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Cadmium and zinc uptake in wheat as affected by nitrogen fertilization and agricultural management

Master thesis
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Acknowledgments

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Abstract

Mineral deficiencies, including zinc (Zn) deficiency are a major risk factor for global human health. Zn deficiency is mainly related to monotonous diets based on cereals and pulses. Wheat grain is a poor source of Zn for humans as they contain anti-nutrients such as phytate. In addition wheat is a major source of toxic cadmium (Cd) in human foods. Zn fertilization is a biofortification option to increase the total Zn concentration in wheat grains. In addition the fertilization of nitrogen (N) has also shown to improve the Zn status of plants. This project investigated the influence of N fertilization on the accumulation of Zn and Cd in wheat plants grown on either organically or conventionally managed soil, as well as the interactions between Zn and Cd in soils and plants.

The soils were spiked with 2mg/kg Cd, 340 mg/kg Zn or both for the combination treatment. Wheat (Fiorina) was grown until full maturity in pots in a climate chamber. Two levels of N (50 mg/kg and 150 mg/kg) were fertilized along with all other essential nutrients. Amongst others the total metal concentrations and N contents in soil and plants were measured.

Spiking of the soils significantly increased the metal uptake of the wheat plants. The Zn concentrations in the plants were not affected by the addition of Cd. However Cd concentrations in the shoots and grains were reduced for the Zn spiked treatments. The wheat grown with high N fertilization did take up more Zn and Cd compared to those with low N fertilization. This effect was only significant for the total contents per plant and not for the concentration results due to growth dilution. The two agricultural management strategies showed only marginal effects.

Direct competition, protection against Cd toxicity and a soil effect that immobilised plant available Cd in the Zn treated soils are the most likely causes of the observed antagonistic interaction between Zn and Cd. N can increase the uptake of metals in plants through larger plant biomass, reducing the soil pH and increasing the activity of metal transport proteins in the cell membranes.

This work has shown that both Zn and N fertilization can significantly increase the amount of Zn in grains and therefore improve its nutritional value. Furthermore Zn fertilization can reduce the amount of Cd in grains. However, the opposite has been shown in other studies. The interactions depend on total and relative Zn and Cd concentrations, the nutritional status of the plants and the plant species.

Zusammenfassung

Zinkmangel (Zn) ist ein Risikofaktor für die Gesundheit der Weltbevölkerung. Zn-Mangel entsteht hauptsächlich durch monotone Ernährung die auf Getreide und Hülsenfrüchten basiert. Weizenkörner sind eine schlechte Zn-Quelle, da sie Antinährstoffe wie Phytate enthalten. Zudem ist Weizen eine der grössten Cadmiumquellen (Cd) der menschlichen Ernährung. Zn-Düngung ist eine Option um den Zn-Gehalt und somit die Qualität im Weizenkorn zu erhöhen. Auch die Düngung von Stickstoff (N) kann den Zn-Status von Weizen erhöhen. Dieses Projekt untersuchte den Einfluss von N-Düngung auf die Cd- und Zn-Aufnahme von Weizenpflanzen, welche auf biologischem uns konventionellem Boden kultiviert wurden, sowie die Interaktionen zwischen Cd und Zn.

Die Böden wurden mit Zn (340 mg/kg), Cd (2mg/kg) oder beidem versetzt. Weizen (Fiorina) wurde bis zur vollen Reife in Töpfen in einer Klimazelle kultiviert. Den Pflanzen wurden zwei unterschiedliche Stufen von N-Düngung (50 mg/kg und 150 mg/kg) sowie alle essentiellen Nährstoffe gegeben.

Die Zugabe von Cd und Zn erhöhte die Metallaufnahme der Weizenpflanzen. Die Zn-Konzentrationen blieben unverändert durch die Zugabe von Cd, während die Cd-Konzentrationen im Stiel und in den Körnern durch die Zn-Zugabe reduziert wurden. Die Weizenpflanzen mit hoher N-Düngung nahmen mehr Zn und Cd auf im Vergleich mit den Pflanzen mit tiefer N-Düngung. Der Effekt war jedoch nur für die Totalgehalte in der Pflanze aber, aufgrund der Wachstumszunahme, nicht für die Konzentrationen signifikant. Die zwei unterschiedlichen Landwirtschaftsmethoden zeigten nur sehr kleine Unterschiede. Die Aufnahme von Zn und Cd wurden davon nicht beeinflusst.

Direkte Konkurrenz, Schutzmechanismen gegen Cd-Toxizität und die Immobilisierung von pflanzenverfügbarem Cd in den Zn-behandelten Böden sind die wahrscheinlichsten Gründe für die antagonistische Interaktion zwischen Cd und Zn. N kann die Metallaufnahme von Pflanzen erhöhen, durch die Senkung des Boden pHs, durch die erhöhte Biomasse und durch die erhöhte Aktivität von Transportproteinen in der Zellmembran.

Diese Arbeit zeigte, dass beides die Düngung von Zn und N die Zn-Konzentration in Weizenkörner signifikant erhöhen kann. Zudem kann die Zn-Düngung, die Aufnahme von Cd senken. Allerdings haben andere Studien das Gegenteil gezeigt. Die Interaktionen zwischen Cd und Zn hängen ab von ihren totalen und relativen Konzentrationen im Boden, vom Ernährungsstatus der Pflanze und der Pflanzenart.

1. Introduction

Zinc (Zn) is an essential trace element required in small but critical amounts by plants and animals including humans (Alloway 2009). The world health organisation has recognised mineral deficiencies, including zinc deficiency, as a major risk factor for global human health. In a report published in 2002 zinc deficiency was ranked as the fifth most important risk factor for illness and death in the developing world (Ezzati et al. 2002). It has been estimated that one in three humans is at risk of marginal to severe zinc deficiency, whereas this risk varies between 4 and 73% between countries.

The effects of zinc deficiency include weak immune systems, stunted growth and impaired maternal health and pregnancy outcomes. As zinc deficiency leads to weakening of the immune system, the risk of diarrhoea, malaria and pneumonia is increased (Hotz and Brown 2004). A full 22-24% of deaths caused by these three diseases are attributable to an inadequate supply of vitamin A and zinc. For children under the age of five, zinc deficiency is estimated to cause 13% of lower respiratory tract infections (mainly pneumonia and influenza), 10% of malaria episodes and 8% of diarrhoea cases worldwide. Apart from children, pregnant women are also at high risk of zinc deficiency (WHO 2009a, WHO 2009b).

Zinc deficiency is mainly caused by an inadequate dietary intake of absorbable zinc, which is the case for diets based on cereals or pulses and little amounts of fish or meat (Hotz and Brown 2004). Cereal grains are the most important part of human foods, with wheat and rice being the two main staple crops worldwide (FAO 2010a). On average 20% of the daily energy intake of the world population is provided by wheat grain. In rural areas of Central Asia and Middle Eastern countries this proportion can exceed 70%. However cereal grains are a poor source of zinc for humans, as they are not only low in total zinc concentration, but also contain anti-nutrients, which are substances reducing the bioavailability of zinc. The estimated bioavailability of zinc in wheat grains is around 25% (Cakmak et al. 2010). Substances with proposed anti-nutrient effects on wheat grain include phytic acid, tannins and other polyphenols, oxalic acid and some heavy metals, in particular cadmium (Graham et al. 2001). The main anti-nutrient for zinc in plant seeds is phytic acid (or phytate). The effect of phytic acid on the zinc bioavailability

depends on the molar phytate:zinc ratio. Frossard et al. (2000) have shown that zinc absorption in the human intestine is reduced at ratios above 20.

Not only is zinc deficiency a worldwide problem in human nutrition, but also the most important micronutrient deficiency in crop plant nutrition (Alloway 2009). Soils with low zinc availability for plant uptake represent nearly half of the cereal-growing areas of the world (Zhao and McGrath 2009). The countries most affected by zinc deficient soils are Pakistan, India, Iran, China and Turkey with 50-70% of arable land classified as zinc deficient (Alloway 2004). Zinc deficiency in soils is either due to a primary deficiency or secondary deficiency. In the case of a primary deficiency the total zinc concentration of the soil is too low to satisfy plant nutritional requirements. This occurs mainly in regions with either sandy or strongly leached tropical soils. Soils affected by secondary zinc deficiency have a high enough zinc concentration to cover plant nutritional requirements but only insufficient amounts of zinc can be taken up by the plants. The main soil factors leading to secondary zinc deficiency are high soil pH, high calcium carbonate content, high concentrations of bicarbonate, phosphate, calcium, magnesium and sodium in the soil solution, and high organic matter content (Alloway 2009).

There is increasing evidence that nitrogen (N) fertilization has potential to increase the uptake of zinc by plants. Cakmak et al. (2010) showed that the plants nitrogen status and the soil nitrogen regime have a major positive influence on the uptake of zinc and its allocation in seeds. Nitrogen fertilization may not only increase zinc concentration but can also increase concentrations of grain proteins and amino acids, which could increase the bioavailability of the accumulated zinc for the human body.

Cadmium (Cd) is a heavy metal with no known essential biological functions in higher plants, animals and humans. Like other heavy metals, including those that are essential at low concentrations, cadmium becomes toxic at elevated concentrations. Toxicity levels depend on the organism, physiological conditions and environmental factors. The joint FAO (Food and Agriculture Organisation of the United Nations) and WHO (World Health Organisation) expert committee on food additives (JECFA) has established a provisional tolerable level for cadmium at 25 µg cadmium intake per kg body weight per month (WHO 2010).

Cadmium is naturally released to the environment either due to volcanic activities on land or under the sea, weathering, erosion and river transport. More important than the natural sources of cadmium are the releases due to human activities. Such activities include mining, smoking, smelting and refining of non-ferrous metals, fossil fuel combustion, incineration of municipal waste, manufacture of phosphate containing fertilizers and the recycling of cadmium containing material. The remobilization of historic sources of cadmium such as the contamination of watercourses by drainage water from metal mines is also an important cadmium source. High levels of cadmium soil pollution are limited to areas with specific input histories such as mining or smelting. But low to medium levels of cadmium pollution in soil are a wide spread issue on agricultural soils. This pollution is mainly caused by the application of cadmium containing phosphate and other fertilizers as well as low quality biowaste and by peri-urban atmospheric deposition. At these levels cadmium is not toxic to plants and does not hinder plant growth or soil fertility but may potentially transfer into humans with food or water. So in many countries, including Switzerland, cadmium is the most important metal pollutant in agricultural soil because of its toxicity risks for humans and its widespread distribution (WHO 2010).

Wheat is a major source of cadmium in human food. According to data from Sweden, about 43% of cadmium ingested with food in Sweden comes from wheat products (Wångstrand et al. 2007). Cadmium is a carcinogenic substance and accumulates in kidneys causing kidney damage. A high intake of cadmium can also disturb the calcium metabolism, which may potentially lead to softening of bones and osteoporosis (WHO 2010).

The application of nitrogen fertilizer may potentially increase cadmium accumulation by crop plants, even if the fertilizer does not contain significant amounts of cadmium. This may be due to acidification and ion exchange effects or due to plant physiological effects (Mitchell et al. 2000, Perilli et al. 2010).

The options for reducing zinc deficiencies in humans include dietary supplementation such as zinc tablets, food fortification, dietary diversification and biofortification (WHO 2009a). Biofortification is an agricultural technique to increase the mineral concentration in edible crops. It can involve agronomic, genetic or transgenic strategies: Agronomic strategies for biofortification include the application of mineral fertilizer and

the improvement of solubilisation and mobilisation of mineral elements in the soil. The genetic strategies for biofortification involve the breeding of crops with an increased ability to acquire and accumulate essential mineral elements while transgenic approaches to biofortification aim to improve the phytoavailability of mineral elements in the soil, their uptake by roots, translocation to the shoot and accumulation in the edible tissue by adding genes from different organism to the wheat genotype. In addition transgenic strategies may be applied to reduce the concentration of anti-nutrients and increase the concentration of promoter substances (White and Broadley 2009). Biofortification is a good approach as no behavioural changes are necessary and the rural population can be more easily reached than in the case of commercially sold dietary supplements. In addition biofortification of crops may lead to increasing yields, as the plants may have also been deficient in micronutrients (Bouis 2003). It has been suggested that biofortification of crops through fertilization of zinc in combination with plant breeding for more zinc efficient crop varieties provides a great short- and long-term solution to combat zinc deficiency in plants and humans (White and Broadley 2009).

Much research has been done on zinc fertilization to abate zinc deficiency in crop plants on soil with low zinc availability and for the purpose of agronomic zinc biofortification of cereal grains (Bouis 2003, Cakmak 2009, Cakmak et al. 2010, Frossard et al. 2000, Graham et al. 2001). Also the effects of macronutrient fertilizers, in particular phosphate but also nitrogen, have been previously studied (Alloway 2004, Cakmak et al. 2010, Erenoglu et al. 2010, Kutman et al. 2010, Moraghan et al. 1999). Furthermore, the uptake of cadmium by crop plants from contaminated soils has received much attention (Cieslinski et al. 1996, Li et al. 2008b, Pan et al. 2001). However, there are only few studies in which interactions between zinc and cadmium have been investigated in this context (Mitchell et al. 2000, Perilli et al. 2010, Wångstrand et al. 2007). We are not aware of any studies in which the role of nitrogen fertilization on zinc and cadmium uptake by cereal crops has been compared between different farming systems.

The objective of this thesis is to investigate the influence of nitrogen fertilization on the accumulation and bioavailability of zinc and cadmium in wheat grains, grown on soil that has been conventionally or organically managed. The general aim is to provide data for the assessment of potential health risks associated with the uptake of zinc and cadmium by wheat grains on soils with elevated cadmium concentrations and low zinc availability and how these risk differ between organically and conventionally managed soils.

Hence, the following questions will be addressed by the thesis:

How do biomass production, grain zinc and cadmium concentrations and other aspects of grain quality (such as protein content) in wheat respond to enhanced zinc and cadmium availability on the experimental soils?

How does nitrogen fertilization affect these responses?

Is there a difference in the responses between the organically and the conventionally managed experimental soils?

The following hypotheses will be tested by the thesis:

- Grain zinc and cadmium concentrations are increased with enhanced zinc and cadmium availability through the addition of available zinc and cadmium to the soils in the relevant treatments.
- There are competition effects between zinc and cadmium for plant uptake whereas zinc is preferably accumulated in wheat grains and shoots for the combination treatment.
- Nitrogen fertilization increases the uptake and accumulation of cadmium and zinc in wheat grains and shoots.
- For the low nitrogen treatment: Wheat grown on the organic soil has higher grain nitrogen and zinc concentrations than wheat grown on the conventional soil, as there is more slowly released nitrogen present in the organic soil.
- The effect of high nitrogen fertilization is therefore less pronounced in wheat grown on the organic soil than in wheat grown on the conventional soil.

The hypotheses will be tested in a pot experiment carried out in parallel on two soils from the long-term DOK experiment in Therwil BL, comparing conventional to organic agricultural management. The two selected DOK treatments are conventional farming with mineral fertilization only and biodynamic farming with composted manure and slurry. The soils will be spiked with cadmium, zinc or both for the combination treatment three months prior to the start of the experiment. A control non-spiked treatment is also included. A Swiss untreated and fungicide free wheat cultivar will be grown on these soils until maturity is reached. In addition the wheat plants will be fertilized with two different levels of nitrogen.

Apart from biomass, concentration of macro- and micronutrient elements as well as cadmium concentrations will be measured in shoots and grains at harvest.

Soil samples will be analysed at the beginning and at the end of the experiment for pH, macronutrients and soluble trace element concentrations, including zinc and cadmium as well as ammonium, nitrate and total nitrogen concentrations.

2. Theoretical Background

2.1. Wheat

Approximately 350'000 plant species are botanically recognised, but only 24 plant species are used as crops to satisfy human requirements for food and fibre (Slafer and Satorre 1999). The cereals wheat (Triticum ssp.), rice (Oryza sativa L.) and maize (Zea mays L.) are the major food crops for all humans across the world (Gustafson et al. 2009). Wheat is the most widely grown crop in the world as approximately one sixth of the total arable land in the world is under wheat cultivation (Slafer and Satorre 1999). This equals an area of about 216 million hectares and the wheat production from this was approximately 651 million tonnes in 2010. The ten largest wheat producers include China, India, Russia, USA, France, Germany, Canada and Australia (FAO 2010b).

Wheat was one of the earliest food crops that were domesticated around 10'000 years ago (8'000 BC) in the fertile crescent of southwest Asia. Einkorn (T. monococcum) and Emmer (T. dicoccum) were the early precursors of the 25'000 different wheat cultivars that are currently cultivated in the world (Gustafson et al. 2009, Winch 2006).

Modern wheat cultivars primarily belong to two polyploid species: Hexaploid bread wheat (Triticum aestivum) and tetraploid hard or durum wheat (Triticum turgidum) (Gustafson et al. 2009). Taxonomically wheat belongs to the Poaceae family and the Triticaea tribe. Wheat is a highly adaptable plant species that can grow in every climatic zone apart from lowland tropics. It is also adapted to grow at altitudes from sea-level to 3'500 meters above sea-level and between latitudes of 60° south and 60° north. Though optimal growth conditions are temperatures between 25°C and 27°C and a yearly rainfall between 350 and 700 mm. Under good conditions and with the right wheat cultivar yields can reach up to 10 MT/ha. However, in less favourable regions the wheat yield can be as low as 300 kg/ha. Wheat is primarily grown for its grains, which are mostly ground to produce flour. Limitations to wheat growth include many diseases such as rusts and pests, bending of plants in the wind, nutrient deficiency and water stress in poorly drained soils (Winch 2006).

All wheat cultivars and each shoot follow the same developmental events, which lead to the definition of growth stages and development stages respectively. The most well-known development stage schemes are the Feekes and the Zadoks scale (Table 1). All of

these growth stages cover basic developmental events such as germination, emergence, leaf production, tillering, shoot elongation, flowering, stages of grain ripening and maturity. Different cultivars and shoots vary in the timing and duration of these developmental events. This results from genotypic differences but also from different responses to environmental conditions (McMaster 2009).

Table 1: Description of the development stages in wheat growth and the according scales by Feekes and Zadoks.

Development Stage	Feekes	Zadoks	Description
Germination	no stage	01-07	
Emergence	no stage	09	First true leaf emerges through the coleoptile and tip is visible above the soil surface
Tillering	01-02	20-29	First tiller is visible
Intermode elongation	06-07	31-36	First node is visible
Flag leaf/booting	08-10	39-49	Flag leaf growth is considered complete when the ligule is visible and lew leaf is emerging
Heading	10.1-10.5	50-58	First spikelet is visible
Anthesis	10.5.1-10.5.4	61-69	First anther (yellow) is visible on inflorescence
Physiological maturity	11.1-11.4	77-99	When all components of the spike, internode tissue, and leaves have lost green colour

All of these development stages can be separated into three phases: The vegetative phase where leaf growth is initiated, tillering and stem elongation takes place, the reproductive phase during which floret development happens and the grain filling phase during which endosperm cells are developed in the grain. In the vegetative phase, several leaves have been initiated in the apex underground by the time of seedling emergence when the first of these leafs appears above ground. Together with the

appearance of the fourth leaf, the first tiller becomes visible. Besides the bud corresponding to the main shoot apex, axillary tiller buds are developed in each phytomer. Each of these buds has the potential to develop into a leafy tiller. A wheat plant keeps tillering until resources become sparse. So to initiate stem elongation, another development stage of the vegetative phase, some tillers may die in reverse order of their appearance. During the reproductive phase spikelets and florets are initiated. The maximum number of floret primordial per spikelet normally ranges between six and twelve. From those, only one to four florets complete their development to produce fertile florets. The grain-filling phase starts once the fertile florets are fertilized through self-pollination (Figure 1). Grain development follows a clear set of steps: lag phase, linear phase and maturation phase. During the lag phase grains slowly accumulate dry matter through cell division. Most of the endosperm cells are developed and all the structures of the grain are formed during the lag phase. The linear phase is driven through maximum cell expansion rates and maturation is mostly the loss of seed moisture (McMaster 2009, Miralles and Slafer 1999).
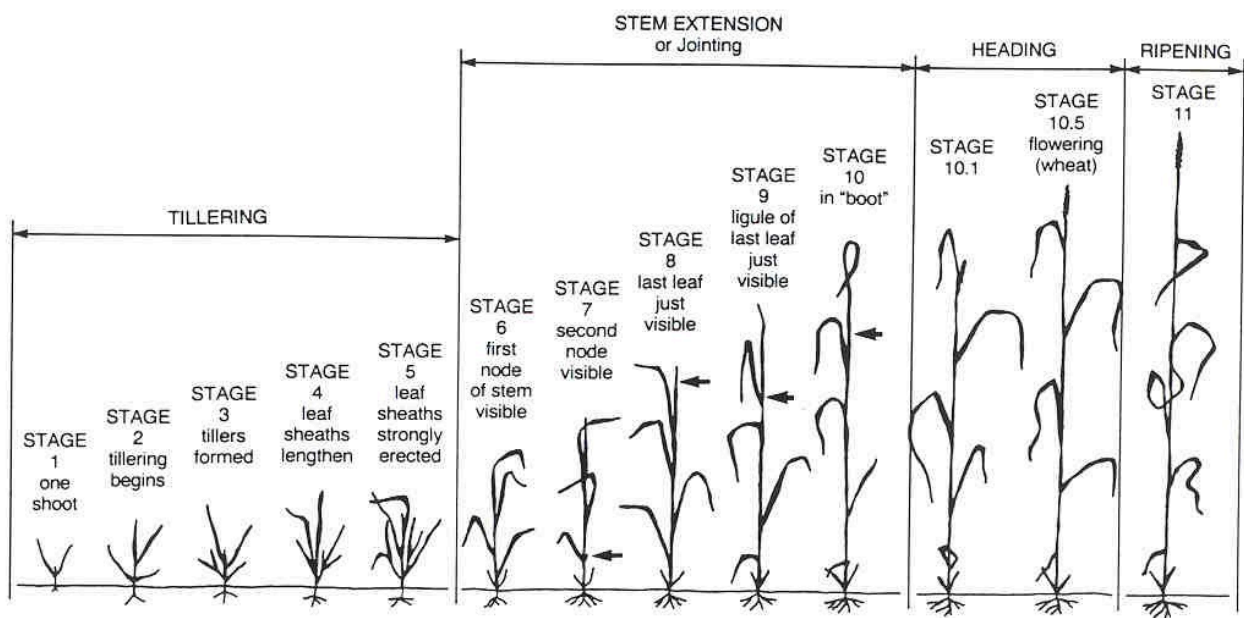
Figure 1: Shown are the growth of a wheat plant and its development stages as described by Feekes (McMaster 2009)

The three main constituents of the mature wheat grain are the endosperm, the embryo and the bran. Figure 2 shows the profile of a mature wheat grain. The endosperm, which contains mostly starch and some protein is surrounded by the aleurone cell layer. The aleurone cell layer connects the developing grain with the maternal tissue and translocates assimilates from the maternal phloem to the embryo (McMaster 2009). Zinc has been shown to co-localize with protein and free amino acids in wheat grains and is mostly contained in the embryo and the bran. Less zinc is contained in the endosperm. This is problematic for human nutrition since processing of wheat removes the zinc rich parts of the grain, therefore reducing its nutritional value (Waters and Sankaran 2011).
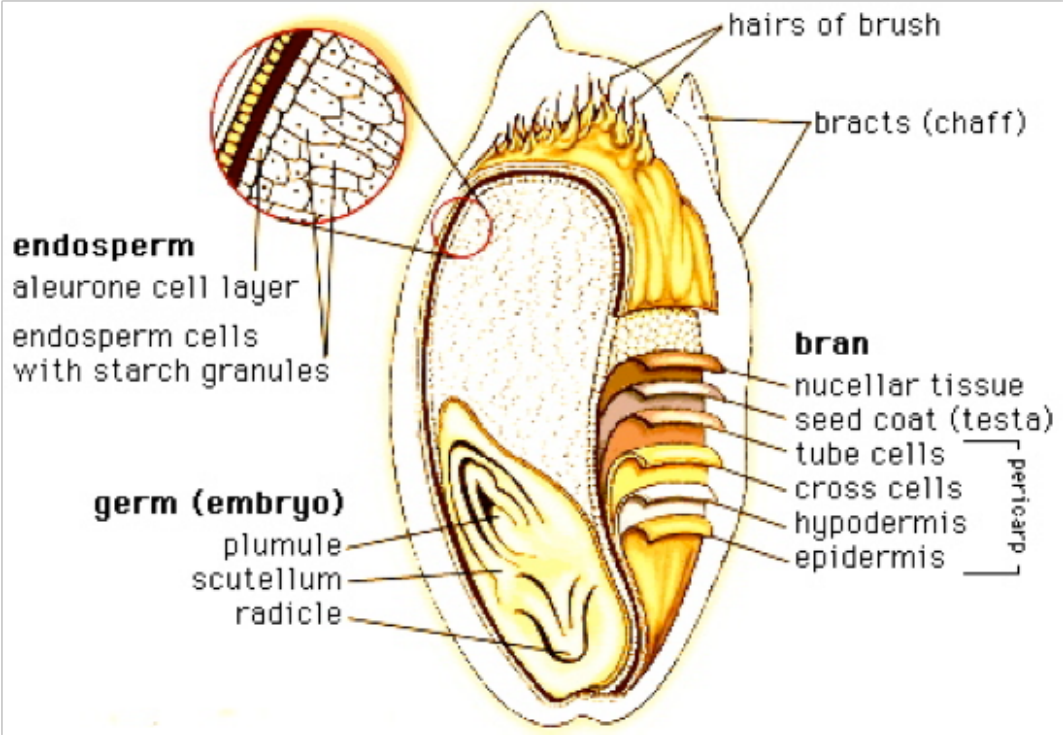
Figure 2: Constituents of the wheat grain. The main parts are the bram, the endosperm and the embryo (Britannica 1996).

2.2. Zinc and cadmium in soils

2.2.1. Zinc

Zinc is a transition metal that shows hard Lewis acid characteristics. Therefore zinc forms strong covalent bonds with sulphur, nitrogen and oxygen donors, which are all hard Lewis bases. These bonds lead to the formation of salts such as sulphates, nitrates and halides (Broadley et al. 2007). Trace elements such as zinc are contained in all soils in measurable amounts. However, these concentrations can vary considerably. The overall mean total zinc concentration in soil is around 55 mg Zn kg^{-1}. A typical range of zinc in soils is from 10 to 300 mg Zn kg^{-1}. These values do not include contaminated soils, which may have much higher zinc concentrations.

The most important natural source of zinc in soils is the geochemical composition of the weathered rock parent material on which the soil has developed. Mafic igneous rocks such as basalts have relatively high zinc concentrations (100 mg Zn kg^{-1}), as they contain ferromagnesian minerals in which zinc has substituted Fe^{2+} and Mg^{2+}. Soils developing on these rocktypes tend to have a relatively high zinc concentration. More silica rich igneous rocks like granite and metamorphic rocks, for example gneiss, contain less zinc (50-60 mg Zn kg^{-1}). Their residual weathering product is usually sand which leads to either sandy soils or sandstones with low total concentrations of zinc and other essential micronutrients. Sedimentary rocks are formed from the weathering products of igneous rocks that are transported and later deposited. Their zinc concentrations depend on the concentrations of the weathered igneous rocks. In addition to these commonly occurring rock types in the earth's crust, high concentrations of zinc can be found in ore minerals in isolated areas. These are generally mined as economic sources of the metal and do not influence the zinc content of agricultural soils apart from those in the immediate vicinity. Zinc can also be imported to soils through atmospheric deposition of small wind-blown particles of soil, rock and sea spray. The sources of zinc in the atmosphere are the burning of coal and oil, waste incineration, industrial processes such as non-ferrous metal smelting and general urban and industrial emissions.

The most widespread anthropogenic inputs of zinc into soils are the agricultural inputs. The application of livestock manure, fertilizers, sewage sludge and agrochemicals are all potential sources of large inputs of zinc. All manure contains zinc as it was part of the

animal's diet. In addition, in intensive livestock production, zinc is often fed to animals for health reasons or as growth promoters. Some fertilizers can contain significant amounts of zinc. Superphosphate for example contains up to 600 mg Zn kg^{-1}. However, the application of superphosphate is declining as, it is replaced by purer phosphorus compounds. In many areas with zinc deficient soils zinc fertilization (such as zinc sulphate) is used to increase the zinc status of crops and livestock (Alloway 2004).

2.2.2. Cadmium

Cadmium is not included in the group of transition metals. Nevertheless, the tendency of cadmium to form complexes with ammonia, amines, halide ions and cyanide indicates the similarity of cadmium with transition metals. Cadmium is a soft Lewis acid, which results in the formation of soluble solution complexes with borderline to soft Lewis bases such as amines, chlorides, sulphhydryls and thiols (McLaughlin and Singh 1999).

Cadmium concentrations in soils vary greatly from relatively low concentrations in uncontaminated materials to high concentrations for local areas receiving large quantities of cadmium through agricultural or industrial activities (750 mg Cd kg^{-1} and higher). The overall mean of total cadmium concentration in soils is around 0.62 mg Cd kg^{-1}. A survey of the cadmium concentrations in agricultural soils in the USA has shown a typical range of cadmium concentrations of 0.037 to 0.98 mg Cd kg^{-1} (Alloway and Steinnes 1999, Traina 1999).

The total concentration of cadmium in soils consists of the contribution from the parent material and inputs from external sources, which are mostly anthropogenic. Cadmium is a trace element in the lithosphere with an average abundance of 0.2 mg kg^{-1}. Typically the highest cadmium concentrations are found in sedimentary rocks. The mean cadmium concentrations in igneous rocks range between 0.07 and 0.25 mg Cd kg^{-1} whereas sedimentary rocks can contain up to 11 mg kg^{-1}. Cadmium is often present in phosphate rocks, which is of particular interest due to the potential as a cadmium-source to agricultural soils through phosphorus fertilization. Characteristic cadmium concentrations in phosphate rocks range from 0.02 to 50 mg Cd kg^{-1} (Alloway and Steinnes 1999, Traina 1999).

The most important anthropogenic sources of cadmium that contaminate soils are atmospheric emissions, direct application and accidental contamination. The

atmospheric emissions of cadmium are caused, by metalliferous mining and smelting, metal-using industries, the manufacturing of fertilizers, the incineration of municipal waste, coal combustion, road dust and general urban and industrial emissions. The application of phosphate fertilizers, sewage sludge and composted municipal waste on soils are the direct application sources of cadmium. Accidental contamination of soils with cadmium can come from chemical factories, mine waste dumps and the corrosion of galvanised metal structures (Alloway and Steinnes 1999).

2.2.3. Distribution of cadmium and zinc within soil

The total content of cadmium and zinc in soils is allocated to different pools or fractions. (I) The water-soluble pool is the fraction of cadmium and zinc, which is present in the soil solution as free ions or as soluble organically complexed metals, (II) an exchangeable pool of cadmium and zinc, which contains the ions bound to soil particles by electrical charges and (III) an organically bound pool, which includes ions adsorbed or complexed with organic ligands in the solid phase. In addition there is (IV) a pool of cadmium and zinc sorbed non-exchangeably onto clay- minerals and insoluble metallic oxides and (V) a pool of weathering primary minerals (Alloway 2004, Helmke 1999). Only the zinc and cadmium present in pools (I)-(III) are available to plants and potentially leachable in water percolating down through the soil profile (Alloway 2004). The distribution of zinc and cadmium between these pools depends on the equilibrium constants of the corresponding reactions in which zinc or cadmium are involved. These reactions are precipitation and dissolution, complexation and decomplexation, and adsorption and desorption. Only a small proportion of the total zinc and cadmium concentration is present in the soil solution. For cadmium this amounts to only 1%. However, the solubility of zinc and cadmium is largely increased under acidic conditions so the proportion of zinc and cadmium in the soil solution is much higher for acidic soils (Alloway 2004, Christensen and Haung 1999, Helmke 1999).

2.2.4. Zinc deficiency in soils

Zinc deficient soils are either low in the total zinc concentration (primary deficiency) or low in bioavailable zinc (secondary deficiency) (Alloway 2009). Zinc deficiency in soils and crops is widespread in different bio-climatic zones of the world and different soil

types. However, zinc deficiency is more common on certain soil types. The soil types most commonly associated with zinc deficiency are calcareous soils, sandy soils, saline and sodic soils, Vertisols and Gleysols. Figure 3 shows the distribution of these soil types in the world. This map cannot be considered as the total area of zinc deficient soils as some of the areas shown are not suitable for crop production due to climatic conditions or a shortage of available water for irrigation (desert areas). In addition in some of these areas indigenous varieties of crop species, which are tolerant to zinc deficiency are cultivated (Alloway 2004).

Alloway (2009) has named the soil factors controlling the plant-available zinc to be "total zinc content, pH and redox conditions, calcium carbonate $CaCO_3$ and organic matter contents, concentrations of all ligands capable of forming organo-zinc complexes, microbial activity in the rhizosphere, concentrations of other trace elements, concentrations of macro-nutrients and the soil moisture status". High soil pH reduces the bioavailability of zinc as the adsorptive capacity of the soil increases resulting in the formation of hydrolysed forms of Zn, chemisorption on calcite and co-precipitation in Fe oxides. The causes of a high soil pH (>7) are a high $CaCO_3$ content, high salt contents and reducing conditions (Alloway 2009).

Figure 3: Zinc deficient soils worldwide. The dark areas indicate the global distribution of soil types frequently associated with zinc deficiency. (Alloway 2004).

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Sandy soils and strongly weathered tropical soils are low in the total zinc concentration, whereas calcareous soils are mostly zinc deficient due to the high pH value of the soil. Calcerous soils are typical soils of semi-arid and arid climatic regions, mostly in Middle Eastern countries, northern Africa and some parts of Australia. Sandy soils occur in arid zones, which include the southern Sahara, Southwest Africa and western Australia. In saline soils zinc deficiency is also related to the high pH of the soil and the high electrical conductivity (Alloway 2009). Examples of countries with saline Soils are Chad, Namibia, Australia, Paraguay and Uruguay. Vertisols are dark clay-rich soils with characteristic shrinking and swelling properties, leading to cracks. They have a high calcium and magnesium content and the soil pH is usually above 7. These soils most occur in hot areas with marked wet and dry seasons such as the semi-arid tropics in Africa, the Deccan Plateau in India and Australia. Gleysols are waterlogged soils with reducing conditions at depth resulting in a high soil pH. They have a permanent groundwater table and are found in valleys (Alloway 2004).

2.3. Mineral and organic nitrogen in soils

Nitrogen together with carbon accounts for 95% of the biosphere and is one of nine essential major nutrients required for plant growth and development. About 90% of soil nitrogen is present in its organic, 6-12% is fixed as non-exchangeable mineral nitrogen in the form of ammonium (NH_4^+) and 1-3% is stored as plant available mineral nitrogen in the form of NH_4^+ and nitrate (NO_3^-) (Nieder and Benbi 2008). The sources of plant available NH_4^+ are fertilizers such as urea and the mineralization of plant residues and organic matter. Non-exchangeable NH_4^+ has the same sources as plant available NH_4^+ but is adsorbed in the interlayer spaces of clay minerals. Nitrate is produced by nitrification of ammonium and is the most important form of nitrogen for plants in non-flooded soils (Bronson 2008). Organic nitrogen is part of the organic matter matrix of a soil and occurs in different biological forms, mainly in polypeptides, amino acids, amino sugars, and their residues (Olk 2008). The global soil organic nitrogen (0-100 cm) pool is estimated to be around 9-13 * 10^{13} tonnes (Nieder and Benbi 2008). Total soil nitrogen usually decreases from top to bottom in the soil profile, as plant biomass is the main building block for soil nitrogen. The nitrogen concentration of an agricultural soil

depends on the soil genesis, the cropping system, tillage, productivity, its susceptibility to erosion, climate, terrain and fertilizer management (Bronson 2008).

2.4. Zinc and cadmium in plants

2.4.1. Plant metal uptake and transport

Water and dissolved minerals, including metals such as zinc and cadmium, are taken up by plants from the soil solution through the epidermis. The epidermis is a single layer of cells covering the root. Root hairs enhance the uptake process as they increase the surface area of the epidermal cells. The uptake of these nutrients is driven by passive transport through diffusion or mass flow. Once passed the epidermis the metals have entered the apoplast, which describes the cell wall continuum (Campbell and Reece 2002). It is also referred to as the apparent free space, which consists of the water free space and the Donnan free space. The water free space is freely accessible to ions, charged and uncharged molecules whereas in the Donnan free space positively charged molecules are accumulated and negatively charged molecules are repelled. The accumulation of positively charged ions in the Donnan free space is caused by the negative charge of the carboxylic groups of polgalacturonic acid, which is contained in pectins in the cell walls. These carboxylic groups consequently act as cation exchangers in the cell wall continuum of roots and other plant tissues. Zinc and cadmium both accumulate in the Donnan free space and therefore accumulate in plant parts that are passed first (root and shoot) (White 2012b). Metals cross the cortex of the root either through the apoplastic or the symplastic lateral transport route. In the apoplastic route metals travel across organs via the cell wall continuum while in the symplastic route metals enter one cell through the plasma membrane and move across organs via the cytosolic continuum. For the apoplastic pathway movement to the stele and vascular tissue is restricted by the endodermis including the casparian strip, a belt made up of suberin, a waxy material that is impervious to water and dissolved minerals. In order to enter the vascular tissue for upward transport in the stele, metals in the apoplast need to enter a cell through the selective plasma membrane to cross the casparian strip while metals in the symplast have already crossed a selective plasma membrane and can therefore directly pass the endodermis (Figure 4) (Campbell and Reece 2002). So the symplastic pathway plays a key role in the transport of most nutrients. Metals either

enter the symplast at the rhizodermis and the root hairs or at the endodermis. In the symplast metals move from cell to cell through plasmodesmata. They connect neighbouring root cells in a complex structure. The transport of any compound through the plasma membranes is facilitated by transporter proteins. There are three known kinds of transporter proteins: (1) primary active transporters (pumps), (2) secondary active transporters or coupled transporters and (3) passive transporters. For primary transporters solute transport is directly coupled to the hydrolysis of an energy substrate such as ATP or pyrophosphate. With the secondary transporters the electrochemical gradient generated by (mostly) hydrogen ions is used to transport a solute either in the same (symport) or the opposite (antiport) direction. Passive transporters catalyze the movement of solutes down their electrochemical gradient through a variety of uniports and channels. For zinc carriers coupled transporters and pumps are relevant (White 2012b). It is assumed that zinc and cadmium are taken up and translocated by similar pathways and transporter proteins as they have very similar chemical properties (Santos et al. 2010, Waters and Sankaran 2011). In the vascular tissue of the stem metals, other essential nutrients and water are transported from the roots to the shoots, leaves and reproductive organs. It consists of the phloem and the xylem. In the xylem metals and water are transported upwards through bulk flow, driven by the tension caused by transpiration. In the phloem organic compounds, such as sucrose made in mature leaves, and some minerals are transported to the roots and other non-photosynthetic parts of the shoot system such as developing leaves and fruits (Campbell and Reece 2002, White 2012a).

From the xylem metals enter the leaf cell apoplastic spaces and are then transported across a plasma membrane via cation channels and transporters to enter the symplasts where they are distributed to the required cells (Longnecker and Robson 1993, Welch and Norvell 1999). As the xylem transport is driven by transpiration, solutes released from the xylem, accumulate in the sites of highest transpiration, which are often not the sites of highest demand for nutrients. On the other hand phloem transport is driven by bulk flow to sites of lower internal pressure, which means sites that act as solute sinks. During long distance transport in the vascular tissue metals are exchanged between the xylem and the phloem. Also nutrients are redistributed within the plant from older tissues to young tissues with high nutrient demand through phloem transport. The phloem transports nutrients to areas of high demand, which are either

utilization sinks such as root tips, shoot apices and stem elongation zones or storage sinks (Engels et al. 2012).
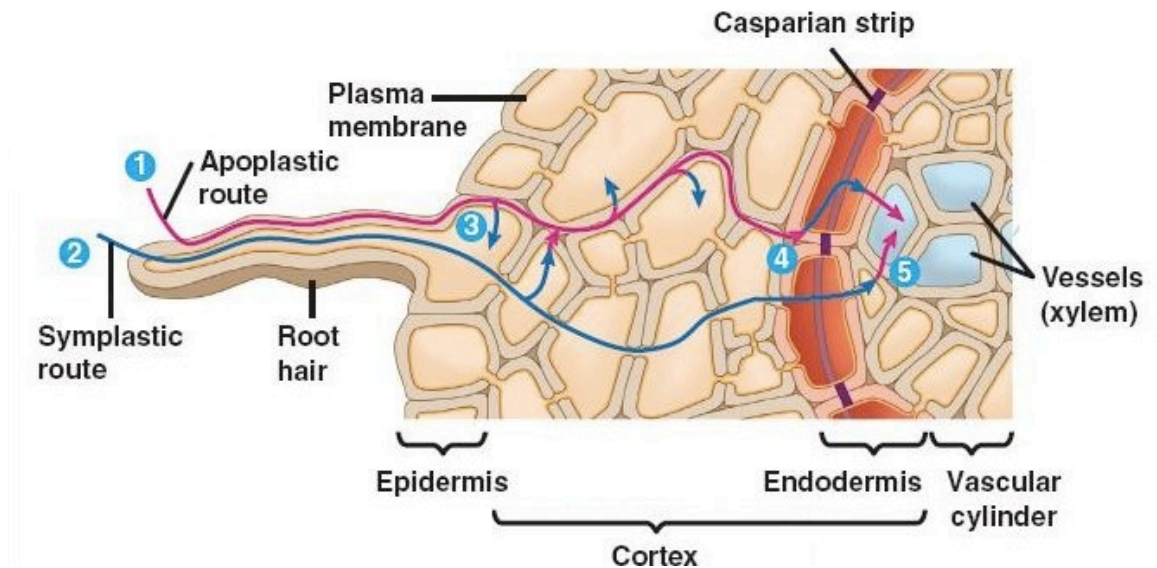

Figure 4: The uptake of water and minerals (e.g. cadmium and zinc) by plant roots and the lateral transport to the vascular tissue. Metals are transported in roots through the apoplastic (1) or the symplastic (2) route; they may change from the apoplastic to the symplastic route during their transport (3). To enter the xylem (5) metals must cross the endodermis (4) and the casparian strip, which act as barriers (Campbell and Reece 2002).

Loading metals into the phloem is a required step for translocation into seeds. During transport metals are either incorporated into proteins, bound to ligands or chelated (with certain oligopeptides and amino acids). Storage organs, such as seeds of cereals contain filial tissues (endosperm and embryo, aleurone) surrounded by maternal tissue (seed coat). The developing seed is connected to the maternal tissue through a single vascular trace. The vascular bundle ends at the seed coat and is not symplastically connected to the filial tissues. So nutrients moving to the seed are unloaded from the phloem and distributed in the maternal tissue surrounding the seed. Eventually the nutrients are effluxed into the apoplastic space that separates the maternal and filial tissues (Waters and Sankaran 2011). Zinc and cadmium are chemically similar and therefore taken up and translocated in plants through similar pathways. In wheat, cadmium was shown to be removed from the xylem, loaded into the phloem and transported to the maturing grain similarly to zinc. To cross cell membranes specific transporter proteins are required. Zinc and cadmium are thought to use the same

transporter proteins as they are so similar. Nitrogen is contained in many chelators that are involved in zinc phloem transport, suggesting that phloem transport of nitrogen and zinc may be directly related. In addition, nitrogen and zinc concentrations in grains show positive correlations for many grass species including wheat. Also, the addition of nitrogen has shown to increase zinc translocation into wheat grains while zinc availability remained the same (Waters and Sankaran 2011).

2.4.2. Cadmium

Within plants cadmium is present as a free ion or as part of metal complexes. It enters the plant mainly by root uptake but foliar uptake is also possible (Santos et al. 2010). Cadmium has no known beneficial effects in plants, but is toxic at higher concentrations. It shows a variety of phytotoxic effects and interferes at several physiological levels through induced oxidative stress, genotoxicity and the inhibition of photosynthesis and respiration. The unspecific symptoms of cadmium stress visible in plants include chlorosis, necrotic lesions, reddish coloration and growth reduction. Cadmium concentrations of 13-35 mg Cd/kg dry weight have been shown to cause growth reduction of more than 25% in soybean, corn and wheat. The critical phytotoxic level for cadmium is set at around 10 mg Cd/kg plant dry weight (Wallnöfer and Engelhardt 1995). The critical phytotoxic level is defined as the concentration of a pollutant in the plant or soil that results in growth depression for a certain plant species of 5 or 10%, respectively.

In the following the main phytotoxicological effects of cadmium are discussed:

- Photosynthesis:
    The toxic effects of cadmium on the photosynthetic system cause several structural and functional disorders. Though the main targets are the photosynthetic pigments biosynthesis pathways: Cadmium reduces chlorophyll production by the inhibition of protochlorophyllide reductase. It can also interfere with the photosynthetic pigments by substituting Mg^{2+} ions with Cd^{2+} ions in chlorophyll molecules. These substituted molecules have much lower fluorescence quantum yields compared to magnesium chlorophylls. These two toxic effects reduce the production of chlorophyll and consequently photosynthesis, which can then lead to senescence and cell death.

Photosystem II and photosystem I, the light-harvesting units in the chloroplasts of plants that transfer light energy to chemical energy in the form of ADP and NADPH, are also affected by cadmium. Here the strong interaction of cadmium with iron leads to iron deficiency, as iron is required in both photosystems and their processes (Campbell and Reece 2002, Santos et al. 2010).

- Carbohydrate metabolism

  The effects of cadmium in the carbohydrate metabolism are mostly due to the inhibition of enzymes such as RuBisCO (Santos et al. 2010).

- Oxidative stress

  An increase of reactive oxygen species (ROS) in plants is a result of abiotic or biotic stress. ROS are partially reduced forms of oxygen (O_2) and typically results from the excitation of O_2 to form singlet oxygen or from the transfer of electrons to oxygen to form O_2^-, H_2O_2 or HO^-. In plants cells ROS are generate during normal metabolic processes such as respiration and photosynthesis. But when the balance between antioxidants and ROS is tilted in favour of ROS, oxidative stress occurs. The exposure of plants to metals such as cadmium can also stimulate the production of ROS. In the case of metals this happens either through direct electron transfers involving the metal cations or as a consequence of the inhibition of metabolic reactions caused by the metal. The effects of ROS are the oxidation of proteins, lipids and nucleic acids, membrane damage, mutagenesis and the inactivation of enzymes (Santos et al. 2010).

2.4.3. Zinc

In plants, zinc's predominant forms are low molecular weight complexes, storage metalloproteins, free ions and insoluble forms present in the cell wall. The metabolic functions of zinc within plants are mostly related to the tendency of zinc to form complexes with nitrogen-, oxygen- and sulphur-ligands. Zinc acts as a functional, structural or regulatory co-factor in many enzymes. More than 70 metalloenzymes containing zinc have been recognised and zinc is the only metal that is present in all six classes of enzymes. Furthermore zinc has shown to play a vital role in the structure and function of biomembranes (Alloway 2004, Brown et al. 1993).

In the following the functions of zinc are discussed in more detail:

- Carbohydrate metabolism

  Zinc affects the carbohydrate metabolism by the involvement in photosynthesis and sugar transformations. In photosynthesis zinc is a constituent of the enzyme carbonic anhydrase. So plants under zinc stress experience a decrease in carbonic anhydrase activity, which affects the carbon dioxide assimilation pathway. Zinc is also a constituent of other enzymes involved in photosynthesis such as ribulase 1,5-biphosphate carboxylae (RuBPC). Sugar transformation enzymes involved in the formation of sucrose, such as aldolase, are affected by zinc deficiency also (Alloway 2004, Brown et al. 1993).

- Protein metabolism

  Zinc has been shown to be necessary for the activity of the enzyme RNA polymerase and zinc also protects the ribosomal RNA. So zinc deficiency leads to a reduction in RNA and the deformation and reduction of ribosomes, which results in reduced protein content in zinc deficient plants (Alloway 2004, Brown et al. 1993).

- Membrane integrity

  The role of zinc in maintaining membrane integrity may involve the structural orientation of macromolecules and the maintenance of ion transport systems. Zinc is also known to interact with phospholipids and sulphydryl groups of membrane proteins. Zinc also plays a key role in controlling the generation and detoxification of free oxygen radicals, which can destroy membrane lipids and proteins (Alloway 2004, Brown et al. 1993).

- Auxin metabolism

  Zinc is required for the synthesis of auxin, which is a growth-regulating compound (indole acetic acid). In zinc deficient plants stunted growth and small leaves are the most distinct visible symptoms and result from the disturbance in the auxin metabolism (Alloway 2004, Brown et al. 1993).

To quantify nutrient deficiencies in plants, critical deficiency values were established. These values give the concentration of a single nutrient for a specified plant species and plant part at which growth is reduced by a predetermined percentage. These values are experimentally determined where all other conditions are at optimum. For zinc the critical value for deficiency (10% growth reduction) ranges from 7-30 mg/kg depending on plant species and plant sample. For wheat the critical deficiency content in grains was established at 10-15 mg Zn/kg dry weight (Alloway 2004, Reuter and Robinson 1986). If zinc contents are lower, zinc deficiency can cause a 50 to 70% reduction in photosynthesis, decreased protein production, loss of membrane integrity and reduced yield. The visual symptoms of zinc deficiency include chlorosis, rosetting of leaves, stunted growth, malformation of leaves and dwarf leaves. Chlorosis is the change of leaf colour from bright green to pale green, yellow or even white. It is caused by the reduced amount of chlorophyll in the plants. Other symptoms of chlorosis include necrotic spots on leaves and bronzing of leaves. Rosetting of leaves happens when stem elongation is disturbed and leaves form close together in clusters instead of being spread out between nodes. In wheat zinc deficiency leads to a reduction in grain yield and grain nutritional quality. Visual symptoms in wheat are chlorotic and necrotic streaks, typically on both sides of leaves mid-rib. In more severe cases the lower leaves tend to be totally chlorotic and short (Alloway 2004).

Zinc can also limit plant growth if it is present in excessive concentrations, due to toxicity. High concentrations of soil zinc are mostly caused by anthropogenic applications of zinc through over fertilization, the application of pesticides, manures and sewage sludge. In addition smelters, incinerators, mines and galvanized products can cause high zinc concentrations in soils. Zinc has been widely dispersed and has reached phytotoxic concentrations in many soils (Chaney 1993). In Switzerland a guide value of 150 mg Zn/kg dry weight soil is put in place for agricultural soils. Soils with zinc concentrations above 2'000 mg/kg dry weight need to be decontaminated (VBBo 1998). The continuous over fertilization of zinc can lead to zinc toxicity in agricultural soils but in general agricultural soils are rarely contaminated enough to cause zinc phytotoxicity. For most economic plants, including wheat, zinc concentrations that exceed 500 mg/kg DW in the shoot cause significant yield reductions. The critical level for phytotoxicity varies between different plant species. For wheat the level is set at around 500 mg/kg zinc in shoots (Chaney 1993, Reuter and Robinson 1986). For wheat grains Reuter and

Robinson (1986) established the toxicity level of zinc at values above 66 mg/kg DW in a soil culture experiment undertaken in a glasshouse. At toxic levels zinc mainly affects carbon fixation and the electron transport during photosynthesis. In addition phloem transport of carbohydrates has shown to be affected by toxic levels of zinc as well. At phytotoxic levels of soil zinc plants accumulate most zinc in roots, the stem and old leaves. The visible symptoms of zinc toxicity are loss of turgescence, necrosis of old leaves, chlorosis and weak growth (Wallnöfer and Engelhardt 1995).

2.5. Interactions of cadmium and zinc in soils and plants

Zinc and cadmium are often associated in nature, because of their similar chemical properties. In soils their main interaction is competition for adsorption and binding sites. In a concentration range of 10^{-6} to 10^{-5} M zinc the cadmium adsorption can be reduced by 25-50% (Christensen and Haung 1999). Shute and Macfie (2006) state that the binding sites in soil such as organic matter and clay particles have a higher affinity to zinc than cadmium. So due to these competition effects increasing soil solution concentrations of zinc result in more cadmium desorption from the soil particles resulting in increased cadmium concentrations in the soil solution. Hence, cadmium becomes more available to plants. On the other hand the competition between cadmium and zinc in plant uptake and translocation are also increased (Grant et al. 1999). In plants cadmium and zinc may interact during plant uptake, transport from root to shoot and/or accumulation in edible tissues. The effect of zinc on cadmium uptake, and vice versa and their concentrations in plants is very controversial in the literature. The interactions between the two metals can be antagonistic and synergistic or they can have no effect on each other depending on plant species, growth conditions, nutritional status of the plants, zinc status of the soil and the plants and cadmium content of the soil and the plant (Köleli et al. 2004, Shute and Macfie 2006).

Generally it is said that the application of zinc decreases cadmium uptake and accumulation in plants due to competition for uptake, because of a common transport system on the plasma membrane (Köleli et al. 2004). This antagonistic interaction has been shown in experiments involving multiple plant species such as wheat (Choudhary et al. 1994, Oliver et al. 1994), rice (Honma and Hirata 1978), and soybean (Shute and Macfie 2006). But synergistic interactions between zinc and cadmium have also been shown. Chaoui et al. (1997) examined zinc and cadmium interactions in hydroponically

grown beans. The beans were exposed to two cadmium treatments (plus 2 or 5 μM Cd), two zinc treatments (plus 10 or 25 μM Zn) and four combination treatments (plus 2 and 10 μM Cd and Zn, 2 and 25 μM Cd and Zn, 5 and 10 μM Cd and Zn, 5 and 25 μM Cd and Zn). The results only showed synergistic and additive interactions and no protective (antagonistic) interactions between the two metals. In the combination treatments less cadmium was retained in roots and more was present in shoots. For zinc the opposite was the case. Both metals were translocated to more susceptible sites: More zinc was retained in zinc-sensitive roots whereas cadmium was translocated in greater amounts to cadmium-sensitive shoots. In the single zinc and cadmium treatments the metals were excluded from these susceptible sites. The authors suggest that the synergistic interaction between cadmium and zinc, which caused increased phytotoxicity, might be related to an inadequate compartmentation of the metal burden (Chaoui et al. 1997). In wheat Nan et al. (2002) showed synergistic cadmium-zinc interactions in a field trial on contaminated soil. The soil contained an average of 3.2 mg/kg cadmium and 146.8 mg/kg zinc. The highest metal concentration in the wheat plants at maturity for both metals was measured in roots followed by stems and leaves and the least concentration was found in seeds. Here the increasing cadmium and zinc contents in soil enhanced the accumulation of the two metals in the crop plant tissues. The high concentrations of zinc and cadmium in this contaminated soil are assumed to be responsible for this interaction. Plant roots exposed to high zinc concentrations in soil loose there membrane integrity because of the phytotoxic effects of zinc. Because of this membrane damage, toxic metals such as cadmium can enter the root uncontrollably (Nan et al. 2002). Dudka et al. (1994) have also shown an increased cadmium uptake at high zinc concentrations in a pot experiment with spiked soil. Shute and Macfie (2006) found synergistic and antagonistic interactions in soybean depending on the level of zinc and cadmium applied in a pot experiment. They spiked the soil with six levels of zinc (between 50 and 2000 mg/kg) and six levels of cadmium (between 2 and 100 mg/kg), plus six combination treatments. When cadmium and zinc were present in low doses antagonistic interactions occurred between the two metals: Less cadmium was taken up by the soybean plants compared to the single cadmium treatments. For zinc, the amount of zinc taken up by plants was not significantly altered in the combination treatment compared to the single treatment at low doses. At high soil concentrations of zinc and cadmium the opposite was the case. More cadmium and less zinc accumulated

in the soybean plant. The antagonistic interactions shown by Shute and Macfie at low doses are related to competition between the two metals for membrane transporters whereas the synergistic interaction might also be related to zinc toxicity to the root membrane and the resulting loss of membrane integrity (Shute and Macfie 2006). Köleli et al. (2004) analysed the interactions of zinc and cadmium in durum and bread wheat in a pot experiment performed with zinc deficient soil. The soil was treated with two levels of zinc (0 and 10 mg/kg) and three levels of cadmium (0, 10 and 25 mg/kg). Cadmium toxicity was more severe in the zinc-deficient treatments. This indicates the importance of zinc in detoxification of cadmium in plant tissues. Zinc is involved in the detoxification of reactive oxygen species, a main toxic action of cadmium. For example zinc is a constituent of the antioxidative enzyme superoxide dismutase. So zinc-deficiency reduces the plant's tolerance to toxic metals such as cadmium. It was also thought that released phytosiderophores from roots in zinc-deficient soils caused an increased cadmium uptake. But this assumption has been proven incorrect recently, as it was shown that phytosiderphores are not involved in the accumulation of cadmium in plants (Köleli et al. 2004).

Generally zinc can prevent plants from taking up cadmium due to competition in uptake mechanisms but also the opposite has been shown, especially at high zinc and cadmium concentrations and in contaminated soils.

2.6. Influence of nitrogen fertilization

Nitrogen is the element plants require the most of after carbon. Between 1 and 5% of total plant matter is nitrogen. It is contained in the plants as part of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites. The amount of nitrogen available to plants is an important factor in plant growth. The most important nitrogen sources for plants are nitrate and ammonium (mineral nitrogen) contained in soils. In agricultural soils the average concentration of mineral nitrogen are 1-5 mM nitrate and 20-200 µM ammonium. Nitrate is not only higher in concentration but also more mobile in the soil solution, making it more available to plants. Organic nitrogen sources in agricultural soils include amino acids that dominate the pool of nitrogen bound to soil particles. As they are bound to particles they are less bioavailable and only taken up by plants in soils that are low in inorganic nitrogen. Organic nitrogen

is slowly degraded into plant available forms of nitrogen. The amino acid concentrations in the soil solution range between 0.1 and 100 µM. The availability of nitrogen to plants is dependent on soil properties such as texture, pH, moisture content and microbial activity and consequently varies in time and space (Hawkesford et al. 2012).

In agriculture large amounts of nitrogen are applied as fertilizers to increase crop production. For example, in 2008 about 100 million tons of nitrogen was used globally (FAO 2008). Apart from increasing plant growth nitrogen fertilization also affects the plant uptake of other nutrients such as zinc.

The zinc status of plants is affected by nitrogen in several ways (Alloway 2004): Nitrogen promotes plant growth, which increases the area in the soil that roots can reach and the surface area of them. The addition of nitrogen also decreases the pH of the soil solution, which leads to increased zinc activity in the soil solution resulting in more zinc uptake. Apart from increased root growth and decreased soil pH nitrogen could also affect (1) the root zinc uptake through the expression level of transport proteins in the root cell membrane, (2) the root-to-shoot transport of zinc by controlling the levels of proteins contributing to xylem loading or the chelation of zinc in the xylem and (3) the remobilization of zinc from vegetative tissue to the grain through the phloem (Cakmak et al. 2010). Furthermore delayed sescence caused by higher nitrogen availability can increase the grain zinc accumulation as the grain-filling period is extended (Kutman et al. 2010). In a recent study Erenoglu et al. (2010) tested if nitrogen affects plant zinc as suggested in effects (1) to (3) with radio-labelled zinc (^{65}Zn). Zinc uptake and root-to-shoot translocation rates showed a clear positive response to increasing nitrogen applications for plants grown under zinc deficient and zinc-sufficient conditions. Also the remobilization of zinc was larger for the plants with increased nitrogen supply. These results demonstrate the importance of the nitrogen nutritional status of wheat for zinc uptake and accumulation in the grain as it affects major steps including its uptake, xylem transport and remobilization via phloem.

Because nitrogen is the limiting factor for growth in many soils, crops often respond to zinc and nitrogen fertilization together but not to zinc fertilization alone. It is also known that the grain concentrations of protein/nitrogen and zinc are correlated positively in many plant species including wheat (Morgounov et al. 2007). This close relationship was confirmed by staining grain zinc and protein in a durum wheat cultivar. Both zinc and protein were highly concentrated in the embryo and aleurone

suggesting a co-localization. These results indicate that grain proteins represent a sink of zinc (Cakmak et al. 2010). Therefore the nitrogen nutritional status of plants is recognised as a critical tool for agronomic biofortification of zinc in wheat (Cakmak et al. 2010, Kutman et al. 2010). Kutman et al. (2010) observed a strong relationship between the grain concentrations of zinc and nitrogen when sufficient amounts of nitrogen and zinc were present in the soil. These results are in agreement with the findings of a field experiment that showed increased zinc concentrations in wheat grains fertilized with nitrogen (Shi et al. 2010).

Experiments in the field and pot experiments have shown a higher grain cadmium concentration with increased nitrogen fertilization (Gray et al. 2002, Li et al. 2011, Mitchell et al. 2000, Perilli et al. 2010, Wångstrand et al. 2007). Mitchell et al. (2000) observed an increase in the ionic strength of the soil solution and a decrease in soil pH with the addition of nitrogen in their pot experiment. They suggest that these changes increased cadmium solubilisation resulting in more cadmium uptake and accumulation in the wheat grain. Perilli et al. (2010) and Gray et al. (2002) support this explanation for the increased plant tissue cadmium concentrations with high nitrogen fertilization. However Li et al. (2011) showed no correlation between plant tissue cadmium concentrations and soil pH. Instead mass flow and transpiration rate in individual plant species and total cadmium in soils showed positive correlations with plant tissue cadmium concentrations (Kashem and Singh 2002). These results suggest that the nutritional nitrogen status of the plant increases the cadmium accumulation in grains in a similar matter to zinc through increased uptake and translocation in the plants.

2.7. Organic and conventional farming

Agricultural production has increased by 160% since the 1950s and is still projected to increase (FAO 2000). The OECD-FAO Agricultural Outlook 2010-2019 estimated 22% growth for the world net agricultural production between 2010 and 2019. Hence, an intensification of agriculture is still in progress and also necessary to cover the future demand for food as world population is expected to reach 9.3 billion people by 2050 (OECD-FAO 2010). On the other hand sustainable agriculture and the demand for organic products is also growing. Consequently, one of the greatest challenges for agriculture in the future is to guarantee food security for the growing world population whilst also ensuring greater sustainability of food production and environmental

protection. Data from 2011 has shown that currently 37.2 million hectares of land are managed organically worldwide, which is 0.9% of the total agricultural land. The land area under organic management has more than tripled in the last ten years (Figure 5). For some European countries, including Switzerland, more than 10% of agricultural land is now managed organically (Wiler 2011).

Figure 5: Organic agricultural land in the different continents between 2000 and 2009 (Wiler 2011)

In 2009 global sales of organic food and beverages had reached 55 billion US dollars with the largest markets being in the USA, Germany and France. In 2000 this market was only half the size with sales of 18 billion US dollars. The outlook for the future of organic farming shows a growth in demand for organic products in regions like Asia and Latin America. In growing economies such as India, Brazil and China people will become more educated and affluent and as a consequence demand more organic products. This development is expected to make sales less concentrated, as now 96% of demand for organic products is located in Europe and North America. This change will make the organic food industry truly global (Sahota 2011). The production of organic products and the landmass under organic management are also projected to grow in the next few years (Wiler 2011).

2.8. The DOK experiment

The DOK (Dynamic, Organic, Konventionell) experiment is a randomized field trial comparing biodynamic, bioorganic and conventional (in german: konventionell) arable farming systems. When the experiment was launched in 1978 the objective was to scientifically examine the feasibility of organic farming. Several organic farms had already been operating in Switzerland for decades, but most people including farmers, researchers and politicians, were convinced that agricultural production without substantial external inputs such as fertilizers and pesticides would not be practical in the long-term. Farmers initiated the DOK experiment in 1974 in cooperation with the former Federal Research Station for Agricultural Chemistry and Hygiene of Environment (FAC) and the Research Institute of Organic Agriculture (FiBL) and four years later the field experiment started.

The DOK experiment is located in Therwil BL, Switzerland (7° 33' E, 47° 30' N). The soil type is a haplic luvisol on deep deposits of alluvial loess. The climate is rather dry and mild with an average yearly precipitation of 785 mm and an average temperature of 9.5°C. Four farming systems are compared differing in their fertilization strategy and the applied plant protection management. The organic systems (BIOORG and BIODYN) were fertilized with farmyard manure (FYM) and slurry corresponding to 1.2 (1st and 2nd crop rotation period) and 1.4 (after 2nd crop rotation period) livestock units (LU) per hectare. One LU equals to approximately 600 kg of FYM and slurry and to an N input of approximately 50 kg N ha^{-1} and yr^{-1}. This fertilization intensity represents the intensity typically found on organic farms in Switzerland. In the bioorganic system slightly aerobically rotted FYM and slurry were used as fertilizer while in the biodynamic system aerobically composted FYM and slurry were used. One of the conventional systems (CONFYM) was fertilized with the same amount of FYM as the organic systems but in addition also received mineral fertilizers up to the recommended level of the plant specific Swiss standard recommendations. The other conventional system (CONMIN) was unfertilized during the first crop rotation but was then amended with mineral fertilizer only according to Swiss regulations (approximately 200 kg N ha^{-1} yr^{-1}) (Table 2). Since 1985 the conventional systems have been farmed according to the Swiss national regulations for integrated plant production (IP-Suisse) representing one type of good agricultural practice. Plant protection was conducted according to the guidelines of the

biodynamic and bioorganic systems. In the conventional systems, pesticides were only applied if economic thresholds for infections were exceeded according to the IP-Suisse scheme of plant protection (Table 2). An unfertilized plot (NOFERT) was also maintained, in which no fertilizer was applied and otherwise managed like the BIODYN system. The single plot size was 5 by 20 meters with a buffer zone strip of 6 meters in between plots, which was planted with grass and regularly mulched. The experiment was designed as a randomized block with four replicates each including three crops planted simultaneously in each system each year.

A seven-year crop rotation including cereals, vegetables, soya beans and grass-clover had been practiced identical in all systems since the start of the experiment (Table 3). Soil tillage was similar in all systems. The soils were ploughed to a depth of 15 to 20 cm before planting potatoes, winter wheat, cabbage, beetroot, soya beans and maize. The grass-clover mixture was sown in drills after rotary harrowing the cereal stubble field. The same varieties and grass-clover mixtures were cultivated for all the treatments.

Table 2: A summary of the fertilization and plant protection methods applied in each treatment of the DOK treatment. BIOORG and BIODYN are the two organic treatments while CONMIN and CONFYM are the conventional treatments of the DOK experiment. The soil used in this experiment was taken from the BIODYN and CONMIN treatments.

	BIOORG	BIODYN	CONMIN	CONFYM
Fertilization				
Type	slightly aerobically rotted FYM and slurry	aerobically composted FYM and slurry	exclusively mineral fertilizer according to official guidelines	stacked FYM and slurry and mineral fertilizer according to official guidelines
Level	1.2/1.4 LU ha^{-1} yr^{-1}	1.2/1.4 LU ha^{-1} yr^{-1}	IP- Suisse guidelines	IP- Suisse guidelines
Plant protection				
Weed control	mechanical	mechanical	mechanical and herbicides	mechanical and herbicides
Disease control	indirect methods, copper	indirect methods	fungicides (according to IP-Suisse guidelines)	fungicides (according to IP-Suisse guidelines)
Insect control	plant extracts, biocontrol	plant extracts, biocontrol	insecticides (according to IP-Suisse guidelines)	insecticides (according to IP-Suisse guidelines)
Special treatments	none	bio-dynamic preparations	plant growth regulators	plant growth regulators

Table 3: The five crop rotation schemes that have been run on the DOK trial since 1978. The soil samples for this experiment were taken during the 5th crop rotation in summer 2011.

1st crop rotation 1978 to 1984	2nd crop rotation 1985 to 1991	3rd crop rotation 1992 to 1998	4th crop rotation 1999 to 2005	5th crop rotation 2006 to 2012
Potatoes catch crop (rye)	Potatoes catch crop (rye)	Potatoes	Potatoes	Maize (silage)
Winter Wheat 1 catch crop (rye)	Winter Wheat 1 catch crop (rye)	Winter Wheat 1 catch crop (rye)	Winter Wheat 1 catch crop (rye)	Winter Wheat 1 catch crop (rye)
White cabbage	Beetroots	Beetroots	Soya beans catch crop (rye)	Soya beans catch crop (rye)
Winter Wheat 2	Winter Wheat 2	Winter Wheat 2	Maize (silage)	Potatoes
Winter Barley	Winter Barley	Grass- Clover 1	Winter Wheat 2	Winter Wheat 2
Grass-Clover 1	Grass-Clover 1	Grass- Clover 2	Grass-Clover 1	Grass-Clover 1
Grass-Clover 2	Grass-Clover 2	Grass- Clover 3	Grass-Clover 2	Grass-Clover 2

In the first phase the focus of the DOK experiment was clearly on agronomic interests. The resulting rich database from 20 years of monitoring has immensely contributed to the acceptance of organic farming. In the last decade, research in the DOK experiment has more focused on key soil processes and on crop quality. Involved research groups are working in several different research fields including soil microbial diversity, soil carbon transformation, phosphorus and nitrogen transformation, soil-plant interface, soil food webs and food quality.

Some key findings from the DOK experiment in terms of fertilizer input, crop yield and soil fertility will be presented in the following. The results cover four crop rotation periods from 1978 to 2005:

- Mean annual fertilizer input (total nitrogen, phosphorus and potassium together) was reduced by 35-40% in the organic systems compared to the conventional systems. The input of nitrogen in mineral form in FYM or synthetic fertilizer was even reduced by 65-70%.

- The mean yields of all seven crops per rotation in the organic systems were 80% of those in the conventional systems. Potato reacted the strongest to the management change with an average reduction in yield of 33-43% whereas winter wheat only showed a reduction of 15%.

- Mycorrhizal root symbioses in the DOK experiment revealed a higher degree of root colonization of crops grown in organic plots and enhanced mycorrhizal species diversity as assessed by morphological spore analysis.

- Several indicators of soil fertility such as pH, soil organic matter, microbial biomass and enzyme activities showed more favourable values for the organic systems. The high production efficiency of the organic system, with only 20% reduction in yield and 30-35% reduction in fertilizer inputs, may be related to the increased soil fertility.

The results presented here are an indication of sustainability of the organic farming systems. However the finding that nutrient balances such as for phosphorous are negative in the organic systems has also to be taken into account.
For this experiment, soil from the BIODYN and the CONMIN treatments was used.

3. Material and Methods

3.1. Material

Table 4 shows the list of chemicals that were used during the analysis of this study. For each chemical the chemical formula and manufacturer is given. Table 5 shows the instruments used during the experiment and the manufacturer of each instrument.

Table 4: List of the chemicals used during this study, including the chemical formula and the manufacturer.

Chemical	Formula	Manufacturer
Ammonium nitrate	NH_4NO_3	Merck, Darmstadt, Germany
Ammonium sulphate	$(NH_4)_2SO_4$	Merck, Darmstadt, Germany
Boric acid	H_2BO_3	Merck, Darmstadt, Germany
Buffer solutions pH		Merck, Darmstadt, Germany
Cadmium chloride	$CdCl_2.H_2O$	Fluka, Buchs, Switzerland
Calcium carbonate	$CaCO_3$	Merck, Darmstadt, Germany
Calcium chloride	$CaCl_2.2H_2O$	Merck, Darmstadt, Germany
Calcium chloride	$CaCl_2.6H_2O$	Merck, Darmstadt, Germany
Copper sulphate	$CuSO_4.5H_2O$	Merck, Darmstadt, Germany
DTPA	$[(HOOCCH_2)_2NCH_2]_2NCH_2COOH$	Fluka, Buchs, Switzerland
Hydrochloric acid	HCl	Merck, Darmstadt, Germany
Hydrogen peroxide	H_2O_2 35%	Merck, Darmstadt, Germany
ICP Standards		Merck, Darmstadt, Germany
Iron sulphate	$FeSO_4.7H_2O$	Merck, Darmstadt, Germany
Magnesium sulphate	$MgSO_4.7H_2O$	Merck, Darmstadt, Germany
Manganese sulphate	$MnSO_4.7H_2O$	Merck, Darmstadt, Germany
Na-Hypochlorite	$NaClO$	VWR, Radnor, USA
Na-nitroprusside	$Na_2[Fe(CN)_5NO]$	Fluka, Buchs, Switzerland
Na.salicylate	$C_7H_5NaO_3$	Merck, Darmstadt, Germany
Na_2-EDTA	$C_{10}H_{14}N_2Na_2O_8.2H_2O$	Merck, Darmstadt, Germany
NEDD	$C_{10}H_7NHCH_2CH_2NH_2.2HCl$	Merck, Darmstadt, Germany
Nitric acid	HNO_3 65%	Merck, Darmstadt, Germany
Potassium chloride	KCl	Fluka, Buchs, Switzerland

Chemical	Formula	Manufacturer
Potassium dichromate	$K_2Cr_2O_7$	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	KH_2PO_4	Merck, Darmstadt, Germany
Potassium nitrate	KNO_3	Merck, Darmstadt, Germany
Potassium phosphate dibasic anhydrous	K_2HPO_4	Fluka, Buchs, Switzerland
Sodium hydroxide	$NaOH$	Riedel-deHaën AG, Seelze, Germany
Sodiummolybdate	$Na_2MoO_4.2H_2O$	Merck, Darmstadt, Germany
Sulfuric acid	$H_2SO_4\,96\%$	Fluka, Buchs Switzerland
Sulphanilamide	$C_6H_8N_2O_2S$	Riedel-deHaën AG, Seelze, Germany
TEA	$C_6H_{15}NO_3$	Fluka, Buchs Switzerland
Vanadium chloride	VCl_3	Aldrich Chemie, Buchs, Switzerland
Zinc sulphate	$ZnSO_4.7H_2O$	Merck, Darmstadt, Germany

Table 5: List of the instruments used during this study including the manufacturer.

Instrument	Manufacturer
Block Digestion System (DigiPrep MS)	SCP Science, Courtaboeuf, France
CNS Analyser (2000)	Leco, Saint Joseph, USA
Electric conductivity meter (LF318)	WTW GmbH, Weilheim, Germany
Fact Balance (PB3002)	Mettler Toledo, Greifensee, Switzerland
Fact Fine Balance (AB2004-S)	Mettler Toledo, Greifensee, Switzerland
Horizontal shaker (K250)	Janke & Kunkel, Staufen, Germany
ICP-MS	Varian, Palto Alto, USA
Mixer mill (MM200)	Retsch GmbH, Haan, Germany
pH Meter (780)	Metrohm AG, Zofingen, Switzerland
Plant grinder (RS1)	Retsch GmbH, Haan, Germany

Instrument	Manufacturer
UV Photometer (50 Scan)	Varian, Palto Alto, USA
Vista-MPX CCS simultaneuous ICP OES	Varian, Palto Alto, USA
X-Ray Fluorescence Spectrometer (X-lab 2000)	Spectro Analytical instruments GmbH, Kleve, Germany

Table 6 shows the detection limits for all measured elements on the ICP-OES and the ICP-MS.

Table 6: Detection limits for all measured elements for the instruments used during analysis.

Element	Detection Limit ICP-OES [ppb]	Detection Limit ICP-MS [ppb]
Ca	10	
Cd		0.02
Cu	5	0.04
Fe	5	
K	5	
Mg	5	
Mn	5	
P	5	
Zn	5	0.02

3.1.1. Wheat (Fiorina spring wheat, untreated and fungicide-free)

For this experiment a wheat variety was required that is currently used in both organic and conventional farming as this experiment aims to compare the two systems. The Swiss variety Fiorina was recommended for organic and conventional (IP-Suisse) farming for the year 2011 and was therefore chosen for the experiment (FiBL 2010, Hiltbrunner et al. 2011). It is a spring wheat variety that has been listed in the recommendations since 2001. Fiorina was given the best grades for its baking quality by the trade organisation swiss granum and it also convinces due to its high yield and

resistance to low temperatures (Hiltbrunner et al. 2011). The germination of the seeds (Semences UFA Samen, Switzerland) was started on the 19th October 2011. Around 350 seeds were used. The seeds were first sterilized by placing them in 10% peroxide for 15 minutes followed by thorough rinsing with nanopure water. The seeds were then left on wet filter papers in petri dishes and covered with wet tissues. The filter papers and tissues were kept moist during the next seven days while germination took place.

3.2. Soil sampling

Soil samples were taken at the DOK field experiment in Therwil, Switzerland (7° 33' E, 47° 30' N) in June 2011. The three crops cultivated at the DOK experiment in 2011 were grass-clover, soya beans (Avline) and potatoes (Desiré). Samples were only collected from the soya bean and potato cultures, as it was easier to access the soil than in the grass-clover culture. Treatments M (conventional) and D2 (organic) were sampled. To achieve the least disturbance to the crops each soya bean and potato plot of the two treatments was sampled.

Table 7 shows the plots that were sampled. In each plot samples were taken from six different locations in between cultures to a depth of 15 cm, as only topsoil was needed for this experiment. The aim was to take around 7 kg dry weight of soil from each plot. All soil was sieved to 5 mm while at field moisture content. Equal quantities of dryweight soil from each plot were mixed together for each treatment (D2 and M). It was important to have equal quantities of soil from the soya and the potato culture, as the cultures may have an effect on the nutrient status of the soil. In order to do this the moisture content of both DOK treatment soils was measured and it was calculated how much dry weight soil was taken from each plot and how much would need to be put into the mix from each sample bag. For both treatments 31.1 kg of potato culture soil and 36.3 kg of soya bean culture soil were required. The calculated amount of soil from each sample bag was put into a container and mixed well. The soil was then put into separate containers according to their further treatment: M Control, M Cd, M Zn, M CdZn, D2 Control, D2 Cd, D2 Zn and D2 CdZn.

Table 7: Treatments, plots and cultures of the DOK trial where soil samples were taken from. Also included in the table is the amount of each sample bag in dry weight that was used to mix the soils for both DOK treatments.

DOK Treatment	DOK Number	Culture	Dry weight used in the mix [kg]
D2	10	Soya	9.3
D2	44	Soya	8.5
D2	54	Soya	8.0
D2	88	Soya	10.5
D2	8	Potato	7.8
D2	48	Potato	7.7
D2	52	Potato	7.0
D2	86	Potato	8.6
M	4	Soya	11.8
M	38	Soya	6.6
M	60	Soya	10.0
M	94	Soya	7.9
M	2	Potato	8.6
M	42	Potato	7.8
M	58	Potato	7.0
M	92	Potato	7.7

3.3. Soil spiking

To approximate the amount of cadmium and zinc required for spiking the soil, a rough estimate of the cadmium and zinc concentration in the soil was performed using XRF. The average zinc concentration was around 60 mg/kg and the cadmium concentration varied between 1 and 2.5 mg/kg for both DOK treatments. Both DOK treatments were spiked with the same amount of cadmium and zinc as the measured metal concentrations were in the same range. For the zinc (Zn) treatment an end

concentration of 400 mg/kg was requested so 340 mg/kg zinc was spiked to both DOK treatment soils. For the cadmium treatment 2 mg/kg was spiked to both DOK treatments. For the cadmium and zinc combination treatment (CdZn) the two DOK soils were spiked with both, 340 mg/kg Zn and 2 mg/kg Cd. The zinc was spiked to the soil as a 124.8 mM $ZnSO_4.7H_2O$ solution and the cadmium was added as a 0.427 mM $CdCl_2.H_2O$ solution. For spiking, the soil was spread out in a large plastic container (1.5x1.5m) mixed and then sprayed with the correct amount of the above solutions or deionised water in the case of the control soils (Table 8). The soils were thoroughly mixed after spiking. The addition of spiking solution and water was the same for each treatment and was kept to the minimum while still achieving homogeneous spiking.

The soils were then stored in covered containers that were not airtight, in a cool room for three months to reach equilibrium conditions. The soils were mixed monthly during this time.

Table 8: Spiking of the soils according to their treatment: Shown are the treatments and the amount of soil to be spiked for each treatment. Also shown is the percent dry weight (DW) before and after spiking and the amount of solution added to each treatment during the spiking. To keep the control soils under the same moisture content as the other soils water was added to these soils.

DOK treatment	% DW	Treatment	DW spiked	solution added [l]	new % DW
D2	82.82	Control	18.63	0.693	80.34
D2	82.82	Zn	12	0.5	80.06
D2	82.82	Cd	12	0.5	80.06
D2	82.82	CdZn	24	1	80.06
M	83.71	Control	16.63	0.788	80.52
M	83.71	Zn	12	0.5	80.89
M	83.71	Cd	12	0.5	80.89
M	83.71	CdZn	24	1	80.89

3.4. Pot experiment

3.4.1. Fertilization

For the fertilization it was vital that all necessary macro- and micronutrient were available for the plants in sufficient amounts (except Zn and N) so the plants' growth and development were not limited. The experiment also consisted of two different nitrogen treatments. The low nitrogen level was selected so that fertilization alone would not be enough for optimal plant growth and the plant may show a response to residual soil nitrogen. The high nitrogen level was chosen at an optimal level so no residual soil nitrogen effect would be seen in the plant response (Figure 6). Swiss farmers fertilize roughly 120 kg N ha^{-1} y^{-1}, which is equal to approximately 40 mg N/kg soil (IP-Suisse 2011). So the low level of nitrogen (50 mg/kg) corresponds to the average nitrogen fertilization on a Swiss farm. For the high nitrogen level three times this average fertilization was chosen (150 mg/kg).
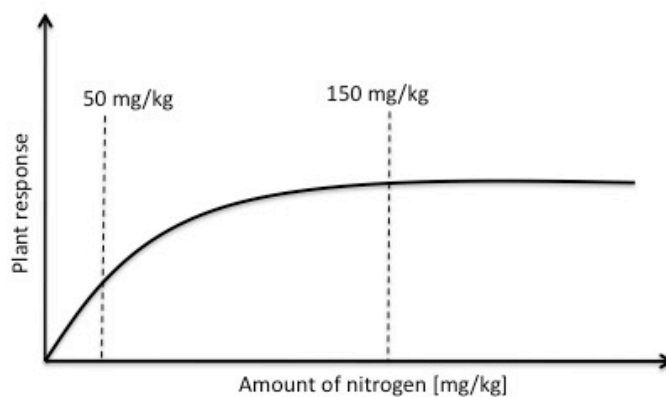
Figure 6: Plot of the amount of nitrogen given to a plant as fertilizer and the plant's response to this fertilization. Also shown in the graph are the two levels of nitrogen fertilization used in this experiment (50 mg/kg and 150 mg/kg) and their location within the plot.

The fertilizer was applied to the soil as a solution. Phosphorus was fertilized from a separate solution and all other required nutrients were mixed in one solution either with the high level of nitrogen or the low level of nitrogen. The amount of each nutrient in the solution was calculated with Hoagland solution rates. The calculations were based on 300 mg/kg potassium (K) and 150 mg/kg phosphorus (P) which are the required amounts by spring wheat as shown in Reuter and Robinson (1986). Zinc was

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not added as fertilizer as it was already spiked to the soils previously. Table 9 shows the amount of

each nutrient added to the soils during the pot experiment. The fertilization was done in three doses: The first dose was added to the pots one week before the wheat seedlings were planted (mid October), the second dose was given at tillering (mid November) and the third dose at flowering (end of December). To each pot 20 ml of either the low or high nitrogen solution and 5 ml of the KH_2PO_4 solution was added.

Table 9: Shown are the compounds and their amounts used to make up the fertilizer solutions for the low and high levels nitrogen treatments. The fertilization was based on 300 mg/kg K and 150 mg/kg P. Zinc was not fertilized as it was already spiked to the soils previously.

Compound	Low N treatment [mMoles/kg]	High N treatment [mMoles/kg]
KH_2PO_4	4.84	4.84
KCl	2.84	2.84
$CaCl_2$	2.5	2.5
$MgSO_4.7H_2O$	2.56	2.56
$FeSO_4.7H_2O$	0.13	0.13
H_2BO_3	0.13	0.13
$MnSO_4.H_2O$	0.03	0.03
$ZnSO_4.7H_2O$	-	-
$CuSO_4.5H_2O$	$2.56 * 10^{-3}$	$2.56 * 10^{-3}$
$Na_2MoO_4.2H_2O$	$1.28 * 10^{-3}$	$1.28 * 10^{-3}$
NH_4NO_3	1.78	5.35

3.4.2. Pots and growth conditions

The pots were prepared in mid October 2011. Each pot was filled with 750 g of soil (dry weight). The soil was fertilized with the first dose of fertilizer solution and mixed thoroughly before putting it into the pots. From previous experience with pot experiments with DOK soils it is known that the soil is best kept at 50% field capacity during the experiment (Fliessbach et al. 2009). Here we started with 57% field capacity,

as we needed to add a certain volume of moisture during fertilization. To reach 57% container capacity a small amount of deionised water was also added to the soil. The pots were then allowed to dry to 50% field capacity and kept at this level for a week. On the 24th of October 2011 two wheat seedlings were planted per pot. After four weeks the plants were thinned out and one plant in each pot was carefully taken out. The pots were kept at 50% container capacity for the rest of the plant growth. Table 10 shows the conditions in the climate chamber during the plant growth.

Table 10: Shown are the conditions present in the climate chamber during plant growth in this experiment. The system had five steps adding up to 24 hours with varying temperature, humidity and light intensity.

Cycle Step	Duration [h]	Temperature [°C]	Humidity [%rH]	Light intensity [%]
1	0.1	22	70	0
2	0.5	22	65	80
3	14	22	65	80
4	0.5	22	70	0
5	9	15	70	0

3.4.3. Plant observations

During plant growth the plants were carefully observed. Every week photos were taken of the plants per treatment. A plant diary was kept in which the number of leaves, tillers, heads and deficiency or toxicity symptoms were written down once a week.

3.5. Plant harvest and pot soil sampling

The wheat plants were harvested at maturity on the 28th of February 2012 (after 19 weeks of growth). The wheat was cut 1 cm above ground, washed thoroughly with deionised water and nanopure water, dried off with tissues and finally dried to constant weight at 60°C in an oven. The heads of each plant were cut off and kept in separate paper bags for drying. The next day the soil from the pot was sampled. Roots were picked out from the soil whenever possible. About 10 g of soil from each pot was frozen straight after sampling for ammonium and nitrate analysis. About 25 g of soil was

weighed and put in the oven at 105°C to determine the moisture content. Another 100 g of soil was sampled for the rest of the analysis and dried until constant weight at 40°C.

3.6. Soil analysis

Some soil analyses described in the following chapter were carried out on the control soils as part of the soil characterisation and some were carried out on each soil treatment prior and/or at the end of the experiment. Table 11 gives an overview of all the methods and on which soils they were performed.

Table 11: This table gives an overview of all the analysis methods performed on soil during this experiment. It shows which method was performed only on the control soils and which one was performed on all the treatment soils during the soil characterisation. Also shown is which methods were performed on the pot soils after the experiment had finished.

Analysis	Soil characterisation: only control soils	Soil characterisation: All treatments	Pot soils
Moisture content		X	X
Container capacity		X	
Org. matter	X		
CaCO$_3$	X		
Texture	X		
pH		X	X
Electric conductivity		X	
DTPA Zn Cd		X	X
DTPA Fe Cu Mn		X	X
XRF		X	
Mineral N	X		X
Total N	X		X

3.6.1. Soil sample preparation

The soil was sieved to 5 mm right after sampling while still moist. After the metal spiking and the storage for three months the soil was dried at either 40 or 60°C and ground depending on the analysis. Table 12 shows each method performed on these soils and what kind of soil preparation was applied.

Table 12: The table shows how the soil samples were prepared for each soil characterisation method performed during this experiment. The soil samples were either dried or used moist and either ground or sieved.

Soil preparation		Soil characterisation method
5 mm sieved	moist	Mineral nitrogen extraction
5 mm sieved	dry (40°C)	pH, EC, DTPA extraction, texture
ground	dry (40°C)	CaCO$_3$, Org. matter. Total N
ground	dry (60°C)	XRF

3.6.2. Moisture content

Moist soil was added to a weighed aluminium container and then also weighed. The soil was left in a drying oven at 105°C for 24 hours or until constant weight and then weighed again. The moisture content in percent was then calculated as follows:

Moisture content (%) = (soil$_{wet}$ [g] − soil$_{dry}$ [g])/ soil$_{wet}$ [g] * 100

(after DIN norm 18121-1).

3.6.3. Container capacity

The container capacity of a soil describes the water-holding capacity or mean (equilibrium) water content of the soil in the container used for growing containerized plants. Container capacity is reached when the hydraulic head becomes constant at each elevation in the container. The time for the soil to drain to container capacity is less than for the same soil to drain to field capacity.

To measure the container capacity moist soil was weighed into a weighed pot. The pot was then placed in a water bath for 12 hours. The pots were drained for 6 hours by rising them above the ground without covering the holes at the bottom of the pot. After 6 hours of drainage the pots were weighed and then dried at 105°C until constant weight

and weighed for the last time. The container capacity is calculated from the obtained data as follows:

$$CC = (M_{drained} - M_{dry})/M_{dry} * 1000$$

CC	Container capacity [g H_2O/kg soil]
$M_{drained}$	Mass of soil after drainage [g]
M_{dry}	Mass of dry soil [g]

(Cassel and Nielsen 1986)

3.6.4. Total metal concentration - XRF

To measure the total metal concentration in soil using X-ray fluorescence (XRF) 4 g of dried and ground soil were weighed into 100 ml plastic tubes with 0.9 g of micro powder wax and two beads for shaking. The soil and wax were mixed well on a mixer mill at 17 Hz for eight minutes. After shaking the beads were removed and the soil and wax mixture was pressed into pellets using 15 tonnes of pressure. The pellets were then analysed using XRF spectroscopy (Evangelou and Studer 2009).

3.6.5. Plant available metal concentration - DTPA extraction

The diethylenetriaminepentaacetic acid-triethanolamine (DTPA-TEA) extraction was developed for the multi-element extraction of copper, iron, manganese and zinc to assess their plant availability, especially for neutral and alkaline soils. In principal DTPA is a chelating agent that complexes metals and is here used as the extractant. The DTPA molecules form water-soluble metal complexes with copper, iron, manganese and zinc and therefore decrease the free metal ion concentration in the soil solution. Then the mentioned metals desorb from soil surfaces to replenish free metal concentration in the soil solution (Reed and Martens 1996).

Reagents:

Diethylenetriaminepentaacetic acid (DTPA):

13.25 ml reagent-grade tetraethanolamine (TEA)

1.97 g DTPA

1.47 g $CaCl_2.2H_2O$

Were dissolved in about 900 ml nanopure water. The solution pH was adjusted to 7.3 with 2M HCl and made up to 1 l.

10 g of air-dried soil was extracted with 20 ml of the DTPA solution in a 125 ml plastic bottle on a horizontal shaker at 120 cycles/min for two hours. Two blank samples containing only 20 ml of DTPA solution, were also carried out. The suspension was filtered through Whatman No 589/3 ashless filter paper.

The filtered solutions were then analysed with ICP-OES for their zinc, cadmium, copper (Cu), manganese (Mn) and iron (Fe) concentrations.

The calibration standards were made up using DTPA as the solution medium and prepared from ICP standard solutions:

1st initial soil measurement:
Zn in mg/l: 0, 0.25, 0.5, 0.75, 1, 25, 50, 75, 100
Cd in mg/l: 0. 0.25, 0.5, 0.75, 1

2nd initial soil and pot soil measurement:
Zn, Cd, Fe, Cu, Mn in mg/l:
0, 0.0125, 0.025, 0.05, 0.125, 0.5, 2.5, 5, 12.5, 25, 50

(Reed and Martens 1996).

3.6.6. Mineral nitrogen concentration (nitrate and ammonium)

Extraction:

Reagents:

2M KCl : 150 g KCl in 1 litre nanopure water.

5 g of moist soil was extracted with 50 ml 2M KCl in 100 ml plastic bottles using a horizontal shaker for one hour. The suspension was filtered through Whatman No 589/3 ashless filter paper. Two blank samples, which only contained 50 ml of KCl were treated in the same way as the samples containing soil. The samples were frozen after the extraction until the analysis was carried out.

Colourmetric nitrate method:

Reagents:

200 mg of VCl_3 in 25 ml 1M HCl. Excess solid was removed with a 0.45 μm syringe filter.

25 mg NEDD in 25 ml of nanopure water.

500 mg sulphanilamide in 25 ml 5% HCl.

NO_3^- standard stock solution: 0.0722 g of KNO_3 in 100 ml of nanopure water.

The following standards were prepared using KCl as the solution medium
in mg/l NO_3^--N: 0, 0.5, 1, 2, 3, 5
The detection limit is 0.01 mg /l NO_3^--N in the soil extract.

For the colourmetric analysis 350 μl of VCl_3 solution was added to 350 μl of sample or standard solution, then 150 μl of NEDD solution and 150 μl of sulphanilamide was also added to the cuvette. The cuvettes were shaken and left for the colour to develop for 40 minutes. The absorbance was read at 540 nm using an UV-Photometer. The results for the standard solutions were plotted using excel to make a linear calibration curve. The sample results were calculated according to the linear regression function.

Colourmetric ammonium method:

Reagents:

3.91 g of Na-salicylate and 0.0625 g Na-nitroprusside in 50 ml of nanopure water.

1.5 g of NaOH and 5 g of K_2HPO_4 were added to 40 ml of water, then 5 ml of Na-hypochlorite was added and pH was adjusted to 13.0 with NaOH and made up to 50 ml with nanopure water.

0.6 g of Na_2EDTA in 10 ml of nanopure water.

NH_4^+ standard stock solution: 0.0471 g of $(NH_4)_2SO_4$ in 100 ml nanopure water.

The following standards were prepared using KCl as the solution medium
in mg/l NH_4^+-N: 0, 0.5, 1, 2, 4, 6
The detection limit is 0.2 mg /l NH_4^+-N in the soil extract.

For the colourmetric analysis 650 μl of sample or standard, 50 μl of EDTA, 200 μl of Na-salicylate-nitroprusside reagent and 100 μl of hypochlorite reagent were added to a 1 ml cuvette. This mixture was shaken and left at room temperature for two hours. Then the absorbance was read at 667 nm with a UV-Photometer. The results for the standard solutions were plotted using excel to make a linear standard curve. The sample results were calculated according to the linear regression function (Keeney and Nelson 1982).

3.6.7. Soil pH (with H_2O and $CaCl_2$)

10 g of soil were added to a plastic bottle along with 25 ml of nanopure water or 0.01 M $CaCl_2$ and shaken on an overhead shaker for one hour. The bottles were removed and allowed to settle for a few minutes. The pH meter was calibrated before use with buffer solutions at pH 4 and pH 7. The sample pH was then measured in the soil slurry (FAL 1996).

3.6.8. Total nitrogen

Dried and ground soil, shoot and grain samples were weighed out accurately into aluminium caps and folded into small balls. For soil samples 40 mg of sample was weighed in and for plant shoot and grain samples 4 mg sample were needed. The samples were then analysed through dry combustion with a CNS Analyser.

3.6.9. Carbonate

To accurately measure the carbonate content of the soil a calcimeter is used. The calcimeter is designed to measure the CO_2 gas released from soil samples when acid is added. The released CO_2 can be related to the carbonate content of the soil using the gas equation. Here a calibration was used to obtain the carbonate content of the soil.

Reagents:

4M HCl

The glass burettes of the calcimeter were filled with water and the soil sample (2 g of dried and ground soil) and 20 ml of distilled water were added to a 500 ml Erlenmeyer flask and mixed well. A small acid filled glass tube (7 ml of 4M HCl) was carefully placed in the sample flask so not to empty the tube. The calcimeter was then attached to the flask in an airtight manner and the burette was closed against outside air. The sample flask was shaken to finally pour the acid over the soil sample. After acid addition, the flask was left for 5 to 30 minutes and then the level of the water in the glass burette of the calcimeter was checked and noted. Before measuring the samples the calcimeter is calibrated using pure $CaCO_3$ samples. For the calibration 0 g, 0.2 g and 0.4 g of $CaCO_3$ were added to an Erlenmeyer flask and were then treated in the same manner as the soil samples described above. The water level found for samples after 30 minutes was converted to % $CaCO_3$ using the calibration curve (Evangelou and Studer 2009).

3.6.10. Organic matter

To measure the organic matter content in soils the following chemical processes are used:

Under acidic conditions dichromate oxidizes organic carbon (Corg) to CO_2 and is reduced to Cr^{3+}:

$$2\ K_2Cr_2O_7 + 3\ Corg + 8\ H_2SO_4 \rightarrow 3\ CO_2 + 2\ Cr_2(SO_4)_3 + 2\ K_2SO_4 + 8\ H_2O$$

After this reaction is completed the left over dichromate is reduced to Cr^{3+} using Fe^{2+}:

$$K_2Cr_2O_7 + 6\ FeSO_4 + 7\ H_2SO_4 \rightarrow 3\ Fe_2(SO_4)_3 + Cr_2(SO_4)_3 + K_2SO_4 + 7\ H_2O$$
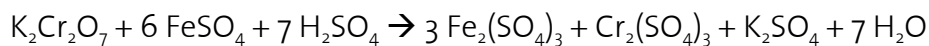
As soon as all of the Cr is reduced residual Fe(II) appears in the solution. Fe(II) in the solution is determined with a colour indicator :

$$Fe^{2+}\ (colourless) + Phenanthrolin\ (colourless) \rightarrow Fe\text{-}Phenanthrolin\ (red)$$

The organic matter content of the soil is then determined from the difference between the added and the residual dichromate.

Reagents:

0.1667 M potassium dichromate: 49.03 g $K_2Cr_2O_7$ in 1000 ml nanopure water.

0.2 M Fe^{2+} solution: 78.42 g $Fe(II)(NH_4)_2(SO_4)_2.6H_2O$ in 500 ml nanopure water, followed by 15 ml of 96% H_2SO_4 and filled up to 1000 ml with nanopure water.

Ferroin colour indicator: 1.485 g o-Phenenthrolin-Monohydrate and 0.695 g $FeSO_4.7H_2O$ in 100 ml warm nanopure water.

1 g of sieved soil was weighed in a 500 ml Erlenmeyer flask and 10 ml of the potassium dichromate were added. The flask was shaken briefly before 20 ml of the 96% H_2SO_4 were added and shaken well. The flask was left for 20 minutes and occasionally shaken carefully. After the solution had cooled 150 ml of nanopure water were added. This solution was then filtered. Two blank samples, which didn't contain any soil were treated in the same manner as the soil samples. Five drops of colour indicator were

added to each sample and then titrated with the Fe(II) solution. The blank samples were also titrated to determine the exact concentration of the Fe(II) solution.

The following equation to determine the organic matter content is deduced from the stoichiometric relationships of the reactions mentioned above:

%Corg. = (10 - 10*y/x) * 300 / z

x: Fe(II) solution used in blank samples (ml)
y: Fe(II) solution used in soil sample (ml)
z: Amount of soil added to sample (mg)

% org. matter = % Corg * 1.724

(Evangelou and Studer 2009)

3.6.11. Texture

The method used to measure soil texture is based on the fact that soil particles reach a certain settling rate when they are dispended in a surrounding medium such as water or air. According to Stoke's law this settling rate is greater the bigger the particle is:

$v = (D-D")*g*d^2/18\eta$

v   settling rate
D   density of the particle
D" density of the fluid
d   diameter of the particle
η viscosity of the fluid
g   apparent gravity

Diameter	Component	Depth	Time
50 μm	Sand and Silt	19 cm	84 sec
2 μm	Clay	2.6 cm	2 hours

The organic matter was removed by oxidization with peroxide. 50 g of soil was added to a 500 ml beaker and placed on a sandbath at 100°C. 35% H_2O_2 was slowly added to the soil. When the reaction was over, the soil was dried on the sandbath and then put in the oven at 105°C.

5 g of the sample (soil without organic matter) was dispersed in a 0.2% calgon solution in a ultrasonic bath and then poured into a 500 ml measuring-cylinder. The cylinder was filled up with the calgon solution and shaken well. After the visible turbulence had passed, time measurement was started. After time t (84 sec and 2 hours) 10 ml of sample were taken out at either 2.6 cm or 19 cm depth of the measuring cylinder. The samples were dried at 105°C and then weighed. One blank sample, which only contained 10 ml of calgon and no soil was also dried and weighed.

For the calculation of the texture components (sand, clay and silt) in percent the following formula has been affiliated:

g(%)= Gp * K = Gp * (100*Vt/Vp*Gt)

Vt Content of the measuring cylinder [ml]
Vp Amount of sample taken out at time t [ml]
Gt Amount of soil without organic matter weighed in [g]
Gp Weight of dried sample taken out at time t [g]

(Evangelou and Studer 2009)

3.6.12. Electrical conductivity

10 g of soil and 25 ml of nanopure water were added to a plastic bottle and shaken in an overhead shaker for one hour. The suspension was allowed to settle for a few minutes before the measurement was performed using an electric conductivity meter (WTW, LF 318) (after DIN norm 11265).

3.1. Plant analysis

3.1.1. Plant sample preparation

Dried shoot samples were ground in a plant grinder and stored in airtight plastic bags. The heads were taken apart to remove the grains. The grain samples were then ground in a mixer mill and stored in airtight containers for later analysis.

3.1.2. Biomass

After the plant material was dry the biomass was recorded for heads, shoots and grains. Furthermore the grains of each plant were counted. With this information the grain dry biomass/100 grains was calculated. To complete the harvest index (HI) was calculated:

HI = dry biomass grains [g]/dry biomass whole plant [g] * 100

3.1.3. Plant total metal concentrations

Plant samples were digested using a digestion block. 200 mg of plant material were weighed into teflon tubes and 15 ml of nitric acid was added. The tubes were then put in the heating block that had reached a temperature of around 80°C. The block was programmed at 95°C for 90 minutes. The tubes were taken out of the block and left to cool before the addition of 8 ml of hydrogen peroxide. The tubes were put back into the digestion block for another 90 minutes at 95°C. After the program finished the tubes were taken out of the block to cool and then diluted to 50 ml using nanopure water. The samples were analysed using ICP-OES and ICP-MS.

Shoots:
ICP-OES: Calcium (Ca), Fe, K, Magnesium (Mg), Mn, P (1:10 dilution)
        Standards: Ca, K, P in µg/l: 0, 0.1, 0.25, 0.5, 1, 2.5, 5, 10
                        Mg, Mn in µg/l: 0, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2
ICP-Ms: Cd, Cu, Zn (1:20 dilution)
        Standards: Cd, Cu in µg/l: 0, 0.5, 1, 2.5, 5, 10, 50, 100
                        Zn in µg/l: 0, 5, 10, 25, 50, 100, 500, 1000

Grains:

ICP-OES: Ca, Cu, Fe, Mn, Zn (undiluted)

        Standards: Ca in mg/l: 0, 0.1, 0.25, 0.5, 1, 2.5, 5, 10

                       Cu in µg/l: 0, 2, 5, 10, 20, 50, 100, 200

                       Fe, Mn, Zn ing mg/l: 0, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2

        Mg, K, P (1:5 dilution)

        Standards: Mg, P in mg/l: 0, 0.1, 0.25, 0.5, 1, 2.5, 5, 10

                       K in mg/l: 0, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2

ICP-MS: Cd (1:2 dilution)

        Standards: Cd: 0, 0.5, 1, 2.5, 5, 10, 50, 100 µg/l

3.1.4.   Total nitrogen, grain protein content

Total nitrogen was measured for the shoot and grain samples as well as the soil samples (see chapter 3.6.8 for detailed description). The grain protein content was calculated by multiplying the total nitrogen content in grain with the factor 5.7 (Zörb et al. 2010).

3.2.   Statistical analysis

All data was checked for statistical significance through two-way anova (for the initial soil dataset) or three-way anova (for the datasets after experiment completion) using R. If the data sets were not normal distributed the statistical model was adjusted accordingly (squared or logarithmised).

4. Results

4.1. Soil

4.1.1. Soil characterisation

The initial soil characterisation was performed on the control soils for the two soil types, organic and conventional. In addition, some of the analysis as part of the soil characterisation was also carried out for the metal spiked soils. The data was obtained at the start of the pot experiment. The soil classified as a silt loam according to US-Soil taxonomy. It contained less than 1% calcium carbonate for both soil types. The organic carbon was 1.3% organic carbon for the organic soil while the conventionally managed soil contained only 1% organic carbon. The conventional soil had a pH of 5.5, which was lower than the pH of the organic soil at 5.7 ($p<0.001$). More total cadmium was contained in the conventionally managed soil whereas DTPA extractable cadmium was about the same for both soil types. The total zinc content was about the same for both soils, with 64 mg/kg total zinc in the organic soil and 59 mg/kg total zinc in the conventional soil. These values for total zinc are close to the average total zinc concentration for non-contaminated soils, which is set at 50 mg/kg soil zinc (Alloway 2004). The plant available zinc concentrations were 1.82 mg/kg for the organic soil and 1.29 mg/kg for the conventional soil. The total nitrogen content was significantly higher for the organic soil compared to the conventional soil ($p<0.001$). It also contained significantly more nitrate ($p<0.05$) (Table 13).

The two different management strategies show a few differences in the soil characterisation. The mineral fertilization in the conventional soil caused a more acidic pH, as mineral nitrogen addition lowers pH and a higher total cadmium concentration in the soil, as mineral fertilizers often contain cadmium as a contamination. The conventional soil was limed in the past but the difference in pH is still present. In the organic soil the total nitrogen content and the organic carbon content were increased through the addition of organic fertilizers such as manure and slurry. So more organic carbon and nitrogen is contained and stored in the organic soil. Plant available zinc was also more concentrated in the organic soil than the conventional soil even though pH was less acidic in the organic soil. This difference is most likely caused by the zinc bound to organic matter as the organic soil contained more organic matter.

Table 13: Shown are the results of the soil characterisation of the two soils, organic and conventional, used in this pot experiment.

	Organic	Conventional
pH (H_2O)	6.16 ± 0.02	5.90 ± 0.01
pH ($CaCl_2$)	5.70 ± 0.01	5.50 ± 0.01
Org. C [%]	1.3 ± 0.015	1.0 ± 0.034
Texture - Sand [%]	4.75 ± 0.04	9.45 ± 1.96
Texture - Silt [%]	72.85 ± 2.95	68.34 ± 1.96
Texture - Clay [%]	22.40 ± 2.95	22.21 ± 1.96
Texture	silt loam	silt loam
$CaCO_3$ [%]	<1.0	<1.0
Electric conductivity [µs/cm]	140.63 ± 11.86	287.77 ± 59.21
Total N [%]	0.17 ± 0.004	0.16 ± 0.013
Total P [g/kg]	0.84 ± 0.02	0.84 ± 0.09
Total K [g/kg]	13.42 ± 0.25	13.10 ± 0.10
Total S [g/kg]	0.301 ± 0.019	0.359 ± 0.009
Total Mg [g/kg]	4.50 ± 0.34	3.62 ± 0.31
Total Ca [g/kg]	6.05 ± 0.15	5.32 ± 0.0032
Total Fe [g/kg]	24.32 ± 0.63	22.98 ± 0.71
Total Cu [mg/kg]	24.17 ± 0.87	23.07 ± 1.38
Total Pb [mg/kg]	28.77 ± 0.31	29.07 ± 1.20
Total Mn [g/kg]	0.77 ± 0.02	0.75 ± 0.02
Total Zn [mg/kg]	64.67 ± 0.60	59.80 ± 0.80
Total Cd [mg/kg]	0.633 ± 0.15	1.167 ± 0.06
DTPA extracted Zn [mg/kg]	1.82 ± 0.30	1.29 ± 0.38
DTPA extracted Cd [mg/kg]	0.071 ± 0.01	0.073 ± 0.02
Nitrate [mg/kg]	81.92 ± 7.92	67.47 ± 2.87
Ammonium [mg/kg]	<2.0	<2.0

4.1.2. Soil pH

Soil pH was measured for all metal spiked soils and the control soils in the initial soil at the start of the experiment and in the pot soil at the end of the experiment in a $CaCl_2$ suspension (Figure 7). Generally soil pH did not change during the pot experiment. The metal addition changed the soil pH as follows. For the metal treatments with zinc addition (CdZn, Zn) pH was reduced by about 0.2 pH units compared to the soils without zinc (Cd, Control) ($p<0.001$). This reduction in pH took place in the initial soil and is still present in the soil after experiment completion. The difference in pH between the organic and the conventional soil was also still visible after the end of the experiment ($p<0.001$). For both soils the high nitrogen treatment was 0.2 pH units more acidic than the low nitrogen treatment ($p<0.001$).

For both, the high nitrogen treatment and the zinc treatments, the acidity was caused by the addition of zinc and nitrogen fertilizer, respectively. To add zinc to the soils zinc sulphate was used and ammonium nitrate was used for the nitrogen fertilization. Both of these cause acidity in soil solutions.

a)
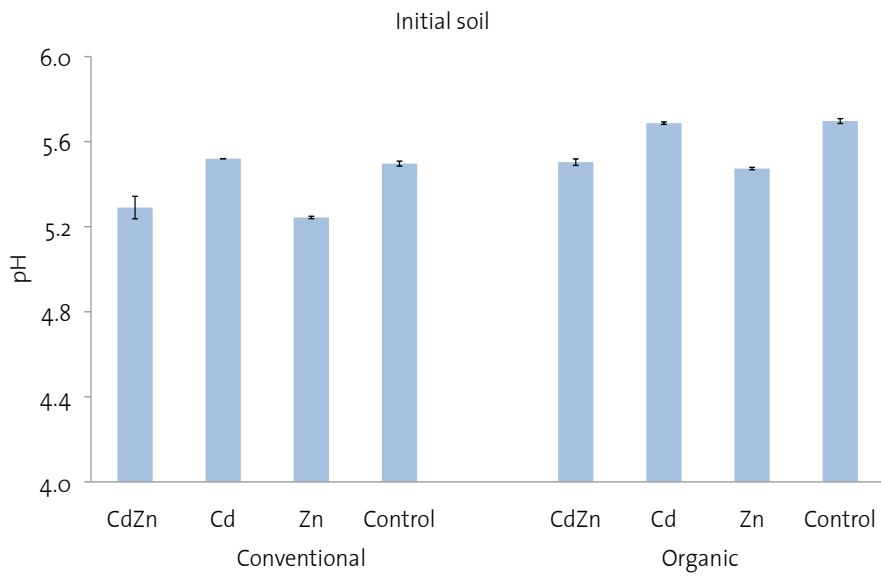
b)
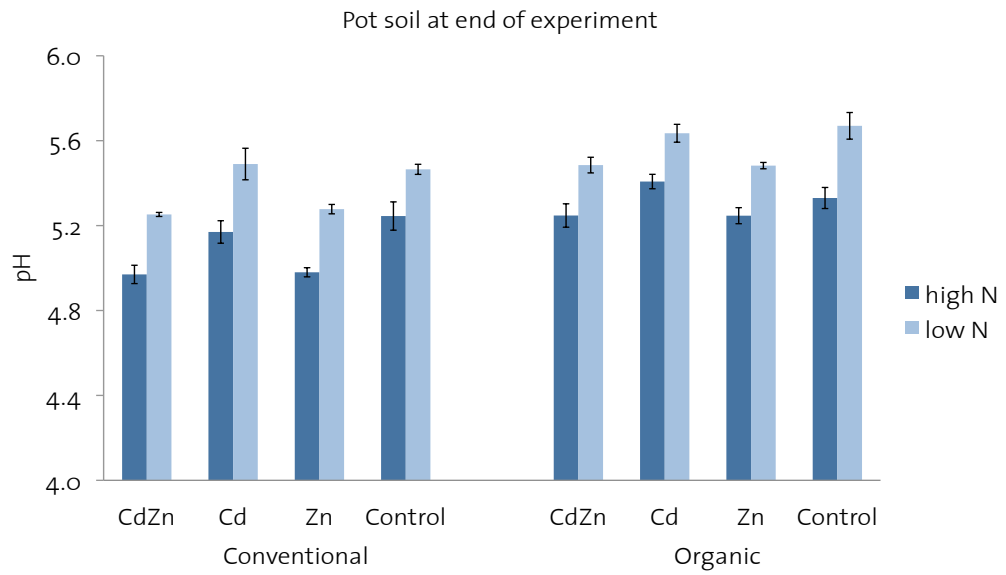
Figure 7: Average values for soil pH: a) pH in the initial soil b) pH after experiment completion.

4.1.3. Cadmium, zinc and iron in soil

Total cadmium concentrations in the soils varied between the treatments because of spiking. For the Cd and CdZn treatments 2 mg/kg cadmium was added to the soil and aged for a couple of months before the experiment started. The addition of cadmium through spiking significantly increased the total cadmium concentration in the soil for the combination and the cadmium treatments compared to the non-cadmium metal treatments (Zn, Control). It seems that the addition of zinc also increased the total amount of cadmium for both soils (Figure 8), but this trend was not significant. There was less cadmium in the organic soil, as there was already less prior to spiking ($p < 0.001$).
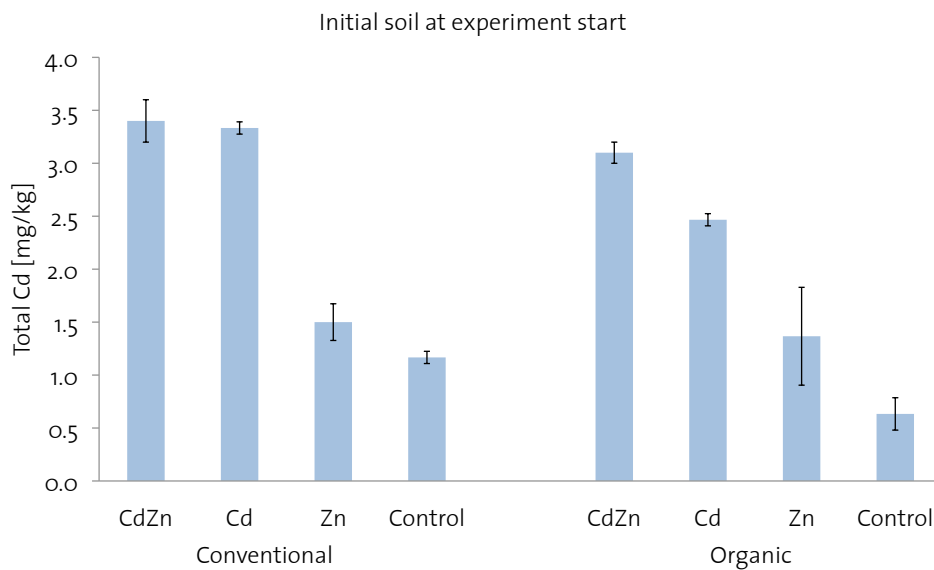
Figure 8: Average values of total cadmium concentrations in the soil.

DTPA extractable cadmium concentrations were measured in the initial soil at the start of the pot experiment and at the end of the pot experiment after storage in airtight-plastic bags at room temperature (Figure 9). DTPA extracted cadmium estimates the cadmium concentration in the soil that is available for plant uptake. At the start of the experiment the trend represented in the total soil cadmium concentrations is also visible for the DTPA extractable cadmium concentrations: Cadmium spiking and the addition of zinc increased the amount of plant available and total cadmium in the soil. There is no difference in plant available cadmium between the two soil types despite more total cadmium in the conventional soil. While the dried soil was stored in airtight plastic bags during the five months of the experiment the DTPA extractable cadmium concentrations changed. After storage the DTPA extractable cadmium in the initial soil was about 50 to 75% lower than in the initial soil at the start of the experiment. The addition of cadmium through spiking significantly increased the DTPA extracted cadmium ($p < 0.001$). For the addition of zinc, the opposite is shown compared to the initial soil at the start of the experiment: The addition of zinc decreased the plant-available cadmium concentration significantly for the CdZn treatment ($p < 0.001$).

After completion of the pot experiment DTPA available cadmium concentrations were also measured in the soil of the pots used in the experiment. The results displayed in Figure 10 show the same trend as the DTPA extraction of the initial soil after storage. The addition of cadmium has increased the plant available cadmium significantly ($p > 0.001$) and the addition of zinc has significantly reduced the plant available cadmium compared to no zinc addition: The CdZn treatment compared to the Zn treatment and the Zn treatment compared to the control treatment ($p < 0.0001$).

a)
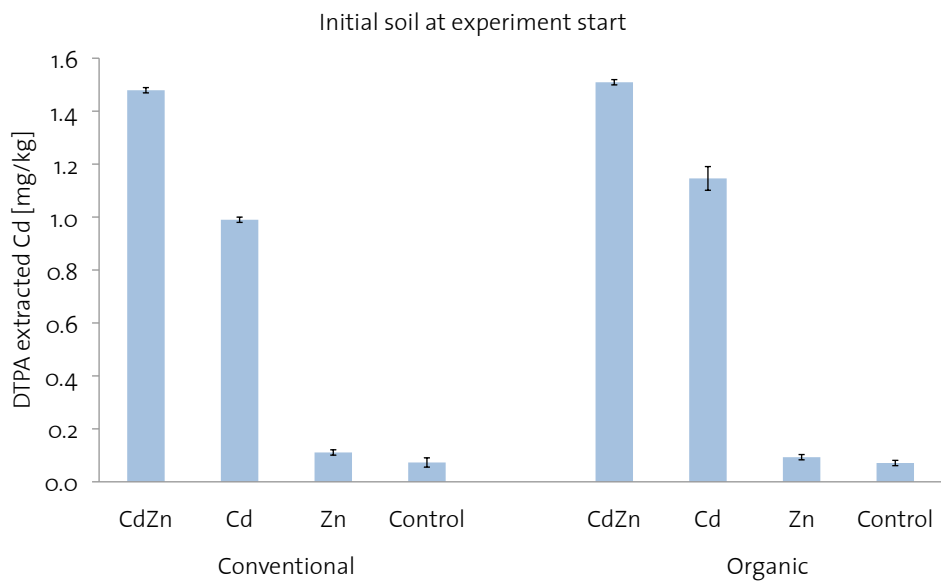
b)
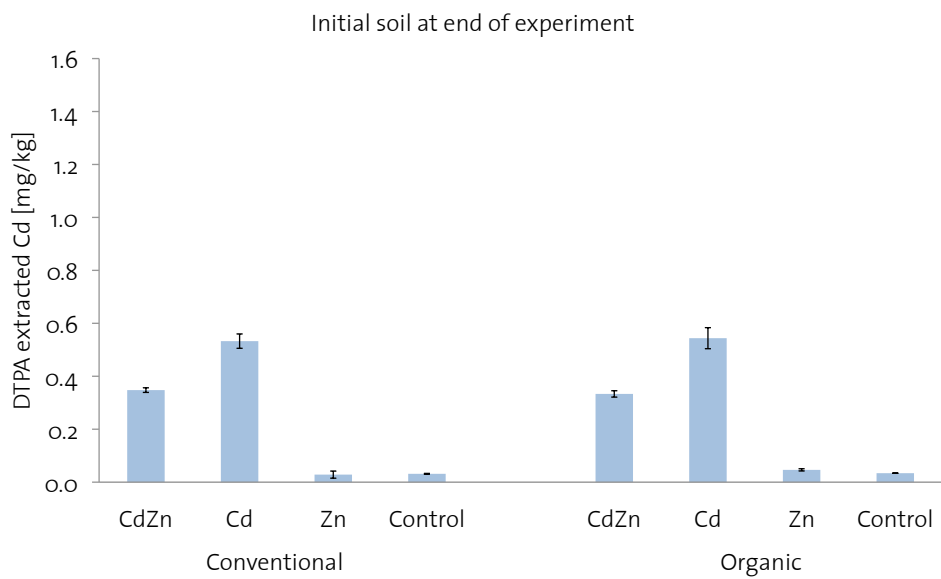
Figure 9: Average DTPA extracted cadmium concentrations in the soil: a) measured in the initial soil at the start of the experiment and b) measured in the initial soil at experiment completion.
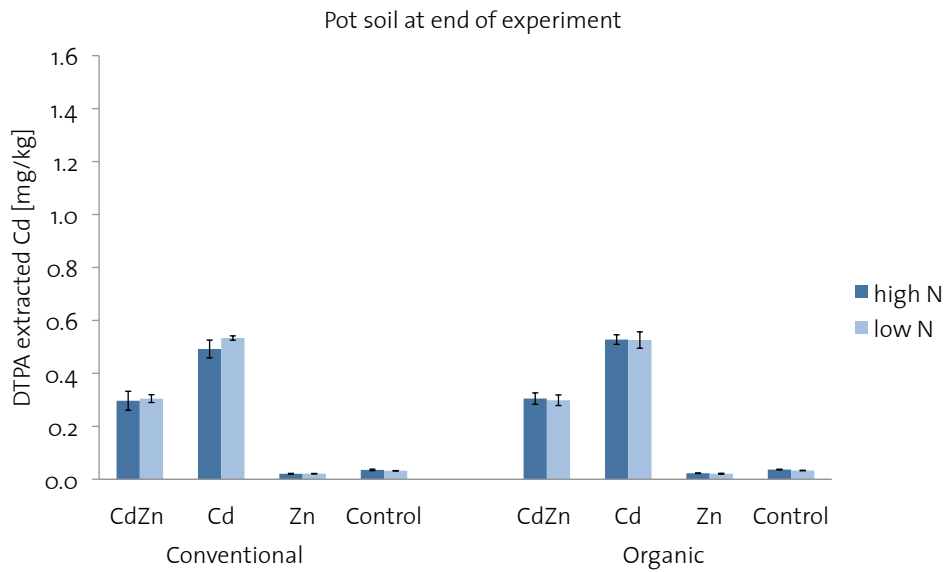
Figure 10: Average DTPA extracted cadmium concentrations in the soil of the pots, measured after harvest of the wheat plants.

For zinc exactly the same measurements were carried out as for cadmium (Figure 11). Total zinc concentrations in the soil were increased by the addition of 340 mg/kg zinc through spiking of the control soils in the CdZn and Zn treatments (p<0.001). Cadmium had no effect on the total zinc concentration and there was no significant difference between the two soil types (conventional, organic). In Switzerland, the guide value (Richtwert), which describes the maximum recommended zinc concentration for agricultural soils is set at 150 mg/kg total soil zinc. The zinc treated soils in this experiment contained more than twice as much (VBBo 1998).
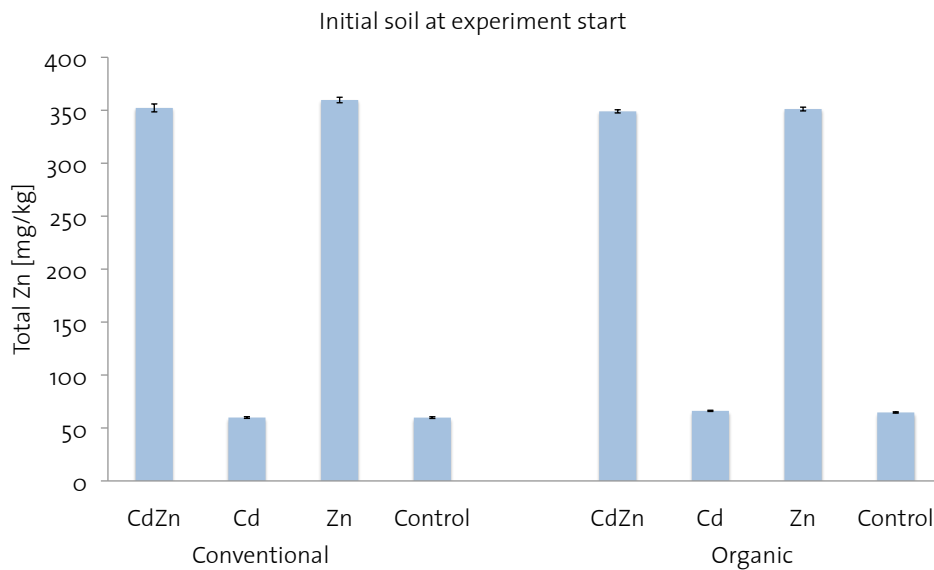
Figure 11: The average total zinc concentrations in the initial soils at the start of the experiment.

The addition of zinc through spiking of the soil significantly increased the plant available zinc concentrations for the zinc treatments (CdZn and Zn) for both soil types. However, the spiking of cadmium led to inconsistent effects in the first measurement of the initial soils carried out at the start of the experiment. For the conventionally managed soil the combination treatment (CdZn) contained less plant available zinc than the zinc only treatment (Zn) whereas for the organically managed soil the opposite was the case. In the organic soil the addition of cadmium in the combination treatment mobilised zinc. The DTPA extractable zinc concentrations changed while the dried initial soils were stored in airtight plastic bags during the 5 months of the experiment: Zinc addition still significantly increased plant available zinc ($p<0.001$) but the addition of cadmium in the combination treatment (CdZn) significantly reduced the plant available zinc concentrations compared to the zinc only treatment (Zn) ($p<0.001$) for both soil types (Figure 12).

After completion of the pot experiment zinc plant availability was reduced to roughly 100 mg/kg zinc for all zinc addition treatments (Figure 13). In the organic soil more zinc was available for plant uptake ($p<0.001$) than in the conventional soil. Between treatments only the addition of zinc compared to no addition of zinc resulted in a significant difference ($p<0.001$). Nitrogen had no consistent and significant effect on the plant available soil zinc concentration and nor did cadmium.

a)
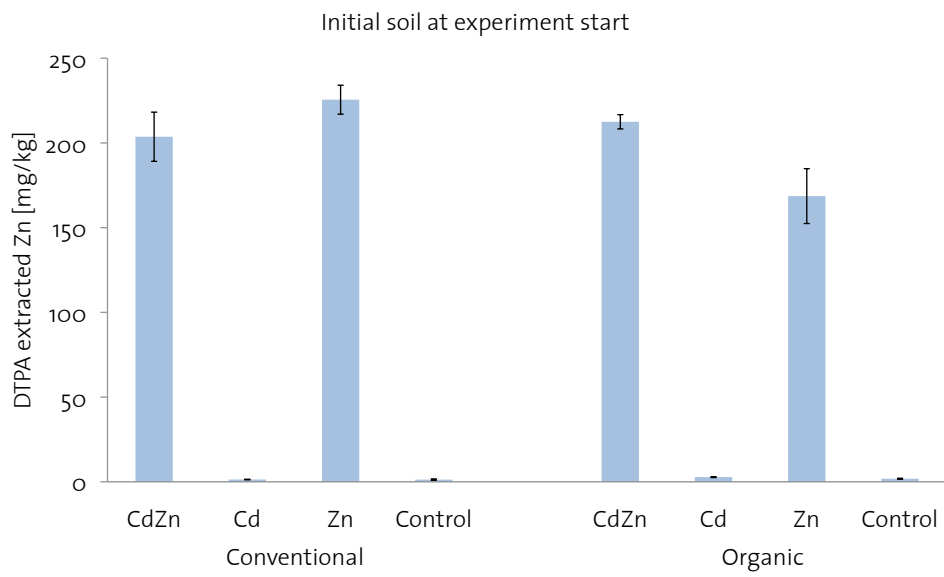
b)
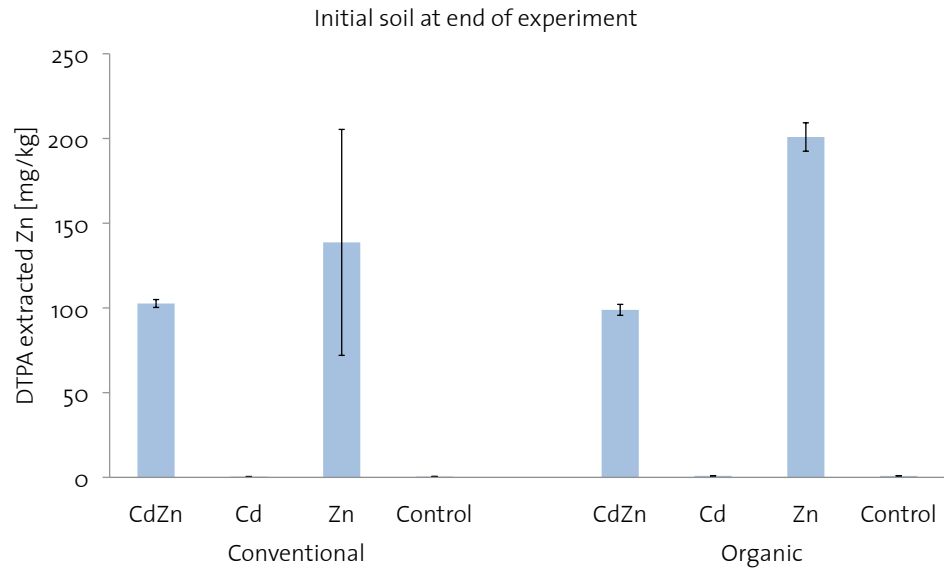
Figure 12: The average DTPA extracted zinc concentrations in the initial soil a) measured at experiment start b) measured at experiment completion.
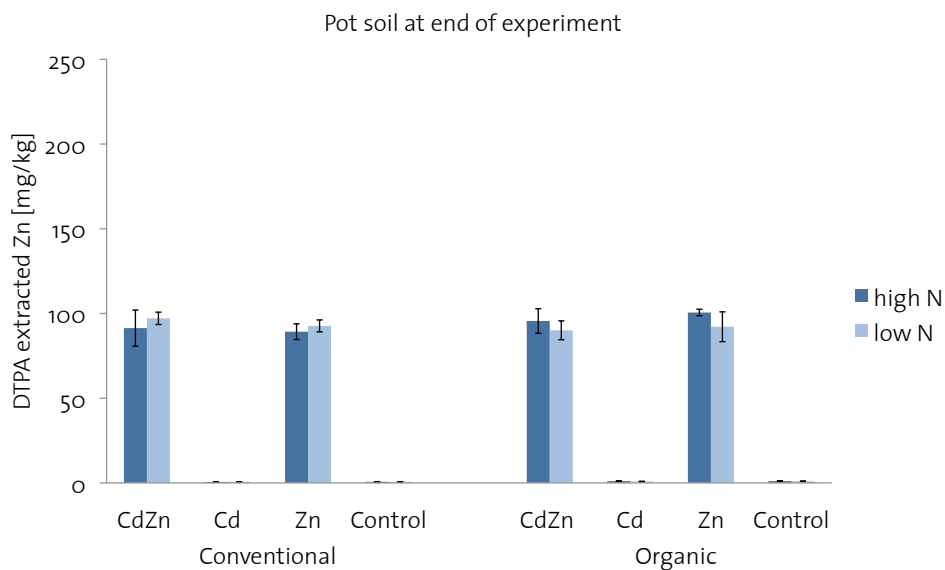
Figure 13: The average DTPA extracted zinc concentrations in the soil of the pots after experiment completion.

DTPA extractable iron was only measured at the end of the experiment in the initial soil and the pot soils (Figure 14). Iron was also immobilised in the combination treatment (CdZn) as already shown for plant available zinc and cadmium. For the initial soil measurements less iron was available in the combination treatment (CdZn) compared to all other treatments in the organic soil, and compared to the Cd and Control treatments for the conventional soil ($p<0.001$). In the pot soils more iron was plant available in the high nitrogen treatment ($p<0.001$). The addition of zinc in the CdZn and Zn treatments immobilised iron for both soils and nitrogen treatments compared to the treatments without zinc addition ($p<0.001$).

DTPA extractable manganese concentrations in soils could not be detected with ICP-OES.

a)
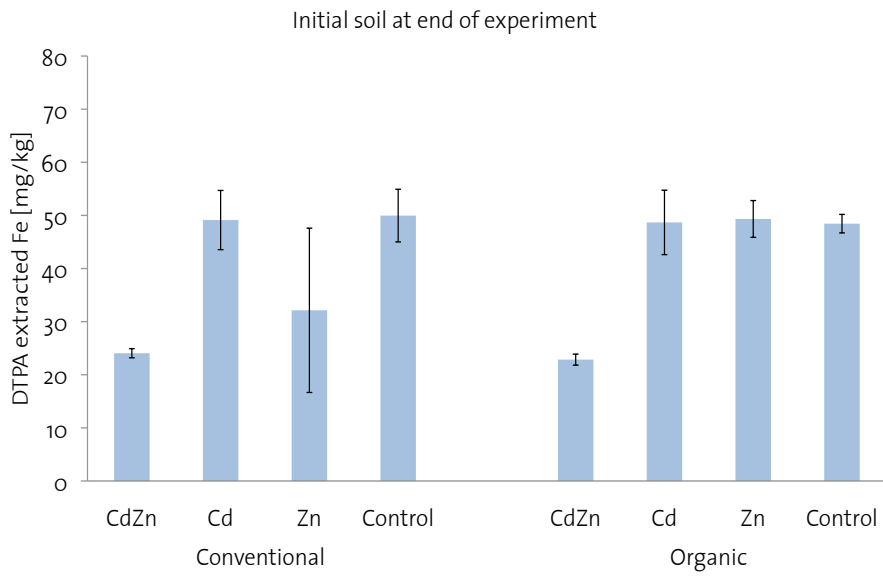
b)
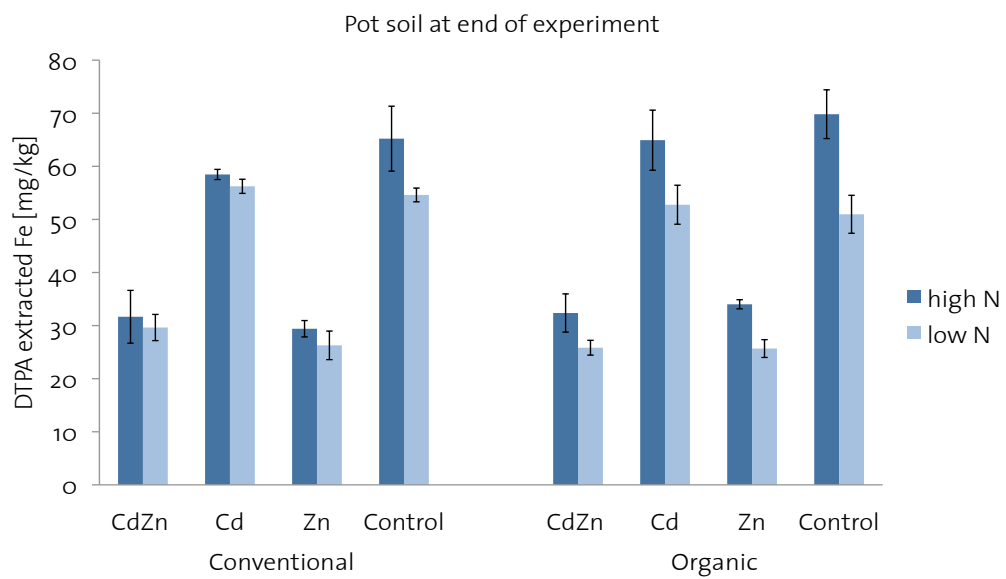
Figure 14: The average DTPA extracted iron concentrations: a) in the initial soil at the end of the experiment b) in the pot soil after experiment completion.

4.1.4. Nitrogen

Total nitrogen was measured at the end of the experiment in the initial soil and the pot soils (Figure 15). For the initial soil the organically managed soil contained more total nitrogen than the conventionally managed soil ($p < 0.001$). As shown in Table 13 the organic soil also contained more nitrate than the conventional soil. Ammonium was also measured in the initial soils but could not be detected as concentrations were below the detection limit of 2 mg/kg. There was no significant effect of metal treatments.

At the end of the experiment the organically managed soil still contained more total nitrogen than the conventionally managed soil ($p < 0.001$). Also there was no visible effect of metal treatments on total soil nitrogen after experiment completion. In the conventional soil the high nitrogen fertilization contained significantly more nitrogen compared to the low nitrogen treatment ($p < 0.001$). In the organically managed soil this difference could not be shown. Ammonium and nitrate could not be detected in the soil extracts carried out at the end of the experiment. The detection limits are 0.2 mg/l for ammonium and 0.01 mg/l for nitrate. This is equivalent to 2 mg/kg ammonium and 0.1 mg/kg nitrate in the soil. Therefore all plant available nitrogen was taken up by the wheat plants for both low and high nitrogen fertilization.

a)
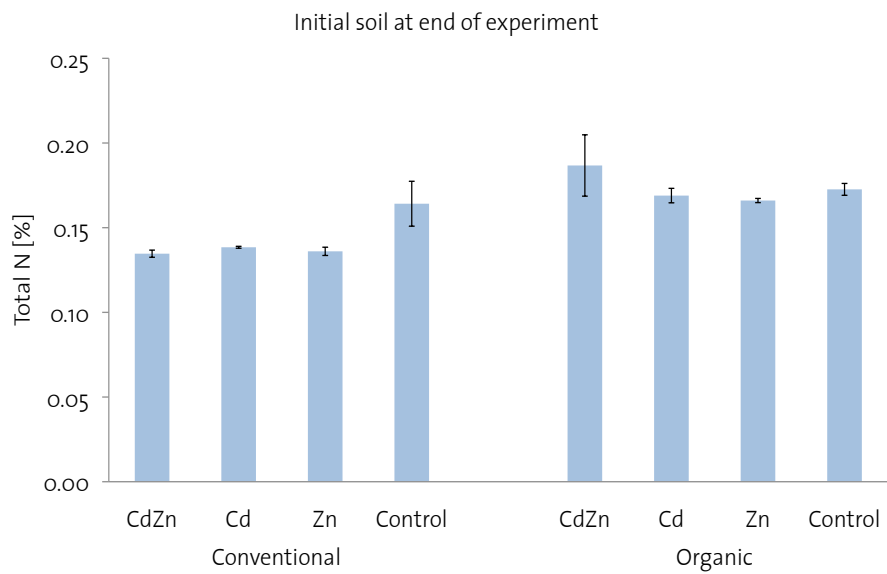
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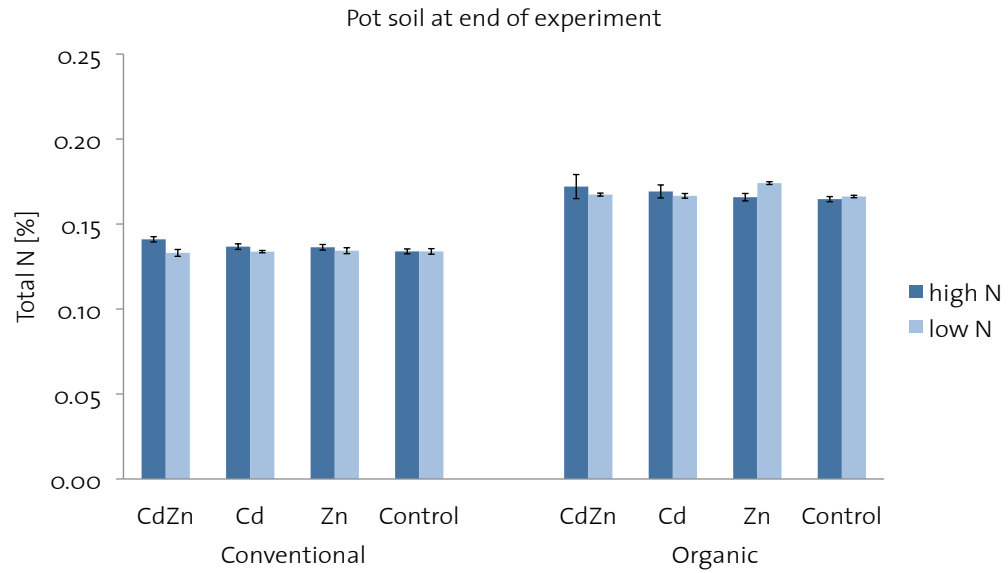
Figure 15: Average values for total nitrogen concentrations in percentage a) in the initial soil and b) in the pot soil after experiment completion.

4.2. Wheat growth and biomass

The wheat growth was not visibly affected by the metal treatments. Generally, the high nitrogen treatment plants developed more heads and therefore also took longer to reach maturity. The low nitrogen plants reached full maturity roughly two weeks before the high nitrogen plants.

The first plants started tillering after three weeks of growth and all plants had two to three tillers after four weeks of growth. The start of tillering was independent of metal treatment and nitrogen fertilization, though the high nitrogen plants did produce more tillers (Figure 16). At week eight the first wheat plants started heading and were flowering between one and three weeks later. During heading all wheat plants had some die back of leaves. This was the case for all plants and therefore not related to metal or nitrogen treatment. By week eleven most low nitrogen plants and some high nitrogen plants finished heading and flowering, and were filling their grains (Figure 17). The plant in pot 44 (D2 Zn high N) had not started heading by then but had already produced 13 tillers instead. For all other plants 9 tillers was the maximum number at that time. During weeks 12 and 13 the low nitrogen wheat plants started drying out while the high nitrogen plants were still heading and flowering. After 18 weeks of growth four plants (pots 13, 19, 44, 45) still had green heads (Figure 18). But due to time limitations of the project harvesting could not be put off any longer and was finally carried out after 19 weeks of wheat growth. At harvest the plant in pot number 44 had produced six heads of which five were still green at harvest. As plant 44 behaved completely differently to all other plants it was excluded from the dataset. Figure 19 shows the number of tillers and heads respectively produced by the plants for each metal treatment (CdZn, Cd, Zn, Control) and two nitrogen treatments in the conventional soil. Figure 20 shows the same for the wheat plants grown in the organic soil. During growth the wheat plants showed some brown spots on the tips of their leaves. These symptoms were not confined to one metal treatment and appeared in most plants. Therefore it was not related to zinc or cadmium toxicity or zinc deficiency.

Week 7

Conventional, high N

CdZn   Cd   Zn   Control

Conventional, low N

CdZn   Cd   Zn   Control

Organic, high N

CdZn   Cd   Zn   Control

Organic, low N
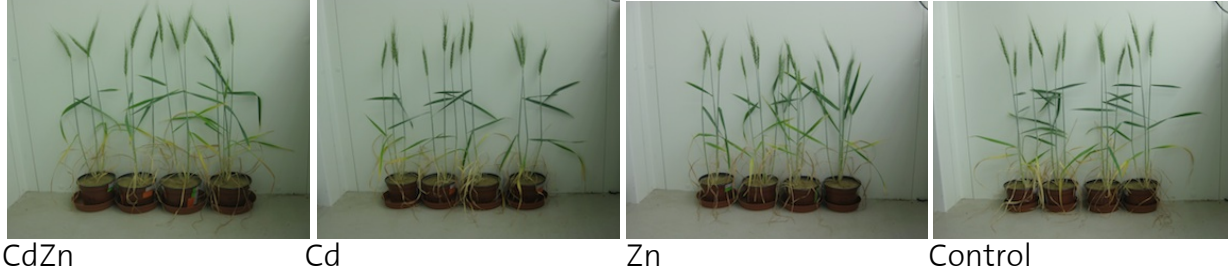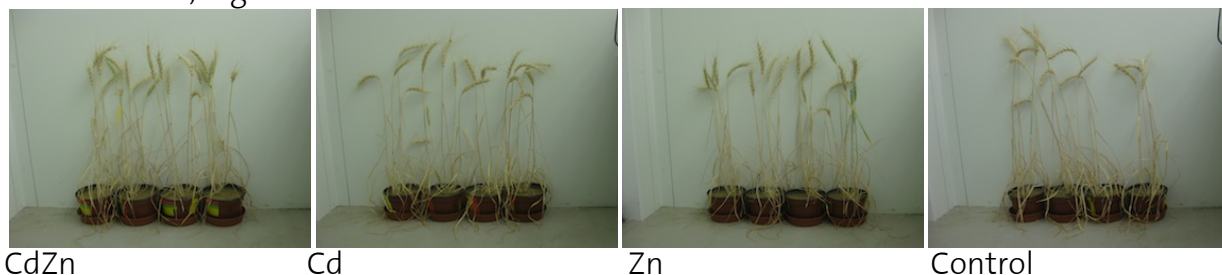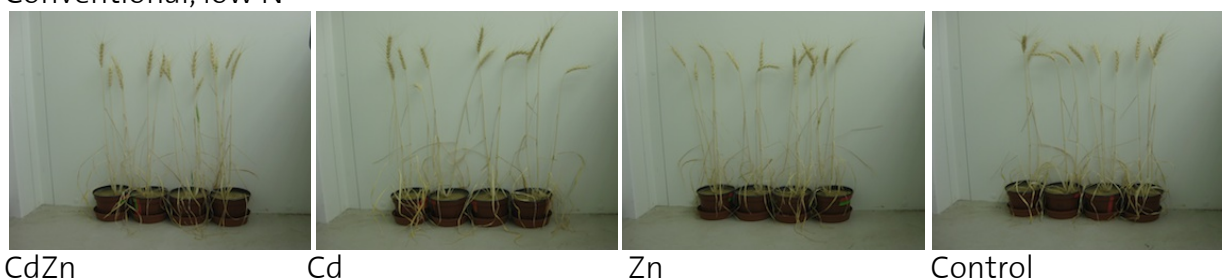

CdZn   Cd   Zn   Control
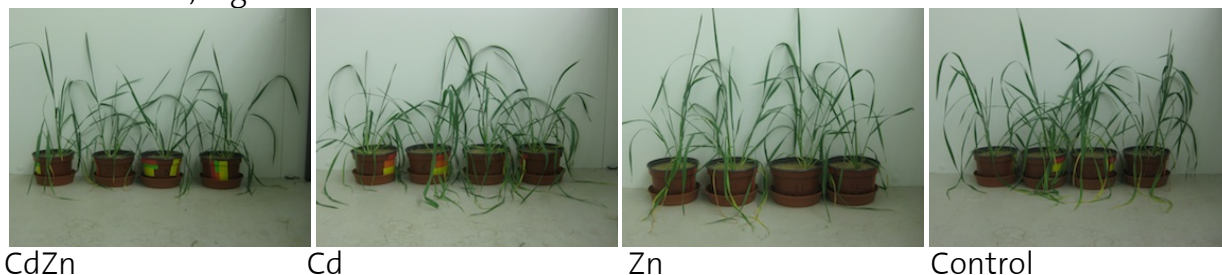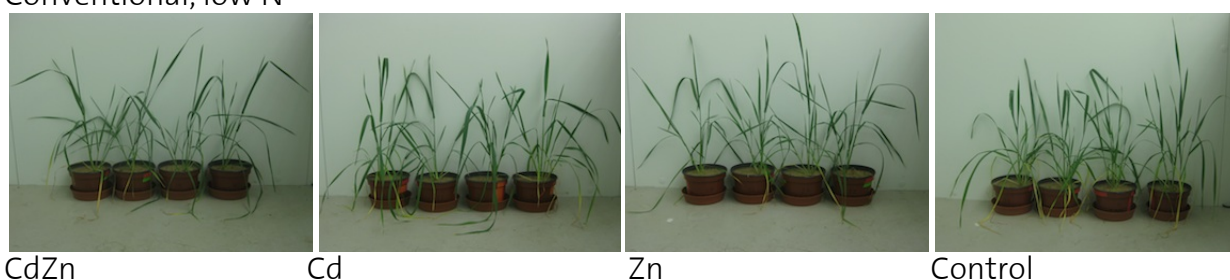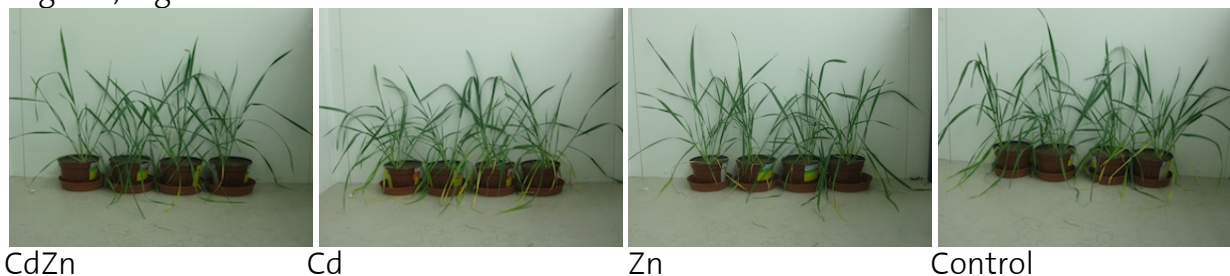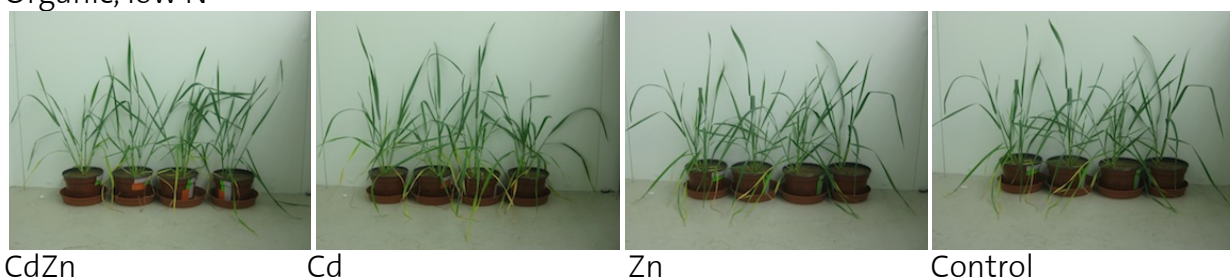
Figure 16: The wheat plants after 7 weeks of growth shown in groups for the two soil types and two levels of nitrogen fertilization, displaying the four replicates per metal treatment.
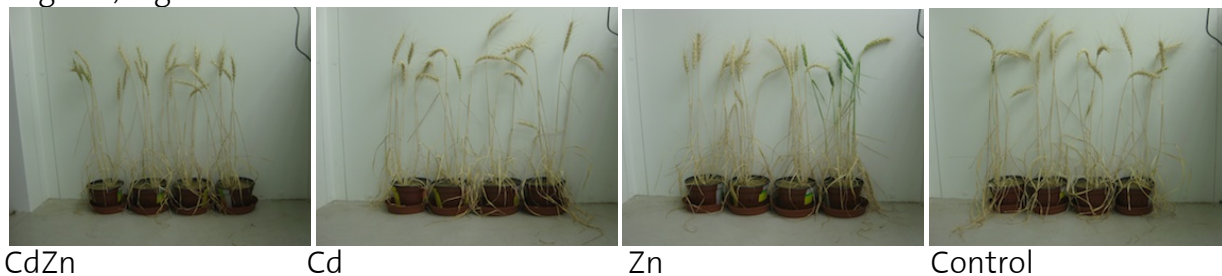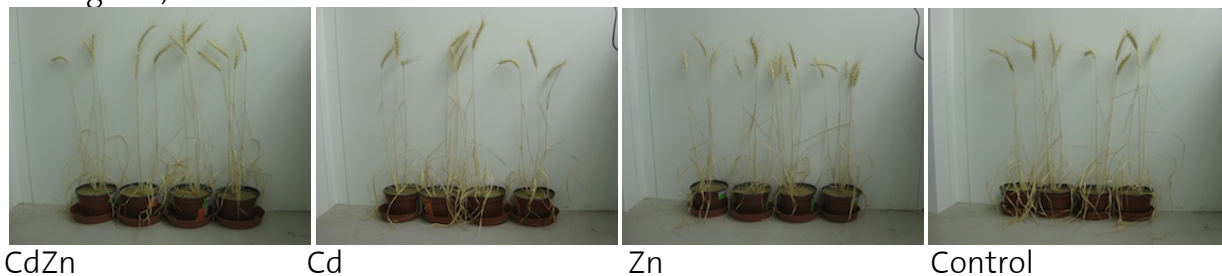
Week 12

Conventional, high N

CdZn   Cd   Zn   Control

Conventional, low N

CdZn   Cd   Zn   Control

Organic, high N

CdZn   Cd   Zn   Control

Organic, low N

CdZn   Cd   Zn   Control
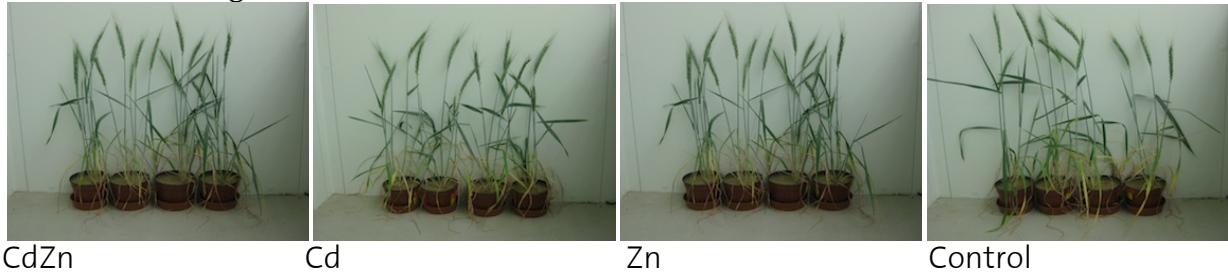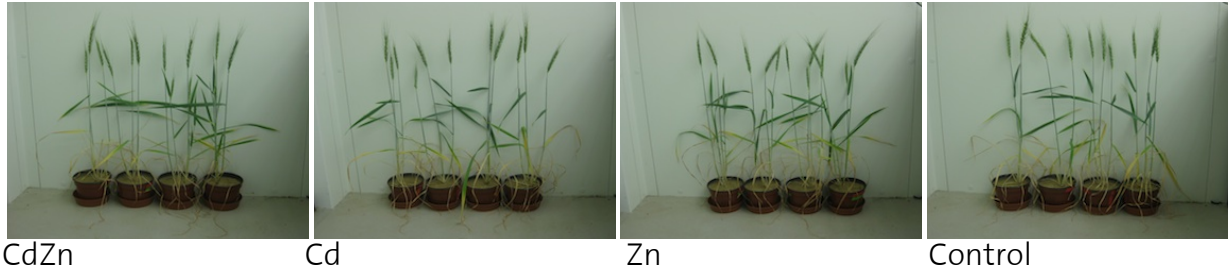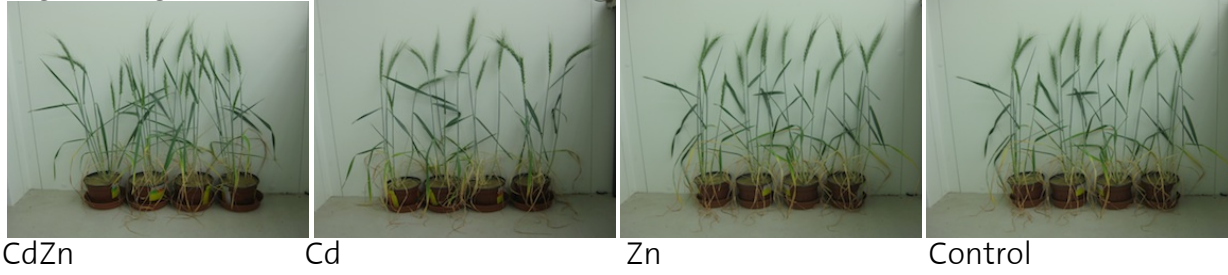
Figure 17: The wheat plants after 12 weeks of growth shown in groups for the two soil types and two levels of nitrogen fertilization, displaying the four replicates per metal treatment.

Week 19:

Conventional, high N

CdZn   Cd   Zn   Control

Conventional, low N

CdZn   Cd   Zn   Control

Organic, high N:

CdZn   Cd   Zn   Control

Organic, low N:

CdZn   Cd   Zn   Control

Figure 18: The wheat plants after 19 weeks of growth shown in groups for the two soil types and two levels of nitrogen fertilization, displaying the four replicates per metal treatment.

a)
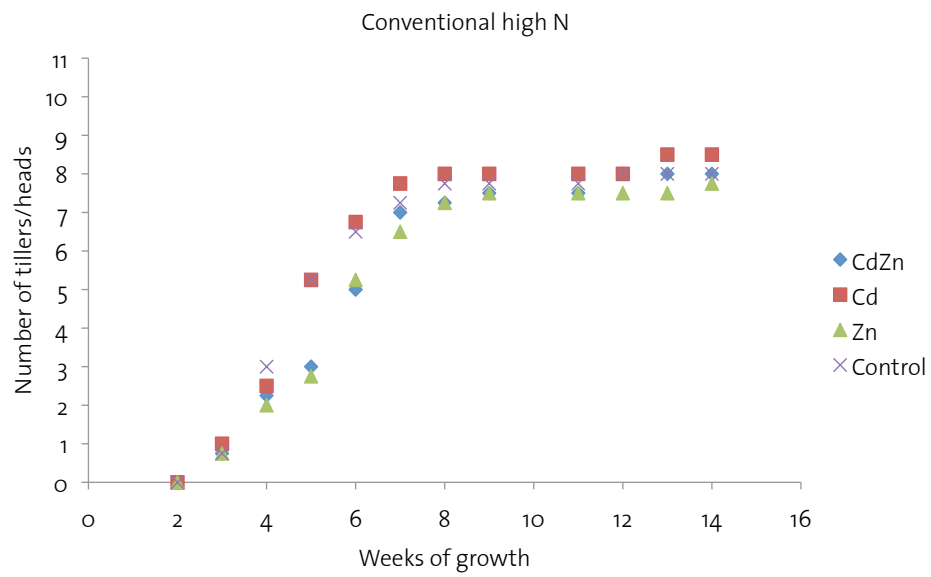
b)
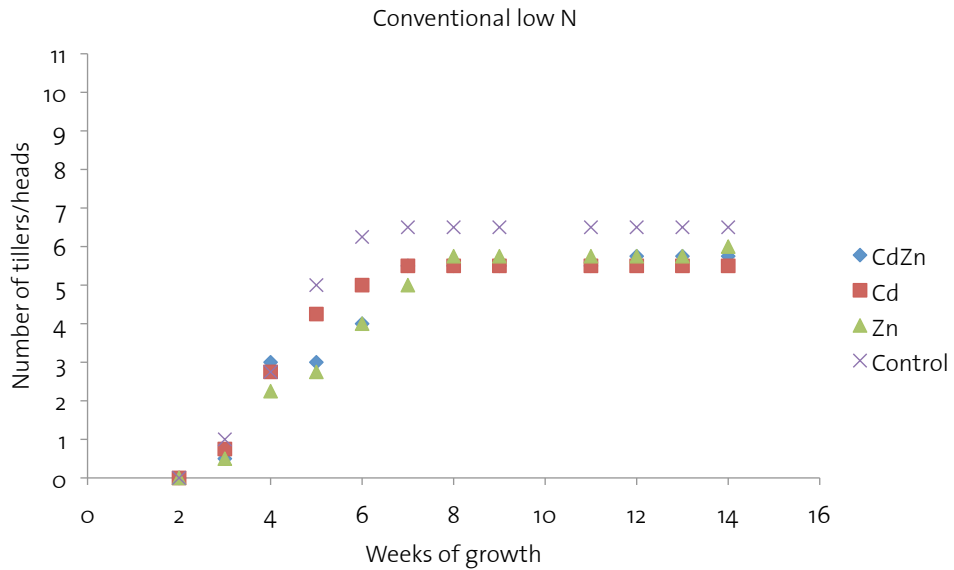
Figure 19: The average number of tillers and heads (sum) per plant for the 18 weeks of growth in the conventional soil with a) high level of nitrogen fertilization and b) low level of nitrogen fertilization.

a)
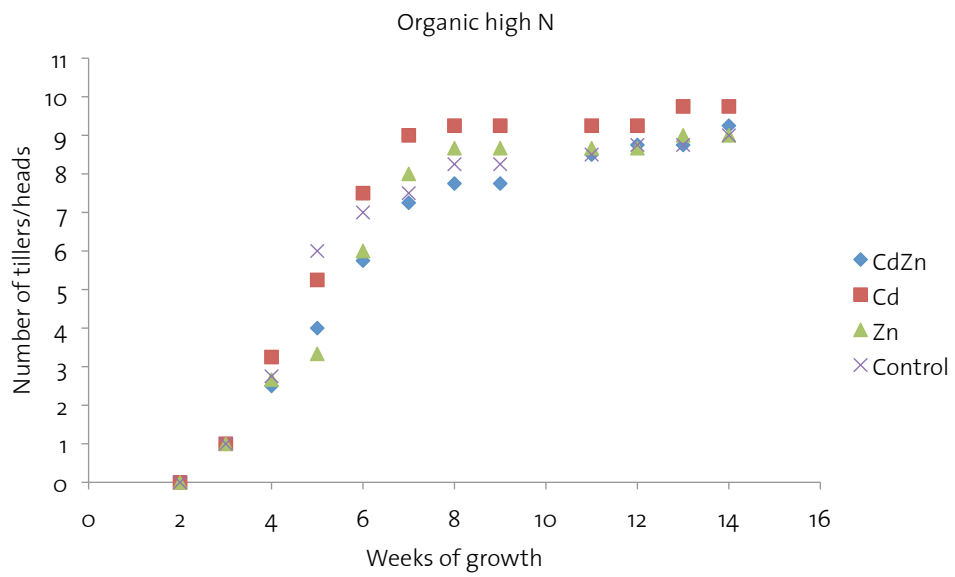
b)
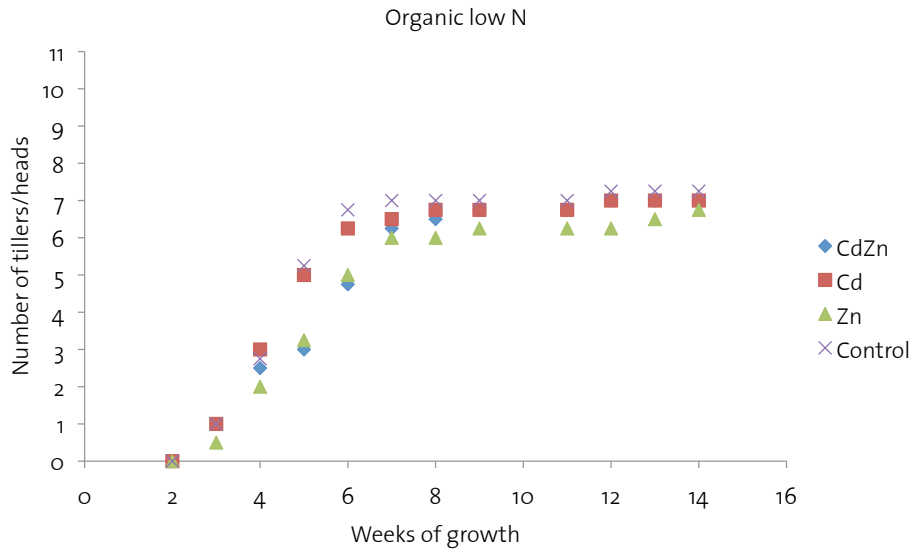
Figure 20: The average number of tillers and heads (sum) per plant for the 18 weeks of growth in the organic soil with a) high level of nitrogen fertilization and b) low level of nitrogen fertilization.

Figure 21 shows the number of heads per plant at harvest for each metal treatment, soil type and nitrogen fertilization. The wheat plants grown in high zinc soils (CdZn, Zn) produced more heads than the ones grown on non-zinc spiked soils (Cd, Control). This effect was statistically significant for all zinc to non-zinc combinations for the conventional soil ($p > 0.01$) and only for the Zn-Cd and Zn-Control combination in the organic soil ($p > 0.001$). Also, the high nitrogen fertilization lead to a higher head production in the wheat plants ($p > 0.001$). After harvest the heads of each plant were taken apart and grains were counted (Figure 22). For the number of grains per plant, there was no significant effect of the metal treatments. High nitrogen fertilization also increased the number of grains developed for each plant ($p < 0.001$). The average number of grains per plant was affected by the soil management strategy. The wheat grown on the organically managed soil produced more seeds per plant than the plants grown on the conventionally managed soil ($p < 0.001$). For the number of heads there was no significant effect of the soil management.
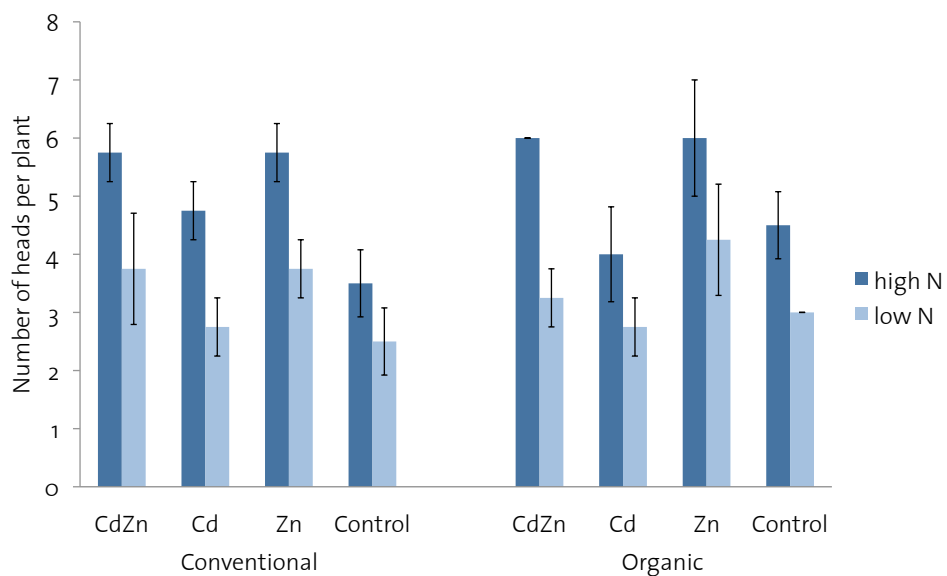
Figure 21: The average number of heads per plant on the day of harvest.
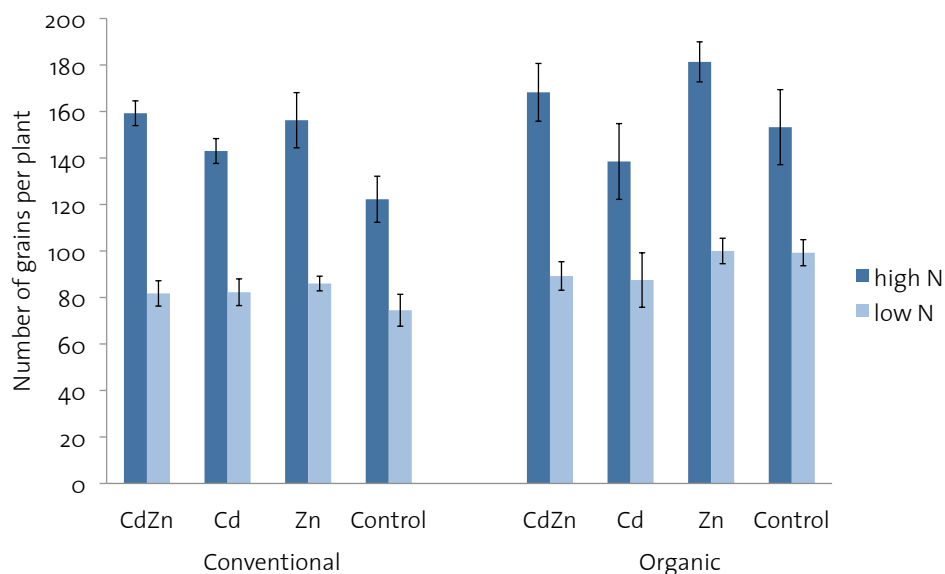
Figure 22: The average number of grains per plant on the day of harvest.

Figure 23, Figure 24 and Figure 25 show the average dry weights for heads, shoots and grains. All of the plants grown on the organically managed soil produced more biomass than the ones grown on conventional soil ($p<0.001$). The high nitrogen treatment also increased the biomass significantly for shoot, heads and grains ($p<0.001$).

For the heads the plants grown on soil of the combination treatment CdZn had significantly lighter heads than the ones grown on the control soil or the cadmium only soil ($p<0.005$).

The grain biomass was affected by metal treatment also. The plants grown on soils spiked with zinc had significantly lighter grains than those grown on non-zinc soils ($p<0.001$). This is interesting as the high zinc plants actually produced more grains. So the wheat plants affected by zinc produced more grains but these grains were smaller leading to less grain biomass compared to the plants grown on non-zinc soils.
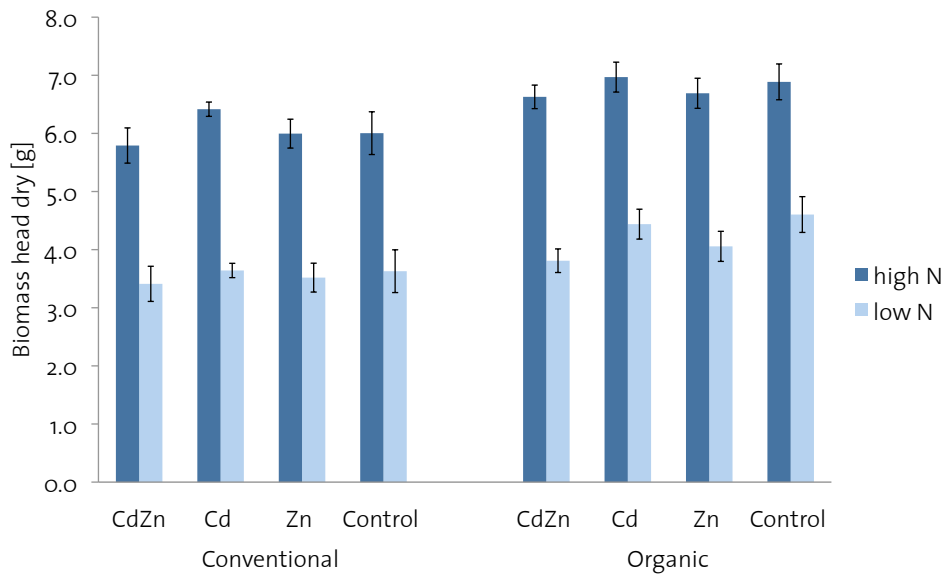
Figure 23: The average dry head biomass per plant after harvest.
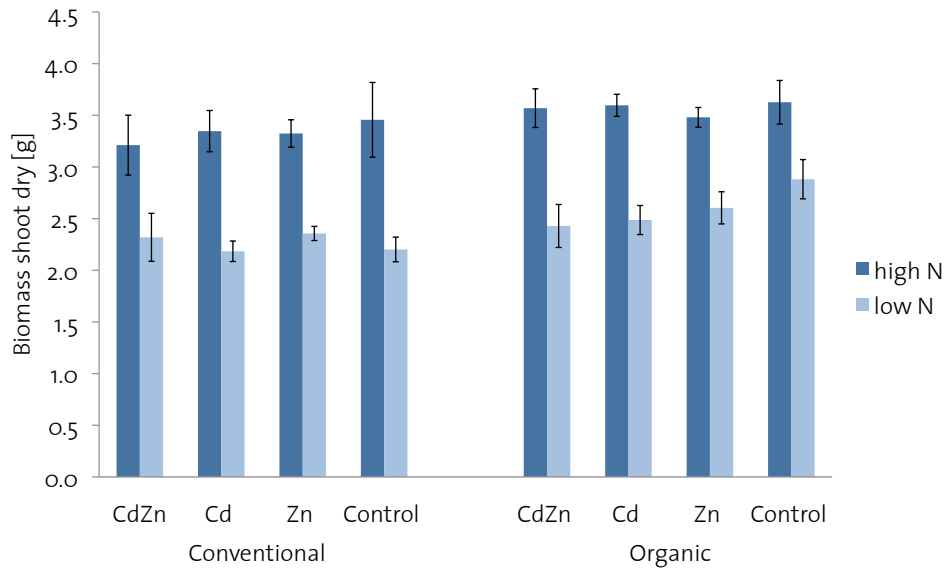
Figure 24: The average dry shoot biomass per plant after harvest.
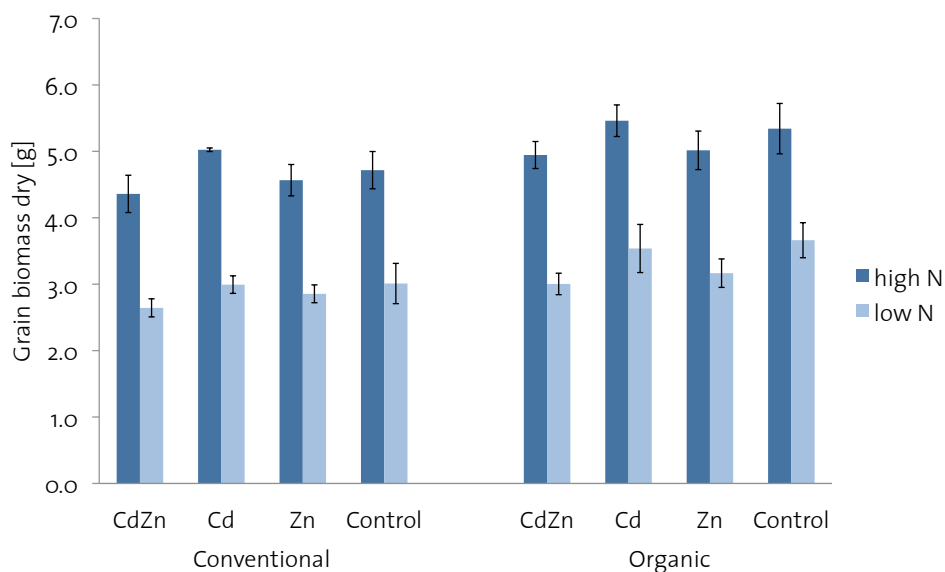
Figure 25: The average dry grain biomass per plant after harvest.

To assess grain biomass in more detail the grain biomass per 100 grains and the harvest index were also calculated. Figure 26 shows the grain biomass per 100 grains for all treatments and soils. The high nitrogen fertilization lead to a smaller grain biomass compared to the low nitrogen fertilization ($p<0.001$). Furthermore it shows the effect of zinc again. The grains developed in plants grown on soils spiked with zinc were lighter compared to the ones of plants grown on non-zinc soils ($p>0.001$).
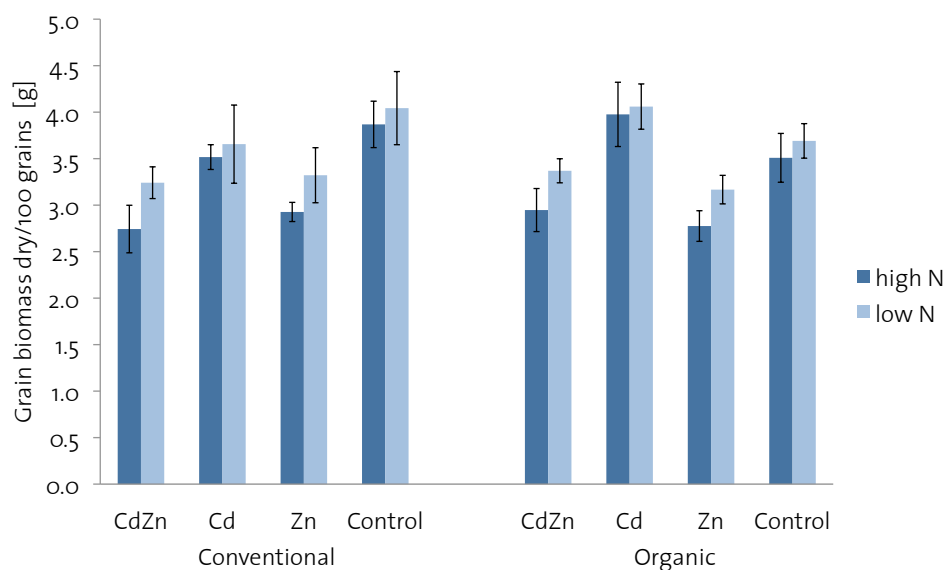
Figure 26: The calculated average grain biomass per 100 grains for each plant.

The harvest index shows the grain biomass as a percentage of the whole plant biomass (head and shoot). Figure 27 shows the result of this calculation for all treatments. Soil and nitrogen fertilization had no significant effect on the harvest index. For the metal treatments a reduction in the harvest index is visible for the wheat plants grown on zinc-spiked soils ($p < 0.001$). Therefore the plants affected by zinc invested less of their biomass into the grain compared to the rest of the plant.
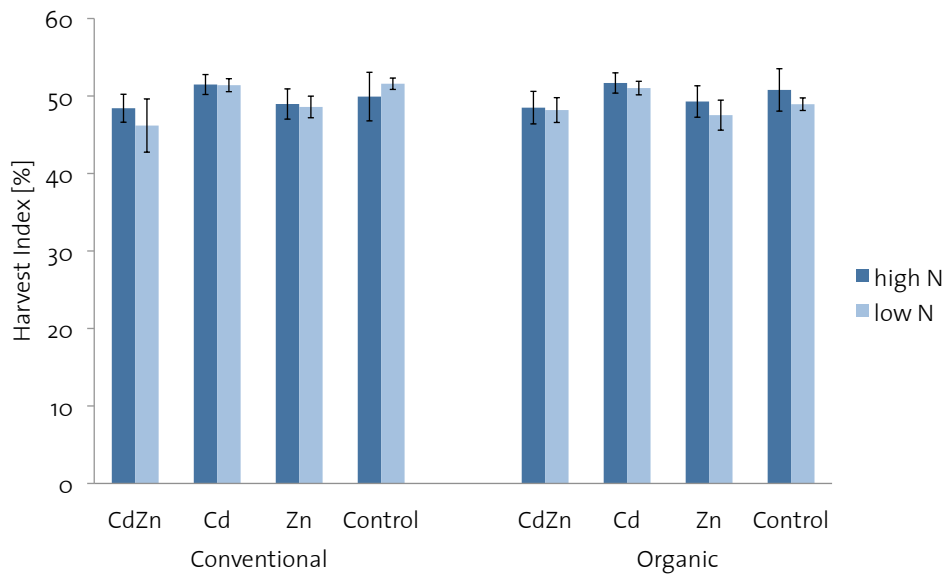
Figure 27: The average harvest index calculated for all plants.

4.3. Wheat analysis

4.3.1. Cadmium and zinc

Cadmium concentrations in the shoot and grain were measured and are displayed per metal treatment (CdZn, Cd, Zn, Control), soil type (conventional, organic) and level of nitrogen fertilization (low N, high N) (Figure 28 and Figure 29). For both shoot and grain the total amount of cadmium per plant was calculated by multiplying the cadmium concentration with the shoot or grain biomass, respectively. Shoot cadmium concentrations were higher for the plants grown on cadmium-spiked soils ($p<0.001$). But zinc also had an effect on the shoot cadmium concentrations ($p<0.001$). Wheat grown on the soils of the combination treatment (CdZn) had significantly less shoot cadmium concentration than the ones grown on cadmium only soil. Also, wheat grown on the zinc-spiked soil (Zn) contained less shoot cadmium than those grown on the control soil. For the low nitrogen treatment the conventionally managed soil shoots contained more cadmium than the organic soil shoots ($p<0.001$). The two levels of nitrogen fertilization had no effect on the cadmium shoot concentrations. The results for the shoot cadmium content per plant followed the trends of the concentration results. Except the zinc effect was not significant for the low level of nitrogen fertilization of the conventional soil for the combination of the zinc and the control treatment. The soil effect shown in the concentration results is no longer significant in the cadmium per plant data. However the amount of cadmium per plant in shoots was significantly higher for the high level of nitrogen fertilization ($p<0.001$). This effect does not show in the concentration data due to growth dilution as the plants with high nitrogen fertilization accumulated more biomass, reducing the shoot cadmium concentration.
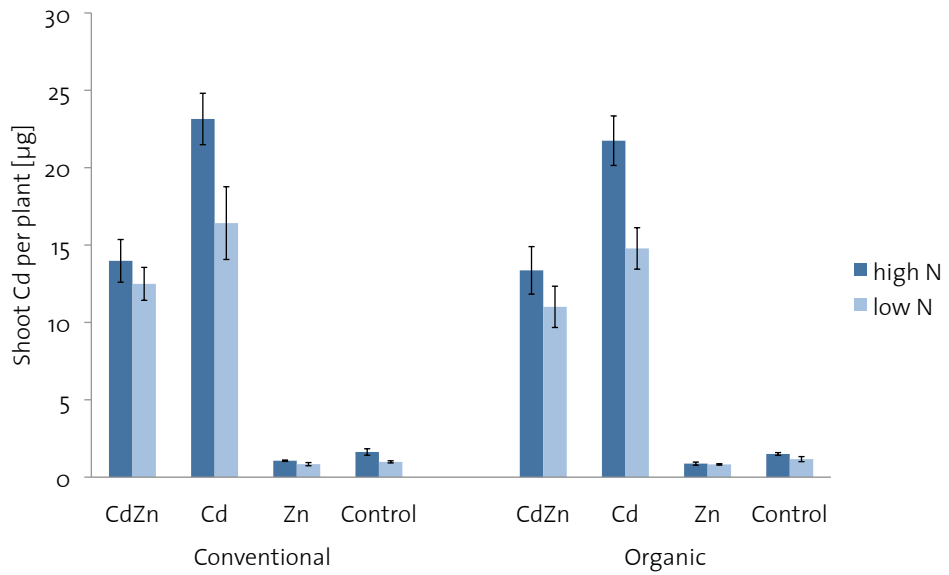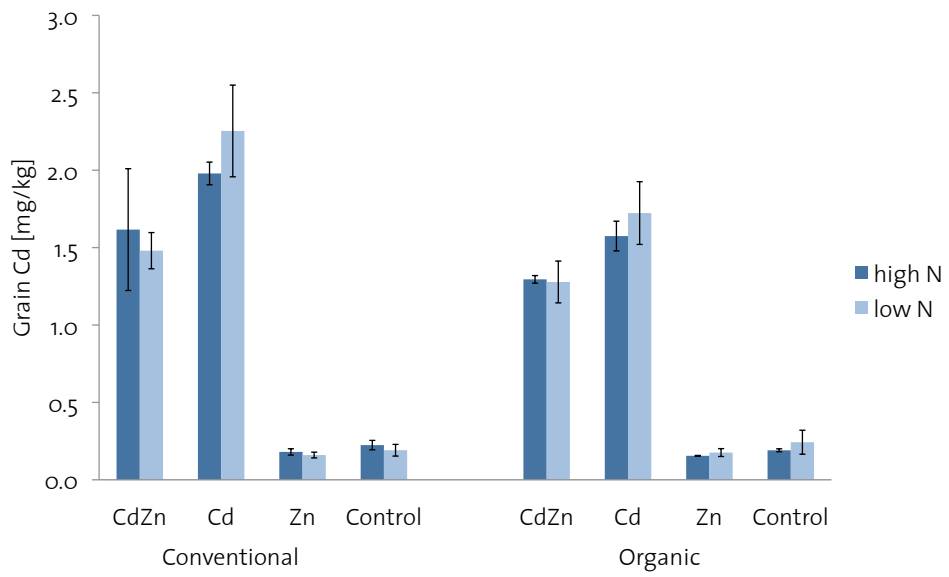
a)
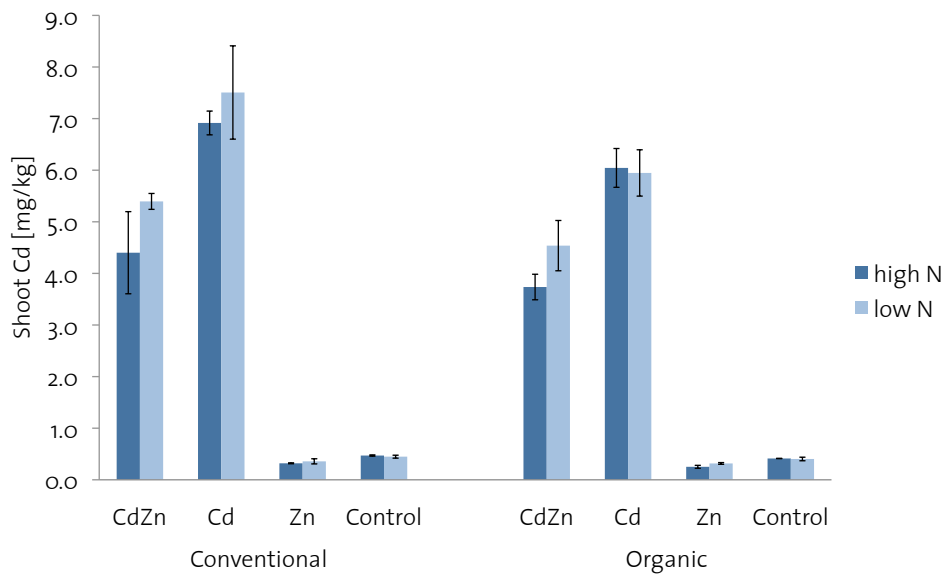
b)

Figure 28: a) The average cadmium concentrations in the shoots and b) the average total shoot cadmium content per plant.

The grain cadmium concentration was increased for the cadmium spiked soils (p<0.001) and zinc spiking reduced the accumulation of cadmium (p<0.001). In the conventional soil the difference in grain cadmium did not prove significant for the zinc treatment compared to the control treatment. Soil and nitrogen had no significant effect on the grain cadmium concentration. The total cadmium per plant was also higher for the plants grown on the cadmium spiked soils and was also negatively affected by zinc spiking, for all metal treatments. The high level of nitrogen fertilization significantly increased cadmium uptake in grains (p<0.001) and as in the shoots this effect does not show in the concentrations due to growth dilution. The total cadmium content in grains was not significantly affected by the soil management strategy (Figure 29).

In addition mass balances were calculated for both DTPA cadmium measurements in the initial soil. Table 14 shows the mass balance calculated with the results of the DTPA measurement at the start of the experiment and the mass balance in Table 15 is calculated for the second Cd DTPA measurement in the initial soil after experiment completion. The mass balance compares the difference in DTPA extracted cadmium in the initial soil and the soil after experiment completion (DTPA Cd lost) with the cadmium plant uptake (grain and shoot). The mass balance for the first DTPA cadmium measurements shows a large discrepancy between the DTPA lost during the experiment and the plant uptake for the soils spiked with cadmium whereas for the second DTPA cadmium measurements this discrepancy is no longer present.
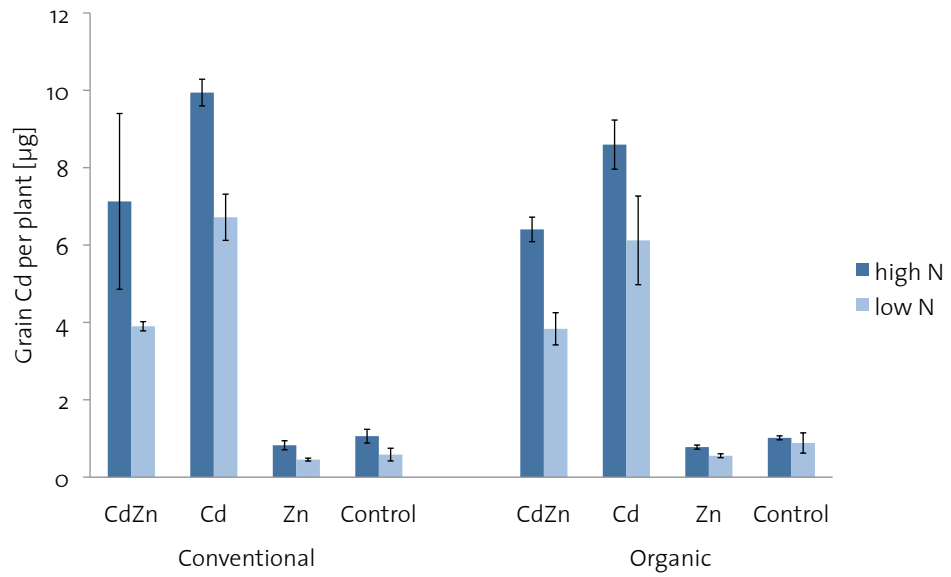
a)

b)

Figure 29: a) The average cadmium concentrations in the grains and b) the average total grain cadmium content per plant.

Table 14: The mass balance for cadmium: The difference between DTPA Cd in the initial soil prior to the experiment start and in the pot soils after experiment completion (DTPA lost), the sum of Cd content in the grains and shoots (plant uptake) and the difference between the two.

		Conventional soil			Organic soil		
		DTPA Cd lost [µg]	Plant uptake [µg]	Difference [µg]	DTPA Cd lost [µg]	Plant uptake [µg]	Difference [µg]
CdZn	high N	886.87	21.11	865.76	903.31	19.77	883.54
CdZn	low N	880.84	16.39	864.44	908.06	16.91	891.15
Cd	high N	373.42	33.09	340.33	463.78	30.34	433.44
Cd	low N	342.33	23.14	319.20	465.06	15.91	449.16
Zn	high N	67.60	1.89	65.71	52.75	1.65	51.10
Zn	low N	67.33	1.29	66.03	54.02	1.68	52.34
Control	high N	28.21	2.69	25.53	25.79	5.17	20.62
Control	low N	30.61	1.57	29.04	28.48	1.39	26.78

Table 15: The mass balance for cadmium: The difference between DTPA Cd in the initial soil and in the pot soils after experiment completion (DTPA lost), the sum of Cd content in the grains and shoots (plant uptake) and the difference between the two.

		Conventional soil			Organic soil		
		DTPA Cd lost [µg]	Plant uptake [µg]	Difference [µg]	DTPA Cd lost [µg]	Plant uptake [µg]	Difference [µg]
CdZn	high N	38.37	21.11	17.26	21.44	19.77	1.67
CdZn	low N	32.34	16.39	15.94	26.19	16.91	9.28
Cd	high N	30.49	33.09	-2.60	12.16	30.34	-18.18
Cd	low N	-0.59	23.14	-23.73	13.44	15.91	-2.46
Zn	high N	5.86	1.89	3.97	17.82	1.65	16.17
Zn	low N	5.59	1.29	4.29	19.10	1.68	17.41
Control	high N	-2.91	2.69	-5.60	-2.03	5.17	-7.20
Control	low N	-0.51	1.57	-2.08	0.66	1.39	-1.04

The wheat plants grown on the zinc-spiked soils showed very high zinc concentrations in the shoots (Figure 30). The average zinc concentrations in the shoots of this experiment were about 550 mg/kg for the plants grown on the zinc-spiked soils (CdZn, Zn). The shoot zinc concentrations of the zinc-treated soils were all significantly higher than all non-zinc treated soil plants ($p < 0.001$), but are not affected by any other factors, such as cadmium addition, soil type and nitrogen fertilization. The total amount of zinc per plant for shoots was significantly affected by the metal treatments in the same way as the concentration results ($p < 0.001$), and in addition nitrogen increases zinc accumulation significantly, which could not be seen in the concentration results, due to growth dilution.
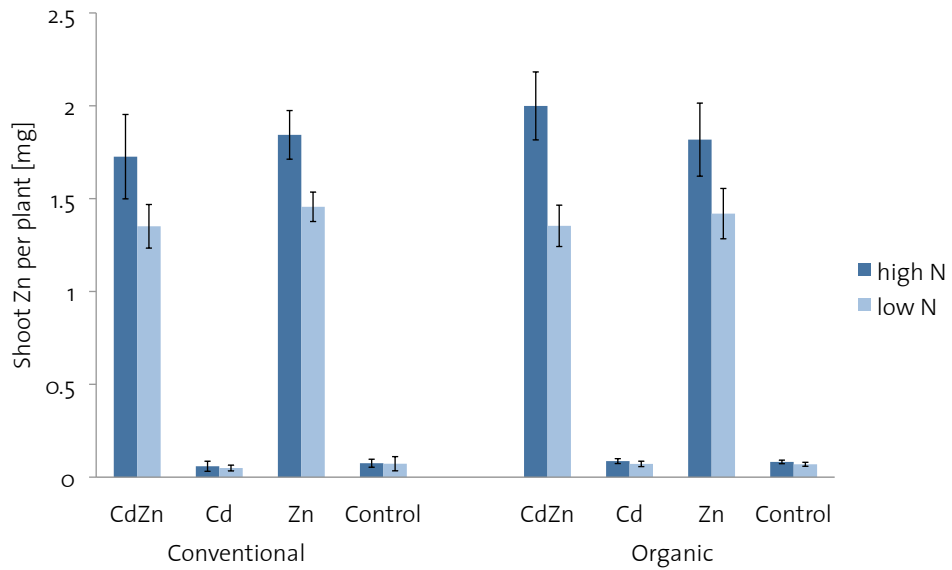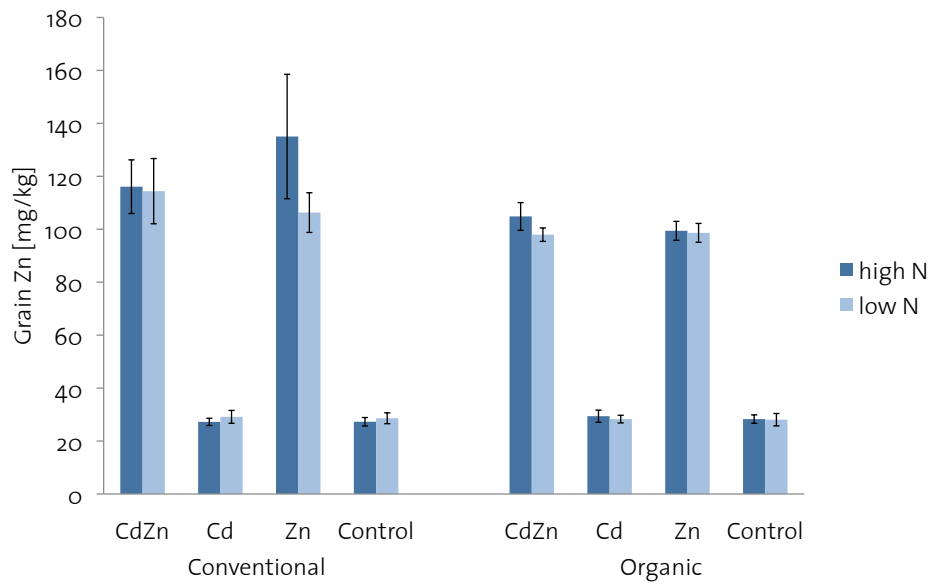
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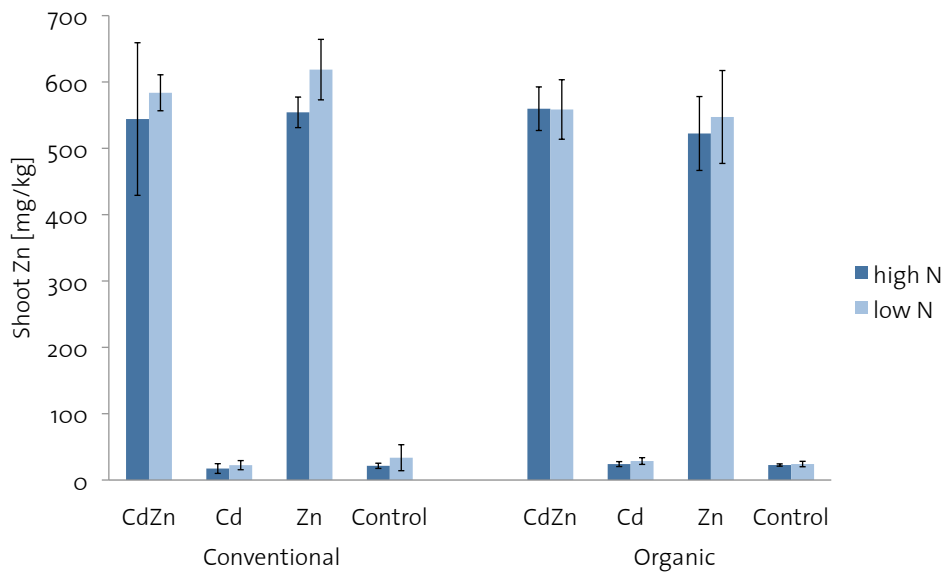
b)

Figure 30: a) The average zinc concentrations in the shoots and b) the average total shoot zinc content per plant.

Grain zinc concentrations and the zinc contents per plant are significantly increased for wheat grown on the zinc and cadmium-zinc treatment soils (p<0.001), whereas cadmium spiking had no effect. Nitrogen did not show a significant effect in the zinc grain concentration but did show a significant increase in total zinc per plant for the high level of nitrogen (p<0.001). Grain zinc concentrations were higher in the conventionally managed soil compared to the organically managed soil (p<0.001), as Figure 31 shows. The grain zinc concentrations measured in this experiment for the high zinc treatment plants averaged at above 100 mg/kg. The average grain zinc concentration for the non-zinc treatment plants was 28.3 mg/kg (Figure 31).

To illustrate the nitrogen effect on the total grain zinc content per plant correlations are displayed in Figure 32. The data was separated according to non-zinc spiked and zinc spiked treatments and correlated with the total grain nitrogen content per plant. The correlations nicely show the growth dilution as mentioned before. For both zinc spiked (R^2= 0.834) and non-zinc spiked (R^2= 0.826) metal treatments there is a positive correlation between total grain nitrogen and total grain zinc.
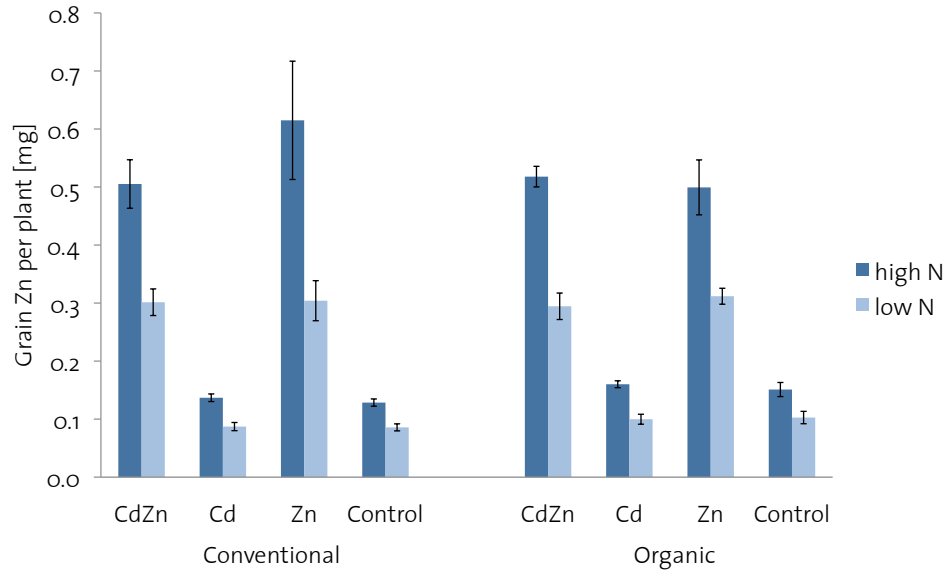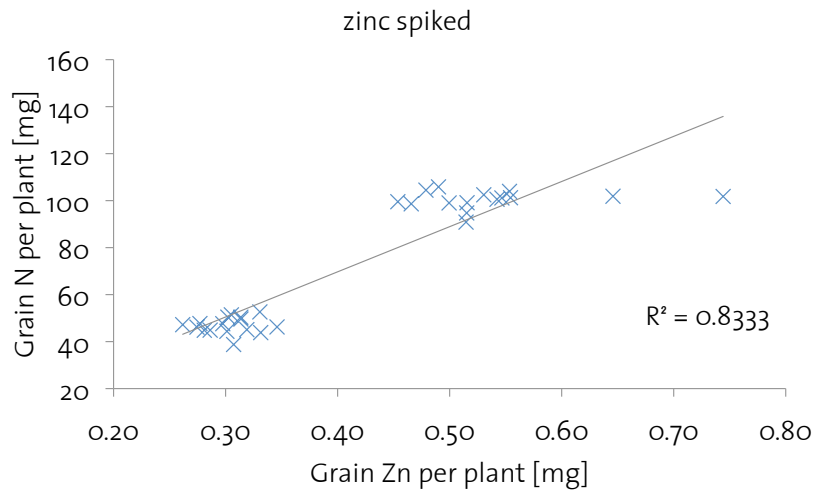
a)

b)

Figure 31: a) The average zinc concentrations in the grains and b) the average total grain zinc content per plant.
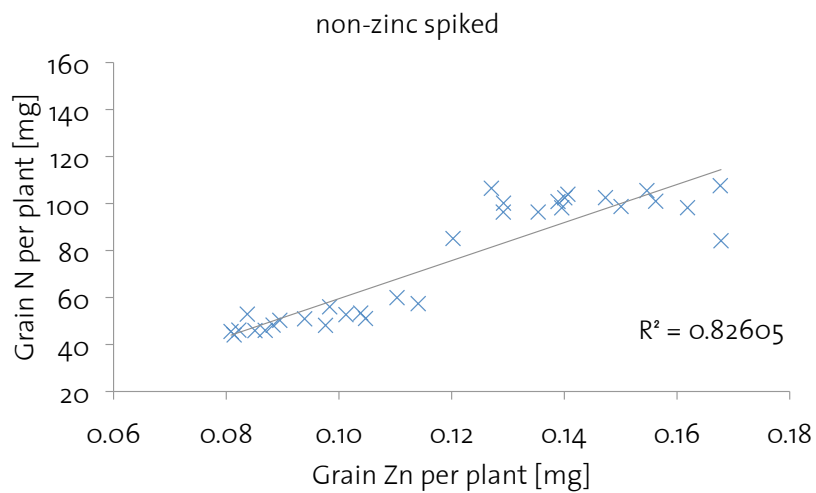
a)

b)

Figure 32: The correlation between the average total grain zinc content per plant and the average total grain nitrogen content per plant for a) the zinc treated soil plants and b) the non-zinc treated soil plants.

For the zinc data mass balances were also calculated for both DTPA Zn measurements in the initial soil. Table 16 shows the mass balance calculated with the results of the DTPA measurement at the start of the experiment and the mass balance in Table 17 is calculated for the second Zn DTPA measurement in the initial soil after experiment completion. The mass balance for the first DTPA zinc measurements shows a large discrepancy between the DTPA lost during the experiment and the plant uptake for the soils spiked with zinc. In the second DTPA zinc measurement this discrepancy is much less for the combination treatment. However, for the zinc treatment the zinc mass balance shows a larger discrepancy for the second than the first measurement.

Table 16: The mass balance for zinc: The difference between DTPA Zn prior to the experiment start in the initial soil and in the pot soils after experiment completion (DTPA lost), the sum of zinc content in the grains and shoots (plant uptake) and the difference between the two.

		Conventional soil			Organic soil		
		DTPA Zn lost [mg]	Plant uptake [mg]	Difference [mg]	DTPA Zn lost [mg]	Plant uptake [mg]	Difference [mg]
CdZn	high N	84.21	2.23	81.98	87.66	2.52	85.15
CdZn	low N	79.88	1.65	78.23	91.81	1.33	90.48
Cd	high N	0.62	0.20	0.42	1.26	0.25	1.01
Cd	low N	0.50	0.14	0.36	1.45	0.54	0.92
Zn	high N	102.21	2.46	99.75	51.04	2.32	48.72
Zn	low N	99.66	1.76	97.90	57.35	1.36	55.99
Control	high N	0.48	0.20	0.28	0.56	0.54	0.02
Control	low N	0.47	0.16	0.31	0.63	0.17	0.46

Table 17: The mass balance for zinc: The difference between DTPA Zn in the initial soil and the pot soils after experiment completion (DTPA lost), the sum of zinc content in the grains and shoots (plant uptake) and the difference between the two.

|		Conventional soil			Organic soil		
Treatment		DTPA Zn lost [mg]	Plant uptake [mg]	Difference [mg]	DTPA Zn lost [mg]	Plant uptake [mg]	Difference [mg]
CdZn	high N	8.43	2.23	6.19	2.45	2.52	-0.07
CdZn	low N	4.10	1.65	2.44	6.59	1.33	5.26
Cd	high N	-0.06	0.20	-0.25	-0.13	0.25	-0.38
Cd	low N	-0.18	0.14	-0.31	0.06	0.54	-0.48
Zn	high N	37.07	2.46	34.61	75.22	2.32	72.90
Zn	low N	34.52	1.76	32.76	81.53	1.36	80.17
Control	high N	-0.10	0.20	-0.30	-0.16	0.54	-0.70
Control	low N	-0.11	0.16	-0.27	-0.08	0.17	-0.25

4.3.2. Nitrogen

The total nitrogen concentrations in the shoots were significantly higher in the conventionally managed soil than in the organically managed soil ($p<0.001$) and shoot nitrogen was more concentrated for the high level of nitrogen in the conventional soil ($p<0.001$). For the organically managed soil there was no influence of nitrogen fertilization. The metal treatments had no effect on the shoot nitrogen concentrations for both soil types. The total shoot nitrogen content per plant followed the same trends as the concentrations (Figure 33).

The shoot samples were prepared and analysed on two separate days according to soil type. As the results are so different for the two soils and the organically managed soil results show no higher nitrogen content in shoots for the high level of nitrogen I doubt the reliability of this data set. During the rest of the nitrogen analysis the machine showed drift and missed out samples that had to be repeated. Linear drift corrections were performed when necessary and worked well according to quality control samples for the grain and soil data. But for the shoot analysis there seemed to be an additional factor influencing the analysis. The total shoot nitrogen concentration and content per plant results will not be further discussed in this thesis. Total nitrogen per plant for the shoot data is also affected by the same uncertainties (Figure 33).

In the grains the nitrogen content was significantly higher for the high level of nitrogen for both nitrogen concentrations and total nitrogen contents per plant ($p<0.001$) (Figure 34). For the nitrogen concentrations the conventionally managed soil plants contained significantly more nitrogen than the organically managed soil plants ($p<0.001$). As this effect could not be shown for total nitrogen per plant growth dilution is expected to be causing this difference as the wheat grown on the organically managed soil had more biomass than the ones grown in the conventionally managed soil. The metal treatments did not affect grain nitrogen concentrations or total grain nitrogen contents per plant.
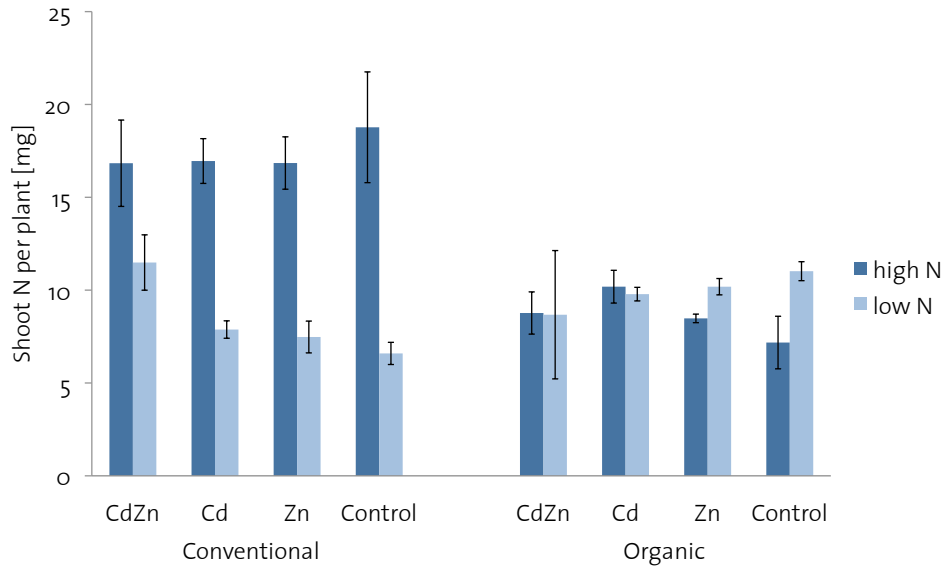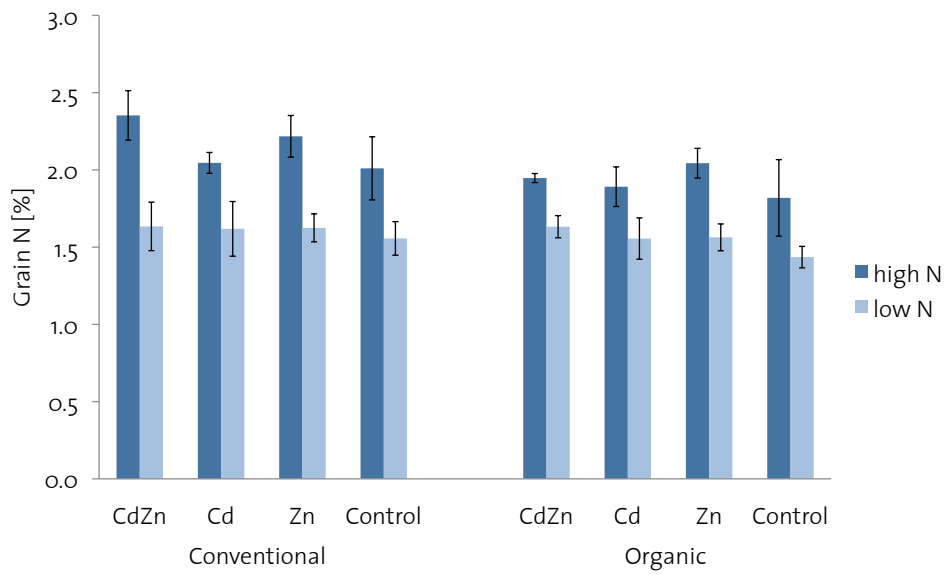
a)
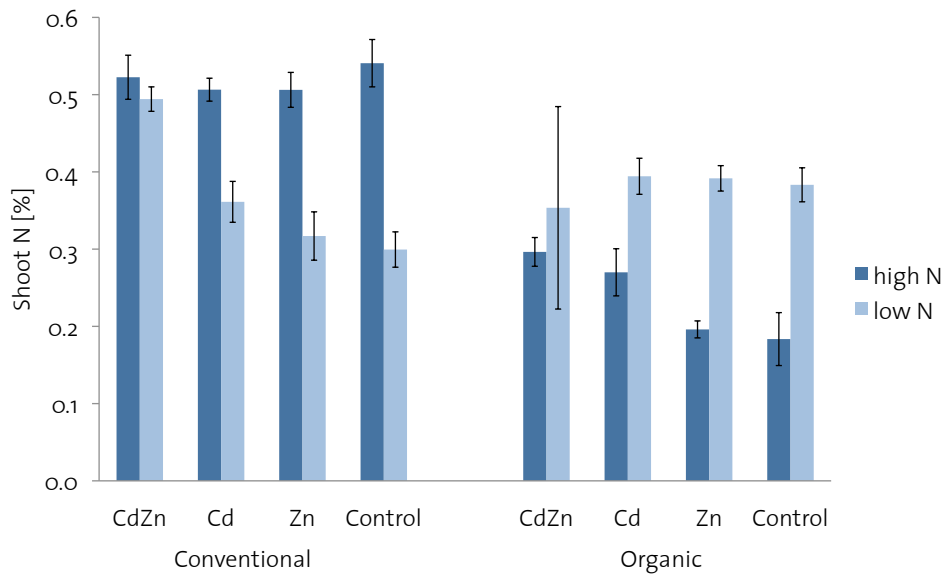
b)

Figure 33: a) The average total nitrogen contents in the shoots in percent and b) the average total nitrogen contents per plant.
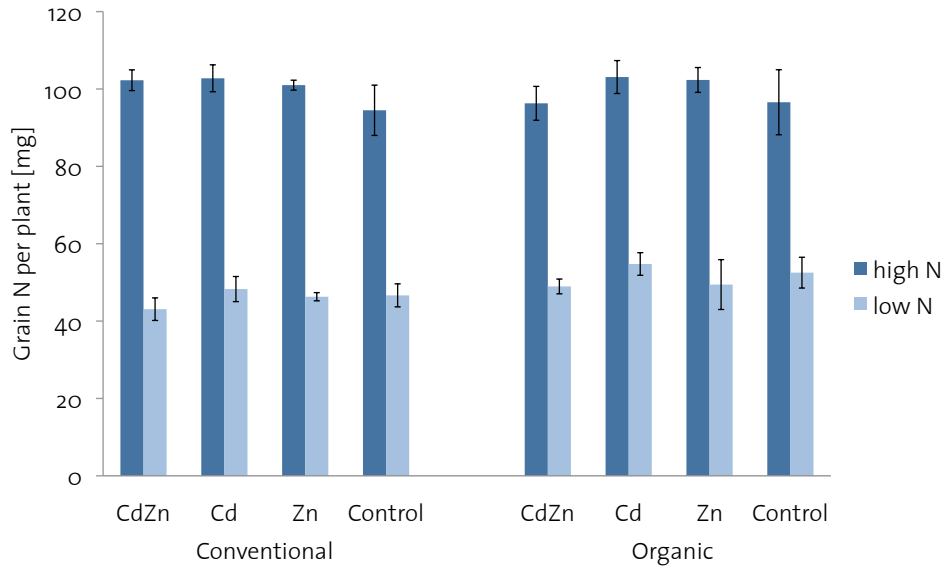
a)

b)

Figure 34: a) The total nitrogen contents in the grain in percent and b) the average total nitrogen grain content per plant.

In addition to total nitrogen, the nitrogen (N) harvest index was calculated and the results are displayed in Figure 35. The N harvest index is the percentage of nitrogen in the grain compared to the amount of nitrogen in the whole plant (shoot and grain). The N harvest index results are also affected by the unreliable shoot nitrogen measurements and are therefore only quickly discussed here. The data shows a higher N harvest index for the organically managed soil (p<0.001) and depending on soil type a higher harvest index for the low nitrogen fertilization (conventional, p<0.05) or the high nitrogen fertilization (organic, p<0.001). The metal treatments did not significantly affect the nitrogen harvest index (Figure 35).
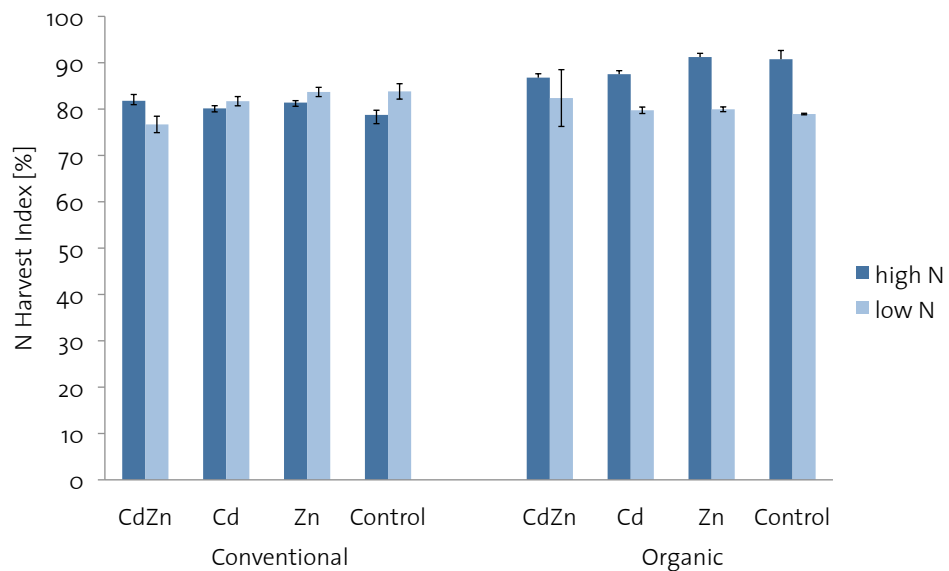

Figure 35: The calculations of the average nitrogen (N) harvest index. The N harvest index indicates the percentage of nitrogen in the grains compared to the nitrogen in the whole plant.

4.3.3. Iron, manganese and phosphorus

For iron the measured concentrations in the shoots were below the detection limit of the ICP-OES and are therefore not shown here. For grains the results are illustrated in Figure 36. The wheat plants grown on the conventionally managed soil had a significantly higher grain iron concentration than those grown in the organically managed soil ($p < 0.001$). Also, plants fertilized with high nitrogen had higher iron concentrations in grains than the ones fertilized with low nitrogen ($p < 0.05$). For the total iron grain content, the effect of soil was no longer significant. Therefore an effect of growth dilution is suggested for the soil effect in the concentration results. The difference between high nitrogen and low nitrogen fertilization is bigger for the total iron content per plant ($p < 0.01$) than for the grain iron concentration. The metal treatments had no significant effect on grain iron concentration or total grain iron content per plant.
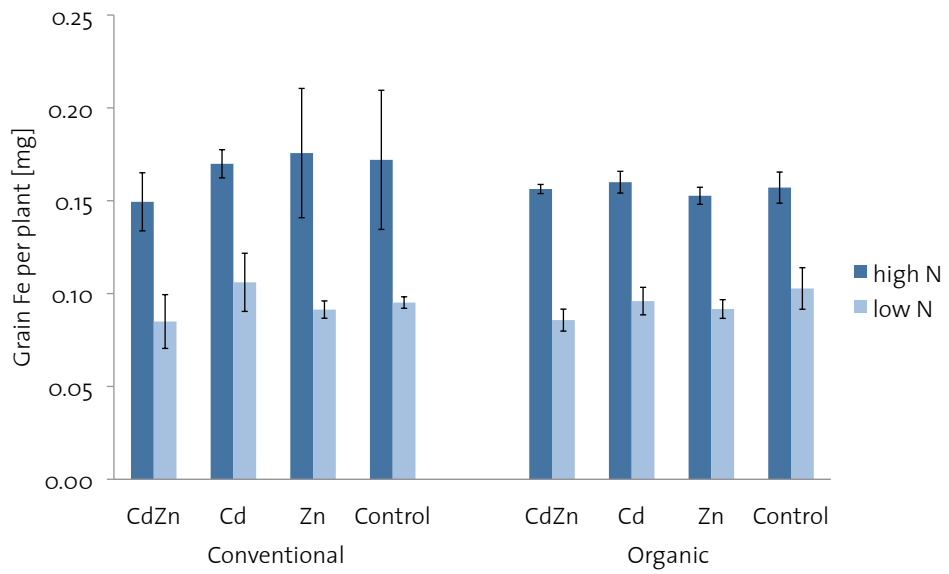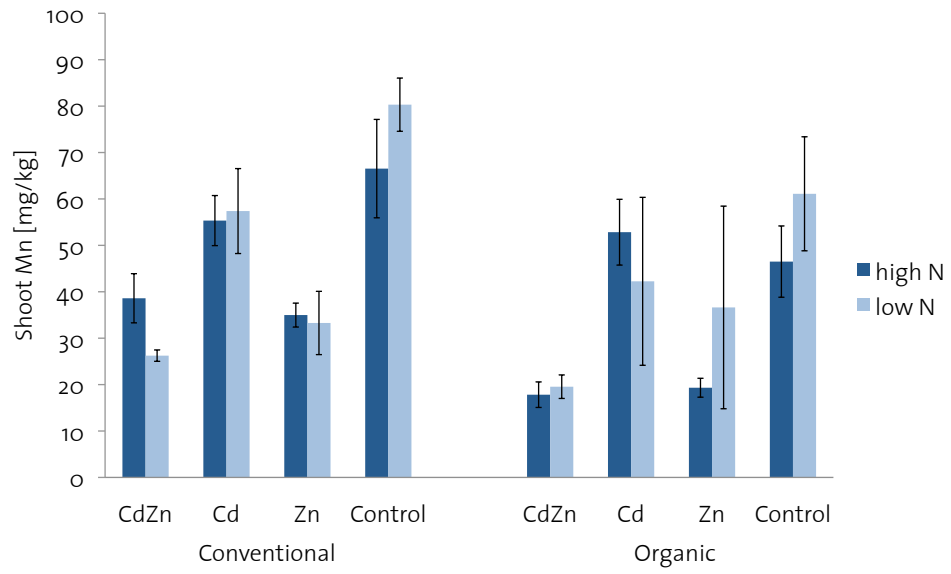
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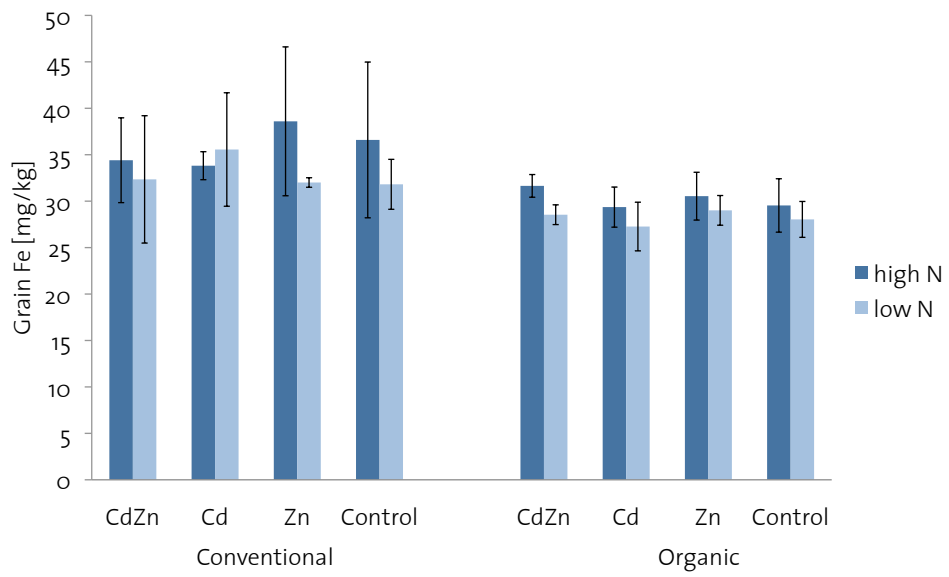
b)

Figure 36: a) The average grain iron concentrations and b) the average total grain iron contents per plant.

The wheat shoot (Figure 37) and grain manganese concentrations (Figure 38) showed similar trends. For both, the plants grown on the conventionally managed soil had significantly higher manganese concentrations in shoot ($p<0.001$) and grain ($p<0.001$) compared to the organically managed soil. Also for both, grain ($p<0.001$) and shoot ($p<0.001$), the wheat plants grown on soils spiked with zinc (Zn and CdZn) had a lower manganese concentration than the wheat plants of the soils not spiked with zinc (Cd, Control). For grains the addition of cadmium significantly lowered the manganese concentration compared to the treatments without cadmium addition (CdZn compared to Zn and Cd compared to Control) ($p<0.001$). Nitrogen had no significant effect on the shoot manganese concentration but for the grains the low nitrogen plants showed a higher manganese concentration than the high nitrogen plants. The total manganese content per plant of grains and shoots both show significant effects for nitrogen. The manganese uptake of high nitrogen fertilized plants was increased for both shoot ($p<0.001$) and grain ($p<0.001$). For the grains the total manganese content per plant was decreased for the plants grown on zinc-spiked soils and the addition of cadmium furthermore decreased the manganese content ($p<0.001$). The additional manganese reduction in the cadmium treatments was not significant for the Cd treatment compared to the control treatment under high nitrogen fertilization. The total manganese content in the shoots was significantly higher for wheat plants grown in the conventionally managed soil, than in the organically managed soil ($p<0.005$) and also showed a significant effect of zinc spiking. The wheat plants grown on zinc-spiked soils (CdZn, Zn) contained less total manganese than the non-zinc spiked soil plants (Cd, Control) ($p<0.001$).
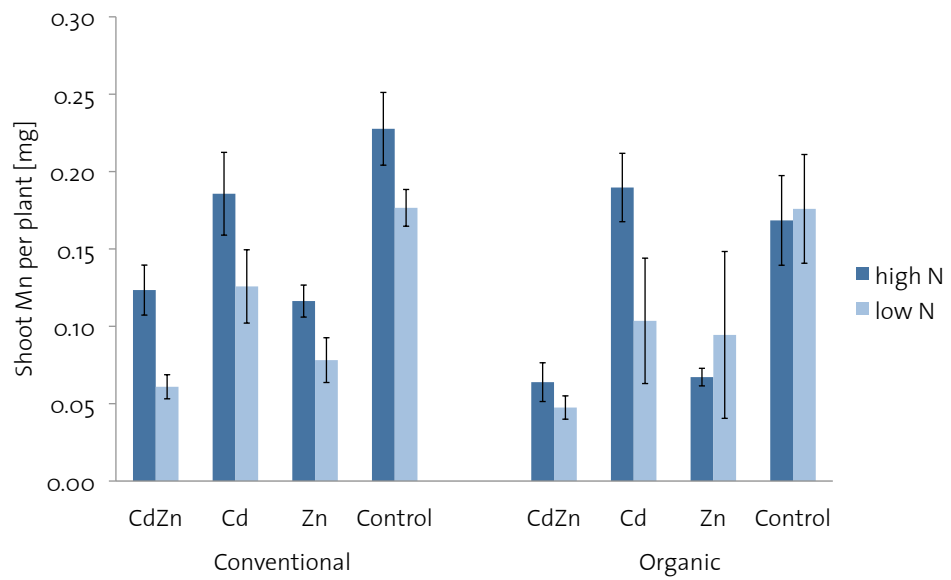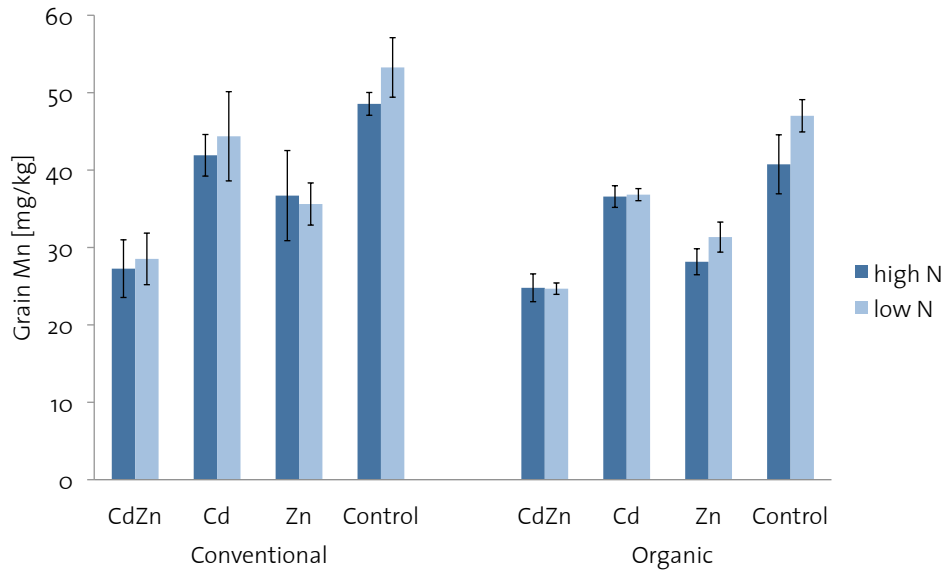
a)

b)

Figure 37: a) The average shoot manganese concentrations and b) the average total shoot manganese contents per plant.
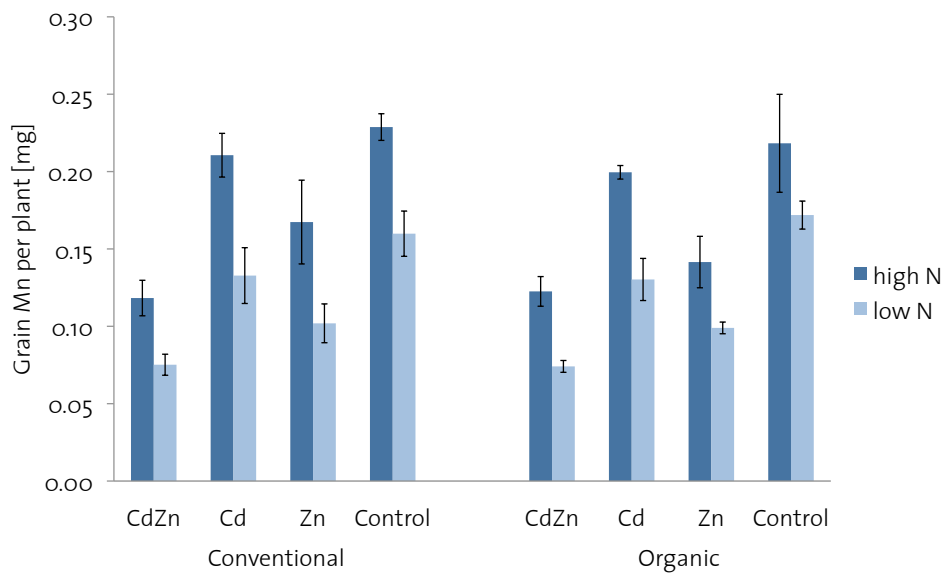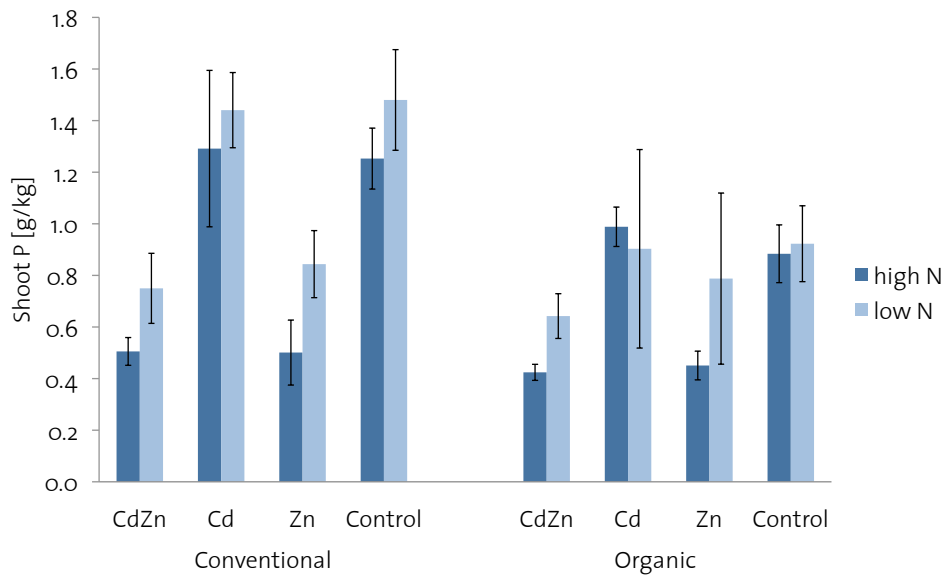
a)

b)

Figure 38: a) The average grain manganese concentrations and b) the average total grain manganese content per plant.

Shoot phosphorus concentrations were higher in the shoots of the wheat plants grown on conventional soil than the ones grown on organic soil ($p < 0.001$) (Figure 39). As seen for the manganese concentration, zinc spiking also decreased the phosphorus shoot concentration significantly ($p < 0.001$), though cadmium had no effect. Low nitrogen fertilization showed more accumulation of phosphorus for both, the conventionally managed ($p < 0.001$) and the organically managed soil ($p < 0.05$). The wheat grown in the organic soil had lower shoot phosphorus concentrations than those grown on conventionally managed soil ($p < 0.001$). For the total phosphorus content per plant in shoots the soil effect resulting in higher phosphorus uptake of the conventionally managed soil was still significant ($p < 0.01$). High nitrogen fertilization increased the uptake of phosphorus showing that the increased concentration of phosphorus in the low nitrogen treatment was the result of less biomass. Zinc spiking decreased the phosphorus uptake for both soil types and nitrogen fertilizations ($p < 0.001$) (Figure 39).

In the grains phosphorus concentrations were independent of metal treatment. The wheat grown on conventionally compared to organically managed soil reached significantly higher phosphorus concentrations ($p < 0.001$). Also, high nitrogen fertilization increased the phosphorus concentration in grains ($p < 0.001$). Total grain phosphorus per plant showed no effect of soil management but still showed the increased phosphorus uptake for the high nitrogen treatment as seen for the phosphorus grain concentration ($p < 0.001$). In contrast to the phosphorus concentration in grain the total phosphorus grain content per plant was affected by the metal treatments. The total grain phosphorus content per plant was decreased in the high nitrogen fertilization treatment for the following metal treatments ($p < 0.001$): For the conventionally managed soil, the Zn treatment compared to the Cd treatment and for the organically managed soil the CdZn treatment compared to all other metal treatments (Figure 40).
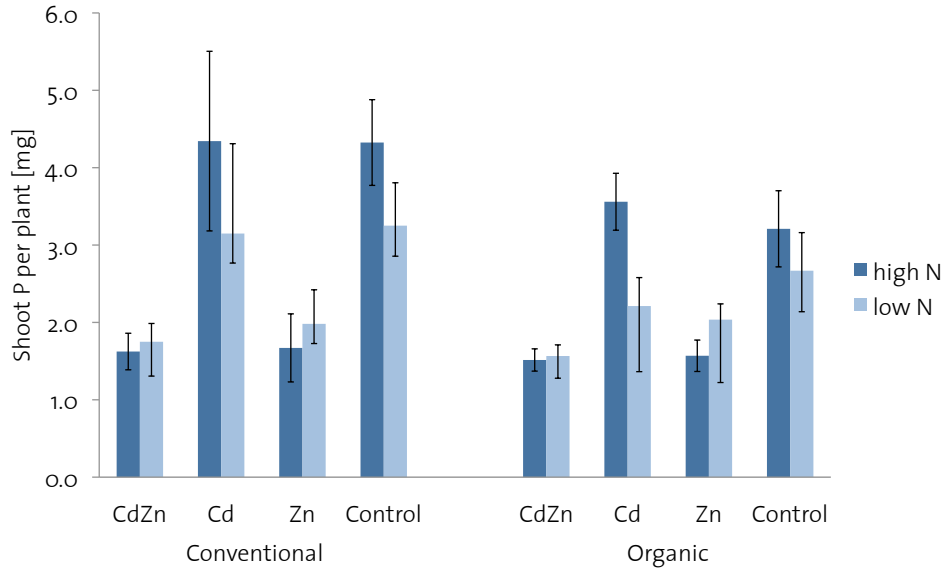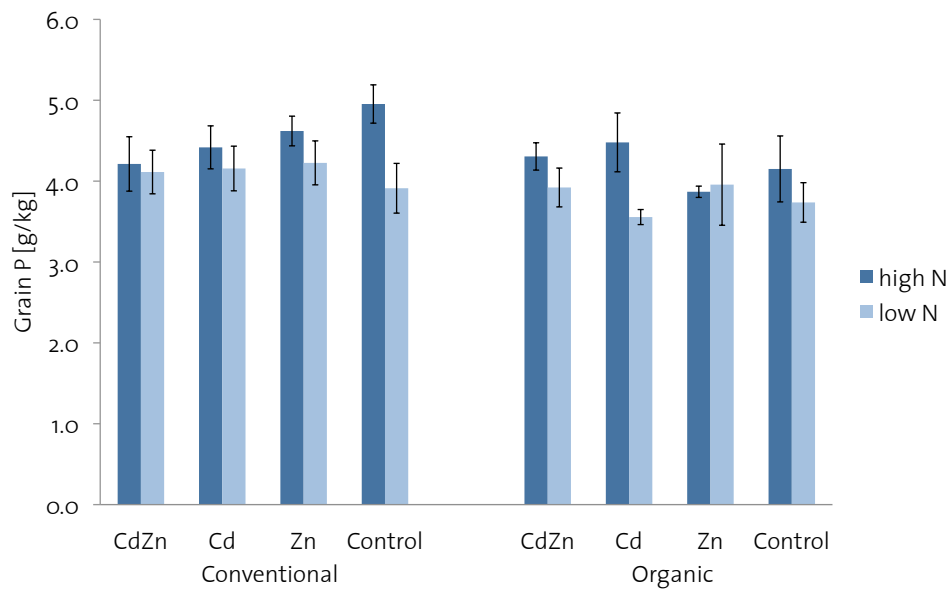
a)

b)

Figure 39: a) The average shoot phosphorus concentrations and b) the average total shoot phosphorus contents per plant.
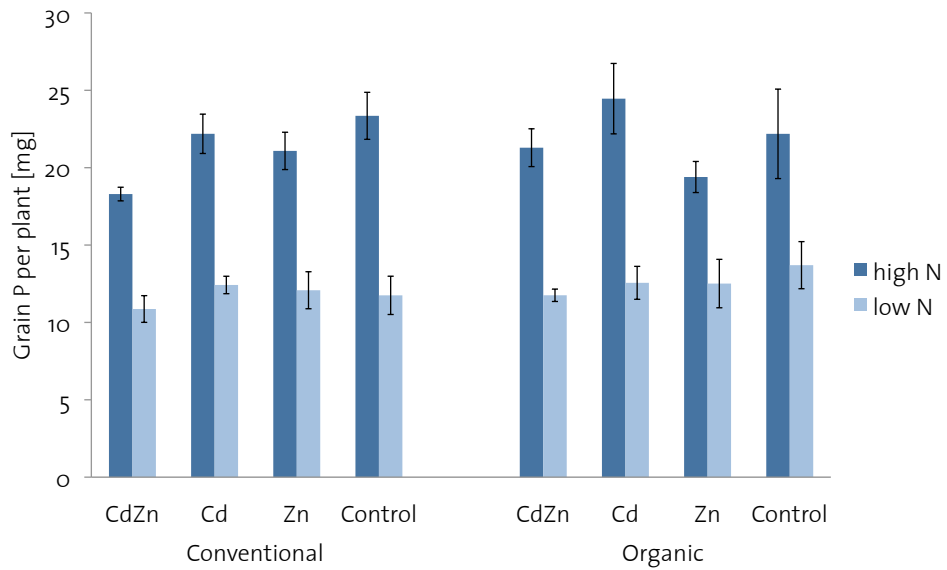
a)

b)

Figure 40: a) The average grain phosphorus concentrations and b) the average total grain phosphorus contents per plant.

4.3.4. Correlations of zinc and other nutrients

To assess the effect of zinc and other nutrients on each other in grain uptake and accumulation, correlations between total grain zinc and total grain iron, manganese and phosphorus were also calculated. The correlations were made in two groups, one for wheat plants grown on zinc spiked soil (zinc spiked, treatments CdZn and Cd) and one for the wheat plants grown on soil not spiked with zinc (non-zinc spiked, treatments Cd and Control). For total grain iron per plant there was a positive correlation for both groups as shown in Figure 41 (non-zinc spiked: R^2= 0.674 , zinc spiked: R^2= 0.821). For the non-zinc group there was no correlation between grain zinc concentration and grain iron concentration (R^2= 0.115) but for the zinc group the grain zinc and iron concentrations also showed a positive correlation (R^2=0.826).

a)
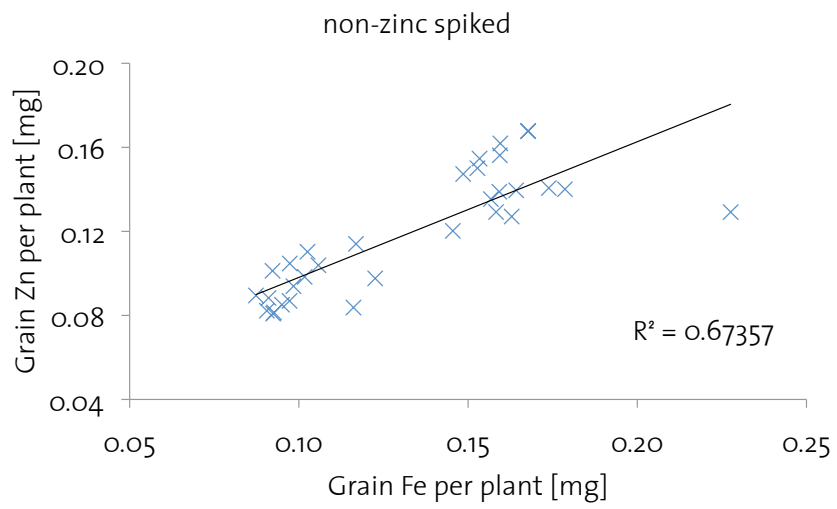
b)

Figure 41: The correlations between the average total grain zinc contents per plant and the average total grain iron contents per plant for a) the non-zinc treated plants and b) the zinc treated plants.

Figure 42 shows the correlations between total grain manganese and total grain zinc. The total manganese content in grains positively correlated with the total zinc grain content for the non-zinc spiked soils (R^2=0.630) and the zinc spiked soils (R^2=0.828). The results for the grain zinc and manganese concentrations did not correlate for both groups (non-zinc spiked: R^2=0.023, zinc spiked: R^2=0.408).

a)

b)

Figure 42: The correlations between the average total grain zinc content per plant and the average total grain manganese content per plant for a) the non-zinc treated plants and b) the zinc treated plants.

The total phosphorus content in grains correlated positively with the total zinc grain content for the non-zinc spiked soils (R^2=0.817) and the zinc spiked soils (R^2=0.836). The results are displayed in Figure 43. The results for the grain zinc and phosphorus concentrations did not correlate for both groups (non-zinc spiked: R^2= 0.0004, zinc spiked: R^2=0.288).
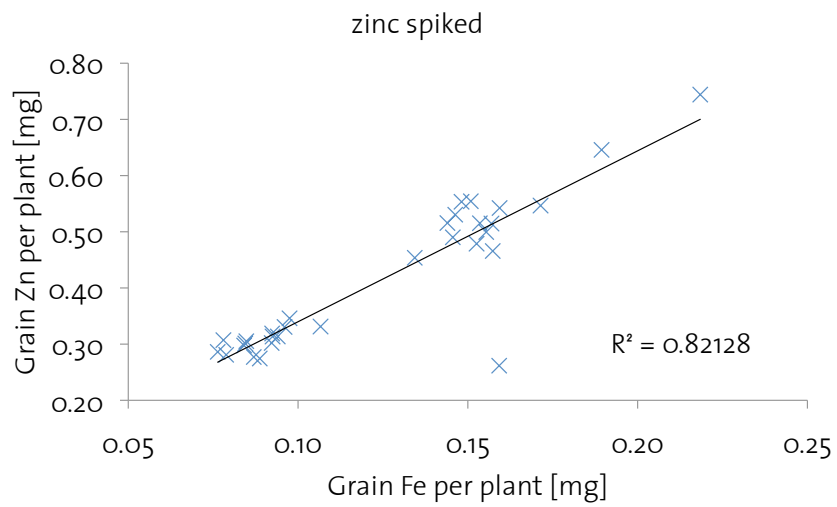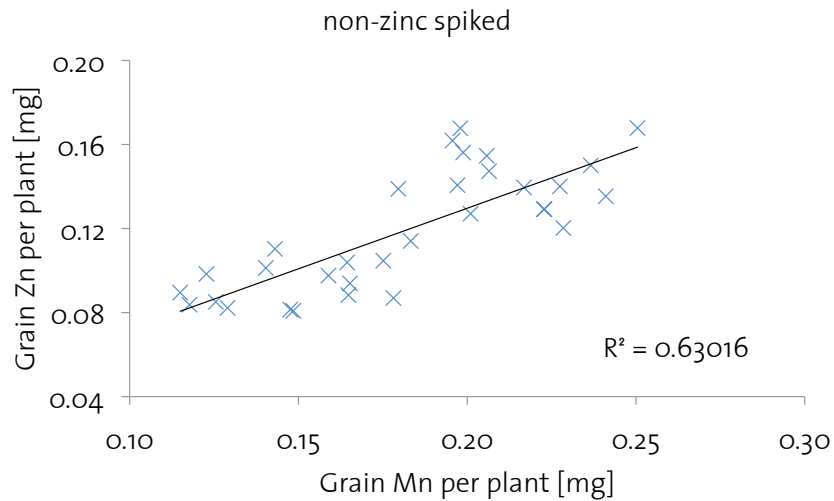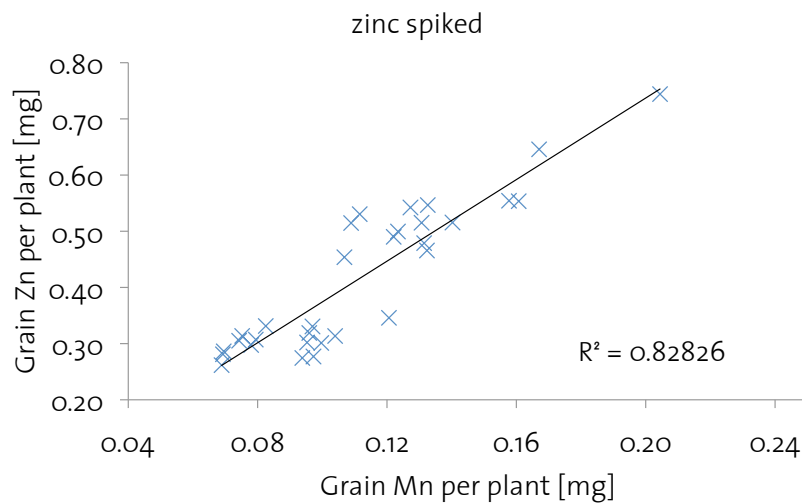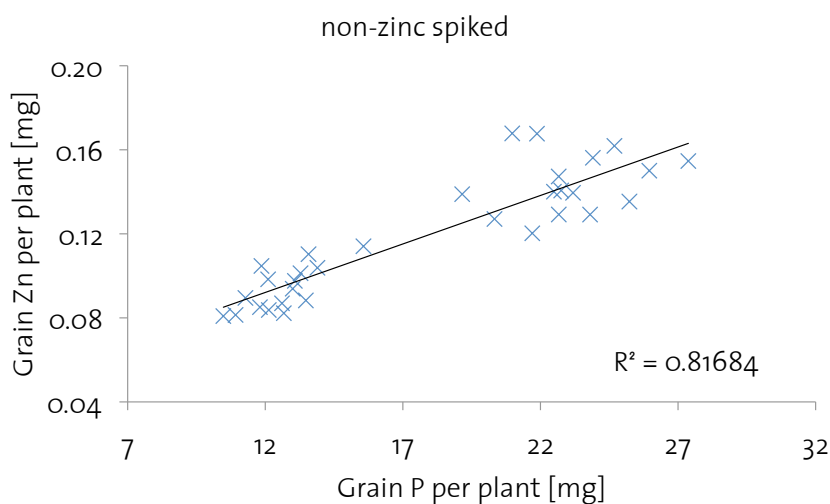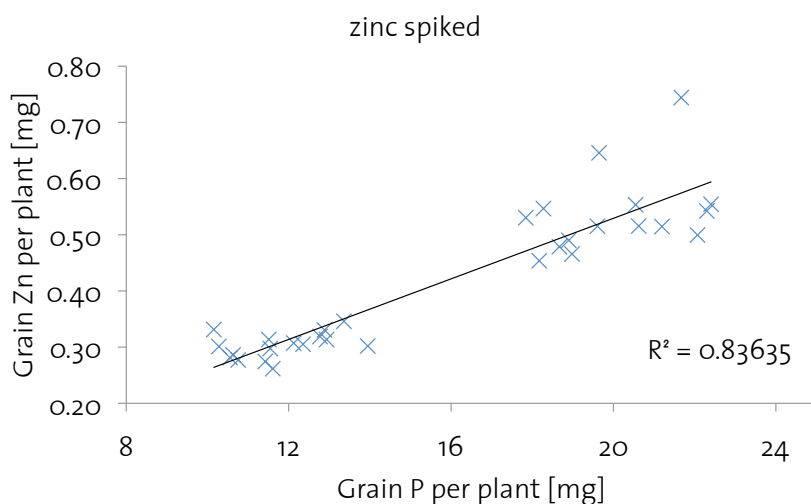
a)

b)

Figure 43: The correlations between the average total grain zinc contents per plant and the average total grain phosphorus contents per plant for a) the non-zinc treated plants and b) the zinc treated plants.

5. Discussion

5.1.   Initial soil characterisation

The two soils used in this experiment showed the same soil characteristics as in previous experiments using soils from the DOK experiment (Fliessbach et al. 2009, Mäder et al. 2006). The soils are silt loam developed on loess in a temperate climate. The differences in soil characteristics between the two management strategies are only marginal. The organic matter content was about 20% lower for the conventionally managed soil, and pH was reduced by 0.2 pH units. Total nitrogen content was slightly higher for the organically managed soil. The management strategy did affect the soils but the differences were probably too small to see a clear effect in the wheat growth or metal uptake.

The characterisation shows that all nutrients were available in sufficient amounts when including the amount of nutrients added to the soil as fertilizer. For the treatments that were not spiked with zinc (Cd, control), there was no zinc added to the soils as fertilizer. These treatment plants were at risk of zinc deficiency. But all in all nitrogen was the limiting factor for plant growth in this experiment.

At the start of the experiment DTPA extractions of the initial soils showed very high concentrations for both zinc and cadmium in the spiked treatments. For the zinc and the combination treatment (CdZn) the average concentration of plant available zinc in the soil was about 200 mg/kg. In the cadmium treatment and the combination treatment (CdZn) plant available cadmium concentrations in soils averaged at around 1.3 mg/kg. This shows that much of the zinc and cadmium added to the soil through spiking was stored in a plant available form and was not yet strongly bound to the soil. It was expected that less cadmium and zinc would be available for plant uptake at the experiment start. Therefore the aging time of the soil after spiking was not sufficient with this high amount of metals spiked to the soil, especially for zinc.

5.2. Soil analysis

As shown in the results for cadmium and zinc the DTPA extracted concentrations in the initial soil were measured twice. The results of these two measurements differ significantly for both cadmium and zinc. The samples were treated in the same way for both measurements. The only difference was the storage time before the extraction and the measurement. The first measurement was carried out as part of the initial soil characterisation at the start of the experiment and the second measurement was carried out together with the pot soils after completion of the experiment. The results show that the DTPA extractable cadmium and zinc concentrations changed in the soil during dry storage in the same way as in the soil used for the experiment. To illustrate this in more detail mass balances for zinc and cadmium were calculated. The mass balances for the first DTPA measurements show a large discrepancy between the DTPA lost during the experiment and the plant uptake for the soils spiked with zinc, and cadmium respectively. In the mass balance for the second DTPA measurement this discrepancy was much less for all spiked soils but especially for the combination treatment. However for zinc the mass balance for the second DTPA measurement showed a larger discrepancy than the mass balance of the first measurement for the zinc treatment in the organic soil.

The mass balances show that the difference between the DTPA extractable soil cadmium and zinc concentrations in the first measurement and in the pot soils after experiment completion cannot be explained with plant uptake. So as already indicated by the difference in the two DTPA measurements on the initial soil, a soil effect is responsible for the change of trends. This soil effect takes place in the soil used during the pot experiment but also in the dried soil stored in airtight plastic bags. Metals are immobilised with time, which could be a process of soil aging.

DTPA extractable cadmium concentrations are about the same for the initial soil (2nd measurement) and the pot soil after experiment completion. For both, cadmium is immobilised in the zinc-spiked soils compared to the soils not spiked with zinc. For zinc there is only a difference between the initial soil (2nd measurement) and the soil after experiment completion for the zinc treatment. The DTPA extractable zinc concentration is higher in the initial soil compared to the pot soils after experiment completion. This difference also shows in the mass balance and can therefore not be explained with

plant uptake. The immobilisation of zinc seems to be promoted in the combination treatment (CdZn) compared to the zinc only treatment. We cannot explain this discrepancy but know that this is an effect that takes place in the soil independent of plant uptake.

There is no effect of soil management or nitrogen treatment on the plant available zinc or cadmium concentrations.

The DTPA extracted iron was also immobilised in the zinc spiked soils compared to the non-zinc spiked soils as was also shown for plant available cadmium. The opposite effect was expected prior to the measurements. It was thought that metals such as cadmium and iron would be more available in the Zn and CdZn treatments because of enhanced competition for uptake sites in the soil structure. Shute and Macfie (2006) have shown this for rhizosphere cadmium. The plant available iron concentrations showed no effect of soil management. The high nitrogen treatment soils had higher concentrations of plant available iron than the low nitrogen ones. This effect can be explained by the more acidic soil pH in the high nitrogen soil making metals such as iron less bound to soil and more plant available.

Total soil nitrogen and nitrate concentrations were significantly higher for the organically managed soil compared to the conventionally managed soil. The organically managed soil was fertilized with manure and slurry only, prior to the pot experiment and therefore received a lot of organic matter input. This organic nitrogen can be stored in the soil and be slowly released by organic matter degradation. This results in a higher total nitrogen concentration for the organically managed soil compared to the conventional soil. After experiment completion ammonium and nitrate concentrations in the soils were below the detection limit. The plants took up all the available nitrogen. As shown in Figure 6 the experiment was set-up for the high nitrogen treatment to reach nitrogen saturation and have plant available nitrogen left over at the end of the experiment. Figure 44 displays the actual nitrogen situation as it was present during the experiment. There was still the possibility of an additional plant response with higher nitrogen fertilization.
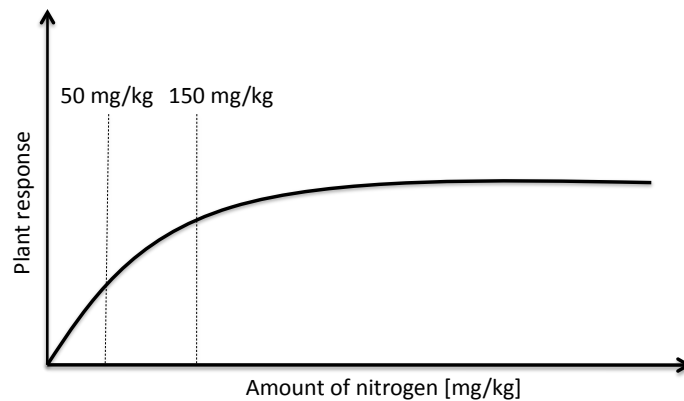
Figure 44: Plot of the amount of nitrogen given to a plant as fertilizer and the plant's response to this fertilization. Also shown in the graph are the two levels of nitrogen fertilization used in this experiment (50mg/kg and 150mg/kg) and their actual location within the plot.

At the end of the experiment still more total nitrogen was present in the organically managed compared to the conventionally managed soil. This is very likely to be the difference in organic nitrogen, already seen between the two soil types in the initial soil. The plants did not take up any of the organic nitrogen that was stored in the soils in either nitrogen treatment. During the experiment, either the mineralisation of organic nitrogen to mineral nitrogen was too slow or there was enough nitrogen available for both the low and the high nitrogen treatment not to take up stored organic nitrogen.

5.3. Wheat growth and biomass

The growth of the wheat plants took longer than expected. This was especially the case for the wheat plants grown with high nitrogen fertilization. The long maturation time shows that the plants really had enough nutrients and were not stressed by any other factor such as water stress, as this would have shortened the maturation time. The high nitrogen plants reached maturity later and had more heads by the time of harvest compared to the low nitrogen plants, probably due to the higher nitrogen availability. This explanation corresponds with the fact that there was no plant available nitrogen left in the soil after the experiment had finished. The number of heads and biomass results show an increased dry weight and grain yield for the high nitrogen wheat plants. The plants grown in the organically managed soil produced more biomass in shoots,

grains and heads than the ones grown in conventionally managed soil. This increased biomass is likely caused by the higher nitrate content in the organic soil compared to the conventionally managed soil.

Generally the wheat grown in zinc spiked soils produced more heads and seeds per plant than the ones grown on soils not treated with zinc. This effect was not significant for all treatments. The grain biomass was reduced in the plants grown in zinc-spiked soils compared to those grown on the cadmium spiked soil and the control soil. There was no such reduction in the shoot biomass. The most obvious explanation for the reduction in grain biomass is zinc toxicity. Zinc toxicity will be discussed in more detail in the wheat analysis chapter.

There was no additional reduction in growth for the combination treatment compared to the zinc only treatment. This is in agreement with Shute and Macfie (2006). Chaoui et al. (1997) and Dudka et al. (1994) found an additional reduction in growth for the combination treatment in their experiment. In the experiment by Dudka et al. (1994) the additional biomass reduction in the combination treatment only occurred for zinc concentrations that were already toxic to the plants when applied alone (from 1000 mg/kg soil zinc), which were actually higher than the ones used in this experiment. Chaoui et al. (1997) used lower zinc concentrations (up to 25 µM/l solution zinc) for an experiment with hydroponically grown beans. Here either the difference in plant species, solution concentration or growing conditions compared to this experiment are responsible for the opposing results.

5.4. Wheat analysis

The zinc concentrations in shoot and grain were increased for the zinc spiked treatments. The average grain zinc concentration was 109 mg/kg for the zinc spiked treatments. The decrease in biomass that was observed in the wheat grains of plants grown in the zinc spiked soils ranged from 3.2 to 13.6%. Data published on phytotoxicity usually declare a certain zinc concentration as toxic if growth is reduced by 5, 10 or even 25%. Though Reuter and Robinson (1986) describe a zinc concentration of 66 mg/kg as toxic for wheat grains, as shown here wheat can tolerate much higher zinc concentrations in the grains. The grain biomass reduction for the plants grown in the

zinc treated soils is in the same range as those used to declare the level of toxicity (5-10%) but the measured concentrations in the grains were much higher than the one given by Reuter and Robinson. This is in agreement with Dudka et al. (1994). They spiked soils with zinc concentrations of up to 5000 mg/kg and grew wheat plants in it. In the soils spiked with up to 300 mg/kg zinc there were no toxic effects such as visible toxicity symptoms or reduced biomass in the wheat plants. The average grain zinc concentration in plants grown on this soil was 98 mg/kg. For soil zinc concentrations of 500 mg/kg the average wheat grain zinc concentration was 140 mg/kg and grain biomass was reduced by about 6%, showing the first toxicity symptoms. Wheat grown in soil with a zinc concentration of 1000 mg/kg grain and shoot biomass was reduced by 30 to 40 % and the grain zinc concentration was 213 mg/kg. Further increase of soil zinc was lethal to the wheat plants.

Reuter and Robinson (1986) give the zinc toxicity level for wheat shoots for zinc concentrations above 550 mg/kg. Broadley et al. (2012) give a general range for zinc phytotoxicity for all plant species for concentrations between 300 and 500 mg/kg. The plants grown in this experiment reached an average shoot zinc concentration of 562 mg/kg in the zinc treatments (CdZn, Zn). The shoots did not show a significant reduction in biomass for the zinc treatments or visible toxicity symptoms on the leaves such as chlorosis.

The wheat plants grown on the non-zinc spiked soils contained an average zinc concentration of 28.3 mg/kg in the grains and 24.3 mg/kg in the shoots. Reuter and Robinson (1986) state marginal deficiencies of zinc for wheat at concentrations below 15 mg/kg in shoots and concentrations below 5-10 mg/kg in grains. Broadley et al. (2012) declare zinc concentrations of 15-20 mg/kg in shoots to be the range below which zinc deficiency occurs. The zinc in the wheat plants grown in the non-zinc spiked soils are just above these deficiency levels and did not show any zinc deficiency symptoms. Therefore an adequate zinc supply was present for the plants grown on non-zinc spiked soils.

Previous experiments have shown positive correlations between plant tissue zinc concentrations and plant tissue nitrogen concentrations (Cakmak et al. 2010, Morgounov et al. 2007). Therefore it was expected that the high nitrogen treatment plants would take up more zinc than the low nitrogen ones in this experiment. The zinc concentrations showed no significant effect of nitrogen. But the total zinc contents

were significantly higher for the high nitrogen treatment plants compared to the low nitrogen treatment plants. So the wheat plants fertilized with the high level of nitrogen did take up more cadmium and zinc, respectively but the effect did not show in the concentrations because of growth dilution. As shown by Erenoglu et al. (2010) nitrogen fertilization increases the zinc uptake, the root to shoot translocation and the remobilization from the vegetative tissue into the grain. In addition to these mechanisms soil pH decreased for the high nitrogen treatment, which would have resulted in higher zinc plant availability. Linked to this the total zinc and nitrogen grain contents per plant showed a positive correlation for both the zinc-spiked and the non-zinc spiked soils. There was no correlation for the concentration results due to growth dilution. Grain protein is calculated by multiplying grain nitrogen by 5.7 so grain zinc also correlated with the amount of protein in the grain. This has been observed in previous studies (Cakmak et al. 2010, Morgounov et al. 2007) suggesting grain protein is a sink for grain zinc.

There was no effect of cadmium addition on the zinc concentration in both shoots and grains. This is in agreement with Dudka et al. (1994) and Shute and Macfie (2006) who also found no interaction between the two for low doses of cadmium (2-10 mg/kg soil Cd). In this experiment the relative cadmium to zinc concentration in the soil for the combination treatment was 1:225. So the cadmium concentration was too small compared to the zinc to give any effects.

Both shoot and grain cadmium concentrations and total cadmium contents per plant, were increased for the wheat plants grown on Cd spiked soils (Cd, CdZn). The measured grain cadmium concentrations ranged from 1.5 to 2.5 mg/kg for the cadmium spiked treatments and the shoot cadmium concentrations varied between 4 and 7 mg/kg in the Cd spiked soils. In an experiment by Dudka et al. (1994) spring wheat plants were exposed to varying concentrations of cadmium (2-50 mg/kg) and zinc (200-5000 mg/kg) in a pot experiment. Even with the highest dose of cadmium at 50 mg/kg the wheat plants showed no toxicity symptoms and yield was not reduced. The cadmium concentrations in the shoot and grain for the 50 mg/kg soil addition were 10.3 and 4.1 mg/kg respectively. The wheat plants grown on the cadmium spiked DOK soils also did not show any toxicity effects and growth/yield was not reduced in any way.

In the high nitrogen treatment the total cadmium contents per plant were increased in grain and shoot. However, the effect did not show in the concentration results due to growth dilution in the high nitrogen plants. The increasing uptake of cadmium with higher nitrogen availability has also been shown in previous experiments (Li et al. 2011, Wångstrand et al. 2007). Most likely similar mechanisms as shown for zinc are responsible for this effect. Especially since the two elements are partly taken up by the same transporter proteins in plant roots.

Zinc addition decreased the cadmium concentrations and the total cadmium contents per plant in grains and shoots compared to the treatments without zinc addition (CdZn treatment, compared to Cd treatment and Zn treatment compared to control treatment). This interaction between cadmium and zinc is antagonistic.

Antagonistic effects of zinc on cadmium accumulation in plants have been shown for wheat plants grown on zinc-deficient soils (Choudhary et al. 1994, Oliver et al. 1994) and in hydroponic cultures (Hart et al. 2005) as well as other plant species such as lettuce and spinach (McKenna et al. 1993). Other experiments have shown synergistic effects where increased zinc enhanced the cadmium accumulation in soybeans in a pot experiment (Shute and Macfie 2006), in a field experiment with wheat (Nan et al. 2002), and in a hydroponic experiment with beans (Chaoui et al. 1997). Furthermore it has been shown that zinc addition to the soil can also have no effect on cadmium uptake by wheat plants (Dudka et al. 1994).

Synergistic effects of zinc on cadmium uptake presented in the literature are mostly related to zinc and cadmium toxicity respectively so high concentrations are used (Chaoui et al. 1997, Dudka et al. 1994, Shute and Macfie 2006). Though Nan et al. (2002) showed synergistic effects in spring wheat and maize in a field experiment with soil zinc and cadmium concentration similar to this study (3.16 mg/kg soil cadmium and 146.78 soil mg/kg zinc). They also related the synergistic effects to high concentrations of cadmium and zinc in the soils, due to soil contamination through polluted irrigation water. They conclude that the interactions may depend on the cadmium and zinc contents in their combination in the soil, the soil characteristics, crop species and plant tissues. Furthermore the experiment was carried out in the field where other factors such as an additional contaminant could have affected the results.

Proposed explanations for antagonistic effects include direct competition between the two metals for plant uptake through common transport proteins, cadmium retention in

the root as protection against cadmium toxicity if the zinc status of the plant is adequate and improved membrane integrity with increasing zinc status in the plant which reduces uncontrolled cadmium uptake and less cadmium uptake through phytosiderophores for zinc-deficient systems (Choudhary et al. 1994, Hart et al. 2005, Köleli et al. 2004, Oliver et al. 1994, Shute and Macfie 2006). In this study wheat plants were not affected by zinc-deficiency. They were at the high end of adequacy so only direct competition and retention of cadmium in the roots can explain the antagonistic effects of increased zinc on cadmium uptake and accumulation in shoot and grain. However, the observed immobilisation of cadmium in the soil for the CdZn combination treatment could also be relevant in this study. Plant available cadmium was reduced in the combination treatment compared to the cadmium only treatment. So the decreased cadmium concentration in the plant tissues could be a direct result of the reduced bioavailable soil cadmium in the combination treatment.

In conclusion it can be said that the interactions between zinc and cadmium are very complex and depend on plant species, the relative and total concentrations of cadmium and zinc in the soil solution and the nutritional status of the plant (Köleli et al. 2004, Shute and Macfie 2006).

The nitrogen content measured in the wheat grains was increased for the high nitrogen treatment as more nitrogen was added to the soil as fertilizer. The metal treatments and soil types had no significant effect on the nitrogen grain concentrations and on the total nitrogen grain contents. The nitrogen shoot data is not reliable and will therefore not be discussed here. The wheat plants grown on the organic soil did not take up more nitrogen than those grown on the conventionally managed soil. Because either the plants did not require more nitrogen than the amount fertilized in a plant available form and therefore did not take up the organic nitrogen of which there was more in the organic soil or the mineralisation of the organic nitrogen was too slow.

Nitrogen significantly affected the manganese and iron contents per plant for shoot and grain. The high nitrogen treatment plants had higher contents per plant compared to the low nitrogen plants but the effect could not be shown in the concentrations. This was caused by growth dilution in the high nitrogen treatment plants. For phosphorus the grain concentrations and total grain phosphorus contents were significantly

increased for the high nitrogen treatment whereas the shoot phosphorus concentrations and contents per plant were not affected by nitrogen.

Therefore iron, manganese and phosphorus are all positively affected by nitrogen fertilization. This could be due to promoted root growth, more bioavailability due to a decrease in soil pH or increased protein activity similar to the effects shown for zinc (Erenoglu et al. 2010). There were positive correlations between the total grain zinc contents per plant and the total grain contents per plant for iron, manganese and phosphorus separated into two groups for the zinc treated soil plants and the non-zinc treated soil plants. These correlations are most likely also related to the increased uptake of ions in the high nitrogen treatments, as all of these nutrients were affected by the high nitrogen treatment. More nitrogen results in higher zinc accumulation, but also higher iron, manganese and phosphorus uptake, which is what can be seen in the positive correlations of zinc and the other metals. For zinc and cadmium there is no positive correlation even though cadmium was also affected by the higher nitrogen fertilization. The spiking of the soil leads to two groups in the squatter plot so the effect of nitrogen can't be shown like for the others.

The grain iron concentrations and also the total iron contents in the grains did not respond to the metal treatments. The plant available iron concentration in the soil showed an immobilisation of iron for the zinc-spiked soils but the iron in the grains did not reflect this. Possibly because iron is dissolved and taken up by phytosiderophores, independent of DTPA extractable iron.

The manganese concentrations and total manganese contents per plant showed reduced manganese in the shoots and grains of the zinc treated plants. Either these results reflect the plant available concentrations of manganese in the soils, which were below the instrument detections limits or there was competition for uptake between zinc and manganese. Competition between zinc and manganese uptake has been shown in a pot experiment with corn. Two levels of zinc (0 and 10 mg/kg) were applied as well as varying concentrations of phosphorus (0, 25 and 75 mg/kg). The application of zinc reduced the manganese content in leaves of phosphorus-supplied plants. But increased the same in roots of zinc-deficient plants. The decrease in manganese concentrations was related to a severe retardation of the manganese absorption in the

roots with zinc addition (Broadley et al. 2012). Fageria (2002) also state that zinc fertilization reduces the uptake of manganese, as shown in rice plants. However, synergistic effects between manganese and zinc have been shown in the uptake in soybean plants (Gray et al. 2002). In the organically managed soil wheat shoots and grains contained less manganese than in the conventionally managed soil. For the grains this was not significant for the total manganese content per plant so the effect can be explained with growth dilution. In the shoots the total manganese content per plant was also significantly higher for the plants grown on the conventionally managed soil. There was no difference in total manganese content between the two soil management strategies in the initial soils. However, manganese is an element that reacts strongly to changes in pH, so the lower pH in the conventional soil probably led to the increased uptake of manganese, just as shown for the higher nitrogen treatment.

The phosphorus shoot concentrations were decreased in the plants grown in the zinc treated soils. The average shoot phosphorus concentrations were 0.6 g/kg in the zinc treated plants and 1.14 g/kg in the non-zinc treated plants. Reuter and Robinson (1986) declare phosphorus concentrations between 1.2 g/kg and 3 g/kg as critical in wheat shoots. Deficient concentrations for shoots of wheat plants in the heading state are set at 1.5 mg/kg. Rashid et al. (2005) found the same critical values in a field experiment with spring wheat. The defined levels for deficiency show that the wheat plants grown on the zinc treated soils experienced a substantial phosphorus deficiency in the shoots. Safaya (1976) showed that zinc application decreases phosphorus concentration and the phosphorus-flux in corn. He speculated that zinc has the ability to control the rate of phosphorus absorption by the roots, through a functional association in the cell membrane. When zinc deficient plants are fertilized with zinc phosphorus uptake also decreases. Zinc deficiency in plants increases the permeability of the plasma membrane therefore leading to uncontrolled phosphorus uptake, which is stopped when plants are zinc fertilized (Hawkesford et al. 2012).

Phosphorus is a building block of important macromolecular structures such as nucleic acids, which are components of the DNA. It is also contained in phospholipids. Phosphates represent the metabolic energy of cells. Energy rich phosphates such as adenosindi- and triphosphate are involved in the energy storage for the carbohydrate, fat and protein metabolism. Phosphorus is very important for the reproduction of

plants. If not enough phosphorus is available to the plant, the maturation time is delayed and the size of the grains is reduced (Bergmann 1992, Hawkesford et al. 2012).

The plants grown on the non-zinc treated soils had shoot concentration just below the level of deficiency. In the grains the phosphorus concentrations ranged from 3.39 to 5.15 g/kg and there was no effect of metal treatment. According to Reuter and Robinson (1986) this range of phosphorus concentrations corresponds to an adequate supply for wheat grains. The adequate supply of phosphorus for the grains in all metal treatments implies that the plants invested more phosphorus into the grains only to expose the shoots to deficiency levels. As phosphorus is very important in the grain development the size of grains is decreased when they are phosphorus deficient so the concentrations remain high enough to ensure the development of the grains.

So the biomass reduction in the grains of the wheat plants grown on the zinc treated plants could also be caused by this phosphorus deficiency. The shoots of the zinc treated plants were deficient in phosphorus resulting in possibly less nutrient uptake and energy accumulation. Therefore these plants could invest less into the grains than the non-zinc treated plants, which then resulted in less biomass and also smaller grains.

6. Conclusion

The hypotheses regarding zinc and cadmium uptake were confirmed by this experiment. The wheat plants grown on the spiked soils took up more cadmium and zinc respectively compared to those grown on the control soils. There were competition effects between cadmium and zinc whereas zinc was preferably taken up in the combination treatment: In the soils spiked with zinc less cadmium was taken up and accumulated in the shoots and grains. Such antagonistic effects have been shown for a variety of plant species including wheat. Possible causes of the interactions are (1) direct competition between the two elements for plant uptake through common transporter proteins, (2) the retention of cadmium in the root as a protection mechanism against cadmium toxicity and (3) the reflection of the plant available cadmium concentrations in the soil as less cadmium was bioavailable in the zinc treated soils. It is unclear what caused the immobilisation of cadmium in the zinc treated soils.

The high level of nitrogen fertilization enhanced the uptake of cadmium, zinc, iron and manganese by the wheat plants. For all these elements the effect of nitrogen was only significant for the metal contents per plant and not for the concentrations. The hypothesis was therefore only proven for the total contents per plant. The wheat plants fertilized high nitrogen achieved higher biomass compared to the low nitrogen plants resulting in growth dilution, for the concentration results. The increased uptake of metals with higher nitrogen fertilization was caused by the increase in biomass and the acidification of the soil pH. The addition of mineral nitrogen fertilizer reduces the pH in the soil solution which results in a higher hydrogen ion activity leading to increased competition for uptake sites on the negatively charged soil minerals and organic matter particles between the positively charged hydrogen ions and the positively charged metals. This competition results in more free metal in the soil solution that is available for plant uptake. The enhanced plant growth caused by the higher nitrogen pool for the plants through fertilization increased the plant requirements for nutrients such as zinc, manganese and iron resulting in higher uptake (also of unwanted metals such as cadmium). For zinc recent experiments have shown additional effects responsible for the increased uptake with higher nitrogen fertilization. These effects are (1) higher expression of transport proteins in the membrane of roots as nitrogen is a main

constituent of proteins, resulting in more uptake, (2) increased expression level of proteins contributing to xylem loading and the chelation of zinc in the xylem leading to a higher root to shoot translocation and (3) more remobilization of zinc from the vegetative tissue to the grain through the phloem. These effects might also be relevant for other metals, especially for cadmium as zinc and cadmium are partly taken up by the same transporter proteins.

The two agricultural management strategies in the soil showed only marginal effects in this experiment. Total nitrogen concentrations, nitrate concentrations and the organic matter content were higher in the organically managed soil compared to the conventionally managed soil and the soil pH was more acidic in the conventional soil. The plants grown on the organically managed soil had more biomass in grains, shoots and heads compared to the conventionally managed soil plants. This is most likely caused by the higher nitrate content for the organic soil prior to the experiment start. The hypotheses for the effects of the two soil management strategies could not be confirmed. The wheat grown on the organic soil did not take up more nitrogen and zinc compared to the wheat grown in the conventional soil, as the organic nitrogen was not used by the wheat plants.

The grain biomass was significantly reduced in the plants grown on the soils treated with zinc. Judging from the results of this experiment this is either caused by zinc toxicity or phosphorus deficiency. All plants showed deficient phosphorus concentrations in the shoots, however the phosphorus concentrations were much lower for the plants in soils treated with zinc compared to the plants in the non-zinc soils. The plants supplied sufficient amounts of phosphorus to the grains but were probably unable to invest as much energy and resources to the grains as the non-zinc treated plants because the shoots were exposed to substantial phosphorus deficiency.

In the literature zinc toxicity in wheat grains is stated for much lower concentrations than the average grain zinc concentrations of the zinc treated plants in this experiment. However as shown here and in other experiments with high zinc soil concentrations wheat plants can tolerate higher zinc grain concentration than the toxicity levels in the literature.

7. Outlook

Research on the uptake of cadmium and zinc in wheat plants has shown contradictory results. Either zinc addition hinders the uptake of cadmium or promotes it. It is yet not clear which is the relevant factor that leads to either more or less cadmium uptake with zinc fertilization. Considering that cadmium is toxic to plants and humans and that zinc fertilization is very important for countries with zinc deficient soils this is still a major task for research. This experiment showed that both zinc and cadmium are more likely to be taken up when nitrogen fertilization is increased and that the addition of zinc reduces the uptake of cadmium under the given experimental conditions. It is known that the interactions between zinc and cadmium depend on the relative and total concentrations of the two ions in the soil solution, the nutritional status of the plant and the plant species.

To further assess the interactions between cadmium and zinc this experiment could be repeated with less zinc spiked to the soil. It is very rare that agricultural soils are polluted with such a high amount of zinc as used in this experiment. It was first assumed that the soil would age faster and therefore maybe only a third of the amount added would be plant available. But at the start of the experiment the DTPA extractable zinc was about 200 mg/kg for the zinc-treated soils. It is suggested to treat the soil with an amount of about 150 mg/kg zinc in a follow on experiment, as this is the maximum recommended soil zinc concentration for agricultural soils in Switzerland. At this concentration it would be more likely to also see effects of cadmium on the zinc concentrations and generally a more realistic agricultural system would be provided for the experiment.

This experiment did not show any effect of the two soil management strategies on the uptake of cadmium and zinc or the nitrogen status of the wheat plants. The soils were probably too similar to show an effect in the experiment. In addition, both soils received the same fertilization during the experiment. So the organic soil had mineral fertilizers such as ammonium nitrate added, which it wouldn't receive under the organic management in the field. The fertilization was carried out in this way so both soils were treated the same and to have more comparable experiment conditions. In order to see a

better effect of the two management strategies both soils should be treated according to their management strategy. This would mean only organic fertilization such as manure and slurry for the organic soil.

Originally this project had a second part with the objective of assessing the role of mycorrhizal fungi in the uptake of cadmium and zinc by wheat. The results of this second experiment could be very valuable to further clarify the interactions of cadmium and zinc. Especially with regards to the two soil management strategies as it is assumed that the organic soil has a higher biological activity and therefore more mycorrhizal fungi than the conventional soil. Here it would also be very important to fertilize the two soil types according to their management strategy in the DOK field experiment.

8. References

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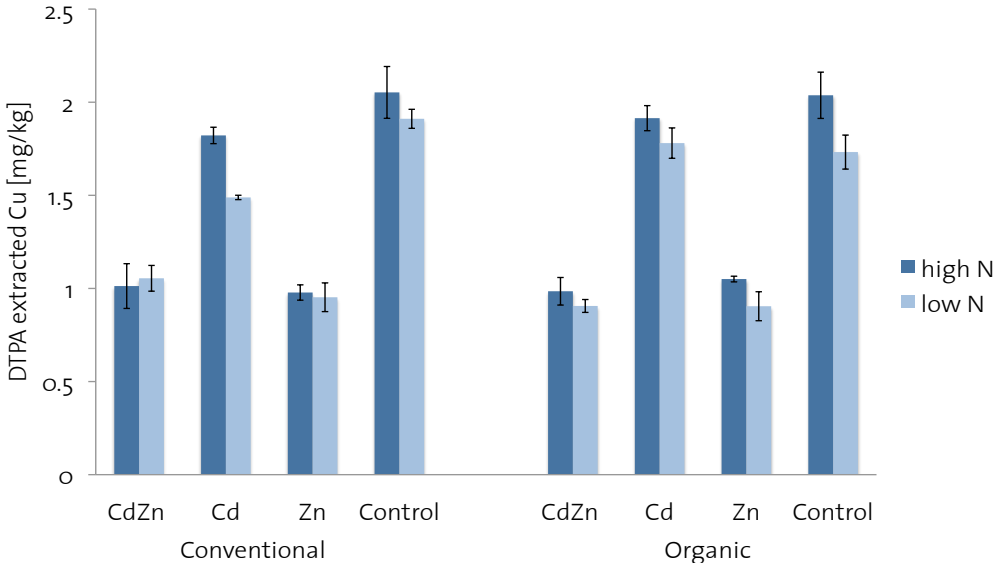
Appendix

Measured average DTPA extracted copper (Cu) concentrations in a) the initial soil and b) the soils after experiment completion.
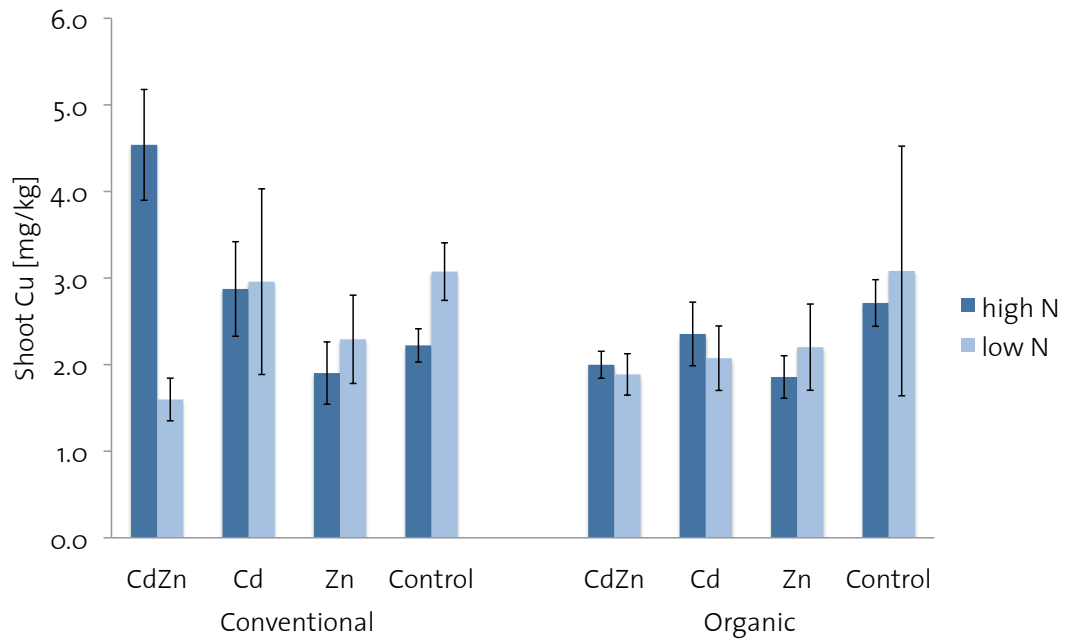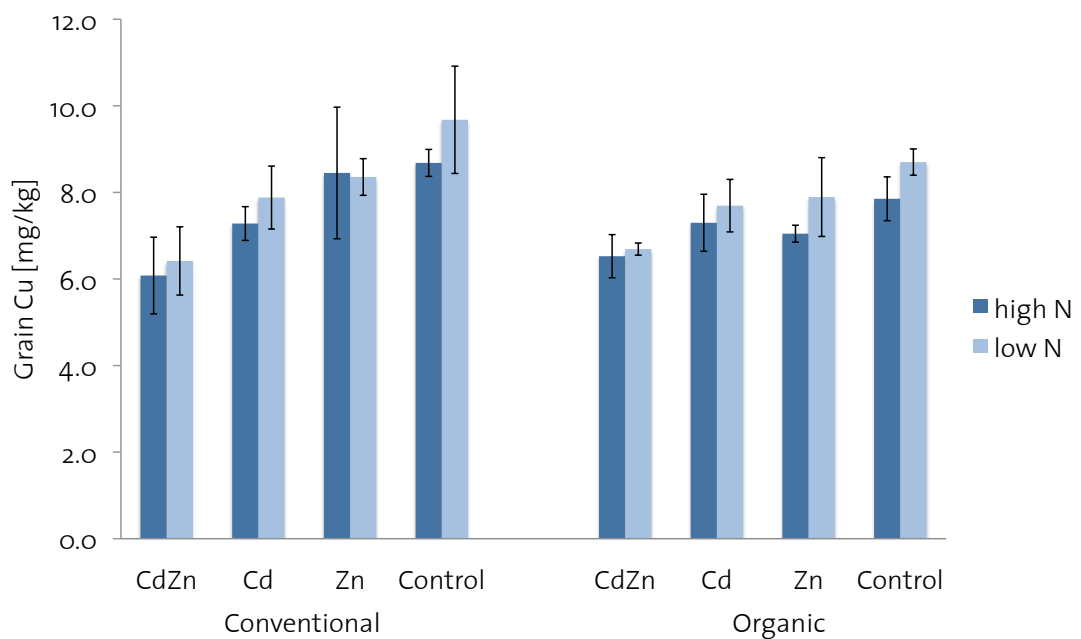
a)

b)
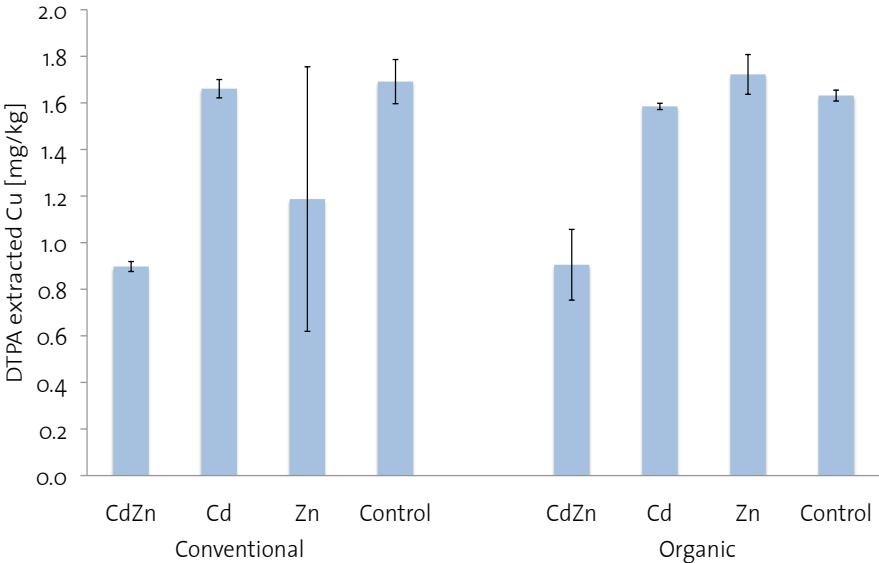
Measured average copper (Cu) concentrations in a) the plant shoots and b) the wheat grains after harvest.
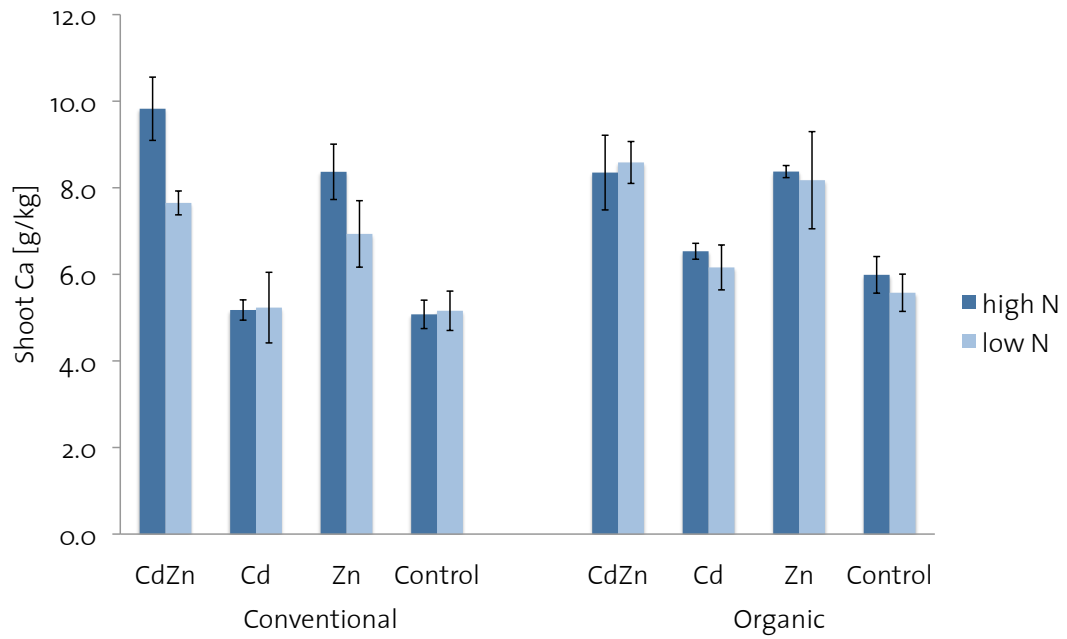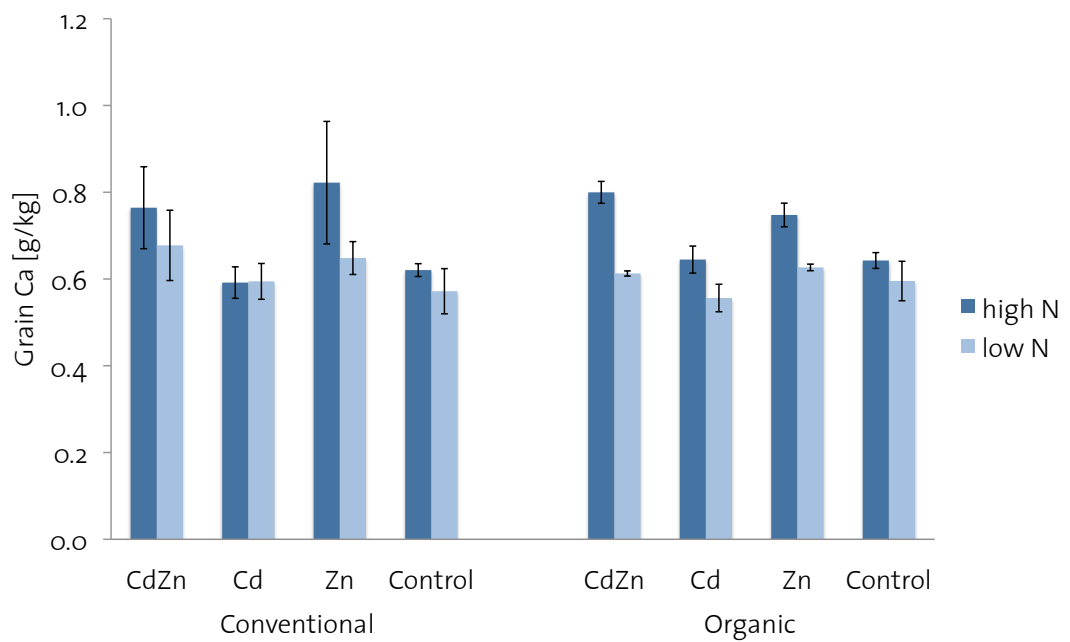
a)

b)

Measured average calcium (Ca) concentrations in a) the plant shoots and b) the wheat grains after harvest.
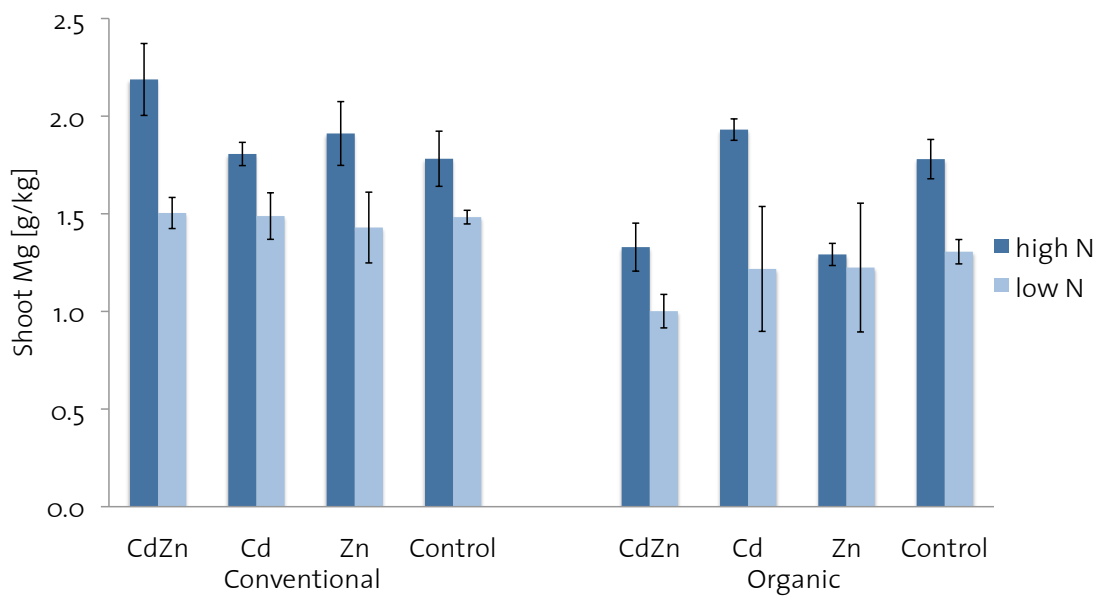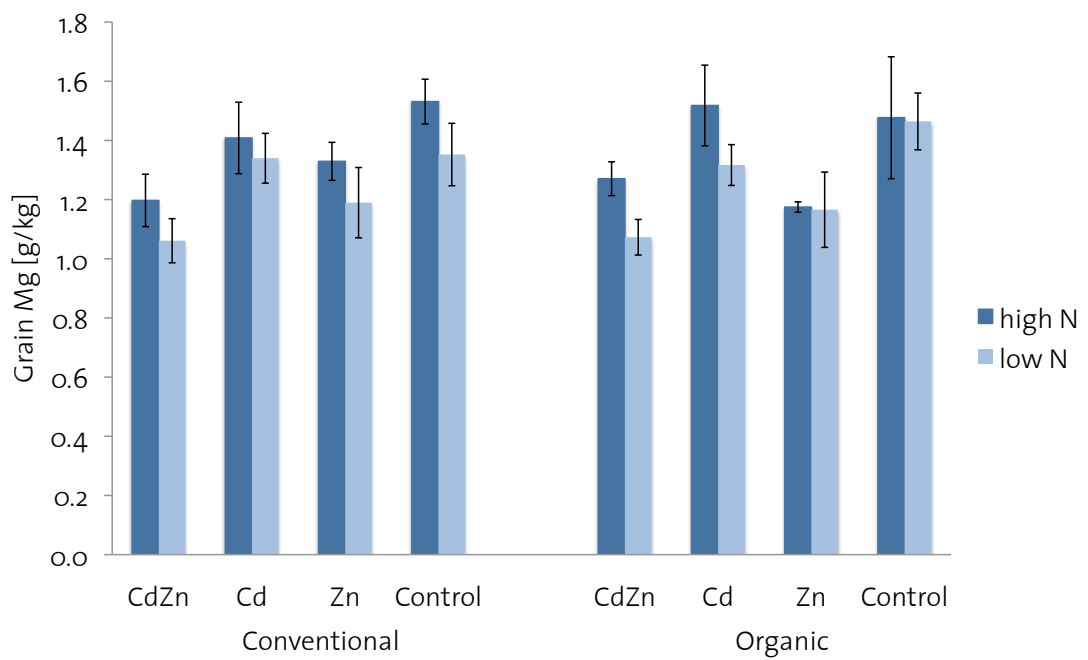
a)

b)

Measured average magnesium (Mg) concentrations in a) the plant shoots and b) the wheat grains after harvest.
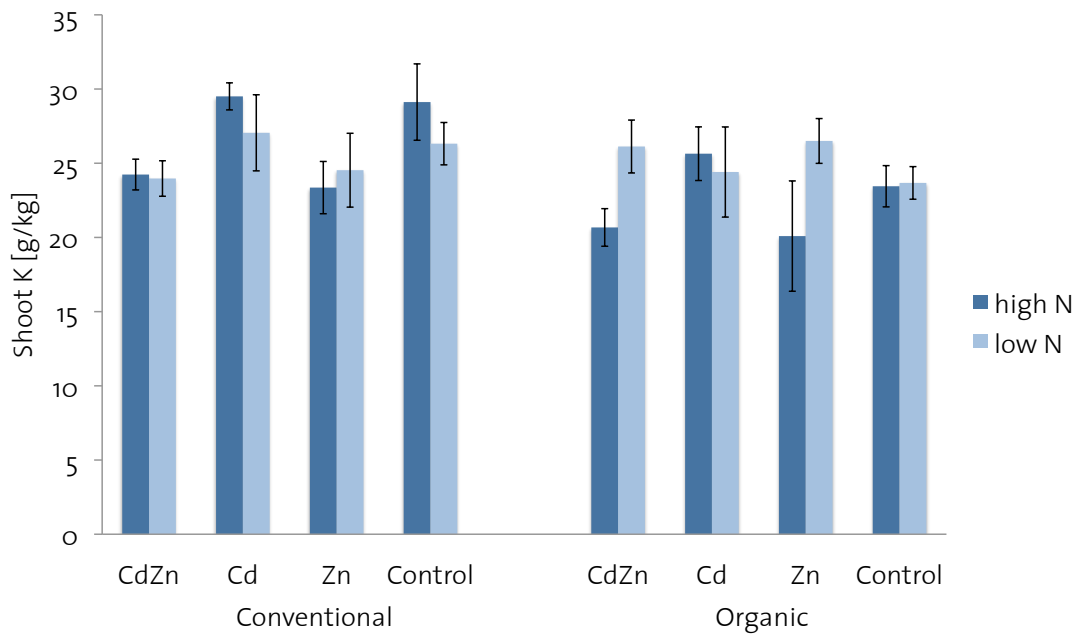
a)

b)

Measured average potassium (K) concentrations in a) the plant shoots and b) the wheat grains after harvest.

a)

b)