutant rice plants. They compared the distribution of Cd forms in roots, leaf sheaths, and leaves of wild type and mutant rice plants. They found that the mutant rice plants were more sensitive to exposure to high Cd concentrations. Cd mainly associated to inorganic components like nitrate ions, chlorides, organic acids, and $M(PO_4)_2$ ($Cd_{EtOH}$ and $Cd_{DI}$) are the most toxic form present in plant tissue as they have a remarkably stronger migration ability compared to the other forms and thus are able to penetrate into the symplasm and reach parts that are used for consumption. The only immobile form is $M(PO_4)_2$ as it has a low solubility. As to my knowledge there is no study available in the relevant literature databases (ISI web of knowledge, SCOPUS) on the effect of phosphate on the chemical form distribution in rice plant tissue so that no comparative study is available. There is only a single study conducted by Yang et al. (2000) claiming to be the first to study the effect of phosphate on the chemical form in wheat and corn. In Figure 4.18 it clearly can be seen that with increasing level of P addition the initial equal distribution between the $Cd_{HAc}$, $Cd_{EtOH}$, and $Cd_{DI}$ forms changed. With increasing P addition there is a significant ($P < 0.05$) increase in $Cd_{DI}$ accompanied by a significant decrease of $Cd_{HAc}$. The decrease of the $Cd_{HAc}$ with increasing P is surprising as this form includes the undissolved cadmium phosphates including $CdHPO_4$ and $Cd_3(PO_4)_2$, representing a less toxic Cd form. It was assumed that with increasing level of P addition more P would be uptaken by plants; thus, with increasing P concentration in plant tissue the Cd ion activity would be decreased due to precipitation as insoluble Cd-phosphates. In a comparable study the effect of P on the chemical form of Cd in wheat and corn plants was studied (Yang et al., 2000). They found that with increasing P addition the $Cd_{HAc}$ significantly increased. They explained that this phenomenon arose by precipitation of the free $Cd^{2+}$ ions as undissolved Cd-phosphates. The contrarily results found in this study could be due to different growth conditions for rice plants and wheat and corn and different genera. In this study the rice plants were grown under anaerob conditions, whereas wheat and corn grew under aerob conditions. However, further experiments are necessary to propose a possible mechanism for a decrease in the $Cd_{HAc}$ form of rice plants with increasing P addition. On the other hand, the $Cd_{DI}$ form in the study of Yang et al.

(1999) increased with increasing P application showing the same trend as in this study. The increase in $Cd_{DI}$ might be an effect of the increased P application as more $M(PO_4)_2$ are formed, which would confirm the augmented P uptake by plants with increasing P found by Yang et al. (1999). A decreasing trend was also found for the $Cd_{NaCl}$ form. The $Cd_{NaCl}$ represents the Cd integrated into proteins and pectates. This form shows less mobility compared to $Cd_{HAc}$, $Cd_{EtOH}$, and $Cd_{DI}$, but it also possesses high toxicity. As Cd has a high affinity to bind with sulfhydryl components (-SH), it prefers to bind with proteins disturbing the enzymatic activity. In other studies it was observed that plants produce so called phytochelatins (section 2.6.3) transporting the Cd to the vacuoles as means of detoxification. In this study with low solution P the uptake of Cd from the nutrient solution was higher than for higher levels of P application (reduction of 28%). The reduced uptake of Cd with increasing P application was also found by Yang et al. (2000) and can be an explanation for the decreasing $Cd_{NaCl}$ form as with decreasing Cd concentration in the plant tissue the production of the phytochelatins and metallothioneins by the plant is reduced as well (compare section 4.4.1).

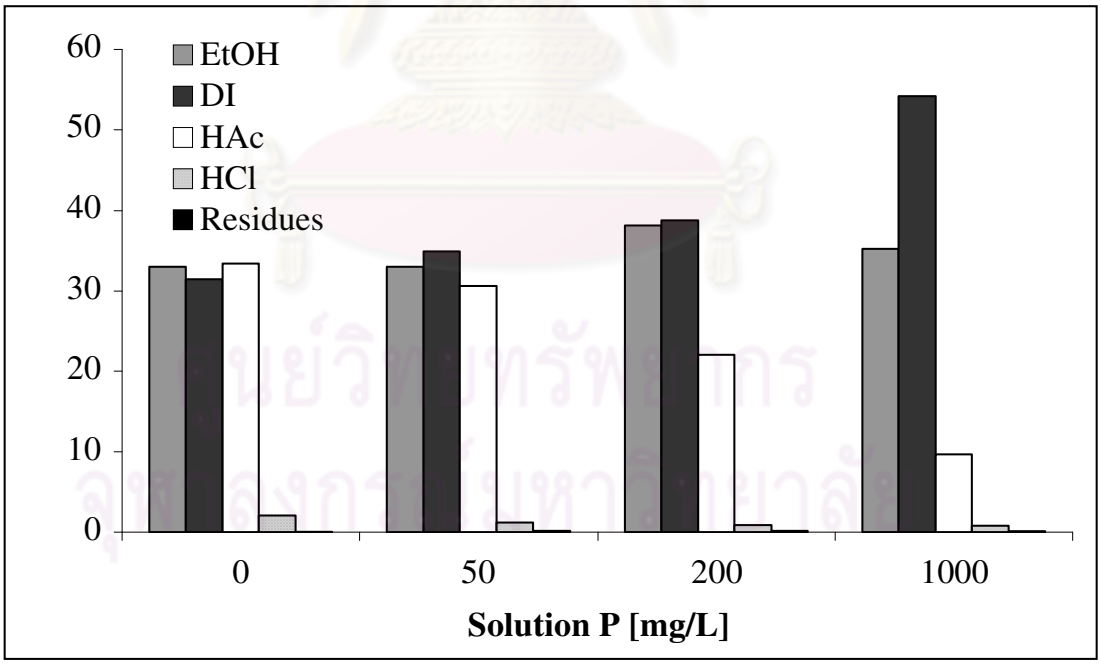



**Figure 4.18.** Percentage of different chemical forms (extraction solution - EtOH: 80% ethanol, DI: d-$H_2O$, HAc: 2% acetic acid, HCl: 0.6 M hydrochloric acid, Residues: residual fraction.

### 4.5.2 Shoots

The pseudototal Cd concentration in the shoots (leaf sheaths and leaves) for different levels of P addition are 1.42 to 5.69 fold lower compared to the root concentration (Table 4.31). This finding is in agreement with other studies (Yang et al., 2000; Wu et al., 2005; He et al., 2008). The initial distribution of the chemical forms is different compared to the roots.

**Table 4.31.** Chemical forms of Cd in the shoots (fresh weight) of the rice plants grown with 0.3 mg/L $Cd^{2+}$ in nutrient solution.

| **Fraction**[a)] | **Level of P addition [mg/L]** | | | |
|---|---|---|---|---|
| **[µg/kg]** | **0** | **50** | **200** | **1000** |
| $Cd_{EtOH}$ | 22.11 ± 0.239 (11.0%)[b)] | 19.74 ± 0.173 (10.8%) | 27.99 ± 0.228 (23.0%) | 14.52 ± 0.160* (40.2%) |
| $Cd_{DI}$ | 32.36 ± 0.408 (16.1%) | 23.07 ± 0.071* (12.7%) | 30.03 ± 0.145 (24.7%) | 8.308 ± 0.474* (23.0%) |
| $Cd_{NaCl}$ | 190.9 ± 9.944 (-) | 46.33 ± 8.393 (-) | 181.8 ± 120.9 (-) | 73.73 ±99.61 (-) |
| $Cd_{HAc}$ | 143.2 ± 10.26 (71.3%) | 137.2 ± 17.86 (75.3%) | 62.11 ± 7.829* (51.1%) | 11.67 ±10.06* (32.3%) |
| $Cd_{HCl}$ | 3.187 ± 0.128 (1.6%) | 2.027 ± 0.097 (1.2%) | 0.499 ± 0.011* (0.4%) | 1.071 ± 0.101* (3.0%) |
| $Cd_{Res}$ | 0.085 ± 0.004 (0.0%) | 0.072 ± 0.001 (0.0%) | 0.962 ± 0.176 (0.8%) | 0.550 ± 0.006 (1.5%) |
| $Cd_{Total}$ | 200.9 (100%) | 182.1 (100%) | 121.6* (100%) | 36.12* (100%) |

Mean ± SD, n=2; [a)]$Cd_{EtOH}$: ethanol-extractable Cd, $Cd_{DI}$: deionized water-extractable Cd, $Cd_{NaCl}$: sodium chloride-extractable Cd, $Cd_{HAc}$: acetic acid-extractable Cd, $Cd_{HCl}$: hydrocloric acid-extractable Cd, $Cd_{Res}$: residual Cd, $Cd_{Total}$: pseudototal Cd concentration (sum of all fractions except $Cd_{NaCl}$); [b)]the values in blankets represent the percentage of each fraction at the given P application; *P < 0.05 (treatment vs. control).

In the shoot samples the most abundant form was $Cd_{HAc}$ representing 71.3% of the pseudototal Cd concentration, whereas $Cd_{EtOh}$ and $Cd_{DI}$ amounted for 11.0 and 16.1%, respectively. It can be assumed that the percentages are biased due to the neglection of the not reliable $Cd_{NaCl}$ results, which would represent one, if not the main, form found in shoot tissue (He et al., 2008). The trends for the single chemical forms are comparable to the roots sample with a significant decrease of $Cd_{HAc}$ and a slightly increase in $Cd_{DI}$. The only difference can be observed for $Cd_{EtOH}$ showing a significant increase with increasing P, whereas in the root samples no change was observable (compare Figure 4.18 and Figure 4.19). The increase in $Cd_{DI}$ and $Cd_{EtOH}$ can be explained by redistribution of the plant's Cd after the great reduction of $Cd_{HAc}$.

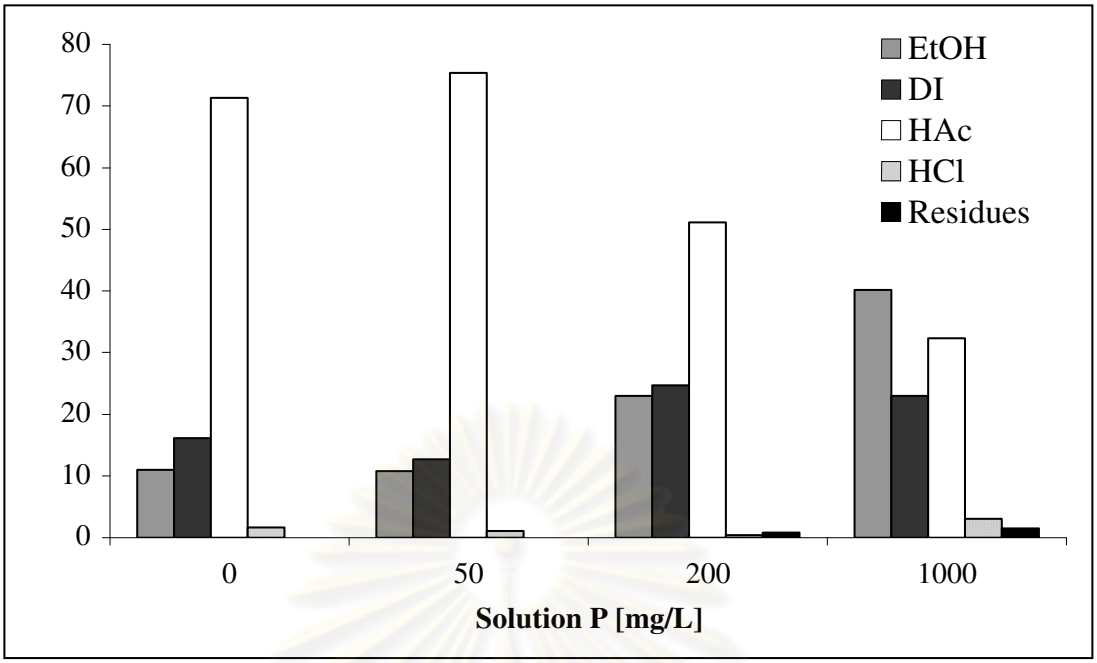



**Figure 4.19.** Percentage of different chemical forms (extraction solution - EtOH: 80% ethanol, DI: d-$H_2O$, HAc: 2% acetic acid, HCl: 0.6 M hydrochloric acid, Residues: residual fraction.

## 4.6 Relating the findings from the pot and field experiments to the plant experiments

The field study revealed a reduced Cd concentration of the plant samples. This reduction was found for the whole rice plants as well as for the different plant parts. A remarkably reduction in Cd was found for the rice grains of the LCS-P as it was even one-third the concentration of the control site, while the Cd concentration in rice grains for the HCS-Ctrl was only half the concentration found for the control site. This finding cannot solely be ascribed to the reduced bioavailable Cd fraction (F1) found in soil, as F1 was not reduced after P treatment for the LCS; for the HCS F1 was still high even a decreased Cd concentration after P addition was observed. Therefore, the reduced Cd concentration found in plant samples cannot solely be explained by reactions occurring on the soil level, but also the plant level has to be taken into account. To understand the results obtained from the field experiment, an additional experiment was conducted studying the events happening on the plants level. As proven by the increased dry matter yield with P application, the plants took up higher amounts of phosphate and hence increasing the plants phosphate concentration. The phosphate directly impacted the subcellular sequestration of Cd in the roots and shoots by means of phosphate depositions on the cell wall and thus reducing the Cd concentration in the more mobile fractions. As a result, less Cd was translocated from the roots to the shoots and from the shoots to the panicle/grains as was also found in the field study.

# CHAPTER V
# CONCLUSIONS AND OUTLOOK

## 5.1 Conclusions

The addition of P-fertilizer at high concentrations (200 and 1000 mg/kg) increased the pH of the soil, whereas it did not have any effect on the redox potential. The increase in pH induced by the addition of anions is expected to result in an increase in the sequestration of Cd to less mobile forms and thereby reducing its phytotoxicity. The results in this study illustrated that P addition enhanced Cd immobilization as shown by the reduction in the F1 fraction and an augmentation in F2. This effect was only observed for high initial Cd concentrations in soil, whereas no effect for low initial concentrations was observed. Zn in soil showed only insignificant, if any, response to Cd addition; thus, the Zn fractions are believed to be unaffected by P application.

Application of P-fertilizer induced rice growth for every level of P addition and increased the yield of the rice root, rice straw, and rice grains biomass. Even though the Cd concentration in the bioavailable fraction was reduced, no clear effect on the Cd concentration in the rice plants for the single growth stages could be observed for the pot experiment, as the plants showed severe symptoms of Cd toxicity. This was different for the field site as plants stayed healthy and showed reduced Cd concentration after P application, suggesting that the levels of P did reduce the uptake by plants for both, low and high initial Cd contents in soil. However, this finding is not confirmed as the HCS showed different initial Cd concentrations in soil. Additionally, the Cd in plants decreased with increasing time being more pronounced for higher levels of P application, but this effect is ascribed to dilution effects evoked by the increased yield of biomass consistent with increasing P application. However, Cd in rice grains obtained from the pot experiment and the HCS exceeded the FAO/WHO standard of 0.2 mg/kg for rice grains.

An effect of different P levels was also proven for the subcellular distribution of Cd within the rice plant. With an augmentation of the P content the Cd uptake was reduced and the immobile Cd fraction in the cell wall increased, whereas a

concomitant decrease in the mobile soluble fraction was determined. This is a hint for a detoxifying effect of P for the uptaken Cd. A reduced Cd uptake for increasing P application was also visible when studying the chemical form of Cd within the rice plants, confirming the results found for the subcellular distribution. Unanticipated was the finding that with increasing P the undissolved Cd-phosphate form was reduced, requiring further experiments to propose a possible mechanism. These findings indicate that P addition does not only have a positive effect on immobilization effects on the soil level, but also seems to have beneficial effects on Cd immobilization on the plant level reducing the translocation to edible plant parts.

## 5.2 Contribution to the scientific knowledge

The main aim of this research was to test a possible remediation technique to enable the rice propagation by local farmers in the Mae Sot District. Previous studies have only focused on the areas and levels of contamination and then on possible alternative crops like sugarcane not used for consumption. The application of a commercial P-fertilizer was studied for this special site, and the rice plants analyzed for Cd at different growth stages. This is not widely done and only a few studies can be found in the common literature of this field analyzing Cd for different growth stages. Even though the application of P-fertilizer at a rate of 50 mg/kg seemed not to be effective for reducing the Cd uptake of rice plants at this area below Cd standards in rice grains established by the FAO/WHO for high concentration sites, it was proven that the main Cd uptake was during the vegetative stage and that possible remediation techniques should prevent the uptake of Cd during that stage. For the pot experiment it was found that the initial Cd concentration in the whole rice plant was higher with increasing P compared to the control for the vegetative stage, but decreased below the plants concentration of the control at the maturity stage. Without the knowledge of the plant's Cd concentration at different growth stages and only knowing the Cd concentration in plants for the maturity stage, one could imply that the P application decreased the Cd uptake. In truth, the decreased Cd content in rice plants for higher levels of P addition at the maturity stage seem to be also an effect of the Cd dilution effect with increasing plant biomass instead of being solely due to reduced uptake.

Also new for this study was the research on the effect of P on the subcellular distribution and chemical form of Cd within rice plants, as it was never preformed before for rice plants and only once for wheat and corn. It is a well-accepted fact that phosphorus is able to reduce the uptake of Cd by plants and also alters the Cd translocation within rice plants, but mechanisms for this altered translocation are not understood yet. The study of the chemical form revealed that P application increased immobilization in the cell wall fraction of roots and shoots and reduced Cd in the more mobile fractions, which is a first step in understanding the effect of P on translocation mechanisms. Same is true for the chemical Cd form as the single forms were greatly affected by phosphorus.

## 5.3 Future research

For future research on this topic the field experiment could be repeated using one single field divided into equal allotments used for different treatments. With this method it is assured that the control and treatment site will have equal conditions allowing a direct comparison and also might clarify if there is an effect of the P application on the Cd uptake at this special site.

Additionally, the experiment on the chemical Cd form within rice plants should be repeated including the $Cd_{NaCl}$ fraction to obtain reliable results. This should also be focus of further studies as the mechanisms for Cd immobilization by P are widely studied, whereas there is only limited information available on the immobilization of Cd on the plant's level.

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# APPENDIX

# APPENDIX

# ANALYSIS DATA

## 1 Pot experiment

### 1.1 pH values

**Table A-1.** pH values obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | pH values of the single treatments and growth stages | | | |
|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage |
| P-0-1 | 7.43 | 7.05 | 6.90 | 7.48 |
| P-0-2 | 7.62 | 7.32 | 7.03 | 7.51 |
| P-0-3 | 7.75 | 7.60 | 7.04 | 6.72 |
| P-0-4 | 7.38 | 6.97 | 7.18 | 7.23 |
| P-0-5 | 7.35 | 7.48 | 7.50 | 7.42 |
| P-1-1 | 7.47 | 6.82 | 7.27 | 7.13 |
| P-1-2 | 7.44 | 7.27 | 7.19 | 7.28 |
| P-1-3 | 7.94 | 7.29 | 7.05 | 7.66 |
| P-1-4 | 7.81 | 6.93 | 6.98 | 7.30 |
| P-1-5 | 7.38 | 7.86 | 7.25 | 7.85 |
| P-2-1 | 7.59 | 7.23 | 7.43 | 7.55 |
| P-2-2 | 7.82 | 7.04 | 7.40 | 7.24 |
| P-2-3 | 7.61 | 7.31 | 7.32 | 7.40 |
| P-2-4 | 7.54 | 7.33 | 7.70 | 7.44 |
| P-2-5 | 7.60 | 7.16 | 7.11 | 7.38 |
| P-3-1 | 7.63 | 7.72 | 7.42 | 7.50 |
| P-3-2 | 7.68 | 7.20 | 7.59 | 7.47 |
| P-3-3 | 7.58 | 7.34 | 7.57 | 7.48 |
| P-3-4 | 7.44 | 7.82 | 7.56 | 7.67 |
| P-3-5 | 7.34 | 7.69 | 7.95 | 7.50 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

## 1.2 Redox potentials

**Table A-2.** Redox potentials obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | Redox potential [mV] values of the single treatments and growth stages | | | |
|---|---|---|---|---|
| | **Initial Stage** | **Vegetative Stage** | **Panicle Stage** | **Maturity Stage** |
| P-0-1 | 1.6 | -147.3 | -171.9 | -199.6 |
| P-0-2 | 81.0 | -15.5 | -145.9 | -241.8 |
| P-0-3 | 154.9 | -78.4 | -208.8 | 64.8 |
| P-0-4 | 128.9 | 4.3 | -181.3 | -115.1 |
| P-0-5 | 153.3 | -192.2 | -154.6 | -151.0 |
| P-1-1 | 70.3 | -171.2 | -257.4 | 60.6 |
| P-1-2 | -126.9 | -106.1 | -105.9 | 3.9 |
| P-1-3 | 127.3 | -158.8 | -181.5 | 37.1 |
| P-1-4 | 81.0 | -81.7 | -38.7 | 107.2 |
| P-1-5 | 116.0 | -213.9 | -193.9 | -242.6 |
| P-2-1 | 67.8 | -193.1 | 16.0 | -64.2 |
| P-2-2 | 97.2 | -214.3 | -217.6 | 40.9 |
| P-2-3 | -183.4 | -232.2 | -232.0 | 7.5 |
| P-2-4 | 107.8 | -98.9 | -237.8 | 27.6 |
| P-2-5 | 30.0 | -146.7 | -233.1 | -273.6 |
| P-3-1 | -225.6 | -211.0 | -38.6 | -318.4 |
| P-3-2 | 102.4 | -227.0 | -29.6 | -192.3 |
| P-3-3 | 27.0 | -240.4 | -272.1 | -221.5 |
| P-3-4 | 171.2 | -233.7 | -283.5 | -277.1 |
| P-3-5 | 127.9 | -258.7 | -279.8 | -264.9 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

## 1.3 Total phosphorus in soil

**Table A-3.** Total phosphorus concentration obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | Total phosphorus [mg/kg] of the single treatments and growth stages | | | |
|---|---|---|---|---|
| | **Initial Stage** | **Vegetative Stage** | **Panicle Stage** | **Maturity Stage** |
| P-0-1 | 426.838 | 250.497 | 246.779 | 312.558 |
| P-0-2 | 734.954 | 313.919 | 268.012 | 324.484 |
| P-0-3 | 399.479 | 394.892 | 358.002 | 235.832 |
| P-0-4 | 378.656 | 282.458 | 265.672 | 259.799 |
| P-0-5 | 542.609 | 336.978 | 369.141 | 275.335 |
| P-1-1 | 341.997 | 526.843 | 338.575 | 470.642 |
| P-1-2 | 439.579 | 377.622 | 540.566 | 519.062 |
| P-1-3 | 665.297 | 747.774 | 386.278 | 424.795 |
| P-1-4 | 433.803 | 501.425 | 448.242 | 410.112 |
| P-1-5 | 360.013 | 537.488 | 374.275 | 474.446 |
| P-2-1 | 546.767 | 763.380 | 731.214 | 496.154 |
| P-2-2 | 855.876 | 582.469 | 503.929 | 596.008 |
| P-2-3 | 510.493 | 469.772 | 522.882 | 585.635 |
| P-2-4 | 494.060 | 718.935 | 684.878 | 650.888 |
| P-2-5 | 499.409 | 606.970 | 537.000 | 712.786 |
| P-3-1 | 1885.920 | 1361.516 | 1147.651 | 1261.682 |
| P-3-2 | 1366.123 | 1359.922 | 1249.788 | 1426.728 |
| P-3-3 | 1767.063 | 1367.861 | 1423.244 | 1400.767 |
| P-3-4 | 1121.614 | 1315.789 | 1408.163 | 1587.000 |
| P-3-5 | 1169.852 | 1401.198 | 1571.988 | 1348.346 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

## 1.4 Available phosphorus

**Table A-4.** Available phosphorus concentration obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | Available phosphorus [mg/kg] of the single treatments and growth stages | | | |
|---|---|---|---|---|
| | **Initial Stage** | **Vegetative Stage** | **Panicle Stage** | **Maturity Stage** |
| P-0-1 | 1.700 | 2.433 | 4.685 | 1.259 |
| P-0-2 | 3.709 | 4.866 | 4.861 | 0.920 |
| P-0-3 | 2.473 | 4.165 | 2.956 | 2.078 |
| P-0-4 | 1.622 | 4.516 | 3.300 | 2.239 |
| P-0-5 | 1.312 | 3.995 | 2.432 | 4.457 |
| P-1-1 | 2.782 | 3.996 | 3.647 | 2.177 |
| P-1-2 | 2.626 | 3.819 | 5.384 | 2.319 |
| P-1-3 | 2.317 | 3.997 | 3.476 | 2.460 |
| P-1-4 | 2.624 | 2.605 | 3.644 | 2.858 |
| P-1-5 | 4.475 | 6.428 | 3.128 | 2.760 |
| P-2-1 | 14.671 | 14.942 | 14.136 | 7.133 |
| P-2-2 | 7.713 | 12.512 | 12.510 | 5.299 |
| P-2-3 | 15.428 | 18.417 | 16.442 | 6.496 |
| P-2-4 | 8.883 | 14.584 | 21.089 | 6.959 |
| P-2-5 | 6.946 | 40.289 | 3.706 | 7.270 |
| P-3-1 | 345.709 | 252.978 | 223.102 | 227.048 |
| P-3-2 | 231.520 | 210.014 | 240.487 | 216.771 |
| P-3-3 | 219.268 | 259.746 | 207.899 | 213.272 |
| P-3-4 | 142.842 | 245.880 | 243.087 | 245.880 |
| P-3-5 | 139.753 | 250.263 | 254.910 | 237.712 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

## 1.5 Total Cd

**Table A-5.** Total Cd concentration obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | Total Cd in soil [mg/kg] for the single treatments and growth stages | | | |
|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage |
| P-0-1 | 74.311 | 86.562 | 80.956 | 73.830 |
| P-0-2 | 76.403 | 85.165 | 82.214 | 84.069 |
| P-0-3 | 73.029 | 76.722 | 82.761 | 71.700 |
| P-0-4 | 88.436 | 84.167 | 89.474 | 79.638 |
| P-0-5 | 84.260 | 81.875 | 92.240 | 87.546 |
| P-1-1 | 74.743 | 81.534 | 77.498 | 78.448 |
| P-1-2 | 68.968 | 78.276 | 82.567 | 84.412 |
| P-1-3 | 71.896 | 77.600 | 86.420 | 84.017 |
| P-1-4 | 68.476 | 79.470 | 81.501 | 90.774 |
| P-1-5 | 77.426 | 80.167 | 88.815 | 82.920 |
| P-2-1 | 71.684 | 82.557 | 82.132 | 81.814 |
| P-2-2 | 70.363 | 78.406 | 80.682 | 83.746 |
| P-2-3 | 71.790 | 79.034 | 87.599 | 85.914 |
| P-2-4 | 71.457 | 89.197 | 79.807 | 82.099 |
| P-2-5 | 71.498 | 83.909 | 83.511 | 82.110 |
| P-3-1 | 74.251 | 79.138 | 80.074 | 90.736 |
| P-3-2 | 69.793 | 83.306 | 79.032 | 81.336 |
| P-3-3 | 73.776 | 82.758 | 77.238 | 84.277 |
| P-3-4 | 71.268 | 86.302 | 81.992 | 84.220 |
| P-3-5 | 70.685 | 76.874 | 79.285 | 80.624 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

## 1.6 Total Zn

**Table A-6.** Total Zn concentration obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | Total Zn in soil [mg/kg] for the single treatments and growth stages | | | |
|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage |
| P-0-1 | 2335.740 | 1635.955 | 2338.471 | 2308.745 |
| P-0-2 | 2258.584 | 2229.021 | 2267.621 | 2400.350 |
| P-0-3 | 2451.770 | 2163.673 | 2313.184 | 2167.298 |
| P-0-4 | 2883.361 | 2333.250 | 2415.049 | 2307.560 |
| P-0-5 | 2610.659 | 2288.662 | 2489.246 | 2432.574 |
| P-1-1 | 2365.468 | 2306.272 | 2264.938 | 2246.760 |
| P-1-2 | 2361.700 | 2342.784 | 2308.150 | 2274.900 |
| P-1-3 | 2364.249 | 2266.850 | 2416.296 | 2178.864 |
| P-1-4 | 2427.747 | 2062.717 | 2262.697 | 2064.270 |
| P-1-5 | 2594.661 | 2229.522 | 2412.676 | 1851.833 |
| P-2-1 | 2228.555 | 2300.632 | 2343.471 | 2299.272 |
| P-2-2 | 2402.497 | 2294.746 | 2297.754 | 2260.891 |
| P-2-3 | 2320.115 | 2300.981 | 2287.153 | 2374.053 |
| P-2-4 | 2405.267 | 2319.200 | 2232.006 | 2280.178 |
| P-2-5 | 2405.870 | 2338.757 | 2266.645 | 2363.205 |
| P-3-1 | 2247.612 | 2271.251 | 2243.058 | 2384.781 |
| P-3-2 | 2492.384 | 2412.870 | 2306.452 | 2360.721 |
| P-3-3 | 2393.849 | 2372.750 | 2215.870 | 2389.956 |
| P-3-4 | 2473.085 | 2474.950 | 2346.937 | 2316.595 |
| P-3-5 | 2369.719 | 2285.488 | 2288.716 | 2335.168 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

## 1.7 Cd and Zn fractions

### 1.7.1 Exchangeable, water, and acid soluble Cd fraction (F1)

**Table A-7.** Cd concentration in F1 obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | Cd in F1 [mg/kg] for the single treatments and growth stages | | | |
|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage |
| P-0-1 | 61.249 | 61.396 | 65.524 | 68.413 |
| P-0-2 | 61.229 | 59.243 | 62.188 | 74.222 |
| P-0-3 | 63.202 | 60.495 | 61.833 | 61.182 |
| P-0-4 | 62.393 | 60.253 | 64.432 | 69.062 |
| P-0-5 | 62.875 | 57.513 | 65.274 | 70.370 |
| P-1-1 | 59.505 | 61.365 | 62.013 | 66.388 |
| P-1-2 | 60.439 | 63.852 | 62.657 | 63.103 |
| P-1-3 | 61.078 | 60.622 | 67.092 | 68.213 |
| P-1-4 | 61.680 | 62.318 | 61.631 | 68.776 |
| P-1-5 | 61.048 | 58.891 | 63.624 | 69.527 |
| P-2-1 | 60.646 | 58.802 | 61.270 | 63.784 |
| P-2-2 | 59.242 | 59.588 | 60.840 | 65.907 |
| P-2-3 | 61.362 | 63.680 | 65.390 | 68.081 |
| P-2-4 | 61.425 | 63.839 | 64.345 | 63.633 |
| P-2-5 | 59.842 | 59.728 | 65.078 | 67.747 |
| P-3-1 | 51.388 | 57.997 | 57.799 | 55.696 |
| P-3-2 | 52.879 | 54.424 | 53.168 | 63.546 |
| P-3-3 | 53.354 | 52.659 | 51.840 | 63.108 |
| P-3-4 | 53.098 | 56.157 | 47.589 | 61.894 |
| P-3-5 | 51.511 | 53.546 | 53.189 | 58.735 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

### 1.7.2 Exchangeable, water, and acid soluble Zn fraction (F1)

**Table A-8.** Total Zn concentration in F1 obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | Zn in F1 [mg/kg] for the single treatments and growth stages | | | |
|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage |
| P-0-1 | 577.919 | 603.084 | 483.539 | 603.119 |
| P-0-2 | 576.010 | 615.788 | 495.261 | 614.844 |
| P-0-3 | 568.019 | 602.562 | 448.494 | 615.096 |
| P-0-4 | 560.247 | 600.855 | 470.442 | 639.042 |
| P-0-5 | 589.143 | 557.792 | 461.588 | 633.931 |
| P-1-1 | 558.706 | 527.782 | 430.597 | 590.692 |
| P-1-2 | 592.934 | 506.389 | 474.145 | 613.522 |
| P-1-3 | 593.054 | 521.993 | 455.923 | 636.526 |
| P-1-4 | 556.704 | 501.472 | 443.669 | 647.522 |
| P-1-5 | 582.324 | 507.348 | 448.885 | 602.254 |
| P-2-1 | 581.738 | 513.682 | 498.714 | 632.725 |
| P-2-2 | 570.060 | 484.607 | 471.168 | 613.909 |
| P-2-3 | 577.316 | 489.168 | 478.133 | 646.435 |
| P-2-4 | 565.438 | 493.190 | 446.884 | 616.146 |
| P-2-5 | 573.077 | 485.849 | 479.409 | 589.684 |
| P-3-1 | 603.065 | 535.653 | 520.911 | 650.020 |
| P-3-2 | 608.886 | 509.107 | 503.410 | 653.785 |
| P-3-3 | 598.072 | 500.714 | 522.936 | 625.697 |
| P-3-4 | 605.607 | 495.848 | 539.325 | 638.166 |
| P-3-5 | 619.672 | 479.433 | 530.462 | 559.328 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

### 1.7.3 Iron-manganese oxides associated Cd fraction (F2)

**Table A-9.** Total Cd concentration in F2 obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | Cd in F2 [mg/kg] for the single treatments and growth stages | | | |
|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage |
| P-0-1 | 20.432 | 16.648 | 16.564 | 19.852 |
| P-0-2 | 20.374 | 20.563 | 15.857 | 24.318 |
| P-0-3 | 21.167 | 19.601 | 16.765 | 21.374 |
| P-0-4 | 23.197 | 19.157 | 17.942 | 22.862 |
| P-0-5 | 21.607 | 17.772 | 20.243 | 22.943 |
| P-1-1 | 21.769 | 17.522 | 15.286 | 19.829 |
| P-1-2 | 20.367 | 16.438 | 17.861 | 23.275 |
| P-1-3 | 20.040 | 17.138 | 15.604 | 24.164 |
| P-1-4 | 19.156 | 18.500 | 15.188 | 22.929 |
| P-1-5 | 19.453 | 16.873 | 16.423 | 18.620 |
| P-2-1 | 24.047 | 19.570 | 19.956 | 23.538 |
| P-2-2 | 23.086 | 16.793 | 16.532 | 21.966 |
| P-2-3 | 21.239 | 17.252 | 19.102 | 22.805 |
| P-2-4 | 22.775 | 18.550 | 16.645 | 24.052 |
| P-2-5 | 22.329 | 18.106 | 17.175 | 20.549 |
| P-3-1 | 31.059 | 20.210 | 22.293 | 30.828 |
| P-3-2 | 24.718 | 25.943 | 23.622 | 29.359 |
| P-3-3 | 28.042 | 22.956 | 22.040 | 27.139 |
| P-3-4 | 23.461 | 23.494 | 25.910 | 29.013 |
| P-3-5 | 24.954 | 21.277 | 23.191 | 25.190 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

### 1.7.4 Iron-manganese oxides associated Zn fraction (F2)

**Table A-10.** Total Zn concentration in F2 obtained for different levels of P application, growth stages, and for every replicate.

| Treatment[a] | Zn in F2 [mg/kg] for the single treatments and growth stages | | | |
|---|---|---|---|---|
| | Initial Stage | Vegetative Stage | Panicle Stage | Maturity Stage |
| P-0-1 | 588.702 | 494.446 | 574.293 | 599.040 |
| P-0-2 | 580.068 | 517.971 | 512.657 | 588.268 |
| P-0-3 | 542.874 | 518.715 | 536.653 | 576.757 |
| P-0-4 | 586.860 | 526.247 | 570.426 | 611.497 |
| P-0-5 | 575.065 | 489.876 | 525.493 | 618.877 |
| P-1-1 | 517.173 | 505.880 | 501.551 | 584.010 |
| P-1-2 | 552.495 | 520.479 | 485.879 | 596.889 |
| P-1-3 | 508.982 | 513.790 | 542.920 | 572.278 |
| P-1-4 | 493.800 | 524.835 | 506.835 | 620.179 |
| P-1-5 | 548.129 | 488.893 | 521.857 | 554.083 |
| P-2-1 | 520.574 | 507.977 | 524.167 | 604.879 |
| P-2-2 | 530.539 | 522.615 | 505.320 | 602.518 |
| P-2-3 | 527.316 | 531.840 | 549.611 | 608.250 |
| P-2-4 | 546.855 | 556.153 | 516.076 | 598.932 |
| P-2-5 | 515.644 | 516.221 | 554.264 | 593.715 |
| P-3-1 | 498.295 | 448.219 | 484.961 | 481.721 |
| P-3-2 | 500.685 | 505.537 | 503.818 | 544.422 |
| P-3-3 | 479.363 | 495.595 | 366.240 | 521.952 |
| P-3-4 | 495.704 | 528.416 | 303.274 | 530.369 |
| P-3-5 | 500.691 | 472.577 | 350.810 | 544.506 |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil.

## 1.8 Dry weight of the plant samples

### 1.8.1 Vegetative Stage

**Table A-11.** Dry weight of plant samples obtained for different levels of P application, plant part, and for every replicate.

| Treatment[a] | Dry weight [g/pot] for the single treatments and plant parts | | | |
|---|---|---|---|---|
| | Root | Straw | Panicle | Grain |
| P-0-1 | 0.20 | 0.50 | -[b] | - |
| P-0-2 | 0.05 | 0.05 | - | - |
| P-0-3 | 0.50 | 2.00 | - | - |
| P-0-4 | 0.10 | 0.70 | - | - |
| P-0-5 | 1.20 | 5.70 | - | - |
| P-1-1 | 1.00 | 3.80 | - | - |
| P-1-2 | 0.05 | 0.20 | - | - |
| P-1-3 | 0.05 | 0.20 | - | - |
| P-1-4 | 1.10 | 4.80 | - | - |
| P-1-5 | 2.80 | 7.20 | - | - |
| P-2-1 | 0.20 | 0.30 | - | - |
| P-2-2 | 0.05 | 0.05 | - | - |
| P-2-3 | 0.05 | 0.05 | - | - |
| P-2-4 | 0.05 | 0.05 | - | - |
| P-2-5 | 4.00 | 13.00 | - | - |
| P-3-1 | 0.05 | 0.05 | - | - |
| P-3-2 | 4.50 | 11.40 | - | - |
| P-3-3 | 0.10 | 0.10 | - | - |
| P-3-4 | 0.70 | 2.20 | - | - |
| P-3-5 | 4.40 | 12.10 | - | - |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil; [b]this part do not exist for this growth stage.

### 1.8.2 Panicle Stage

**Table A-12.** Dry weight of plant samples obtained for different levels of P application, plant part, and for every replicate.

| Treatment[a] | Dry weight [g/pot] for the single treatments and plant parts | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| P-0-1 | 1.10 | 8.40 | 1.20 | -[b] |
| P-0-2 | 4.40 | 19.30 | 2.70 | - |
| P-0-3 | 2.40 | 7.50 | | - |
| P-0-4 | 1.70 | 12.90 | 3.00 | - |
| P-0-5 | 1.80 | 9.60 | | - |
| P-1-1 | | | | - |
| P-1-2 | 5.20 | 23.60 | 5.60 | - |
| P-1-3 | 1.80 | 7.90 | 0.50 | - |
| P-1-4 | 1.90 | 11.60 | 3.90 | - |
| P-1-5 | 3.70 | 23.80 | 5.00 | - |
| P-2-1 | 4.10 | 29.40 | 13.90 | - |
| P-2-2 | 7.00 | 26.40 | 5.60 | - |
| P-2-3 | 1.20 | 3.00 | | - |
| P-2-4 | | | | - |
| P-2-5 | 11.00 | 23.80 | 6.7 | - |
| P-3-1 | 6.50 | 28.10 | 9.90 | - |
| P-3-2 | 13.10 | 39.40 | 7.40 | - |
| P-3-3 | | | | - |
| P-3-4 | | | | - |
| P-3-5 | 3.20 | 12.00 | 0.50 | - |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil; [b]this part do not exist for this growth stage.

### 1.8.3 Maturity Stage

**Table A-13.** Dry weight of plant samples obtained for different levels of P application, plant part, and for every replicate.

| Treatment[a] | Dry weight [g/pot] for the single treatments and plant parts | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| P-0-1 | 1.70 | 6.30 | -[b] | 4.80 |
| P-0-2 | 1.60 | 5.20 | - | 1.80 |
| P-0-3 | 3.40 | 23.40 | - | 7.60 |
| P-0-4 | 2.00 | 16.60 | - | 7.00 |
| P-0-5 | 1.10 | 10.30 | - | 2.10 |
| P-1-1 | 2.90 | 26.10 | - | 17.30 |
| P-1-2 | 3.50 | 12.70 | - | 5.80 |
| P-1-3 | 6.20 | 34.50 | - | 16.60 |
| P-1-4 | 2.80 | 24.90 | - | 20.80 |
| P-1-5 | 4.60 | 13.30 | - | 7.00 |
| P-2-1 | 11.90 | 31.80 | - | 15.90 |
| P-2-2 | 9.70 | 38.90 | - | 16.80 |
| P-2-3 | 8.10 | 33.90 | - | 10.30 |
| P-2-4 | 7.80 | 30.70 | - | 6.10 |
| P-2-5 | | | - | |
| P-3-1 | | | - | |
| P-3-2 | | | - | |
| P-3-3 | | | - | |
| P-3-4 | | | - | |
| P-3-5 | | | - | |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil; [b]this part do not exist for this growth stage.

## 1.9 Cd and Zn concentration in plants

### 1.9.1 Vegetative stage

**Table A-14.** Cd concentration for plant part samples obtained for different levels of P application and for every replicate.

| Treatment[a] | Cd concentration [mg/kg] for the single treatments and plant parts | | | |
|---|---|---|---|---|
| | Root | Straw | Panicle | Grain |
| P-0-1 | 0.254 | 0.770 | - | - |
| P-0-2 | 49.703 | 8.853 | - | - |
| P-0-3 | 0.129 | 0.826 | - | - |
| P-0-4 | 0.221 | 1.305 | - | - |
| P-0-5 | 0.666 | 1.020 | - | - |
| P-1-1 | 1.406 | 0.952 | - | - |
| P-1-2 | 2.436 | 7.660 | - | - |
| P-1-3 | 0.609 | 4.152 | - | - |
| P-1-4 | 1.407 | 0.141 | - | - |
| P-1-5 | 1.407 | 0.770 | - | - |
| P-2-1 | 11.022 | 1.262 | - | - |
| P-2-2 | 0.201 | 3.049 | - | - |
| P-2-3 | 0.513 | 2.574 | - | - |
| P-2-4 | 0.069 | 4.576 | - | - |
| P-2-5 | 1.623 | 0.866 | - | - |
| P-3-1 | 22.780 | 5.548 | - | - |
| P-3-2 | 0.956 | 1.007 | - | - |
| P-3-3 | 0.978 | 1.276 | - | - |
| P-3-4 | 1.801 | 0.891 | - | - |
| P-3-5 | 1.535 | 0.958 | - | - |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil; [b]this part do not exist for this growth stage.

**Table A-15.** Zn concentration for plant part samples obtained for different levels of P application and for every replicate.

| Treatment[a] | Zn concentration [mg/kg] for the single treatments and plant parts | | | |
|---|---|---|---|---|
| | Root | Straw | Panicle | Grain |
| P-0-1 | 2545.348 | 50.946 | - | - |
| P-0-2 | 2690.520 | 176.757 | - | - |
| P-0-3 | 775.993 | 86.547 | - | - |
| P-0-4 | 2459.375 | 125.299 | - | - |
| P-0-5 | 814.513 | 85.528 | - | - |
| P-1-1 | 686.300 | | - | - |
| P-1-2 | 2187.615 | 715.675 | - | - |
| P-1-3 | 1833.102 | 188.763 | - | - |
| P-1-4 | 648.439 | 56.358 | - | - |
| P-1-5 | 634.738 | 63.919 | - | - |
| P-2-1 | 1854.867 | 60.050 | - | - |
| P-2-2 | 1715.152 | 163.444 | - | - |
| P-2-3 | 1928.105 | 495.238 | - | - |
| P-2-4 | 749.746 | 216.721 | - | - |
| P-2-5 | 567.821 | 67.662 | - | - |
| P-3-1 | 4314.286 | 475.000 | - | - |
| P-3-2 | 1074.432 | 77.900 | - | - |
| P-3-3 | 2469.202 | 51.038 | - | - |
| P-3-4 | 1102.169 | 54.936 | - | - |
| P-3-5 | 788.003 | 61.509 | - | - |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil; [b]this part do not exist for this growth stage.

### 1.9.2 Panicle stage

**Table A-16.** Cd concentration for plant part samples obtained for different levels of P application and for every replicate.

| Treatment[a] | Cd concentration [mg/kg] for the single treatments and plant parts | | | |
|---|---|---|---|---|
| | Root | Straw | Panicle | Grain |
| P-0-1 | 0.042 | 0.631 | 0.108 | -[b] |
| P-0-2 | 0.144 | 0.965 | 0.994 | - |
| P-0-3 | 0.064 | 0.445 | | - |
| P-0-4 | 0.466 | 0.753 | 0.564 | - |
| P-0-5 | 1.301 | 0.845 | | - |
| P-1-1 | | | | - |
| P-1-2 | 1.262 | 0.759 | 0.817 | - |
| P-1-3 | 1.519 | 0.446 | 0.106 | - |
| P-1-4 | 1.487 | 0.005 | 0.201 | - |
| P-1-5 | 1.335 | 0.615 | 0.752 | - |
| P-2-1 | 1.376 | 1.184 | 1.095 | - |
| P-2-2 | 1.314 | 0.625 | 0.376 | - |
| P-2-3 | 1.961 | 0.768 | | - |
| P-2-4 | | | | - |
| P-2-5 | 1.541 | 0.853 | 0.695 | - |
| P-3-1 | 1.136 | 1.133 | 0.577 | - |
| P-3-2 | 1.044 | 0.956 | | - |
| P-3-3 | | | | - |
| P-3-4 | | | | - |
| P-3-5 | 0.532 | 0.124 | | - |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil; [b]this part do not exist for this growth stage.

**Table A-17.** Zn concentration for plant part samples obtained for different levels of P application and for every replicate.

| Treatment[a] | Zn concentration [mg/kg] for the single treatments and plant parts | | | |
|---|---|---|---|---|
| | Root | Straw | Panicle | Grain |
| P-0-1 | 834.503 | 63.793 | 42.251 | -[b] |
| P-0-2 | 1006.254 | 60.931 | 75.685 | - |
| P-0-3 | 821.447 | 39.214 | | - |
| P-0-4 | 972.727 | 54.440 | 48.119 | - |
| P-0-5 | 1066.724 | 61.085 | | - |
| P-1-1 | | | | - |
| P-1-2 | 999.467 | 51.206 | 144.916 | - |
| P-1-3 | 844.748 | 47.788 | 62.701 | - |
| P-1-4 | 920.388 | 102.365 | 66.286 | - |
| P-1-5 | 803.782 | 39.662 | 105.974 | - |
| P-2-1 | 813.476 | 97.588 | 78.772 | - |
| P-2-2 | 1096.605 | 49.947 | 38.313 | - |
| P-2-3 | 636.183 | 34.778 | | - |
| P-2-4 | | | | - |
| P-2-5 | 821.547 | 40.153 | 42.389 | - |
| P-3-1 | 999.068 | 95.346 | 31.711 | - |
| P-3-2 | 824.643 | 54.191 | | - |
| P-3-3 | | | | - |
| P-3-4 | | | | - |
| P-3-5 | 836.081 | 12.193 | | - |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil; [b]this part do not exist for this growth stage.

### 1.9.3 Maturity Stage

**Table A-18.** Cd concentration for plant part samples obtained for different levels of P application and for every replicate.

| Treatment[a] | Cd concentration [mg/kg] for the single treatments and plant parts | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| P-0-1 | 1.338 | 1.417 | -[b] | 0.153 |
| P-0-2 | 1.785 | 0.871 | - | 0.212 |
| P-0-3 | 1.112 | 1.709 | - | 0.627 |
| P-0-4 | 1.325 | 0.058 | - | 0.324 |
| P-0-5 | 1.307 | 0.027 | - | 0.317 |
| P-1-1 | 1.029 | 0.009 | - | 0.378 |
| P-1-2 | 1.015 | 0.007 | - | 0.499 |
| P-1-3 | 0.858 | 0.001 | - | 0.215 |
| P-1-4 | 0.817 | 0.045 | - | 0.528 |
| P-1-5 | 1.461 | 0.003 | - | 0.223 |
| P-2-1 | 0.512 | 0.034 | - | 1.523 |
| P-2-2 | 0.677 | 0.012 | - | 0.025 |
| P-2-3 | 0.750 | 0.019 | - | 1.561 |
| P-2-4 | 0.787 | 0.027 | - | 1.009 |
| P-2-5 | | | - | |
| P-3-1 | | | - | |
| P-3-2 | | | - | |
| P-3-3 | | | - | |
| P-3-4 | | | - | |
| P-3-5 | | | - | |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil; [b]this part do not exist for this growth stage.

**Table A-19.** Zn concentration for plant part samples obtained for different levels of P application and for every replicate.

| Treatment[a] | Zn concentration [mg/kg] for the single treatments and plant parts | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| P-0-1 | 825.604 | 60.535 | -[b] | 21.375 |
| P-0-2 | 627.787 | 55.367 | - | 70.870 |
| P-0-3 | 1002.396 | 96.311 | - | 50.325 |
| P-0-4 | 841.841 | 44.556 | - | 30.728 |
| P-0-5 | 569.376 | 75.086 | - | 36.576 |
| P-1-1 | 983.726 | 82.218 | - | 37.765 |
| P-1-2 | 964.770 | 57.797 | - | 40.549 |
| P-1-3 | 972.880 | 90.409 | - | 38.867 |
| P-1-4 | 987.802 | 172.154 | - | 29.383 |
| P-1-5 | 932.713 | 56.212 | - | 27.999 |
| P-2-1 | 1034.674 | 102.894 | - | 33.042 |
| P-2-2 | 938.254 | 148.998 | - | 57.366 |
| P-2-3 | 913.211 | 157.246 | - | 101.219 |
| P-2-4 | 886.291 | 165.250 | - | 134.476 |
| P-2-5 | | | - | |
| P-3-1 | | | - | |
| P-3-2 | | | - | |
| P-3-3 | | | - | |
| P-3-4 | | | - | |
| P-3-5 | | | - | |

[a]P-0: 0 mg P/kg soil, P-1: 50 mg P/kg soil, P-2: 200 mg P/kg soil, P-3: 1000 mg P/kg soil; [b]this part do not exist for this growth stage.

# 2 Field experiment

## 2.1 pH values

**Table A-20.** pH values for the different field sites and growth stages.

| Growth Stage | pH values for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | LCS[a]-Ctrl | LCS[b]-P | HCS[c]-Ctrl | HCS[d]-P |
| **Background** | 6.28 | 6.30 | 7.66 | 7.23 |
| | 6.33 | 6.48 | 7.50 | 7.64 |
| | 6.17 | 6.44 | 7.70 | 7.24 |
| | 5.78 | 6.58 | 7.75 | 7.69 |
| | 6.38 | 6.61 | 7.42 | 7.64 |
| **Background-P** | 6.60 | 6.99 | 7.33 | 7.38 |
| | 6.76 | 6.63 | 7.22 | 7.39 |
| | 6.43 | 6.72 | 7.10 | 7.42 |
| | 6.63 | 6.76 | 7.23 | 7.47 |
| | 6.66 | 6.71 | 7.24 | 7.36 |
| **Vegetative Stage** | 6.59 | 6.59 | 7.02 | 6.86 |
| | 6.34 | 6.68 | 7.05 | 6.92 |
| | 6.51 | 6.55 | 6.96 | 7.04 |
| | 6.57 | 6.74 | 7.00 | 7.05 |
| | 6.64 | 6.70 | 6.88 | 6.97 |
| **Panicle Stage** | 6.30 | 6.34 | 7.21 | 7.16 |
| | 6.40 | 6.16 | 7.11 | 7.04 |
| | 5.62 | 5.54 | 7.03 | 7.06 |
| | 6.30 | 6.73 | 7.20 | 7.09 |
| | 6.38 | 6.05 | 7.26 | 7.21 |
| **Maturity Stage** | 5.81 | 5.65 | 7.23 | 7.07 |
| | 6.44 | 6.48 | 7.50 | 6.81 |
| | 6.57 | 5.84 | 7.39 | 6.78 |
| | 5.89 | 5.70 | 7.54 | 6.75 |
| | 5.78 | 6.07 | 7.55 | 7.37 |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg.

## 2.2 Redox potentials

**Table A-21.** Redox potentials for the different field sites and growth stages.

| Growth Stage | Redox potential [mV] values for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | **LCS[a]-Ctrl** | **LCS[b]-P** | **HCS[c]-Ctrl** | **HCS[d]-P** |
| **Background** | 467.30 | -107.90 | -9.40 | -85.20 |
| | 135.60 | -170.20 | -144.70 | 64.50 |
| | 11.70 | -166.40 | -83.30 | 41.20 |
| | -16.30 | -160.60 | -82.90 | -41.60 |
| | -34.60 | -98.60 | 90.90 | -19.10 |
| **Background-P** | -118.90 | -154.10 | -163.30 | -216.60 |
| | -235.10 | -172.20 | -195.40 | -251.50 |
| | -216.40 | -244.70 | -132.20 | -231.10 |
| | -164.70 | -227.20 | -116.90 | -176.70 |
| | -242.20 | -189.80 | -172.00 | -198.10 |
| **Vegetative Stage** | -64.60 | -154.10 | 26.60 | -60.80 |
| | -75.30 | -172.20 | -121.00 | -196.00 |
| | -197.30 | -244.70 | -62.50 | 166.90 |
| | -211.30 | -227.20 | 48.00 | -138.20 |
| | -184.60 | -189.80 | 2.90 | -137.60 |
| **Panicle Stage** | 7.80 | -118.30 | -25.90 | -272.40 |
| | 240.40 | 109.40 | -254.70 | -268.10 |
| | 133.50 | 130.20 | -61.70 | -233.10 |
| | -36.30 | -281.90 | -39.00 | -205.00 |
| | -172.80 | -139.90 | -135.40 | -433.30 |
| **Maturity Stage** | 228.90 | 255.10 | 110.30 | 173.60 |
| | 196.00 | 261.30 | 160.10 | 134.40 |
| | 217.30 | 252.50 | 175.30 | 127.50 |
| | 187.50 | -74.90 | 220.30 | 185.40 |
| | 498.90 | 101.30 | 212.10 | 120.80 |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg.

## 2.3 Total phosphorus

**Table A-22.** Total phosphorus concentration for the different field sites and growth stages.

| Growth Stage | Total phosphorus [mg/kg] concentration for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | **LCS[a]-Ctrl** | **LCS[b]-P** | **HCS[c]-Ctrl** | **HCS[d]-P** |
| **Background** | 274.013 | 228.797 | 382.467 | 387.247 |
| | 287.811 | 275.650 | 529.135 | |
| | 276.753 | 254.011 | 181.954 | 435.429 |
| | 734.336 | 567.745 | 688.440 | 388.488 |
| | 199.081 | 220.696 | 387.734 | 591.921 |
| **Background-P** | 268.750 | 275.494 | 459.157 | 485.715 |
| | 393.380 | 249.951 | 626.729 | 485.715 |
| | 279.816 | 214.067 | 544.620 | 905.632 |
| | 588.990 | 621.813 | 873.966 | 493.100 |
| | 315.233 | 261.367 | 514.004 | 983.087 |
| **Vegetative Stage** | 318.452 | 411.531 | 450.442 | 455.053 |
| | 383.222 | 651.026 | 426.211 | 447.683 |
| | 258.094 | 311.348 | 469.617 | 443.590 |
| | 310.734 | 395.280 | 456.458 | 444.925 |
| | 382.728 | 417.722 | 451.779 | 449.861 |
| **Panicle Stage** | 280.788 | 232.597 | 423.901 | 472.582 |
| | 275.558 | 234.770 | 464.067 | 416.930 |
| | 425.665 | 237.335 | 408.493 | 436.389 |
| | 274.038 | 244.058 | 426.635 | 389.041 |
| | 262.987 | 235.952 | 416.372 | 534.692 |
| **Maturity Stage** | 254.354 | 191.747 | 439.746 | 399.254 |
| | 271.542 | 218.208 | 435.777 | 440.176 |
| | 269.268 | 224.114 | 434.563 | 418.248 |
| | 271.554 | 355.566 | 418.625 | 419.655 |
| | 270.577 | 253.846 | 380.847 | 439.444 |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg.

## 2.4 Available phosphorus

**Table A-23.** Available phosphorus concentration for the different field sites and growth stages.

| Growth Stage | Available phosphorus [mg/kg] concentration for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | **LCS[a]-Ctrl** | **LCS[b]-P** | **HCS[c]-Ctrl** | **HCS[d]-P** |
| **Background** | 14.406 | 14.976 | 3.563 | 6.965 |
| | 9.576 | 6.972 | 1.864 | 13.206 |
| | 8.672 | 25.056 | 4.622 | 13.205 |
| | 11.341 | 29.396 | 2.512 | 50.999 |
| | 6.324 | 21.248 | 1.782 | |
| **Background-P** | 12.885 | 43.792 | 3.482 | 31.467 |
| | 13.298 | 23.724 | 4.215 | 11.905 |
| | 10.688 | 36.425 | 3.731 | 24.294 |
| | 13.459 | 30.405 | 7.779 | 31.583 |
| | 13.864 | 14.509 | 6.245 | 72.894 |
| **Vegetative Stage** | 10.731 | 57.857 | 3.782 | 11.976 |
| | 14.905 | 46.793 | 2.317 | 11.342 |
| | 11.116 | 41.521 | 2.395 | 6.096 |
| | 10.354 | 39.499 | 6.257 | 5.638 |
| | 12.825 | 43.532 | 2.162 | 1.312 |
| **Panicle Stage** | 8.398 | 45.821 | 1.702 | 15.567 |
| | 12.276 | 39.273 | 1.216 | 1.946 |
| | 14.173 | 38.345 | 1.621 | 9.893 |
| | 14.835 | 41.033 | 2.098 | 10.931 |
| | 11.741 | 40.337 | 1.538 | 12.070 |
| **Maturity Stage** | 6.099 | 38.754 | 1.899 | 10.638 |
| | 11.975 | 22.580 | 1.897 | 14.609 |
| | 8.298 | 41.518 | 0.680 | 9.407 |
| | 14.831 | 50.519 | 2.758 | 16.462 |
| | 13.753 | 47.323 | 2.439 | 11.674 |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg.

## 2.5 Total Cd

**Table A-24.** Total Cd concentration for the different field sites and growth stages.

| Growth Stage | Total Cd [mg/kg] concentration for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | **LCS[a]-Ctrl** | **LCS[b]-P** | **HCS[c]-Ctrl** | **HCS[d]-P** |
| **Background** | 0.246 | 0.248 | 74.615 | 28.099 |
| | 0.250 | 0.265 | 72.227 | 30.264 |
| | 0.446 | 0.306 | 81.902 | 7.509 |
| | 0.357 | 0.276 | 72.380 | |
| | 0.270 | | 73.365 | 27.941 |
| **Background-P** | 0.332 | 0.248 | 70.054 | 24.215 |
| | 0.248 | 0.166 | 56.433 | 24.121 |
| | 0.349 | 0.241 | 75.794 | 14.328 |
| | 0.288 | 0.269 | 56.622 | 34.053 |
| | 0.380 | 0.253 | 75.732 | 27.838 |
| **Vegetative Stage** | 0.249 | 0.166 | 70.671 | 20.919 |
| | 0.166 | 0.083 | 66.058 | 24.396 |
| | 0.378 | 0.237 | 64.461 | 29.068 |
| | 0.365 | 0.280 | 65.968 | 33.004 |
| | 0.488 | 0.283 | 76.871 | 36.911 |
| **Panicle Stage** | 0.115 | 0.093 | | 29.214 |
| | 0.134 | 0.194 | 62.457 | 39.730 |
| | 0.286 | 0.338 | 55.088 | 32.899 |
| | 0.444 | 0.372 | 75.660 | 38.950 |
| | 0.523 | 0.319 | 67.430 | 39.882 |
| **Maturity Stage** | 0.161 | 0.035 | | 22.289 |
| | 0.091 | 0.040 | 61.005 | 27.207 |
| | 0.312 | 0.293 | 70.967 | 32.880 |
| | 0.337 | 0.357 | 71.056 | 37.575 |
| | 0.549 | 0.284 | 76.019 | 38.919 |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg.

## 2.6 Total Zn

**Table A-25.** Total Zn concentration for the different field sites and growth stages.

| Growth Stage | Total Zn [mg/kg] concentration for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | **LCS[a]-Ctrl** | **LCS[b]-P** | **HCS[c]-Ctrl** | **HCS[d]-P** |
| **Background** | 139.130 | 62.165 | 3302.044 | 1244.393 |
| | 127.828 | 78.170 | 2905.316 | 1428.924 |
| | 105.311 | 71.040 | 3316.504 | 0.000 |
| | 94.512 | 69.128 | 3579.993 | |
| | 85.736 | | 3358.246 | 1353.856 |
| **Background-P** | 108.056 | 61.496 | 2634.559 | 1247.709 |
| | 84.270 | 53.821 | 1970.603 | 1307.260 |
| | 91.771 | 67.481 | 2762.897 | 1377.424 |
| | 84.272 | 78.906 | 2158.751 | 1382.309 |
| | 88.974 | 79.797 | 3163.720 | 1435.855 |
| **Vegetative Stage** | 89.286 | 55.247 | 3189.202 | 959.520 |
| | 76.950 | 53.671 | 2821.790 | 1212.998 |
| | 82.701 | 49.851 | 2786.872 | 1540.693 |
| | 87.173 | 66.412 | 3213.218 | 1682.127 |
| | 86.895 | 65.542 | 3138.113 | 1643.092 |
| **Panicle Stage** | 155.758 | 98.723 | | 1217.136 |
| | 134.634 | 102.718 | 2577.384 | 1455.490 |
| | 123.818 | 96.581 | 2388.031 | 1404.471 |
| | 124.363 | 82.231 | 2957.261 | 1612.655 |
| | 125.414 | 95.452 | 2353.181 | 1463.722 |
| **Maturity Stage** | 105.376 | 75.206 | | 1019.432 |
| | 93.854 | 80.228 | 1880.420 | 1249.959 |
| | 93.138 | 78.241 | 2176.965 | 1410.935 |
| | 100.166 | 82.772 | 2317.341 | 1533.052 |
| | 109.495 | 75.640 | 2464.712 | 1362.043 |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg.

## 2.7 Cd and Zn fractions

### 2.7.1 Exchangeable, water, and acid soluble Cd fraction (F1)

**Table A-26.** Cd concentration in F1 for the different field sites and growth stages.

| Growth Stage | Cd concentration in F1 [mg/kg] concentration for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | **LCS[a]-Ctrl** | **LCS[b]-P** | **HCS[c]-Ctrl** | **HCS[d]-P** |
| **Background** | 0.032 | 0.418 | 54.810 | 17.900 |
| | 0.069 | 0.417 | 53.416 | |
| | 0.037 | 0.299 | 68.517 | 18.774 |
| | -[e] | - | - | - |
| | - | - | - | - |
| **Background-P** | 0.201 | 0.155 | 50.973 | 14.190 |
| | 0.145 | 0.156 | 36.038 | 20.473 |
| | 0.175 | 0.097 | 39.302 | 15.445 |
| | - | - | - | - |
| | - | - | - | - |
| **Vegetative Stage** | 0.196 | 0.174 | 51.076 | 13.659 |
| | 0.177 | 0.137 | 53.855 | 16.284 |
| | 0.392 | 0.120 | 82.738 | 16.206 |
| | - | - | - | - |
| | - | - | - | - |
| **Panicle Stage** | 0.177 | 0.156 | 34.891 | 14.459 |
| | 0.207 | 0.173 | 45.658 | 23.214 |
| | 0.232 | 0.147 | 35.269 | 17.338 |
| | - | - | - | - |
| | - | - | - | - |
| **Maturity Stage** | 0.164 | 0.184 | 47.014 | 11.689 |
| | 0.197 | 0.227 | 34.558 | 14.214 |
| | 0.180 | 0.158 | 50.306 | 18.293 |
| | - | - | - | - |
| | - | - | - | - |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg; [e]this experiment was performed in triplicates.

### 2.7.2 Exchangeable, water, and acid soluble Zn fraction (F1)

**Table A-27.** Zn concentration in F1 for the different field sites and growth stages.

| Growth Stage | Zn concentration in F1 [mg/kg] concentration for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | LCS[a]-Ctrl | LCS[b]-P | HCS[c]-Ctrl | HCS[d]-P |
| **Background** | 8.565 | 6.978 | 519.178 | 219.405 |
| | 8.410 | 7.541 | 534.199 | |
| | 7.429 | 6.034 | 619.844 | 242.134 |
| | -[e] | - | - | - |
| | - | - | - | - |
| **Background-P** | 6.883 | 6.544 | 547.932 | 190.197 |
| | 6.736 | 7.038 | 422.855 | 252.021 |
| | 6.643 | 6.810 | 465.051 | 230.414 |
| | - | - | - | - |
| | - | - | - | - |
| **Vegetative Stage** | 9.588 | 7.736 | 549.378 | 150.634 |
| | 8.129 | 7.657 | 537.766 | 207.356 |
| | 8.304 | 8.128 | 551.854 | 235.065 |
| | - | - | - | - |
| | - | - | - | - |
| **Panicle Stage** | 8.425 | 7.662 | 2565.157 | 156.599 |
| | 7.659 | 8.195 | 609.258 | 204.467 |
| | 8.493 | 7.854 | 518.371 | 155.432 |
| | - | - | - | - |
| | - | - | - | - |
| **Maturity Stage** | 8.714 | 8.408 | 715.008 | 197.923 |
| | 8.669 | 9.322 | 599.057 | 262.215 |
| | 7.333 | 7.660 | 667.338 | 317.035 |
| | - | - | - | - |
| | - | - | - | - |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg; [e]this experiment was performed in triplicates.

### 2.7.3 Iron-manganese oxides associated Cd fraction (F2)

**Table A-28.** Cd concentration in F2 for the different field sites and growth stages.

| Growth Stage | Cd concentration in F2 [mg/kg] concentration for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | LCS[a]-Ctrl | LCS[b]-P | HCS[c]-Ctrl | HCS[d]-P |
| **Background** | 0.023 | 0.281 | 26.221 | 12.565 |
| | 0.048 | 0.268 | 28.278 | |
| | 0.022 | 0.234 | 22.611 | 12.002 |
| | -[e] | - | - | - |
| | - | - | - | - |
| **Background-P** | 0.104 | 0.073 | 21.374 | 9.780 |
| | 0.093 | 0.089 | 21.529 | 14.808 |
| | 0.132 | 0.080 | 20.278 | 10.918 |
| | - | - | - | - |
| | - | - | - | - |
| **Vegetative Stage** | 0.131 | 0.075 | 29.124 | 9.777 |
| | 0.119 | 0.087 | 21.260 | 10.062 |
| | 0.127 | 0.090 | 31.139 | 12.041 |
| | - | - | - | - |
| | - | - | - | - |
| **Panicle Stage** | 0.165 | 0.131 | 21.744 | 9.350 |
| | 0.173 | 0.162 | 18.493 | 13.910 |
| | 0.176 | 0.154 | 20.731 | 12.029 |
| | - | - | - | - |
| | - | - | - | - |
| **Maturity Stage** | 0.131 | 0.132 | 26.170 | 8.375 |
| | 0.119 | 0.148 | 20.035 | 10.348 |
| | 0.141 | 0.102 | 24.635 | 12.993 |
| | - | - | - | - |
| | - | - | - | - |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg; [e]this experiment was performed in triplicates.

### 2.7.4 Iron-manganese oxides associated Zn fraction (F2)

**Table A-29.** Zn concentration in F2 for the different field sites and growth stages.

| Growth Stage | Zn concentration in F2 [mg/kg] concentration for the single field sites and growth stages | | | |
|---|---|---|---|---|
| | LCS[a]-Ctrl | LCS[b]-P | HCS[c]-Ctrl | HCS[d]-P |
| **Background** | 7.981 | 8.222 | 791.108 | 371.571 |
| | 12.426 | 8.166 | 763.004 | |
| | 10.628 | 7.717 | 747.455 | 422.269 |
| | -[e] | - | - | - |
| | - | - | - | - |
| **Background-P** | 8.968 | 7.176 | 699.349 | 331.393 |
| | 9.620 | 7.947 | 505.061 | 387.335 |
| | 8.352 | 7.449 | 501.329 | 391.469 |
| | - | - | - | - |
| | - | - | - | - |
| **Vegetative Stage** | 9.608 | 7.939 | 667.371 | 256.206 |
| | 11.816 | 8.698 | 496.065 | 321.736 |
| | 9.600 | 7.295 | 527.080 | 418.169 |
| | - | - | - | - |
| | - | - | - | - |
| **Panicle Stage** | 9.214 | 7.949 | 614.608 | 366.231 |
| | 9.048 | 9.004 | 673.947 | 490.768 |
| | 9.884 | 9.412 | 624.667 | 461.639 |
| | - | - | - | - |
| | - | - | - | - |
| **Maturity Stage** | 9.237 | 9.177 | 866.919 | 324.321 |
| | 9.402 | 9.685 | 641.139 | 427.389 |
| | 8.248 | 7.752 | 715.708 | 505.363 |
| | - | - | - | - |
| | - | - | - | - |

[a]LCS-Ctrl: Low concentration site without P addition; [b]LCS-P: Low concentration site with an addition of 50 mg P/kg; [c]HCS-Ctrl: High concentration sites without P addition; [d]HCS-P: High concentration site with an addition of 50 mg P/kg; [e]this experiment was performed in triplicates.

## 2.8 Dry weight of the plant samples

### 2.8.1 Vegetative Stage

**Table A-30.** Dry weight of the different plant parts for the different field sites.

| Field Site[a] | Dry weight [g/pot] for the single field sites and plant parts | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| **LCS-Ctrl** | 6.62 | 26.70 | -[b] | - |
| | 5.80 | 22.70 | - | - |
| | 5.89 | 18.80 | - | - |
| | 8.81 | 39.30 | - | - |
| | 8.40 | 27.20 | - | - |
| **LCS-P** | 7.16 | 19.30 | - | - |
| | 9.59 | 36.40 | - | - |
| | 9.25 | 28.50 | - | - |
| | 7.69 | 20.40 | - | - |
| | 6.32 | 20.90 | - | - |
| **HCS-Ctrl** | 5.37 | 23.60 | - | - |
| | 3.03 | 20.00 | - | - |
| | 4.14 | 28.20 | - | - |
| | 7.01 | 26.70 | - | - |
| | 5.15 | 32.00 | - | - |
| **HCS-P** | 6.23 | 34.00 | - | - |
| | 7.47 | 35.00 | - | - |
| | 8.75 | 48.00 | - | - |
| | 7.26 | 47.30 | - | - |
| | 4.70 | 26.60 | - | - |

[a]LCS-Ctrl: Low concentration site without P addition; LCS-P: Low concentration site with an addition of 50 mg P/kg; HCS-Ctrl: High concentration sites without P addition; HCS-P: High concentration site with an addition of 50 mg P/kg; [b]this part do not exist for this growth stage.

## 2.9 Panicle Stage

**Table A-31.** Dry weight of the different plant parts for the different field sites.

| Field Site[a] | Dry weight [g/pot] for the single field sites and plant parts | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| **LCS-Ctrl** | 13.80 | 128.40 | 19.10 | -[b] |
| | 10.50 | 93.30 | 13.80 | - |
| | 11.80 | 98.50 | 11.80 | - |
| | 26.50 | 194.00 | 26.90 | - |
| | 10.80 | 116.90 | 17.00 | - |
| **LCS-P** | 17.70 | 152.40 | 19.90 | - |
| | 15.60 | 126.90 | 19.20 | - |
| | 14.20 | 112.80 | 15.30 | - |
| | 8.50 | 97.90 | 14.00 | - |
| | 7.90 | 89.40 | 16.50 | - |
| **HCS-Ctrl** | 18.30 | 185.20 | 25.40 | - |
| | 12.40 | 162.40 | 22.10 | - |
| | 13.80 | 182.70 | 22.00 | - |
| | 12.50 | 168.30 | 26.40 | - |
| | 20.70 | 180.40 | 19.60 | - |
| **HCS-P** | 16.40 | 164.20 | 16.00 | - |
| | 29.20 | 282.80 | 34.20 | - |
| | 33.80 | 294.50 | 31.20 | - |
| | 30.10 | 233.40 | 26.00 | - |
| | 20.90 | 136.30 | 14.90 | - |

[a]LCS-Ctrl: Low concentration site without P addition; LCS-P: Low concentration site with an addition of 50 mg P/kg; HCS-Ctrl: High concentration sites without P addition; HCS-P: High concentration site with an addition of 50 mg P/kg; [b]this part do not exist for this growth stage.

## 2.10 Maturity Stage

**Table A-32.** Dry weight of the different plant parts for the different field sites.

| Field Site[a] | Dry weight [g/pot] for the single field sites and plant parts | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| **LCS-Ctrl** | 18.50 | 89.60 | -[b] | 60.30 |
| | 19.00 | 92.80 | - | 61.20 |
| | 16.10 | 53.60 | - | 34.00 |
| | 18.90 | 119.70 | - | 71.40 |
| | 18.90 | 78.20 | - | 73.00 |
| **LCS-P** | 19.10 | 144.70 | - | 84.50 |
| | 19.30 | 88.60 | - | 64.80 |
| | 20.50 | 102.40 | - | 73.40 |
| | 21.80 | 120.40 | - | 61.30 |
| | 21.00 | 120.40 | - | 87.20 |
| **HCS-Ctrl** | 19.50 | 143.10 | - | 72.40 |
| | 15.60 | 131.70 | - | 53.70 |
| | 19.20 | 124.40 | - | 67.60 |
| | 17.50 | 131.70 | - | 58.50 |
| | 16.30 | 107.00 | - | 64.90 |
| **HCS-P** | 21.40 | 121.50 | - | 93.00 |
| | 21.80 | 125.30 | - | 77.40 |
| | 18.00 | 108.30 | - | 70.10 |
| | 20.30 | 162.80 | - | 78.20 |
| | 17.00 | 89.60 | - | 62.50 |

[a]LCS-Ctrl: Low concentration site without P addition; LCS-P: Low concentration site with an addition of 50 mg P/kg; HCS-Ctrl: High concentration sites without P addition; HCS-P: High concentration site with an addition of 50 mg P/kg; [b]this part do not exist for this growth stage.

## 2.11 Cd and Zn concentration in plants

### 2.11.1 Vegetative stage

**Table A-33.** Cd concentration in plant parts for different sites.

| Field Site[a] | Cd concentration [mg/kg] in different plant parts and for the single field sites | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| **LCS-Ctrl** | 0.133 | 0.133 | -[b] | - |
| | 0.596 | 0.000 | - | - |
| | 0.527 | 0.000 | - | - |
| | 0.132 | 0.000 | - | - |
| | 0.532 | 0.000 | - | - |
| **LCS-P** | 0.528 | 0.133 | - | - |
| | 0.100 | 0.066 | - | - |
| | 0.464 | 0.000 | - | - |
| | 0.399 | 0.000 | - | - |
| | 0.335 | 0.000 | - | - |
| **HCS-Ctrl** | 42.635 | 15.087 | - | - |
| | 12.127 | 12.810 | - | - |
| | 13.829 | 2.320 | - | - |
| | 42.247 | 7.780 | - | - |
| | 12.797 | 3.976 | - | - |
| **HCS-P** | 11.424 | 10.222 | - | - |
| | 17.542 | 10.489 | - | - |
| | 17.886 | 4.882 | - | - |
| | 11.356 | 6.362 | - | - |
| | 15.299 | 7.800 | - | - |

[a]LCS-Ctrl: Low concentration site without P addition; LCS-P: Low concentration site with an addition of 50 mg P/kg; HCS-Ctrl: High concentration sites without P addition; HCS-P: High concentration site with an addition of 50 mg P/kg; [b]this part do not exist for this growth stage.

**Table A-34.** Zn concentration in plant parts for different sites.

| Field Site[a] | Zn concentration [mg/kg] in different plant parts and for the single field sites | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| **LCS-Ctrl** | 67.463 | 89.008 | -[b] | - |
| | 86.528 | 28.707 | - | - |
| | 83.241 | 29.536 | - | - |
| | 38.497 | 24.669 | - | - |
| | 38.554 | 24.054 | - | - |
| **LCS-P** | 30.633 | 23.513 | - | - |
| | 40.271 | 28.269 | - | - |
| | 43.319 | 20.894 | - | - |
| | 48.677 | 24.301 | - | - |
| | 36.238 | 23.238 | - | - |
| **HCS-Ctrl** | 516.669 | 155.523 | - | - |
| | 450.630 | 50.845 | - | - |
| | 451.073 | 97.441 | - | - |
| | 483.486 | 113.712 | - | - |
| | 451.847 | 63.610 | - | - |
| **HCS-P** | 221.870 | 72.348 | - | - |
| | 236.085 | 57.754 | - | - |
| | 299.417 | 60.694 | - | - |
| | 334.706 | 74.221 | - | - |
| | 311.923 | 70.069 | - | - |

[a]LCS-Ctrl: Low concentration site without P addition; LCS-P: Low concentration site with an addition of 50 mg P/kg; HCS-Ctrl: High concentration sites without P addition; HCS-P: High concentration site with an addition of 50 mg P/kg; [b]this part do not exist for this growth stage.

### 2.11.2 Panicle stage

**Table A-35.** Cd concentration in plant parts for different sites.

| Field Site[a] | Cd concentration [mg/kg] in different plant parts and for the single field sites | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| **LCS-Ctrl** | 0.089 | 0.532 | 0.033 | -[b] |
| | 0.089 | 0.333 | 0.033 | - |
| | 1.613 | 0.992 | 0.033 | - |
| | 0.054 | 0.000 | 0.033 | - |
| | 0.054 | 0.033 | 0.033 | - |
| **LCS-P** | 0.051 | 0.066 | 0.000 | - |
| | 0.051 | 0.399 | 0.066 | - |
| | 0.051 | 0.664 | 0.099 | - |
| | 0.051 | 0.066 | 0.000 | - |
| | 0.052 | 0.066 | 0.000 | - |
| **HCS-Ctrl** | 125.349 | 1.996 | 0.665 | - |
| | 1.987 | 0.598 | 0.099 | - |
| | 43.408 | 1.931 | 0.662 | - |
| | 57.309 | 1.987 | 0.929 | - |
| | 91.845 | 1.193 | 0.099 | - |
| **HCS-P** | 38.183 | 0.132 | 0.066 | - |
| | 42.453 | 0.330 | 0.066 | - |
| | 81.065 | 0.331 | 0.067 | - |
| | 45.565 | 0.099 | 0.066 | - |
| | 20.573 | 0.927 | 0.000 | - |

[a]LCS-Ctrl: Low concentration site without P addition; LCS-P: Low concentration site with an addition of 50 mg P/kg; HCS-Ctrl: High concentration sites without P addition; HCS-P: High concentration site with an addition of 50 mg P/kg; [b]this part do not exist for this growth stage.

**Table A-36.** Zn concentration in plant parts for different sites.

| Field Site[a] | Zn concentration [mg/kg] in different plant parts and for the single field sites | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| **LCS-Ctrl** | 83.444 | 56.804 | 26.535 | -[b] |
| | 85.105 | 45.935 | 22.639 | - |
| | 90.166 | 75.784 | 31.813 | - |
| | 66.769 | 33.890 | 23.298 | - |
| | 62.597 | 30.414 | 25.257 | - |
| **LCS-P** | 68.943 | 46.725 | 22.247 | - |
| | 75.979 | 50.219 | 29.982 | - |
| | 77.030 | 54.469 | 29.231 | - |
| | 68.743 | 29.360 | 23.047 | - |
| | 61.670 | 40.266 | 26.110 | - |
| **HCS-Ctrl** | 475.063 | 100.253 | 38.635 | - |
| | 320.021 | 51.355 | 32.910 | - |
| | 703.822 | 89.214 | 49.372 | - |
| | 563.633 | 75.364 | 40.900 | - |
| | 1107.717 | 70.170 | 28.077 | - |
| **HCS-P** | 379.766 | 50.217 | 24.769 | - |
| | 417.875 | 36.193 | 28.130 | - |
| | 716.173 | 34.649 | 36.011 | - |
| | 718.471 | 44.053 | 25.771 | - |
| | 448.502 | 48.709 | 19.199 | - |

[a]LCS-Ctrl: Low concentration site without P addition; LCS-P: Low concentration site with an addition of 50 mg P/kg; HCS-Ctrl: High concentration sites without P addition; HCS-P: High concentration site with an addition of 50 mg P/kg; [b]this part do not exist for this growth stage.

### 2.11.3 Maturity stage

**Table A-37.** Cd concentration in plant parts for different sites.

| Field Site[a] | Cd concentration [mg/kg] in different plant parts and for the single field sites | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| **LCS-Ctrl** | 0.124 | 0.538 | -[b] | 0.096 |
| | 0.325 | 0.080 | - | 0.096 |
| | 0.352 | 0.081 | - | 0.095 |
| | 0.317 | 0.081 | - | 0.096 |
| | 1.388 | 0.081 | - | 0.096 |
| **LCS-P** | 0.937 | 0.042 | - | 0.082 |
| | 0.116 | 0.337 | - | 0.056 |
| | 0.113 | 0.119 | - | 0.363 |
| | 0.310 | 0.006 | - | 0.007 |
| | 0.084 | 0.006 | - | 0.007 |
| **HCS-Ctrl** | 23.183 | 3.443 | - | 0.761 |
| | 10.294 | 1.649 | - | 0.448 |
| | 20.128 | 4.508 | - | 0.823 |
| | 16.472 | 2.588 | - | 0.633 |
| | 22.872 | 3.605 | - | 0.866 |
| **HCS-P** | 10.697 | 1.811 | - | 0.440 |
| | 9.186 | 0.770 | - | 0.271 |
| | 11.539 | 1.345 | - | 0.511 |
| | 8.075 | 0.733 | - | 0.273 |
| | 4.745 | 0.430 | - | 0.087 |

[a]LCS-Ctrl: Low concentration site without P addition; LCS-P: Low concentration site with an addition of 50 mg P/kg; HCS-Ctrl: High concentration sites without P addition; HCS-P: High concentration site with an addition of 50 mg P/kg; [b]this part do not exist for this growth stage.

**Table A-38.** Zn concentration in plant parts for different sites.

| Field Site[a] | Zn concentration [mg/kg] in different plant parts and for the single field sites | | | |
|---|---|---|---|---|
| | **Root** | **Straw** | **Panicle** | **Grain** |
| **LCS-Ctrl** | 77.051 | 67.748 | -[b] | 16.114 |
| | 84.429 | 57.685 | - | 13.852 |
| | 98.542 | 71.873 | - | 17.355 |
| | 55.367 | 47.927 | - | 15.115 |
| | 86.540 | 60.124 | - | 14.011 |
| **LCS-P** | 99.578 | 75.367 | - | 15.244 |
| | 58.591 | 64.341 | - | 13.495 |
| | 93.033 | 83.069 | - | 14.647 |
| | 57.150 | 20.580 | - | 12.794 |
| | 72.375 | 55.233 | - | 14.915 |
| **HCS-Ctrl** | 685.620 | 119.814 | - | 14.456 |
| | 697.095 | 88.853 | - | 13.572 |
| | 541.650 | 136.328 | - | 16.358 |
| | 522.101 | 90.214 | - | 13.701 |
| | 671.021 | 122.013 | - | 16.056 |
| **HCS-P** | 260.318 | 74.248 | - | 13.638 |
| | 322.542 | 146.178 | - | 15.937 |
| | 363.754 | 68.263 | - | 15.398 |
| | 342.755 | 62.920 | - | 13.279 |
| | 317.131 | 80.098 | - | 13.431 |

[a]LCS-Ctrl: Low concentration site without P addition; LCS-P: Low concentration site with an addition of 50 mg P/kg; HCS-Ctrl: High concentration sites without P addition; HCS-P: High concentration site with an addition of 50 mg P/kg; [b]this part do not exist for this growth stage.

# 3 Subcellular Fractionation

## 3.1 Roots

**Table A-39.** Subcellular fractionation of Cd in roots.

| Level of P addition [mg/L] | Cd concentration [µg/kg fresh weight] for different levels of P addition and in different fractions | | | |
|---|---|---|---|---|
| | F I[a] | F II[b] | F III[c] | F IV[d] |
| **0** | 268.244 | 381.556 | 140.935 | 352.195 |
| | 269.340 | 242.747 | 174.488 | 282.992 |
| | 263.991 | 400.832 | 201.271 | 315.164 |
| **50** | 281.429 | 283.857 | 196.571 | 174.000 |
| | 256.103 | 218.273 | 137.573 | 311.635 |
| | 247.857 | 483.000 | 261.429 | 395.857 |
| **200** | 265.690 | 247.130 | 142.253 | 263.406 |
| | 272.054 | 284.628 | 260.766 | 257.766 |
| | 261.906 | 334.254 | 173.180 | 263.473 |
| **1000** | 208.214 | 138.534 | 50.556 | 360.240 |
| | 234.966 | 150.836 | 51.621 | 395.086 |
| | 277.730 | 145.904 | 105.845 | 373.720 |

[a]F I: cell wall fraction; [b]F II: chloroplast fraction; [c]F III: membranes and organelles; [d]F IV: soluble fraction.

## 3.2 Shoots

**Table A-40.** Subcellular fractionation of Cd in shoots.

| Level of P addition [mg/L] | Cd concentration [µg/kg fresh weight] for different levels of P addition and in different fractions | | | |
|---|---|---|---|---|
| | F I[a] | F II[b] | F III[c] | F IV[d] |
| **0** | 275.552 | 147.092 | 96.194 | 193.273 |
| | 262.686 | 68.359 | 33.888 | 138.439 |
| | 256.623 | 77.544 | 52.798 | 131.154 |
| **50** | 196.173 | 61.600 | 49.986 | 161.571 |
| | 162.219 | 27.537 | 23.740 | 113.248 |
| | 226.210 | 65.257 | 57.600 | 116.900 |
| **200** | 157.381 | 26.826 | 13.235 | 84.433 |
| | 243.798 | 130.526 | 163.604 | 228.903 |
| | 238.489 | 71.408 | 58.448 | 191.694 |
| **1000** | 99.486 | 15.231 | 5.804 | 48.674 |
| | 109.268 | 15.641 | 9.984 | 31.853 |
| | 82.692 | 16.581 | 7.139 | 41.340 |

[a]F I: cell wall fraction; [b]F II: chloroplast fraction; [c]F III: membranes and organelles; [d]F IV: soluble fraction.

# 4 Chemical Form

## 4.1 Roots

**Table A-41.** Chemical form of Cd in roots.

| Level of P addition [mg/L] | Cd concentration [μg/kg fresh weight] for different levels of P addition and in different fractions | | | | | |
|---|---|---|---|---|---|---|
| | EtOH[a] | DI[b] | NaCl[c] | HAc[d] | HCl[e] | Res[f] |
| 0 | 93.136 | 89.057 | 0.000 | 91.664 | 6.118 | 0.206 |
| | 95.360 | 90.405 | 0.000 | 99.310 | 5.675 | 0.197 |
| 50 | 87.777 | 93.008 | 627.412 | 78.095 | 3.485 | 0.598 |
| | 87.912 | 92.906 | 595.820 | 84.971 | 2.911 | 0.570 |
| 200 | 87.050 | 89.056 | 731.764 | 52.228 | 1.960 | 0.384 |
| | 85.310 | 86.268 | 409.407 | 47.520 | 2.039 | 0.369 |
| 1000 | 72.330 | 108.186 | 45.311 | 19.076 | 1.643 | 0.212 |
| | 72.575 | 114.476 | 41.478 | 20.739 | 1.623 | 0.196 |

[a]EtOH: ethanol-extractable Cd, [b]DI: deionized water-extractable Cd, [c]NaCl: sodium chloride-extractable Cd, [d]HAc: acetic acid-extractable Cd, [e]HCl: hydrocloric acid-extractable Cd, [f]Res: residual Cd.

## 4.2 Shoots

**Table A-42.** Chemical form of Cd in shoots.

| Level of P addition [mg/L] | Cd concentration [μg/kg fresh weight] for different levels of P addition and in different fractions | | | | | |
|---|---|---|---|---|---|---|
| | EtOH[a] | DI[b] | NaCl[c] | HAc[d] | HCl[e] | Res[f] |
| 0 | 22.276 | 32.647 | 197.880 | 150.432 | 3.096 | 0.082 |
| | 21.938 | 32.070 | 183.816 | 135.925 | 3.277 | 0.087 |
| 50 | 19.866 | 23.024 | 40.392 | 124.582 | 1.958 | 0.072 |
| | 19.621 | 23.124 | 52.262 | 149.836 | 2.095 | 0.071 |
| 200 | 28.150 | 29.931 | 96.252 | 56.573 | 0.507 | 1.086 |
| | 27.827 | 30.136 | 267.265 | 67.646 | 0.491 | 0.837 |
| 1000 | 14.410 | 7.973 | 144.168 | 4.559 | 1.142 | 0.554 |
| | 14.636 | 8.643 | 3.284 | 18.788 | 0.999 | 0.545 |

[a]EtOH: ethanol-extractable Cd, [b]DI: deionized water-extractable Cd, [c]NaCl: sodium chloride-extractable Cd, [d]HAc: acetic acid-extractable Cd, [e]HCl: hydrocloric acid-extractable Cd, [f]Res: residual Cd.

# BIOGRAPHY

Nina Siebers was born on the 22nd May, 1985 in Wesel, Germany. She started to study at the University Duisburg-Essen Faculty of Chemistry in 2004 and graduated in the Bachelor Degree of Science in the major of Water Science. Then, she continued her further education for the Master Degree at the International Post-graduated Program in Environmental Management, a joint program of National Center of Excellence for Environmental and Hazardous Waste Management (NCE-EHWM) in May 2008.

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