# Heavy metals from phosphate fertilizers in maize-based food-feed energy systems

**Dissertation to obtain the doctoral degree of Agricultural Sciences (Dr. sc. agr.)**

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submitted by

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## Table of content

## 1. General Introduction

Maize (*Zea mays* spp.) is one of the most important cereal crops worldwide, cultivated under several conditions and for numerous purposes, including food, feed, and biofuels. Maize was probably domesticated in Mexico around 9,000 years ago from its ancestor and close wild relative teosinte (*Zea perennis*). In developing countries, 20% to 30% of daily calorie intake in the human diet comes from maize grain consumption (Smith et al., 2017). In 2020, the worldwide production reached 1162 million metric tons, while in Europe, the maize production was 123 million metric tons, with 4.02 million metric tons produced in Germany. Meanwhile, in the East Asia, specifically in China, maize production reached 260 million metric tons in 2019 and 2020, corresponding to 23.42% of the global maize production. Thus, China become the second most important country with respect to maize production after the United States (FAO, 2022; Shahbandeh, 2020). Besides being a multipurpose crop, maize is also distributed worldwide due to its nutritional properties. Maize derivate products such as maize oil have a high nutritional value with high content of essential and unsaturated fatty acids and low amounts of saturated fatty acids, controlling cholesterol and blood pressure levels in humans. However, maize is also categorized as a starchy crop with deficiencies in essential amino acids such as lysine and tryptophan. Moreover, around 80% of the P content in maize is found as phytate, i.e. phytic acid, which is considered an anti-nutrient by reducing the bioavailability of essential minerals, such as Zn and Fe (Chaudhary, 2017; Zhang et al., 2017).

In European countries, maize is primarily ensiled for feed and biogas uses rather than for food purposes. By 2020, the worldwide production of silage maize reached 10.5 million metric tons, while in Europe, the silage production was 2.32 million metric tons. In China, the production of silage maize doubled in the last years from 78,021 metric tons in 2010 to 211, 61 metric tons in 2020 (FAO, 2022). However, local German databases indicate that the actual production of silage maize reached 97 million metric tons in Germany in 2020, indicating discrepancies between worldwide and local databases (Statistisches Bundesamt, 2022) .

As a result of anthropogenic activities and as one of the main cultivated crops around the globe, maize can be prone to heavy metal pollution, including cadmium (Cd). Cd is a heavy metal with an atomic weight of 112.411 g $mol^{-1}$, atomic number 48, and is found in nature as $Cd^{2+}$, an oxidation state that facilitates its soil bioavailability and plant uptake. Cd has no known essential function in humans, animals, and plants. In humans, high Cd levels can cause damage at the DNA level and trigger reactive oxidative species production in cells as far as inducing damage to several organs resulting in osteoporosis and cancer (Genchi et al., 2020; Zwolak, 2020). In plants, Cd can also produce slight to severe damage. Specifically, elevated soil Cd concentrations can cause decreased nutrient uptake, biomass, growth, and photosynthesis in maize plants and, therefore, lower harvested yields of this crop (Haider et al., 2021). Under extremely polluted conditions, Cd can induce chlorosis, inhibit growth, and even induce necrosis in maize. However, maize can grow in contaminated arable soils and accumulate hazardous Cd concentrations without visible damages, posing health and environmental risks. The maize tolerance to certain Cd levels without any toxicity symptoms might stem from its high biomass production, a consequent dilution effect, and a low translocation from roots to aboveground biomass (Rizwan, Ali, & Qayyum et al., 2017).

Agricultural soils are often polluted with Cd as the product of anthropogenic activities, including mining, smelting, and Cd polluted-P fertilizers in agriculture (Genchi et al., 2020). In this regard, high amounts of P fertilizers are often applied to arable soils due to the high nutrient demand for high yields in maize production.

As a macronutrient, phosphorus is one of the most critical elements involved in essential processes in microorganisms, plants, animals, and humans. However, 80% of the phosphate is lost from the mines to the consumers' tables, including farming and transportation stages. One of the reasons for these nutrient losses is that mixed farms were the primary farming type in the past, allowing P to return to soils in the form of manure. After the green revolution, significant losses in agriculture of P were and still are being produced due to the separated and intensive livestock farming and crop production (Childers et al., 2011).

In soil, P can be present in sufficient quantities for plant nutrition. However, its bioavailability is often limited by soil characteristics such as soil pH, clay content, temperature, water content, and leaching. Consequently, only 15 to 30% of the applied P fertilizer is taken up by the crops, while the rest is assumed to stay in the soil for future crop production or is leached to deeper horizons or water bodies, resulting in other environmental issues. Additionally, P is not efficiently recycled, thus preventing a closed cycle of this nutrient (Bindraban et al., 2020; Childers et al., 2011).

The raw and finite material for the production of mineral P fertilizers, i.e., rock phosphate, is mainly concentrated in Morocco's phosphorus mines. Germany and several countries of the European Union depend on the import of these P fertilizers. However, many of these imported mineral P fertilizers are frequently polluted with heavy metals, such as Cd and uranium (Kratz et al., 2016). In 2019, aware of this problem, and to prevent further soil Cd pollution and Cd bioaccumulation in the food chain, the European Parliament agreed on new limits for Cd concentrations in several fertilizers. The regulation (EU) 2019/1009 will come into force from July 15$^{th}$, 2022, and it significantly impacts mineral

P fertilizers, allowing a maximum concentration of 60 mg Cd $kg^{-1}$ $P_2O_5$ when the fertilizer contains equal or more than 5% $P_2O_5$ (Table 1.1). Furthermore, voluntary labeling for P fertilizer with a content equal to or lower than 20 mg Cd $kg^{-1}$ $P_2O_5$ might be used to ensure the application of low Cd-P fertilizers in arable soils (European Parliament, 2019b).

Table 1.1 Limit of Cd concentration in fertilizing and soil amending products (European Parliament, 2019b)

| **Fertilizers and soil amendments** | **Limit (mg Cd $kg^{-1}$ dry mass)** |
|---|---|
| Organic fertilizer | 1.5 |
| Organo-mineral fertilizers | 3 |
| | 60 mg $kg^{-1}$ $P_2O_5$ when fertilizer contains ≥ 5% $P_2O_5$ |
| Inorganic macronutrient fertilizers | 3 |
| | 60 mg $kg^{-1}$ $P_2O_5$ when fertilizer contains ≥ 5% $P_2O_5$ |
| Lime material | 2 |
| Organic soil improver | 2 |
| Inorganic soil improver | 1.5 |
| Growing medium | 1.5 |
| Plant biostimulant | 1.5 |

As foreseen in the limits given by the European Union, organic P fertilizers might also be polluted with heavy metals (see Table 1.1) (Roskosch & Heidecke, 2019; Xu et al., 2015). The application of animal manure can enhance Cd levels in arable land (Tian et al., 2016; Wajid et al., 2020), a major concern in China compared to the Cd input by mineral P fertilizers (Wu et al., 2012). Furthermore, industrial and mining activities (which in turn cause atmospheric Cd deposition) and irrigation with polluted water rather than Cd input by fertilizers might increase Cd levels in Chinese soil-crop systems (Zhuang et al., 2022).

The micronutrient zinc (Zn) is an essential heavy metal with atomic number 30, related and chemically similar to Cd. After iron (Fe), Zn is the most abundant metal in microorganisms, plants, animals, and humans, with important enzyme and metabolism activity (Broadley et al., 2007). In plants, Zn is involved in vital metabolic processes such as enzyme activity, protein synthesis, gene expression regulation, membrane lipid protection, photosynthesis, and stress tolerance (Rehman et al., 2012; Rehman et al., 2018). Zn bioavailability in the soil is limited, with more than 90% of total Zn unavailable for plants and microorganisms. The bioavailable Zn fraction is controlled mainly by soil pH, and it is present in soil solution as $Zn^{2+}$ or bond to soluble organic fractions (Broadley et al., 2007).

Due to their chemical similarities, the interactions between Cd and Zn are relevant for Cd availability in soils and plant uptake. Thus, two processes can happen when Cd and Zn interact in soils (Lambert et al., 2007):

1. A higher Zn concentration enhances the desorption of Cd in soil adsorption sites, increasing Cd bioavailability.
2. Zn competes directly with Cd for plant uptake.

Furthermore, Cd can enter the plant using Zn transporters when the soil is polluted with Cd or Zn deficient (Haider et al., 2021). Due to this last condition in soil, new crop genotypes capable of taking up high Zn levels have been developed in recent times; however, high Cd uptake by these cultivars has been observed in polluted-Cd soils, despite sufficient foliar Zn fertilization (Hussain et al., 2019). Zn concentration within the plant can also be negatively affected by the application of P fertilizers, diminishing the Zn quality of the harvested crop by dilution effect or direct interference on the Zn uptake process, a phenomenon also known as "P-induced Zn deficiency" (Alloway, 2009; Gao & Grant, 2012; Suganya et al., 2020).

Consequently, this dissertation hypothesizes that the accumulation, transportation, and translocation of Cd decrease in soil-maize systems by optimizing the P cycle as a result of crop and fertilizer management strategies. Thus, the principal aims of this dissertation were to identify the agricultural practices that enhance the Cd accumulation in maize crops, such as P fertilization rate and placement. A further objective was to compare the soil characteristics and the metal content (total and bioavailable fraction) to the actual Cd uptake by silage and grain maize under different P management.

The first publication provides a broad knowledge about Cd behavior in agricultural soils and crops, introducing the topic of the dissertation. More than 100 scientific publications dealing with soil Cd pollution derived from agricultural practices, Cd toxicity in humans and plants, long-term field experiments, and modeling of inputs and outputs of Cd in arable soils were reviewed in the first paper. Thus, the research questions were the following:

1) Which main soil and plant characteristics influence Cd accumulation in arable soils?
2) How do P fertilizers affect the Cd accumulation in arable land?
3) Which are the main inputs and outputs of Cd in soil-crop systems?
4) How does crop management influence Cd accumulation in soil and Cd uptake?

In food production, not only maize is widely cultivated and consumed. Wheat (*Triticum aestivum* L.) is equally among the most consumed grains around the globe, reaching a production of 760 million metric tons and 219 million harvested hectares in 2020 (FAO, 2022; Rizwan et al., 2016). In the same year, wheat production reached 134 million metric tons (one-fifth of the worldwide production) and 23 million harvested hectares in China (FAO, 2022). Regarding Cd pollution, wheat consumption is one of the most significant sources of Cd intake (Rizwan et al., 2016; Yang, Y. et al., 2020). Considering the importance of wheat in agriculture and human nutrition, Cd accumulation in arable soils, wheat grains, and along the food chain has been of great interest (Abedi & Mojiri, 2020; Rizwan et al., 2016).

The second paper collected and analyzed data from several field experiments studying Cd pollution for diverse sources in China to model Cd content in maize and wheat grain, employing soil characteristics as predictors. This publication focused on maize and wheat grain due to the importance of these staple crops in China and worldwide. Consequently, the research questions were the following:

1) Which are the mean Cd levels in maize and wheat grain in China?
2) How do the combined effects of the soil characteristics and soil Cd concentration affect the Cd accumulation in maize and wheat grain?
3) Are the present Chinese thresholds for Cd concentration in soil adequate for the actual Cd concentration in maize and wheat grain?

Several field experiments were established in 2019 within the scope of the International Research Training Group (IRTG) "AMAIZE-P". The central field experiment, which aims to last until the end of the IRTG program (9 years), is located in Hirrlingen, Baden-Württemberg, Germany (48° 24' N 8° 53' E). Several research subjects (RS) worked in close cooperation in this central field experiment, including RS 2.1 "Genotype to phenotype modeling of phosphate acquisition and related biomass and yield traits of maize", RS 2.2 "Increasing soil phosphate availability and phosphate fertilizer efficiency", RS 4.2 "Synthesis and field experiments" and the present RS 2.4 “Heavy metals from phosphate fertilizers in maize-based food-feed-energy systems”. The main objective of the central field experiment was/is to study P efficiency in soil-maize systems. In 2019, the field experiment had a random complete block design (RCBD), consisting of four main blocks, with 30 plots each (n=120). Several crops, including maize, wheat, and legumes, were cultivated according to the planned crop rotation, focusing on silage maize. Data from the central field experiment were used in the third and fourth publication included in the present dissertation.

In the third scientific publication, the behavior and the accumulation of Cd and Zn were analyzed under diverse fertilization P management in soil-maize systems, including different crop rotations, P placements, and P application rates. The variable of crop rotation was included as crop management to observe the effect of a previous crop on the accumulation of Cd and Zn in soil-maize systems. The crop rotation included silage maize following summer wheat and a legume crop. Furthermore, the P fertilizers applied to all crops contained a relatively high Cd level. Two different metal bioavailability methods and the bioconcentration factors were assessed and compared to the concentration of Cd and Zn in silage maize at an early development stage under field conditions. Thus, the research questions were the following:

1) Does P fertilizer containing relatively high Cd levels enhance Cd accumulation in soil and silage maize in the short term?
2) Does a previous crop influence the accumulation of labile Cd in soil and Cd uptake by silage maize?
3) Do the bioavailability methods mimic the actual Cd and Zn uptake by silage maize at an early development stage?
4) Does P fertilizer management influence Zn concentration on young silage maize?

The fourth publication tackles a recent problem stated in the second publication (Zhuang et al., 2021): most field experiments studying Cd accumulation in arable soils and crops are performed under polluted conditions, leading to a bias in the available data. This publication studied the effect of P fertilizer management and soil characteristics on the uptake of Cd and Zn by silage maize at two different growth stages under field conditions. The Cd levels in the soil and the applied P fertilizer were not manipulated, providing a realistic agronomic approach and fulfilling the knowledge gap for Cd and Zn behavior in unpolluted arable soils. Therefore, the research questions were the following:

1) Does P fertilizer rate application affect Cd and Zn concentrations in soil and silage maize in the short term?
2) Does P fertilizer placement affect Cd and Zn concentrations in soil and silage maize in the short term?
3) Does the exchangeable Cd fraction relate to the actual Cd uptake by silage maize at different growth stages?

## 2. Cadmium pollution from phosphate fertilizers in arable soils and crops: an overview [a]

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**Abstract:**

The application of mineral and organic phosphorus fertilizers to arable land has greatly increased crop yield to meet the world food demand. On the other hand, impurities in these fertilizers, such as heavy metals, are being added to agricultural soils, resulting both from the raw materials themselves and the processes used to obtain the final product. Cadmium, a non-essential and toxic heavy metal, has been found in relatively high amounts in common P fertilizers obtained from sediments. This metal poses a high risk for soil fertility, crop cultivation, and plants in general. Furthermore, human health might be compromised by the cadmium concentrations in agricultural and livestock products, due to the bioaccumulation effect in the food web. The accumulation in the different matrixes is the result of the high mobility and flexible availability of this harmful metal. This review summarizes risks to human health, the factors influencing cadmium movement in soils and crop uptake, as well as common plant responses to its toxicity. In addition, it summarizes cadmium balances in soils, trends, long-term experiments, and further studies. Cadmium inputs and outputs in arable soil, together with their calculated concentrations, are compared between two different regions: the European countries (in particular Germany) and China. The comparison appears useful because of the different proportions in the inputs and outputs of cadmium, and the diverse geographical, environmental and social factors. Moreover, these variables and their influences on cadmium contamination improve the understanding of the pollution from phosphate fertilizers and will help to establish future mitigation policies.

**Keywords:** soil pollution, arable land, crop production, cadmium balance, P fertilizer, cadmium toxicity.

## 2.1 Introduction

Phosphorus is a scarce essential element for plant, animal and human life. It forms part of the sugar phosphates in the structures of DNA and RNA, as well as component of other biomolecules including ATP and phospholipids (Childers et al., 2011). Thus, the element is involved in virtually all metabolic processes. The availability of this scarce macronutrient is the product of its cycle and its pools. The soil pools include P in solution (available for plants in organic or orthophosphate form), active (depending on soil pH bound as Ca-, Fe, Al-phosphate, metal hydroxides or as organic phosphate, but potentially mobilized by biogenic activity) and fixed (in deposits or sediments) (Gupta et al., 2014).

Due to its deficiency in many soils and the human need for higher crop yields, mineral phosphorus fertilizers are produced via the treatment of phosphate rock (PR) from sedimentary or igneous origin (Linderholm et al., 2012; Taylor et al., 2016). The final products of the process include phosphoric acid, superphosphate (SP) and triple superphosphate (TSP) (Taylor et al., 2016). Other common P fertilizers include monoammonium (MAP) and diammonium (DAP) phosphate, the result of nitrogen addition in ammonium form to phosphoric acid (Selim, 2015).

The production and consumption of P fertilizers have been rising and will rise further to meet the food demand of the increasing global population (Table 2.1) (Dikilitas et al., 2016). Phosphorus slowly accumulates in P mineral deposits, which are renewed over a time-scale of thousands to millions of years. The intense mining activity for agricultural purposes is rapidly decreasing these high-quality rock phosphate deposits, leading to a probable scarcity or depletion in the next 50–100 years, although other studies claim that the actual reserves will last for 400 years or more (Childers et al., 2011; Desmidt et al., 2015; Rosemarin & Ekane, 2016). According to the most recent survey by the Geological Survey in 2018 (Jasinski, 2019), the world P reserves will last around 260 years, taking into account the phosphate mine production (270 kt $yr^{-1}$) and reserves (70000 kt).

Beside potentially exhausting P stocks, another problem is the presence of toxic heavy metals in the fertilizer input added to otherwise uncontaminated arable soils (Bigalke et al., 2017; Dikilitas et al., 2016; Mar & Okazaki, 2012; Shi et al., 2018). These non-essential heavy metals, including cadmium, may disturb human, animal and plant life even at low concentrations (Dikilitas et al., 2016; Kabata-Pendias & Pendias, 2001; Kabata-Pendias & Szteke, 2015).

Table 2.1 Phosphate rock production and reserves (kt; data from Geological Survey (US Geological Survey, 1996, 2000, 2006, 2011, 2016, 1996, 2001, 2006, 2012, 2016)), and production and demand of P fertilizers (kt; data from International Fertilizer Industry Association (International Fertilizer Industry Association))

| **Year** | **Country** | **Phosphate Rock USGS 2019** | | | **IFAData 2019** | |
|---|---|---|---|---|---|---|
| | | **Production** | **Reserves**[a] | **$P_2O_5$ content** | **Production ($P_2O_5$)** | **Demand ($P_2O_5$)** |
| **2010** | All countries | 181 000 | 65 000 000 | 56 000 | 42 532 | 41 663 |
| | China | 68 000 | 3 700 000 | 20 400 | 15 998 | 13 092 |
| | Germany | - | - | - | 3 | 286 |
| | Morocco and Western Sahara | 26 600 | 50 000 000 | 8 800 | 1 875 | 191 |
| | United States | 25 800 | 1 400 000 | 7 400 | 6 297 | 3 890 |
| **2015** | All countries | 223 000 | 69 000 000 | 73 900 | 44 139 | 43 912 |
| | China | 120 000 | 3 700 000 | 36 000 | 17 224 | 12 111 |
| | Germany | - | - | - | 25 | 225 |
| | Morocco and Western Sahara | 30 000 | 50 000 000 | 9 100 | 2 169 | 221 |
| | United States | 27 600 | 1 100 000 | 7 710 | 5 257 | 4 302 |
| **2018** | All countries | 270 000 | 70 000 000 | - | - | - |
| | China | 140 000 | 3 200 000 | - | - | - |
| | Germany | - | - | - | - | - |
| | Morocco and Western Sahara | 33 000 | 50 000 000 | - | - | - |
| | United States | 27 000 | 1 000 000 | - | - | - |

[a] The estimated reserves correspond to the survey done the year after (2011, 2016 and 2019)

## 2.2 Cadmium in the environment and its health risks

### 2.2.1 Health risks by cadmium consumption

Cadmium is known as a toxic heavy metal with high mobility and hazardous effects for human life and the environment (Fig. 2.1) (Godt et al., 2006; Gray et al., 2003; Kabata-Pendias & Pendias, 2001; Kabata-Pendias & Szteke, 2015; Roberts, 2014).For human health, the tolerable weekly intake given by the World Health Organization is 7.00 µg $kg^{-1}$ body weight (Phillips & Prankel, 2011; Roberts, 2014; World Health Organization / Food and Agriculture Organization of the United Nations, 2010). However, an intake above 75.00 µg $d^{-1}$ Cd by an average adult person is considered a hazardous consumption, since cadmium has a half-life about 20 years in the human body (Kabata-Pendias & Szteke, 2015; Phillips & Prankel, 2011).

Cadmium can cause damage to DNA and disturbances to enzyme activities. As a consequence, cadmium can trigger failure or cancer in different organ systems, including the reproductive system, muscles, bones (by demineralization and Ca replacement), heart, lungs, liver, and kidneys. The kidneys accumulate most of the cadmium and it often binds to proteins, due to its affinity for sulfhydryl and phosphate groups (Godt et al., 2006; Kabata-Pendias & Szteke, 2015; Khan et al., 2017; Roberts, 2014; Yu et al., 2017).

### 2.2.2 Cadmium in soil

In general, cadmium concentrations in surface soils range from 0.06 to 1.10 mg $kg^{-1}$ with an average of 0.41 mg $kg^{-1}$ (Kabata-Pendias & Szteke, 2015). The cadmium concentrations in arable land in Germany are on average 0.31 mg $kg^{-1}$ (0.30 to 1.20 mg $kg^{-1}$) (Grant et al., 1998; Zhang et al., 2014), with concentrations varying with the soil type. Arable soils in China contain an average concentration of 0.27 mg Cd $kg^{-1}$, with higher amounts in soils near areas of mining and industrial activity, where the values can reach 150.00 mg $kg^{-1}$(Zhang et al., 2014).

The bioavailability of heavy metals (including Cd) via plant roots depends on various factors: abiotic factors include the metal concentration in soil and the physicochemical characteristics (pH, clay content, salinity, humidity, mineral, and organic matter); and biotic factors including the presence of metal-releasing microorganisms and the substances (enzymes, organic acids and hydrogen ions) released into the rhizosphere (Grant et al., 1998; Jiao et al., 2012; Kumar et al., 2016; Roberts, 2014).

Due to its high mobility, cadmium can be transferred from soil to plants including crops, thereby increasing the risk of bioaccumulation along the food chain (Khan et al., 2017). Cadmium solubility and bioavailability in soils is strongly dependent on pH (Grant et al., 1998; Gray et al., 1999b; Guan et al., 2018; Kabata-Pendias & Szteke, 2015; Lambert et al., 2007; Mclaughlin et al., 1996; Roberts, 2014; Sarwar et al., 2010). Lower mobility is observed when the pH is above 7.5, and a higher availability under lower pH conditions. The critical pH range is between 4.0 to 4.5 where a decrease of 0.2 pH units can cause up to five times higher mobilization and bioavailability (Kabata-Pendias & Pendias, 2001; Lambert et al., 2007). However, cadmium uptake by plants can be reduced or suppressed regardless of the suitability of the pH for mobilization by simultaneous competition with other metallic cations ($Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$) and hydrogen ions (Hatch et al., 1988).

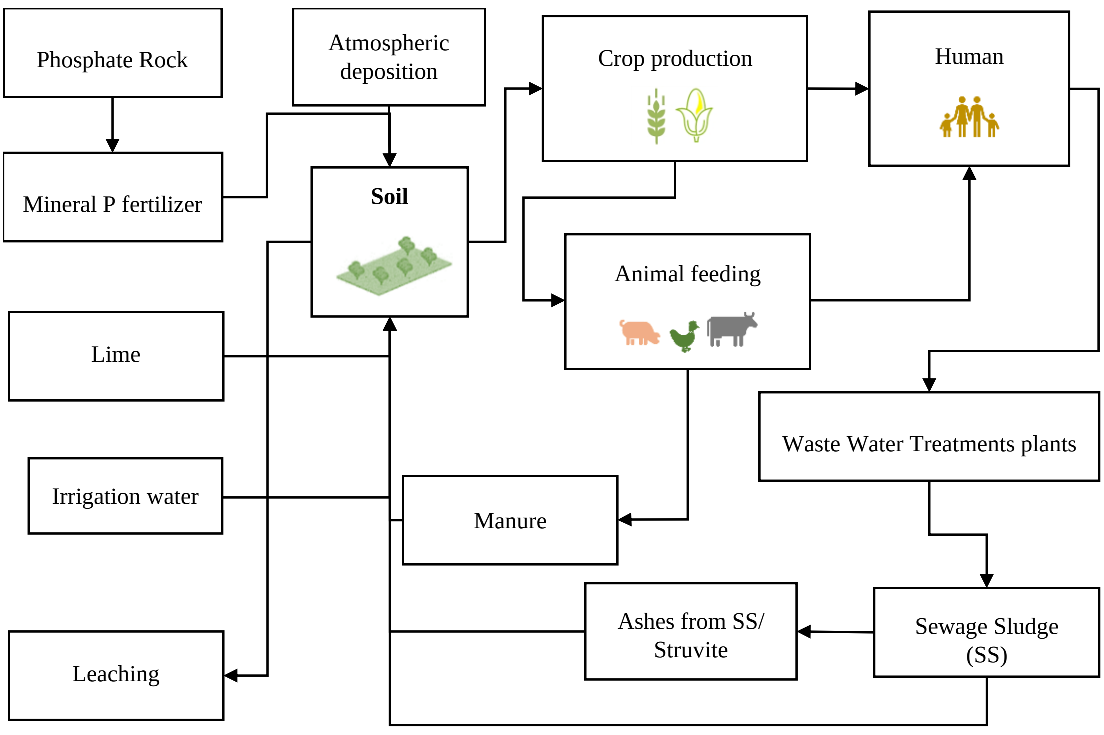


Fig. 2.1 General scheme of cadmium balance in an air-soil-crop system

Other physicochemical characteristics of soil, such as the high organic matter content in arable soils (for instance crop residues or input of farmyard manure) may form insoluble organic complexes with cadmium, diminishing its phytoavailability and increasing crop yield (Grant et al., 1998; Grant & Sheppard, 2008; Roberts, 2014; Wang et al., 2014).

In general, the processes of sorption-desorption, precipitation, and complexation reactions control the retention of metals in soils. The sorption-desorption equilibrium is the predominant process if heavy metals (such as Cd) are present at a low concentration. In contrast, when heavy metal concentrations are relatively high, or the pH is low, the precipitation-dissolution reactions are likely to regulate availability of heavy metal in the soil solutions (Mclaughlin et al., 1996; Selim, 2015).

In addition, cadmium behavior in acidic soils can be controlled by the amount of soluble organic matter. In alkaline soils, however, cadmium mobility is dominated by precipitation processes involving phosphates and carbonates (Kabata-Pendias & Szteke, 2015).

The biotic factors influencing cadmium bioavailability include organic acids in the rhizosphere, which can form complexes with cadmium, facilitating plant uptake (Kumar et al., 2016). Further biotic factors are the microorganisms. For example, mycorrhizal fungi can decrease cadmium phytoavailability by adsorbing cadmium in their hyphae and bacteria, can take up metallic cations and release them in a less mobile form (Kumar et al., 2016). However, according to Vig et al. (2003), many studies regarding plant-microbe-metal interactions are based on soils amended with sewage sludge (SS) or on polluted soils after bioremediation. In such cases, high concentrations of cadmium, as well as other heavy metals and organic pollutants, are employed. Furthermore, the role of microbes is focused on augmenting tolerance to heavy metals in plants or reducing cadmium uptake by plants (Vig et al., 2003). In another review related to soil microorganisms, Wyszkowska et al. (2012) point out that cadmium and other heavy metals can affect the microbial community, especially bacteria, by damaging cellular structure (protein or lipid bonding structures), denaturalization of proteins or affecting enzyme activity, and thereby, influence the microbial population and its interactions with plants.

### 2.2.3 Cadmium and fertilizers

The fertilizer type, the fertilization rate, the quantity per application, crop rotation, crop residues management and liming, along with the plant species and genotype, as well as changes in pH and plant growth , can all affect the cadmium concentration and availability in soils (Grant et al., 1998; Roberts, 2014). To illustrate this, chloride ions ( e.g. from KCl fertilizers) may form soluble Cd-Cl complexes, reducing cadmium sorption in soils and thus increasing the Cd mobilization and bioavailability (Grant & Sheppard, 2008; Mclaughlin et al., 1996).

The combination of high pH and high fertilization rates with nitrate compounds, such as $Ca(NO_3)_2$, can enhance the cadmium concentration in soil solution, since calcium in solution competes with $Cd^{2+}$ for adsorption by soil particles, thus increasing cadmium phytoavailability (Sarwar et al., 2010).

Mineral P fertilizers are considered the main input source of cadmium in arable soils in Europe (European Commission, 2016). The cadmium comes from the raw materials used to produce the fertilizers, i.e. PR, which is often sourced from materials of sedimentary (with higher Cd concentration), rather than igneous origin (with lower Cd concentration). Unfortunately, only 13% of the global P sources is found in igneous rock (Linderholm et al., 2012; Mclaughlin et al., 1996; Selim, 2015). Dependent on their origin, the cadmium concentrations in PR range from 0.10 to 60 mg $kg^{-1}$, with the highest values found in PR from North Africa (~60.00 mg $kg^{-1}$)(Aydin et al., 2010). However, other studies have found cadmium concentrations above 500.00 mg $kg^{-1}$ in PR from Morocco (Table 2.2) (Mar & Okazaki, 2012).

In Europe cadmium concentrations in P fertilizers generally range from trace amounts to 300.00 mg $kg^{-1}$, with an average of 7.40 mg $kg^{-1}$(Nziguheba & Smolders, 2008) , and 36.00 mg Cd per kg $P_2O_5$ considering the phosphate content (Smolders & Six, 2013). The current permitted limit for cadmium in fertilizers in Germany is 50 mg (kg $P_2O_5$)$^{-1}$ according to the German Fertilizer Ordinance (Kratz et al., 2016). Nevertheless, P fertilizers including PR, SP and TSP might exceed this value (Aydin et al., 2010; Kratz et al., 2016; Mar & Okazaki, 2012).

P fertilizers containing ammonium, such as MAP and DAP, can temporally acidify soils as a consequence of the natural process of nitrification, thereby, releasing hydrogen ions (Mclaughlin et al., 1996; Sarwar et al., 2010; Selim, 2015). However, the acidification of soils caused by nitrate is only relevant when this is lost by leaching (Bolan et al., 1991).

$$NH_2H_2PO_4 \rightarrow NH_4^+ + H_2PO_4^-$$

$$NH_4^+ \rightarrow \text{conversion of ammonia to nitrate} \rightarrow NO_3^- + 2H^+ \rightarrow \text{decreased pH and soil acidification}$$

Another effect of ammonium fertilizer may be rhizosphere acidification when ammonium is taken up directly by the plant root, which may compensate this cation uptake by proton release. If ammonia is taken up, the proton release by conversion of ammonium to ammonia also acidifies the rhizosphere. In the case of neutral or alkaline soil solution pH, both these variants mobilize phosphate and cadmium in the rhizosphere.

In contrast, P fertilizers, such as TSP and PR, can induce changes in pH and cadmium allocation into less available compartments after several applications (Kassir et al., 2012;

Lambert et al., 2007). These fertilizers can enhance the formation of insoluble cadmium-phosphate compounds, e.g., $Cd_3(PO_4)$, in soils, thus immobilizing cadmium and reducing plant uptake (Wang et al., 2014), due to the shift in soluble-exchangeable Cd distribution towards more stable bound phosphate forms (Lee et al., 2018; Sarwar et al., 2010; Selim, 2015).

Table 2.2 Cadmium concentrations (mg kg$^{-1}$) in inorganic and organic P fertilizers from different countries

| **Inorganic P fertilizers** | **China** | **Brazil (sold)** | **Germany (sold)** | **Morocco** | **Russia** | **South Africa** | **USA** |
|---|---|---|---|---|---|---|---|
| Phosphate Rock | 5.00[1] | 20.00[2] | 19.00[b][3] | 30.00-60.00[4] | 0.25[a][5] | 1.00[6] | 60.00-340.00[1] |
| | <2.00[6] | - | - | 12.00-38.00[1,6] | 1.00[6] | - | 6.00-92.00[6] |
| | 4.48[a][7] | - | - | 46.00-120.00[a][5] | 0.15[8] | - | 1.45-199.00[8] |
| | 2.60[b][9] | - | - | 507[8] | - | - | - |
| DAP | 5.10[5] | - | 28.10[a][10] | 29.50-68.00[a][5] | 2.10[a][5] | 2.20[a][5] | 18.20-185.40[5] |
| | 2.20[11] | - | 61.00[a][10] | 9.36[11] | 0.84[12] | - | - |
| MAP | 5.30[a][5] | 17.12[2] | - | 30.60-70.60[a][5] | 2.20[a][5] | 2.20[a][5] | 18.80-192.40[a][5] |
| | - | - | - | - | 0.14[11] | - | 50.92[11] |
| NPK | 0.60-1.51[11] | 5.80[2] | 15.80[b][13] | 0.80-11.45[11] | 3.23-3.66[11] | - | - |
| | - | - | 2.30[14] | - | - | - | - |
| PK | - | - | 55.60[13] | - | - | - | - |
| SP | 0.22[b][15] | 8.50[2] | 34.00[14] | - | - | - | - |
| TSP | - | - | 24.40[14] | 31.50-72.70[a][5] | 2.30[a][5] | 2.30[a][5] | 13.30-198.10[a][5] |
| | - | - | 62.00[3] | - | - | - | - |
| | - | - | 36.70-73.10[b][13] | - | - | - | - |
| | - | - | 28.10[b][10] | - | - | - | - |

[a] Given in mg (kg $P_2O_5$)$^{-1}$

[b] Given in mg (kg dry mass)$^{-1}$

[1] Mclaughlin et al. (1996), [2] Vieira da Silva et al. (2017), [3] Weissengruber et al. (2018), [4] Aydin et al. (2010), [5] European Commission (2016), [6] Roberts (2014), [7] Qingqing et al. (2016), [8] Mar and Okazaki (2012), [9] Jiao et al. (2012), [10] Schütze et al. (2003), [11] Lugon-Moulin et al. (2006), [12] Bošković-Rakočević et al. (2017), [13] Dittrich and Klose (2008), [14] Kratz et al. (2016), [15] Huang, Q. et al. (2018).

### 2.2.4 Cadmium in plants

Cadmium can have detrimental effects on enzyme activities in plants, leading to lower photosynthesis. As a result, plant growth and development including germination, root elongation, and leaf expansion can be affected by cadmium (Hameed et al., 2016; He et al., 2017; Kabata-Pendias & Szteke, 2015). Under higher concentrations in soils, cadmium can also produce phytotoxicity symptoms such as chlorosis, and reduced vigor and performance (for instance, water and nutrient uptake) (He et al., 2017; Kabata-Pendias & Pendias, 2001; Lux et al., 2011). Reduced growth and proliferation is also a consequence of the additional C skeletons required for defense and repair, which usually causes the size of the most tolerant plants to be very small (see e.g. hyperaccumulators such as *Arabidopsis helleri* (Zhao et al., 2006)). Therefore, in agricultural production cadmium can be responsible for damage to crop, decreasing yield and protein content in seeds (Hameed et al., 2016; He et al., 2017).

Plants have developed resistance mechanisms against heavy metal pollution, and some species are hyperaccumulators of certain heavy metals. Even in non-accumulating species, such as maize, responses can be detected. For example, when maize is grown under cadmium presence, phytochelatins (Cd-binding polypeptides) are released as a detoxification response to avoid cadmium binding to important enzymes or proteins (Grant et al., 1998; Keltjens & van Beusichem, 1998). Another reaction is the storage of cadmium-binding peptides in the vacuole, removing the cadmium from essential and sensitive metabolic activities (Gupta et al., 2014; Hameed et al., 2016).

Several environmental factors can activate an increased production of reactive oxygen species (ROS) in plants, including stress by heat, drought, air pollutants, organic chemicals or heavy metals. These compounds include hydroxyl radicals, hydrogen peroxide, singlet oxygen and, superoxide radicals, which are highly reactive products of an incomplete reduction of $O_2$ to $H_2O$ for energy production (Anjum et al., 2015; Dikilitas et al., 2016; Hameed et al., 2016; Sarwar et al., 2010). The oxidative stress triggered by high ROS concentrations impacts negatively on plant metabolism, including potential DNA damage, inhibition of enzyme activity, protein oxidation, lipid peroxidation and cell membrane damage (Hameed et al., 2016).

Thus, a further plant defense mechanism against elevated ROS production derived from heavy metal stress is the production of antioxidants. For example, some studies have detected an increased enzyme production with antioxidant activity in some maize cultivars under high cadmium concentrations, such as peroxidase, catalase, ascorbate peroxidase and superoxide dismutase (Anjum et al., 2015).

## 2.3 Cadmium balance in arable soil

The cadmium balance in surface soils as well as the bioaccumulation along the food chain involving crop production, are determined by the inputs and the outputs in arable land. Currently, the main cadmium input into European arable soils derives from mineral P fertilizers (Bigalke et al., 2017; Shi et al., 2018; Sterckeman et al., 2018). Nonetheless, other cadmium contributions from animal manure, SS, lime, and atmospheric deposition also need to be considered (Table 2.3) (European Commission, 2016; Mclaughlin et al., 1996; Six & Smolders, 2014).

For atmospheric deposition, cadmium can be present in the air as particulate matter, facilitating its mobility through the atmosphere and to other parts of the ecosphere (Kabata-Pendias & Szteke, 2015). However, this input has been reduced since 2002 in European countries through environmental policies (Six & Smolders, 2014), while in China atmospheric deposition still has a larger Cd contribution compared to P fertilizers (Table 2.3) (Selim, 2015; Shi et al., 2019).

Table 2.3 Atmospheric cadmium deposition (g $ha^{-1}$ $y^{-1}$) in different regions of China

| **Location in China** | **Cadmium Deposition** | **Reference** |
|---|---|---|
| Heilongjiang, Northeast | 1.46 | Deng et al. (2012) |
| Mongolian Plateau, Northwest | 1.04 | Wan et al. (2019) |
| Beijing, North China | 4.75 | Pan and Wang (2015) |
| Tianjin, North China | 5.30 | Pan and Wang (2015) |
| Hebei, North China | 5.57 | Pan and Wang (2015) |
| Henan, North China | 4.93 | Zhang et al. (2018) |
| Shanxi, North China | 2.04 | Zhang et al. (2018) |
| Fujian, Southeast | 0.91 | Zhang et al. (2018) |
| Lianyuan, Southeast | 17.00 | Liang et al. (2017) |
| Shenzhen, Southeast | 7.42 | Liang et al. (2017) |
| Guizhou, Southwest | 2.01 | Zhang et al. (2018) |
| Jiaozhou Bay, Central Yellow Sea | 1.30 | Xing et al. (2017) |
| Daya Bay, South China Sea | 1.60 | Wu et al. (2018) |
| East China Sea | 1.78 | Changling et al. (2005) |
| Southern Yellow Sea | 1.80 | Changling et al. (2005) |

Another important source of cadmium inputs to the soil are recycled P fertilizers. Due to the decline of PR reservoirs (Childers et al., 2011) and the increase of organic farming, the development and use of non-mineral fertilizers had intensified (Weissengruber et al., 2018). These fertilizers include animal manure, dewatered SS, chemical and thermally treated SS and anaerobically digested wastes (Weissengruber et al., 2018).

Manure and SS, which offer a large number of benefits to agricultural soils, are also important for the cadmium pathway through the P cycle in crop production, since cadmium is still found in these fertilizers, especially in regions of China (Table 2.4). Cadmium concentrations in manure usually differ according to the animal origin, with higher amounts in swine manure regardless of the region (Li et al., 2010; Schütze et al., 2003; Six & Smolders, 2014). As a consequence of its nature, the nutrient concentration in manure is lower and more variable compared to mineral P fertilizer, leading to a higher field application rate (Selim, 2015), and thereby, a higher cadmium input rates. For SS in

Germany, the cadmium concentration in dewatered and stabilized SS should not exceed 1.00 mg $kg^{-1}$ (Fijalkowski et al., 2017). Due to this reasonably low cadmium concentration in SS and the controlled application rate (Wiechmann et al., 2015), the estimated input through SS to arable land in Europe is relatively low (Six & Smolders, 2014).

Another cadmium input to soils is the addition of lime, given that it can contain cadmium as an impurity. Usually liming of soils increases pH and reduces cadmium availability in acidic soils, thus the uptake by plants is reduced (Grant et al., 1998; Guo et al., 2011; Rochayati et al., 2010; Sarwar et al., 2010; Tlustos et al., 2006). Several studies have found that 50% to 70% less cadmium accumulates in maize and some vegetables (amaranth, cabbage, and lettuce), most likely due to the supply of calcium from lime and its absorption competition with cadmium (Guo et al., 2011; Rochayati et al., 2010; Tan et al., 2011). However, this lime addition might not diminish cadmium uptake in alkaline soils, under deeper rooting or due to the antagonism with $Ca^{+}$ in the soil solution (Grant et al., 1998).

Table 2.4 Cadmium concentrations (mg kg$^{-1}$) in organic P fertilizers from China, European region and Germany

| Organic P fertilizers | China | Europe | Germany |
|---|---|---|---|
| **Manure** | 0.67a[1] | 0.20[2] | 0.30a[3] |
| **Swine Manure** | 1.30[4] | - | - |
| | 12.05[5] | 0.46[2] | 0.74[3] |
| | 0.64-21.02[6] | - | - |
| **Cattle Manure** | 0.92[4] | - | 0.43a[3] |
| | 5.61[7] | - | 0.80[8] |
| **Poultry Manure** | 1.48[4] | - | 0.25a[3] |
| | 15.38[5] | - | - |
| **Sewage Sludge** | 1.65[9] | 1.80[2] | 1.00a[8,9] |
| | - | 0.30-5.10[9] | 1.50-4.50[10] |

a Given in mg (kg dry mass)$^{-1}$

[1] Yang et al. (2017), [2] Six and Smolders (2014), [3] Schütze et al. (2003), [4] Wang et al. (2013), [5] Li et al. (2010), [6] Xu et al. (2015), [7] Wu et al. (2012), [8] Weissengruber et al. (2018), [9] Fijalkowski et al. (2017), [10] Wiechmann et al. (2015).

When calculating the cadmium outputs from the soil, crop uptake and leaching must be considered (Table 2.5) (Schütze et al., 2003; Shi et al., 2018; Sterckeman et al., 2018). While crop harvest contributes to the output, leaching most likely represents a significant output from agricultural soils (Six & Smolders, 2014). Several factors influence cadmium leaching, including the sorption-desorption processes, which, as specified in section 2.2, regulate the cadmium retention in soils. One of the most important sorption types is non-specific sorption. This occurs when cadmium is weakly bound to negatively charged surfaces by electrostatic attraction, and can be easily replaced by other ions (exchangeable) and predisposed to leaching and bioavailability (Loganathan et al., 2012; Mclaughlin et al., 1996). Another influencing factor is the pH, which could cause increased leaching when the value is lower, while higher pH values decrease this variable

(Houben et al., 2013). For instance, data obtained by He et al. (2005) indicate that the cadmium quantity adsorbed in soils was higher, the more acidic these became. This is due to the likely release of hydrogen ions from adsorption sites and their replacement with cadmium (He et al., 2005). Paradoxically, as mentioned before, a lower pH can increase the cadmium leaching to deeper layers. Furthermore, periods with elevated precipitation (water surplus or excess of precipitation) contribute to leaching to deeper layers since cadmium leaching is coupled to water leaching (Legind et al., 2012). Other factors such as soil density, total cadmium concentration in the soil, temperature, distribution coefficient ($K_D$), cation exchange capacity (CEC), which is influenced by organic matter and clay content, are known to influence cadmium leaching (He et al., 2005; Römkens & Salomons, 1998; Six & Smolders, 2014; Sterckeman et al., 2018). Thus, leaching rates are usually modeled or calculated (Shi et al., 2018). In 2014, Six and Smolders (2014) calculated a relatively high leaching rate for European soils, which represents the main output from arable soils (Table 2.5). This agrees with some Chinese studies, in which leaching has been found to be the main output mechanism rather than crop harvesting (Shi et al., 2018; Xia et al., 2014). However, another recent study has indicated that the high value for leaching for European soils could be an overestimation due to the equation used (Sterckeman et al., 2018) since lower rates of cadmium leaching were measured or calculated in other studies done in soils from Europe and New Zealand (Gray et al., 2003; Legind et al., 2012; Römkens & Salomons, 1998). Furthermore, soils in Europe are usually limed (Six & Smolders, 2014), which should be sufficient to buffer soil and avoid cadmium leaching.

Table 2.5 Cadmium inputs and outputs (g ha$^{-1}$ y$^{-1}$) in arable land from studies in Europe and China

| Type | Finland[1] | Austria[2] | Germany[3] | Northern Sweden[4] | Europe[5] | Central Europe[6] | France[7] | China[8] | Heilongjiang[9] | Hunan[10] | Yangtze River delta [11] | Hainan island[12] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Inputs** | | | | | | | | | | | | |
| **Atm. Dep.** | 0.3 | 2.10 | 1.70 | 0.34 | 0.35 | 0.35 | 0.20 | 4.04 | 0.36 | 6.85-40.25 | 2.66 | 0.91 |
| **P fertilizers** | 0.025 | 0.79 | 5.60 | - | 0.80 | 3.40 | 2.84 | 0.20 | 0.4 | 0.06-2.39 | 0.11 | 3.20 |
| **Manure** | 0.32 | 0.46 | 0.64 | 0.47 | 0.06-0.14 | 1.40 | 0.25 | 6.38 | | | | |
| **Sewage Sludge / Irrigation water[a]** | 0.023 | 0.04 | - | - | 0.05 | 0.30 | - | 1.80 | <0.1 | 0.002-8.19 | 5.65 | 0.11 |
| **Lime** | 0.035 | - | - | - | 0.02-0.09 | 0.15 | 0.02 | - | - | - | - | - |
| **Total input** | **0.71** | **3.39** | **7.94** | **0.81** | **1.43** | **5.60** | **3.31** | **12.42** | **0.76** | **6.91-50.83** | **8.42** | **4.22** |
| **Outputs** | | | | | | | | | | | | |
| **Crop offtake** | 0.14 | 0.13 | 0.68 | 0.17 | 0.20 | - | 0.99 | - | 0.2 | 15.66-61.45 | 0.61 | 0.41 |
| **Leaching** | 0.06 | 0.26 | 0.28 | 0.61 | 2.56 | - | 3.00, 0.56 and 0.28 | - | - | 0.033-0.412 | 1.11 | 0.64 |
| **Total output** | **0.20** | **0.39** | **0.96** | **0.78** | **2.76** | **0.50** | **3.99, 1.55 and 1.28** | **1.46** | **0.2** | **15.66-61.66** | **1.72** | **1.05** |

[a] Sewage sludge is banned for arable use in China. The input from irrigation water is considered instead of sewage sludge.

[1]Louekari (2000); [2]Zethner and Goodchild (2000); [3]Schütze et al. (2003); [4]Bengtsson et al. (2006); [5]Six and Smolders (2014); [6]Weissengruber et al. (2018); [7]Sterckeman et al. (2018); [8]Luo et al. (2009); [9]Xia et al. (2014); [10]Yi et al. (2018); [11]Hou et al. (2014); [12]Jiang et al. (2014)

## 2.4 Long-term studies

### 2.4.1 Field studies

One single application of fertilizer may not cause a significant accumulation of heavy metals in arable land. However, repeated fertilizer application over the long-term can result in harmful heavy metal concentrations for crops and for bioaccumulation potential in the food chain, thus representing a health risk (Gray et al., 1999a; Wu et al., 2012; Zhou et al., 2015). Nevertheless, contrasting studies reviewed by Jiao (2012) indicate that cadmium concentrations in soils might not be affected by P fertilization addition in the long-term.

Long-term studies by Gray et al. (1999a) in pastures of New Zealand indicated that after 44 years of P mineral fertilization, an increase in total soil cadmium had occurred. The P fertilizer used (single SP) had relatively high concentrations of cadmium, ranging between 34.00 and 69.00 mg $kg^{-1}$. However, after the long period of fertilization, a higher proportion of cadmium was in the residual soil fraction, which represents the residual and least mobile fraction from the sequential extraction method used, indicating that cadmium moves to less plant-available forms with time (Gray et al., 1999a).

Results from other long-term fertilization experiments (>22 years) suggest that animal manure could cause cadmium accumulation in soils as a consequence of the mixture of mineral and non-mineral fertilizers (Guan et al., 2018; Zhou et al., 2015). In one of these studies, cadmium concentrations were 10 to 25 times higher than prior to fertilization with manure. However, the manure decreased the uptake of cadmium by the maize crop (Zhou et al., 2015), while in other field experiments under swine manure and NPK fertilization, the plant-available cadmium fraction in soils decreased compared to the total concentration (Guan et al., 2018). This may be due to the formation of insoluble cadmium-complexes with organic compounds originating from the manure (Grant et al., 1998; Grant & Sheppard, 2008). In other words, the application of amendments, namely manure and SS, does not decrease (or increase) the total cadmium concentration, but their application can reduce its bioavailability for crops (Khan et al., 2017).

In another long-term experiment (17 years) by Wu et al.(2012), the application of pig manure together with mineral fertilizer (NPK) increased the total cadmium concentration in different soil types: 'black' soils, which have a higher quality humus and a moderate to high organic matter content (Delvaoux & Brahy; Food and Agriculture Organizations of the United Nations [FAO], 2019), and 'red' soils, which have high contents of Al- and Fe-oxides, and a lower cation exchange capacity (Delvaoux & Brahy). In this long-term experiment, the cadmium bioavailability (reducible and exchangeable fractions) was determined by the soil type: the 'red' soil had a lower cadmium bioavailability and a higher residual fraction, while the 'black' soil had a higher cadmium percentage in the exchangeable and reducible fraction. This is likely due to the mineral differences between both soils. Still, for mineral P fertilizer, application in the form of calcium superphosphate did not result in any difference in cadmium concentration compared to the unfertilized soils (Wu et al., 2012).

### 2.4.2 Models and trends

The problem of relatively high cadmium concentrations in fertilizers is not just a recent concern and several papers have approached this challenging situation in the past.

Cupit et al. (2002) studied the economic aspects, concluding that the lowest cost option for decreasing cadmium risks was to use low-cadmium phosphate rock, since taxing the fertilizers with high cadmium concentrations would impact largely on the farmer. Another suggested option was to the limit concentration gradually from 60.00 mg Cd per $kg^{-1}$ $P_2O_5$ by 2006 to 20.00 mg Cd per $kg^{-1}$ $P_2O_5$ by 2015 (Cupit et al., 2002; Roberts, 2014).

However, a lower threshold would affect important producers and exporters (European Commission, 2016; European Parliament, 2019a), for instance, producers from Morocco, where cadmium concentrations in PR are usually higher than 20.00 mg Cd per $kg^{-1}$ $P_2O_5$ (Aydin et al., 2010; European Commission, 2016; Mar & Okazaki, 2012), and in 2018 the flexible limit was still 60 mg Cd per $kg^{-1}$ $P_2O_5$ (Ulrich, 2019).

In a study by Smolders and Six (2013), European soils with different P fertilizers concentrations (with 0, 40, 60 and 80 mg Cd per $kg^{-1}$ $P_2O_5$) were modeled . It was predicted that soil cadmium will stay constant over the long-term (100 years), even if fertilizers with the highest concentrations of cadmium were to be applied. Additionally, under low or medium fertilizer application, the cadmium concentrations in the soil will decrease in most of the scenarios after 100 years of P fertilizers application. This prediction was based on lower P fertilization rates, leaching rates, the $K_D$ models used and the strong reduction in atmospheric deposition of cadmium in European countries, compared to other mass balances done previously.

Another mass balance model, for accumulation of cadmium and other hazardous substances after 200 years of different P fertilizer application, was developed by Weissengruber et al. (2018). The authors assumed two different pH values and different rainfall scenarios to test the influence of different fertilizers on hazardous substances accumulation. These fertilizers included mineral fertilizers (e.g., TSP), recycled P fertilizers allowed in organic farming (e.g., compost), and other emerging options (e.g., treated biosolid ashes). The results indicated that there is likely to be a decline in cadmium accumulation in soils even when high cadmium concentration fertilizers are applied, which agrees with the study of Six and Smolders (2014). However, there is a probability of cadmium output by leaching and crop harvests, which is higher under TSP, PR and compost application. Recycled fertilizer had a higher probability of cadmium output than struvite or biosolids ashes, as the result of the fertilizer application rate, which is dominated by the P concentration in the fertilizers. In other words, if the P concentration is lower, such as in green compost, the application frequency will increase to meet the crop P demand, as will the addition of hazardous substances as a consequence. Meanwhile, if the P concentration is relatively high, as in struvite or biosolid ashes, the P fertilization rate decreases and therefore the pollutant input to soils will also decrease, despite cadmium concentration of these fertilizers being higher (Selim, 2015; Shi et al., 2018; Weissengruber et al., 2018).

In contrast to these results, Qian et al. (2018) indicated that cadmium concentrations in Chinese soils will increase, reaching the acceptable threshold for agricultural soils in 50 years. This is the result of the cadmium background concentrations in soil, the atmospheric deposition and the continuous application of animal manure to fields, which are the main heavy metal inputs to arable soils in China (Shi et al., 2018; Shi et al., 2019), especially from swine manure, which has higher cadmium concentrations than the current approved limit (Cd < 3.00 mg $kg^{-1}$ DM) (Li et al., 2010).

Another mass balance model for actual P fertilizer application rates in soils in France was developed by Sterckeman et al. (2018). In their study, the authors indicated that under high leaching rates (using the equation from Six and Smolders (2014)), the cadmium concentration in the soil would decrease in the long term (100 years) from 0.31 to 0.29 mg $kg^{-1}$. Meanwhile, considering a medium and low leaching rate, cadmium would increase from 0.31 to 0.35 and 0.36 mg $kg^{-1}$ respectively. In addition, the increase of cadmium in soils under the actual P rates would lead to proportionally higher crop uptake, increasing the cadmium exposure of animals and humans through dietary intake (Sterckeman et al., 2018).

There are numerous model studies, however, and the results of these cadmium accumulation models can be imprecise, due to the many assumptions and generalizations that are made to simplify the complexity of reality, including social, agricultural, climatic and regional factors (Ulrich, 2019). Moreover, the lack of consistency in the leaching rate estimates indicates that its determination in different regions and environmental variables should be a priority to eliminate further uncertainties around this factor.

## 2.5 Conclusion and outlook

Cadmium input from phosphate fertilizers represents an environmental and health risk due to soil pollution, crop uptake, and bioaccumulation along the food chain. A decrease in mineral P fertilizer dependence, along with the use of non-polluted recycled fertilizers could alleviate the gap in the P cycle and the cadmium pollution of arable land, in countries such as Germany, where atmospheric deposition does not represent an important cadmium contribution to the soil. In countries like China, however, where atmospheric deposition and the manure application are the main cadmium inputs to agricultural land, environmental policies, and trace metals limits in animal waste could be used to decrease the pollution of arable land.

Future work should focus on cadmium balances in arable land, considering the soil properties (e.g., pH and CEC), crop and soil management (e.g., liming) and therefore the potential leaching, which seems to be an important but also inconsistent output regarding cadmium balance models. The social, climatic and economic differences and circumstances among countries should also be taken into account. Furthermore, there is a lack of knowledge about the potential accumulation of cadmium from P fertilizers via crops in the various elements of the food chain and in the P cycle. Hopefully, an improved, highly efficient P input and a more closed P cycling can mitigate the problem of cadmium pollution, due to a higher recycling and a lower dependency on mineral P fertilizer from sedimentary origin, but this remains to be investigated.

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## 2.7 Compliance with ethics guidelines

Andrea Giovanna Niño-Savala, Zhong Zhuang, Xin Ma, Andreas Fangmeier, Huafen Li, Aohan Tang, and Xuejun Liu declare that they have no conflicts of interest or financial conflicts to disclose.

This article is a review and does not contain any studies with human or animal subjects performed by any of the authors.

## 3. Cadmium accumulation in wheat and maize grains from China: Interaction of soil properties, novel enrichment models and soil thresholds [b]

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**Abstract:**

The cadmium (Cd) activity in soil has been widely studied. However, the interactive effects of soil properties (e.g. soil pH, CEC, and SOM) on Cd transfer from soil to grain are generally overlooked. In total 325 datasets including soil pH, CEC, SOM, and soil Cd content were used in this study. The descriptive statistics indicated that Cd content in wheat and maize soils ranged from 0.05 to 10.31 mg/kg and 0.02 —13.68 mg/kg, with mean values of 0.87 and 1.14 mg/kg, respectively. Cd contents in wheat and maize grains were 0.01—1.36 mg/kg and 0.001—1.08 mg/kg with average values of 0.15 and 0.10 mg/kg, respectively. The results of SEM demonstrated that the interactive effects of soil properties contributed more to Cd transfer from soil to wheat grain than the soil Cd content. Subsequently, CITs-MLR indicated that the critical factors, including soil pH and total soil Cd content, could mask the contribution of other soil properties on Cd accumulation in grain; soil CEC may prevent Cd from leaching and therefore improve grain Cd level of wheat especially at acidic soil condition. The result of derived Cd thresholds revealed that current Cd thresholds are not completely suitable to wheat and maize grain at different soil conditions. This study provides a new model for further investigation on relationships between soil properties, soil Cd content and grain Cd level.

**Keywords:** Conditional inference trees, Cd pollution, Structural equation model, Soil Cd threshold, Soil properties

## 3.1 Introduction

The rapid economic development in China in the last thirty years has also created concerns about heavy metal pollution in soils. Chinese soils, especially arable soils, are moderately to highly

polluted with Cd in recent years (Hou, Zheng, Tang, Ji, & Li, 2019; Hou, Zheng, Tang, Ji, Li, & Hua, 2019; Huang et al., 2019; Zhao et al., 2020). This heavy metal can enter the agricultural soils via atmospheric deposition, irrigation with polluted water, or application of contaminated fertilizer (Li et al., 2020; Luo et al., 2009). With non-essential function, Cd is a toxic heavy metal for plants, animals, and human beings (Hu et al., 2009; Kirkham, 2006; Rizwan, Ali, & Adrees et al., 2017). For human health, Cd is a concern due to its high mobility and potential for bioaccumulation in organs, being stored mostly in the kidney and liver. High dietary Cd levels can cause damage in the kidney, while low Cd levels in the diet can cause renal dysfunction (Andjelkovic et al., 2019).

One of the pathways by which Cd enters the human body is through the consumption of polluted staple crops. Soil is the first contact of edible plants with Cd in the food production, thereby, not only Cd content in soil but as well as the soil properties, e.g. pH, soil organic matter (SOM) or cation exchange capacity (CEC), are relevant for Cd content in crops and vegetables (Kim et al., 2020; Lu et al., 2017). Among these variables, pH is remarkable since it generally has an inverse and strong relationship with the Cd up- take: the lower the soil pH, the higher is the Cd content found in plants (Ge et al., 2000; Li, L. et al., 2014; Li, Y. et al., 2014). Another important factor to acknowledge is that not all Cd contained in soils is taken up by plants, rather, bioavailable fractions have a higher correlation with the real Cd content in crops. In addition, these metal fractions can be significantly influenced by the pH (Li et al., 2017; Zeng et al., 2011), and they can provide more valuable information about the Cd content taken up by plants, rather than the total Cd content in soils. Nevertheless, the pH along with the total Cd content can alleviate the lack of bioavailable fraction data, helping to predict the available Cd in soils and the Cd uptake (Khaliq et al., 2019; Zhang et al., 2011). These predictive models are relevant to assess the risk sur- rounding Cd content in edible plants. Some studies have used linear regression to correlate the total soil Cd and the Cd uptake by crops (Ran et al., 2016). As mentioned above, soil pH and total Cd content as the commonly accepted soil factors get more attention in most of the predictive models. Other soil characteristics such as SOM, CEC or clay content have also been used for more precise predicting Cd models for plant uptake (Jafarnejadi et al., 2011; Lu et al., 2017). Generally, the effect of individual soil properties on Cd uptake by crops has been clearly studied. While soil pool is a complex system, the interactions between soil properties and total Cd content on Cd transfer from soil to grain are often overlooked in current studies. Moreover, most of the predictive models of Cd content in grains in present studies are derived by using integrated data without any classification, which may not suit for certain cases (Jafarnejadi et al., 2011; Rafiq et al., 2014; Zhang et al., 2011). In addition, the influential variables such as soil pH or total Cd content in models may mask other variables' contribution, which leads to discrepancies between modeling and practice.

In this meta-analysis, we aim to synthesize and recapitulate the research done in China related to Cd content in two important crops: wheat and maize. Grain wheat is a worldwide staple crop, with a high potential of Cd accumulation (Rezapour et al., 2019; Wang et al., 2014; Wang et al., 2017). Since China is the most important producer and consumer of wheat (Rehman & Jingdong, 2017), the Cd content of the wheat grain is of interest. On the other hand, maize is one of the most relevant cereal crops around the world due to its versatile applications including food, feed, and bioenergy. Although maize crops are displaying an efficient root defense system against Cd uptake (Vatehová et al., 2016), it can still accumulate Cd in the grain to the degree of being hazardous for consumption (Lux et al., 2015). Consequently, recapitulating and modeling the Cd contents in wheat and maize grain using soil properties is imperative for a better understanding, monitoring, and management of these soil- crop systems. Therefore, the aims of the present study are: (1) to analyze and summarize the mean Cd contents in wheat and maize grain, (2) to explore the interactive effects of soil properties (including pH, SOM and CEC) and soil total Cd content on Cd transfer from soil to grain and obtain the Cd enrichment model, (3) to derive more precise

Cd threshold for wheat and maize cultivated soils, respectively. This study will provide new ideas for understanding the deeper and complex relationships between soil properties, total Cd content and Cd content in grain.

## 3.2 Materials and methods

### 3.2.1 Data collection

To investigate the relations between soil properties and Cd content in wheat and maize grains, meta-analysis was used as an effective approach. The data were obtained from literature published on web of science and China National Knowledge Infra- structure (CNKI) during the last two decades, by using the key words “heavy metal”, “cadmium”, “soil properties”, “agriculture soil”, “wheat” and “maize”. In order to filter the data, there are some criterions as follows: (I) field experiments conducted on wheat or maize soils of China; (II) the results of Cd content in soil and grain were given, and the soil properties (pH, SOM and CEC) were not mandatory but beneficial to have; (III) soil and grain samples have at least three repetitions; (IV) cultivars of wheat and maize were not distinguished; (V) soil and grain samples should be digested completely by strong acid (such as nitric acid or aqua regia); (VI) Cd content should be determined by inductively coupled plasma mass spectrometry (ICP-MS) or graphite furnace atomic absorption spectrometry (GFAAS). The establishment of database was described in Fig. 3.1.

### 3.2.2 Structural equation models (SEM)

SEM as a tool has been widely used to investigate the causation between the latent and manifest variables of ecological systems in recent years (Beaumelle et al., 2016; Grace et al., 2010; Wang et al., 2018). The latent variables cannot be measured directly, but can rather be reflected by several observable variables. In this study, we defined the “soil properties” as a latent variable, which can be reflected by soil pH, SOM and CEC. Cd content in soil as well as the wheat and maize grain were regarded as the manifested variables, which can be measured directly. In SEM, the casual relationships between soil properties, soil Cd content and grain Cd level were modeled based on the means of linear regression and tested by comparing the structure of covariances. Including the path analysis and the effects of soil properties on Cd contents in soil and wheat or maize grain could be demonstrated in the model.

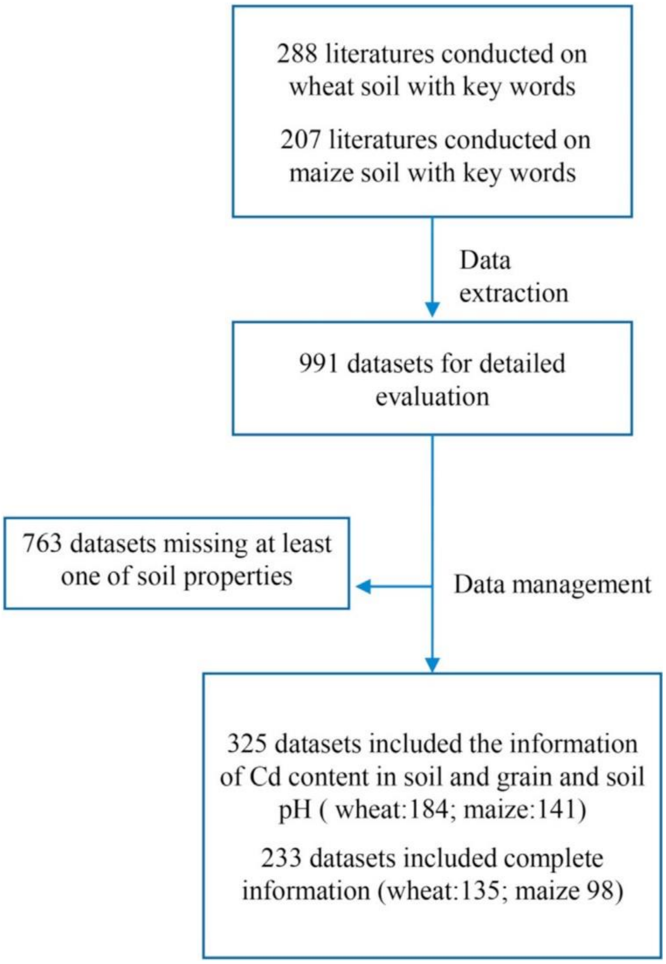



Fig. 3.1 Database compiling.

### 3.2.3 Conditional inference trees (CITs)

CITs is commonly used to classify the samples based on the binary recursive partitioning in a conditional inference framework (Zheng et al., 2020). The relations between Cd content in grain and soil properties were identified by two steps: first, by selecting the crucial soil environmental factors, which have an impact on Cd content in grains by significant testing, and secondly, by classifying the datasets into several degrees based on the significant differences in soil properties (including pH, SOM and CEC). The specific processes are as follows: (I) to calculate the P value between the Cd grain (output variable) and soil properties (pH, SOM and CEC; predictor variable) respectively; (II) try binary recursive partitioning between their dependent variable and the selected variable which has the minimum P value and then select the most significant segmentation; (III) classify the datasets into two categories, and repeat the above steps in the subset until the smallest node is implemented.

### 3.2.4 Quality control and data analysis

To ensure that the results were reliable, the Cd bio- accumulation factors (BCF) were calculated. Samples with extremely higher or lower BCF (e.g. outliers) were removed to minimize the effect of cultivar on Cd transfer from soil to wheat or maize grain. Data statistics and plots were completed in RStudio®. The structural equation models were performed in RStudio® by using the "lavaan" package. The conditional inference trees analysis was implemented by the "party" package of RStudio® version 3.6.2.

## 3.3 Results and discussion

### 3.3.1 Soil properties and cadmium contents in wheat or maize grain

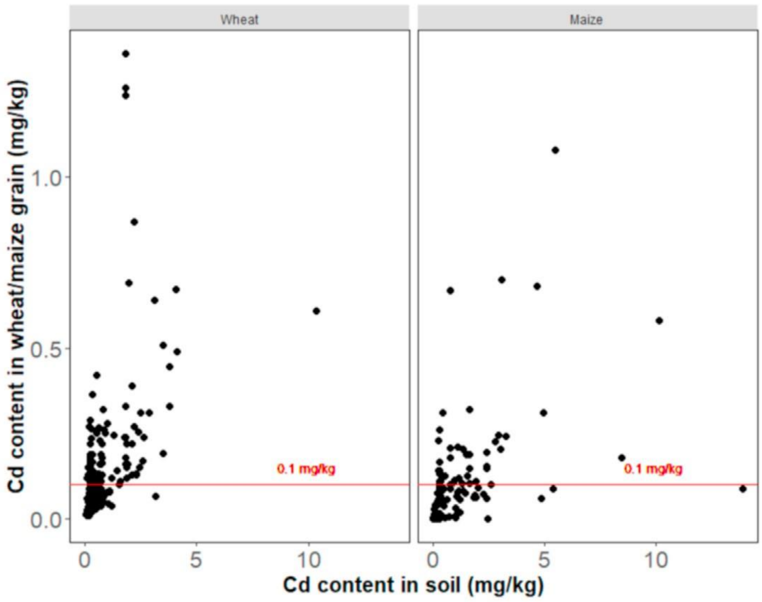
As described in Table 3.1, the results of soil properties where wheat and maize are grown derived from several publications, which covered the main areas of wheat and maize production (Li, L. et al., 2014; Li, Y. et al., 2014; Ran et al., 2016; Xue et al., 2019). Especially, the China North Plain area, which has a suitable climate for both wheat and maize production (Xiao et al., 2020) is the major component of the database. The soil pH ranges of wheat and maize fields are 5.02 — 8.66 and 4.42 to 8.92, with average values of 7.53 and 7.49 units, respectively. This indicates that most of the wheat and maize soils are neutral or slightly alkaline. The SOM and CEC of wheat and maize soils have wider ranges as compared to those from other studies which were conducted on regional scales (Huang, J. et al., 2018; Jinqiu et al., 2019; Ran et al., 2016), suggesting that the data in this study is more representative to do the analysis on national scale. The bioaccumulation factors (BCF) of wheat and maize were between 0.02 and 1.35 and 0.0001 and 0.87 with average values of 0.16 and 0.10, respectively. These results are consistent with other reports indicating that wheat has a higher potential for Cd accumulation in grains than maize (Wang et al., 2017; Xue et al., 2019).

Table 3.1 Descriptive statistics of soil properties and bio-accumulation (BCF) factors in soil- wheat/maize system.

| Crops | Variables | Min | Median | Max | Mean | SD |
|---|---|---|---|---|---|---|
| Wheat | pH | 5.03 | 7.79 | 8.66 | 7.53 | 0.82 |
| | SOM (g/kg) | 1.81 | 23.10 | 60.20 | 22.50 | 8.40 |
| | CEC (cmol/kg) | 5.75 | 15.70 | 32.31 | 15.74 | 6.31 |
| | BCF | 0.02 | 0.16 | 1.35 | 0.25 | 0.24 |
| Maize | pH | 4.42 | 7.87 | 8.92 | 7.49 | 1.06 |
| | SOM (g/kg) | 3.28 | 18.10 | 43.30 | 20.35 | 9.97 |
| | CEC (cmol/kg) | 3.47 | 15.85 | 32.31 | 14.96 | 4.36 |
| | BCF | 0.0001 | 0.10 | 0.87 | 0.16 | 0.17 |

The Cd content in wheat and maize soils ranged from 0.05 to10.31 mg/kg and 0.02 — 13.68 mg/kg with mean values of 0.87 and 1.14 mg/kg, respectively. According to the risk screening value of Chinese soil environmental qualities, which gives the Cd thresholds based on soil pH (Mee, 2018), the wheat and maize soils surpassing the Cd content thresholds accounted for 44.8% and 44.7%, respectively. Comparatively, both the average soil Cd content and overlimit ratio of contaminated samples in this study are significantly higher than those reported in other studies (Chen et al., 2015; Huang et al., 2019; MEP, 2014), which may due to the publication biases. Frequently, the aim of current studies was focused on food safety of the contaminated soil (Huang, J. et al., 2018; Huang, Q. et al., 2018; Lv & Liu, 2019; Qiu et al., 2020; Rezapour et al., 2019), and the significant results could have been obtained from heavy metal enriched soils. Thus, more and more studies conducted on contaminated soils are published and the available information about the uncontaminated area is decreasing, influencing the percentage of wheat and maize soils surpassing the Cd thresholds. The Cd contents in wheat and maize grains were 0.01 — 1.36 mg/kg and 0.001 — 1.08 mg/kg with average values of 0.15 and 0.10 mg/kg, respectively. Referring to the Chinese national food quality standard (NHFC & S.F.A.D.A., 2017), 45.3% and 29.8% of wheat and maize grain samples exceeded the limit value (0.1 mg/kg). It is worth noting that the high Cd content in grains is not significantly related to the Cd content in soil (Fig. 3.2a). Consistently, the Cd content in wheat or maize grain did not have a good linear relationship with corresponding Cd content in soil (Fig. 3.2a). Freundlich-type models have demonstrated that the log-transformed soil Cd contents have a good correlation with Cd uptake by plants, which has been widely used to predict the Cd content in grain (Liu et al., 2015; Yang et al., 2016). However, in this study it was found that there were non-linear relations between Cd content in grain and soil after log-transformation. In addition, most of the samples were outside of the 95% confidence intervals (Fig. 3.2b), which indicates that simply relying on the soil Cd content may not fit in the prediction of Cd content in wheat and maize grains. As previous studies revealed, the Cd uptake by crops is not only determined by the total soil Cd content but it is also affected by the different Cd fractions and soil properties such as pH, SOM and CEC (Li et al., 2017; Liu et al., 2018; Zheng et al., 2020). The critical factors, which affect the Cd content in grain should be therefore further analyzed.

(a)




(b)

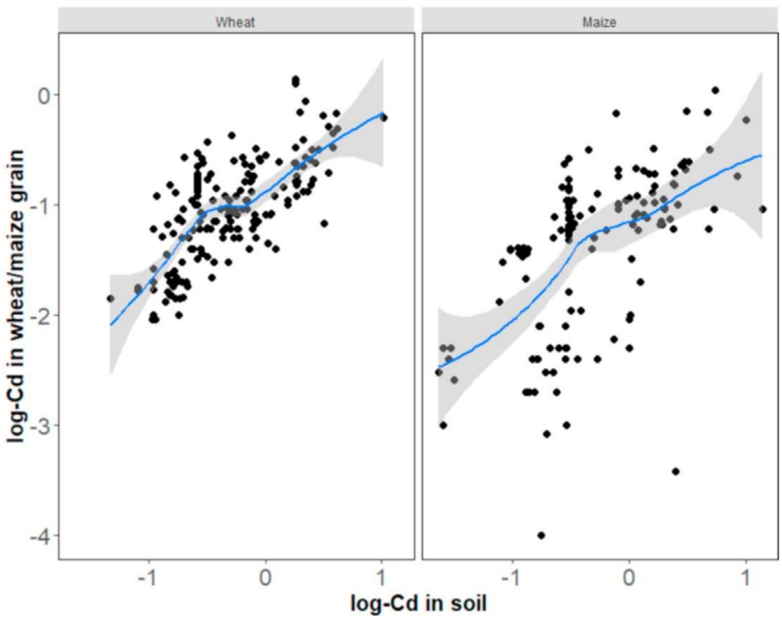



Fig. 3.2 (a) Cd content in soil-wheat and maize systems (red line is the threshold value of Cd content in wheat and maize grain based on Chinese food quality standard GB 2762 — 2017) (b) log-transformed Cd content in soil-wheat/maize systems (the blue line is nonlinear regression curve; the shadow is 95% confidence intervals). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

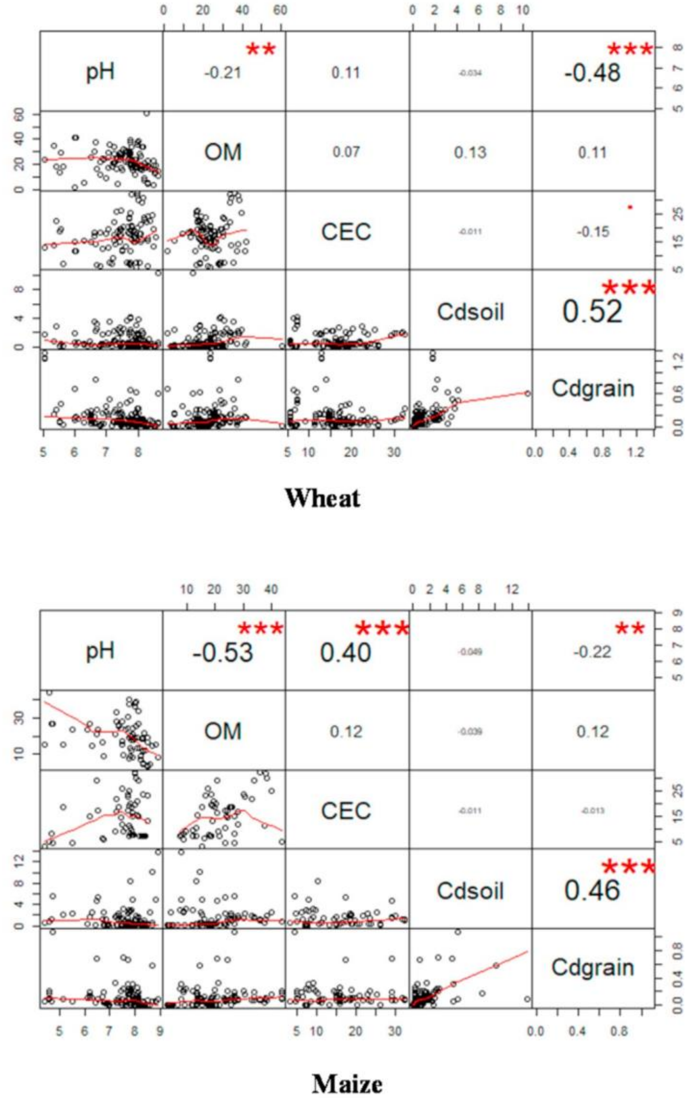
### 3.3.2 Critical factors of Cd transfer from soil to grain

Pearson correlations were performed to identify the critical factors in determining the Cd content in wheat and maize grains (Fig. 3.3a). The results showed that the soil pH (r = −0.48, $p < 0.01$) and soil Cd (r = 0.52, $p < 0.01$) were significant, with negative and positive correlation with Cd content in wheat grain, respectively. In addition, CEC was also negatively correlated with the Cd content in wheat grain, while the correlation coefficient (r = −0.15) was lower compared to soil pH and soil Cd. Similarly, the soil Cd content (r = 0.46, $p < 0.01$) was significantly and positively correlated with Cd content in maize grain. And the soil pH had a negative effect on the Cd content on maize grain Cd with the correlation coefficient −0.22 ($p < 0.05$).

Previous studies have demonstrated that soil pH, SOM, CEC and soil Cd content are considered to be important factors that affect the Cd transfer from soil to plants (Ding et al., 2013; Liu et al., 2018; Soriano-Disla et al., 2014). In this study, there is no significant correlation among SOM, CEC and Cd content of wheat and maize. This may be because of the strong influence of soil pH or soil Cd mask the contribution of SOM or CEC on Cd transfer in certain cases. Consistently, Li et al. (2003) reported that soil texture, mineral substances, and SOM are also likely to affect the bioavailability of Cd. However, due to the few available data for these parameters, there are limited tests of their significant relation to Cd uptake by plants. Cd transfer from soil to grain is a complicated process including the precipitation/dissolution and adsorption/desorption of Cd in soil, uptake by root, translocation by xylem, and other influencing factors (Li et al., 2017). Apart from the transport capacity of plants, soil pH is a relevant factor on Cd uptake by plants compared to SOM and CEC (Hough et al., 2003; Kirkham, 2006). Further, it has been observed that Cd is readily available to plants in acidic soils but is strongly immobilized under alkaline conditions (Hu et al., 2016). Also, a high correlation coefficient between soil Cd and grain Cd has been found, suggesting soil Cd is another important factor on Cd uptake by wheat and maize grain. Although the Cd uptake is commonly controlled by the bioavailable form of Cd rather than the total soil Cd, an increase in total soil Cd content may disproportionately increase the Cd taken up by crops, when all other factors are constant (Six & Smolders, 2014).

SEM models of Cd uptake by wheat and maize were used to identify the critical factors between integrated soil properties and soil Cd. The results indicated that SOM did not fit with the covariance matrix of SEM model, and therefore variable SOM was removed, while the latent variable of "soil properties" was reflected by soil pH and CEC. In wheat SEM models, the standard path coefficient of manifest variables pH and CEC were 0.61 and 0.18, respectively, suggesting that soil pH contributes more to the interactive effects of soil properties of wheat compared to CEC. This was consistent with the results of Pearson correlations, in which soil pH had a higher and more significant correlation with wheat grain Cd than that of CEC. The standard path coefficients of soil properties and soil Cd for wheat grain Cd were 0.42 and −0.84, respectively (Fig. 3.3b). This result could be explained by the fact that the integrated soil properties negatively correlated to Cd content in wheat grain; while soil Cd content had a positive effect on Cd uptake, and this effect was weaker than the interactive effects of soil properties.

(a)




(b)

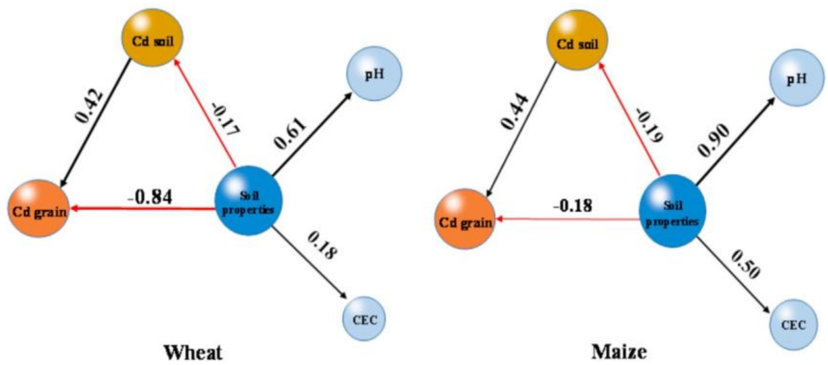



Fig. 3.3 (a) Pearson correlations between soil properties, soil Cd and grain Cd; (b) SEM models for describing the relations between soil properties, soil Cd and grain Cd. (standardized path coefficients shown on the edge of path representing partial regression coefficients).

As previous studies demonstrated, the bioavailable forms of metals in soil such as water-extractable, $CaCl_2$-extractable, EDTA-extractable, DPTA-extractable and DGT-metal are easily taken up by plants rather than the total metal content (Pérez & Anderson, 2009; Qu et al., 2020; Zhang et al., 2016); in addition the Cd bioavailability can be significantly controlled by soil properties including soil pH, SOM and CEC (Li, L. et al., 2014; Li, Y. et al., 2014; Rafiq et al., 2014; Yu et al., 2016; Zhao et al., 2014). This demonstrates that the interaction of soil properties may play more important role than soil total Cd on Cd uptake by wheat grain in Cd contaminated or accumulation soil. As for maize, the standard path coefficients of soil properties and soil Cd for maize grain Cd were −0.18 and 0.44, respectively, suggesting soil total Cd has a stronger influence on Cd uptake by maize in comparison to soil properties.

In this study, we used SEM to model the casual chain between soil properties, soil Cd content and grain Cd level. Different from Pearson correlations, SEM could connect soil pH and CEC to build a comprehensive model of soil properties, and compare their contributions on Cd transfer from soil to grain with total soil Cd content. The contrasting results of maize and wheat may be due to the different Cd uptake potential since maize exhibited a lower bio- accumulative potential compared to wheat (Chen et al., 2015). We therefore assume the response of Cd uptake to changes in soil properties for maize may be not as sensitive as for wheat. However, this assumption should be further confirmed by a target-oriented study.

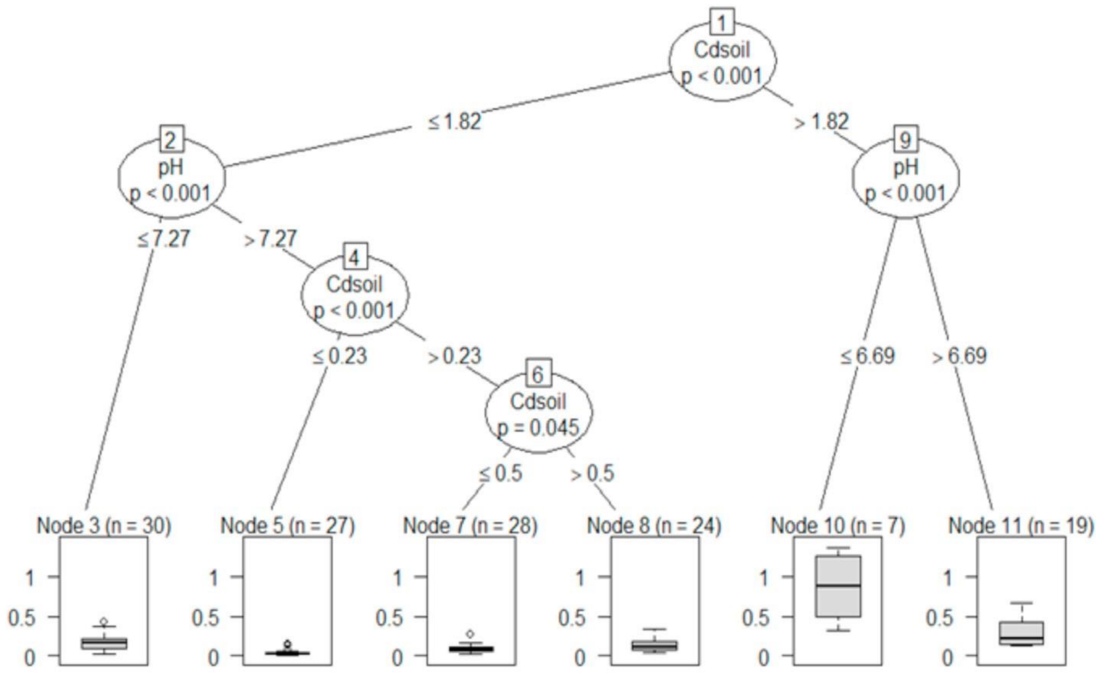
### 3.3.3 Conditional inference trees-based enrichment models

Based on the approach of CITs, Cd content in wheat and maize grain were characterized by soil properties and soil Cd (Fig. 3.4). Table 3.2 gives the average Cd contents in wheat and maize grains under different categories. For wheat, soil Cd content including 0.23, 0.50 and 1.82 mg/kg, and soil pH values including 7.27 and 6.69 were selected as the critical values by the significant testing of CITs models. The details are as follows: When soil Cd content was ≤ 1.82 mg/kg and soil pH was ≤ 7.27, respectively, the average Cd content in wheat grain was 0.17 mg/kg (node 3 in Fig. 3.4 wheat). When soil pH was > 7.27, the datasets were classified into three groups (Table 3.2, group 2, 3 and 4) with the average value 0.03, 0.09 and 0.13 mg/kg, respectively (node 5, 7 and 8 in Fig. 3.4 wheat). Extreme cases were when soil Cd was greater than 1.82 mg/kg and soil pH was less than or equal to 6.69. Then, the average Cd content in wheat could reach to 0.86 mg/kg (node 10 in Fig. 3.4 wheat). While, in high soil pH value soils (≥ 6.69) the average wheat Cd content was 0.29 mg/kg (node 11 in Fig. 3.4 wheat).

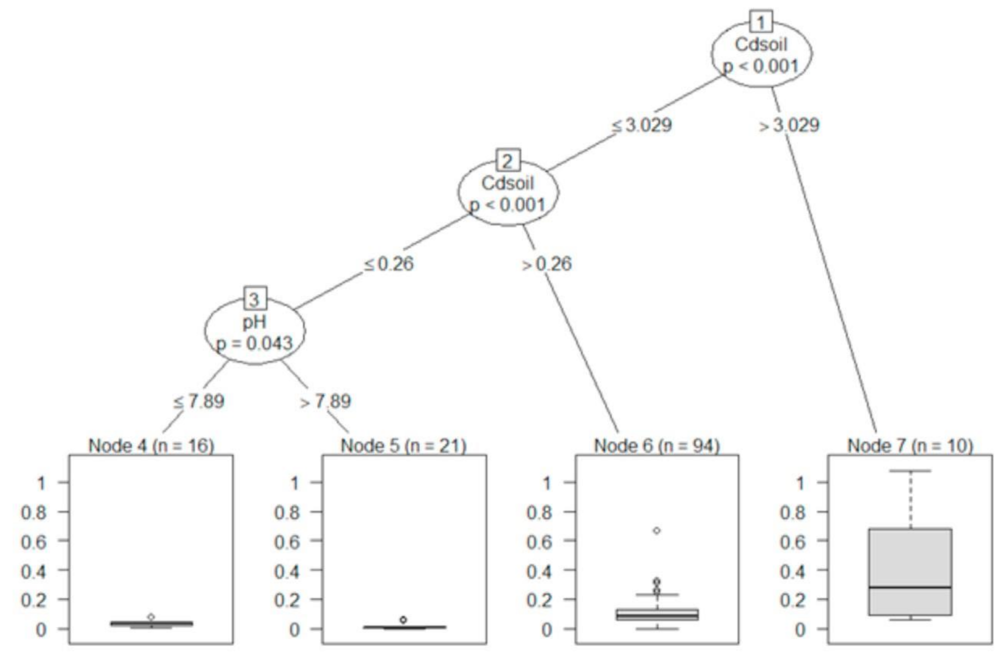
The results of CITs for maize were classified into four groups (Fig. 3.4 maize). When soil Cd content and pH were below 0.26 corresponding group 7 and 8 (Table 3.2), the average Cd contents in maize grain were 0.03 and 0.008 mg/kg, respectively (node 4 and 5 in Fig. 3.4 maize). When soil Cd contents were between 0.26 and 3.03 mg/kg, the average Cd content in maize grain was 0.10 mg/kg (node 6 in Fig. 3.4 maize). The extreme cases were when soil Cd content was greater than 3.03 mg/kg. Here, the average Cd content in maize grain could reach up to 0.40 mg/kg (node 7 in Fig. 3.4 maize).

The CITs results of both crops suggested that Cd content in grain is strongly correlated with the interactive effect of soil total Cd content and the soil pH. The Cd contents in wheat and maize grains commonly exhibited a high value under acidic conditions and high soil Cd content conditions. For example, the Cd content in wheat grain when soil pH was ≤ 6.69 and the soil Cd content was > 1.82 mg/kg is about 9 times higher than when the soil pH was > 7.27 and soil Cd content was ≤ 1.82 mg/kg. Similar results were also found for maize. Our results are consistent with other studies, which indicate that the interactive effect of lower pH and higher soil Cd content would lead to a higher release of free Cd cations from soil particles to soil pore water. Cd was therefore much more available to plants and Cd accumulation in grain was increased (Li, L. et al., 2014; Li, Y. et al., 2014; Zheng et al., 2020). Moreover, Cd content in wheat and maize also exhibited higher values in polluted soil as compared to that in uncontaminated soil, except for the effect of soil pH on Cd mobility. This could reveal that the Cd contaminated soils caused by mining activities, industrial emissions, and agricultural practices, normally have high contents of available metals. Consistent results have been found in other studies (Ge et al., 2000; Liu et al., 2013; Zhen et al., 2020). Thus, more awareness should be given to the impact of anthropogenic activities on soil contamination.

In several previous studies which used these multiple linear regression (MLR) models of metal uptake by plants, the models were fitted commonly by using the integrated data (Bešter et al., 2013; Chaudri et al., 2007; Liu et al., 2018; Yang et al., 2016). However, in fact, Cd content in plants normally exhibited significant differences under various soil conditions, which was also demonstrated by the results of CITs above. It is critical to build targeted MLR models for metal uptake by plants to fit different soil conditions. Therefore, a new method (CITs-MLR) was investigated to obtain the multiple linear regression models of wheat and maize in this study, respectively, based on CITs (Table 3.3). All the subset regressions are given in Fig. S1 to get the optimal regression equations. Due to the small data capacity, the groups of $Cd_{soil}$ > 1.82 mg/kg and pH ≤ 6.69 for wheat and $Cd_{soil}$ > 3.03 mg/kg for maize were not discussed. As shown in Table 3.3, Eq (1) fitted by using integrated data, which had a high regression coefficient value ($R^2_{adj}$ = 0.70) and significant parameters, statistically exhibiting a good performance for predicting the overall Cd content in wheat grain. However, the strong effects of soil Cd and pH may create an illusion that other factors such as soil CEC and SOM are unnecessary in predicting models (Li et al., 2003). Besides, the high values of variables and intercept indicated Eq. (1) may be not suitable for certain cases. Thus, the CITs-MLR models with their corresponding soil conditions were analyzed and showed in Table 2.3. According to the results of the subset regressions (Fig S2.1 b and d), soil CEC was added as a variable in model (Eq. 2 and 4) to improve the model suitability. As compared to Eq. (1), the lower coefficient of soil pH and higher coefficient of soil CEC in Eq. (2) revealed that the narrow range of soil pH, classified by CITs, would weaken its contribution on Cd transfer, which directly highlights the effects of soil CEC especially in acid soil. Further, positive and negative coefficient values of the independent variable CEC were found in Eq (2) and Eq (4), respectively. The result indicated that soil CEC has different effects on Cd uptake by wheat. Commonly, the soil CEC has a protective effect on Cd uptake by crops, due to the strong ability for immobilizing cations (XU et al., 2017; Yang, H. et al., 2020). However, soil CEC exhibited opposite effects in Eq. (2), which may be attributed to the interactive effects between soil properties, while the high soil CEC content may prevent Cd from leaching, and more cations could be retained in soil under acidic conditions. In addition, some metal cations such as $Zn^{2+}$, $Cu^{2+}$, $Fe^{3+}$ and $Mn^{4+}$ may compete with Cd for the binding sites of soil particles, increasing the $Cd^{2+}$ concentration in soil solution (Khaliq et al., 2019; Sanderson et al., 2019; Sebastian & Prasad, 2015). Subsequently, Eq. (5) showed higher explained variability ($R^2_{adj} = 0.66$) and significant level ($p < 0.001$) for Cd uptake by wheat, which was consistent with other studies, showing that MLR models are prone to Cd contaminated soil with varied pH ranges (Adams et al., 2004; Ran et al., 2016).




**Wheat**




**Maize**

Fig. 3.4 Conditional interference trees of wheat and maize (the values on the edge of the path are the corresponding criteria, the unit for soil Cd content is mg/kg; box plots represent the distribution of Cd content in wheat or maize grain under different categories).

Table 3.2 The classified average Cd contents in wheat and maize grains.

| Crops | Groups | Cd content (mg/kg) |
|---|---|---|
| **Wheat** | $Cd_{soil} \leq 1.82$; $pH \leq 7.27$ (1) | 0.17 |
| | $pH > 7.27$; $Cd_{soil} \leq 0.23$ (2) | 0.03 |
| | $pH > 7.27$; $0.23 < Cd_{soil} \leq 0.50$ (3) | 0.09 |
| | $pH > 7.27$; $0.50 < Cd_{soil} \leq 1.82$ (4) | 0.13 |
| | $Cd_{soil} > 1.82$; $pH \leq 6.69$ (5) | 0.86 |
| | $Cd_{soil} > 1.82$; $pH > 6.69$ (6) | 0.29 |
| **Maize** | $Cd_{soil} \leq 0.26$; $pH \leq 7.89$ (7) | 0.03 |
| | $Cd_{soil} \leq 0.26$; $pH > 7.89$ (8) | 0.008 |
| | $0.26 < Cd_{soil} \leq 3.029$ (9) | 0.10 |
| | $Cd_{soil} > 3.029$ (10) | 0.40 |

As for maize, the significant coefficient ($p < 0.001$) of soil Cd in the MLR model of Eq. (6) suggests that Cd content in maize grain was predominantly controlled by soil Cd content. And similar to Eq. (3), the weak $R^2_{adj}$ value in Eq. (7) confirmed that the extended Freundlich-type model was not appropriate for predicting Cd uptake by plants in low Cd content soil. Additionally, Eq. (8) with a high level of significance ($p < 0.001$) and comparative highest $R^2_{adj}$ value, exhibited the best performance for predicting Cd content in maize grain when soil content was between 0.26 and 3.03 mg/kg. Generally, CITs-MLR models reveal that soil factors, such as soil pH and CEC, exhibit diverse effects on Cd transfer at different soil conditions when the interaction of soil properties is considered. And the soil CEC is a potential factor on determining grain Cd level in wheat. In addition, the results also confirm that the response of Cd uptake by maize to changes in soil conditions is not as sensitive as wheat. In comparison with other studies that predict Cd content in wheat or maize grain (Hou, Zheng, Tang, Ji, & Li, 2019; Hou, Zheng, Tang, Ji, Li, & Hua, 2019; Novotná et al., 2015; Tudoreanu & Phillips, 2004; Viala et al., 2017), the CITs-MLR model would weaken the interference of variables with strong effects on modeling, and could be used for a more precise prediction of Cd content in plants.

Table 3.3 Multiple linear regression equations for Cd content in wheat and maize grains on the basis of CITs.

| Crops | Groups ($Cd_{soil}$ mg/kg) | Regression models log $Cd_{grain}$ = | | n[a] | $R_{adj}^2$ | $P$ |
|---|---|---|---|---|---|---|
| Wheat | Total | 0.71 log $Cd_{soil}$ – 0.25 pH – 0.003CEC + 1.18 | (1) | 135 | 0.70 | < 0.001 |
| | $Cd_{soil}$ ≤ 1.82; pH ≤ 7.27 | 0.70 log $Cd_{soil}$ – 0.11 pH + 0.03 CEC – 0.25 | (2) | 30 | 0.47 | < 0.001 |
| | pH > 7.27; $Cd_{soil}$ ≤ 0.23 | 0.87 log $Cd_{soil}$ – 0.49 pH + 3.08 | (3) | 27 | 0.35 | < 0.001 |
| | pH > 7.27; 0.23 < $Cd_{soil}$ ≤ 1.82 | 0.76 log $Cd_{soil}$ + 0.17 pH – 0.004 CEC – 2.16 | (4) | 41 | 0.51 | < 0.001 |
| | $Cd_{soil}$ > 1.82; pH > 6.69 | 1.61 log $Cd_{soil}$ – 0.20 pH + 0.29 | (5) | 19 | 0.66 | < 0.001 |
| Maize | Total | 0.68 log $Cd_{soil}$ – 0.13 pH – 0.19 | (6) | 141 | 0.36 | < 0.001 |
| | $Cd_{soil}$ ≤ 0.26 | 0.53 log $Cd_{soil}$ – 0.58 pH + 2.9 | (7) | 35 | 0.28 | 0.002 |
| | 0.26 < $Cd_{soil}$ ≤ 3.029 | 1.01 log $Cd_{soil}$ – 0.08 pH – 0.59 | (8) | 63 | 0.44 | < 0.001 |

[a] Outliers were removed to improve the $R^2_{adj}$ values of MLR models

### 3.3.4 Soil thresholds for wheat and maize

The log-transformed ratio of soil Cd content and current soil threshold combined with log-transformed ratio of grain Cd content and grain threshold was used to evaluate the validity of the current soil Cd threshold. As shown in Fig. 3.5, the four parts were respectively represented: (I) Cd content in both soil and grain surpassed their corresponding threshold, which means that the current soil Cd threshold is effective, and this part accounted for 28.5%; (II) soil Cd content below the threshold while grain Cd content exceeded its limit value indicating that the current soil threshold overlooked its associated risk for crops, which accounted for 11.4%; (III) accounted for 44.9% both Cd content of soil and grain were lower than their corresponding thresholds, indicated the current soil Cd threshold is a suitable indicator for health risk; (IV) accounted for 15.2%, soil Cd content exceed its threshold while Cd content in grain below its limit value, which indicted current soil overestimated the soil Cd risk for crops. Interestingly, according to the current soil Cd threshold, in some cases the contaminated soil can grow safe crops and vice versa, which accounted for 26.6% of total datasets (Fig. 3.5 II and IV), indicating that the current national soil quality standard may be not a good indicator for soil quality assessment in these cases.

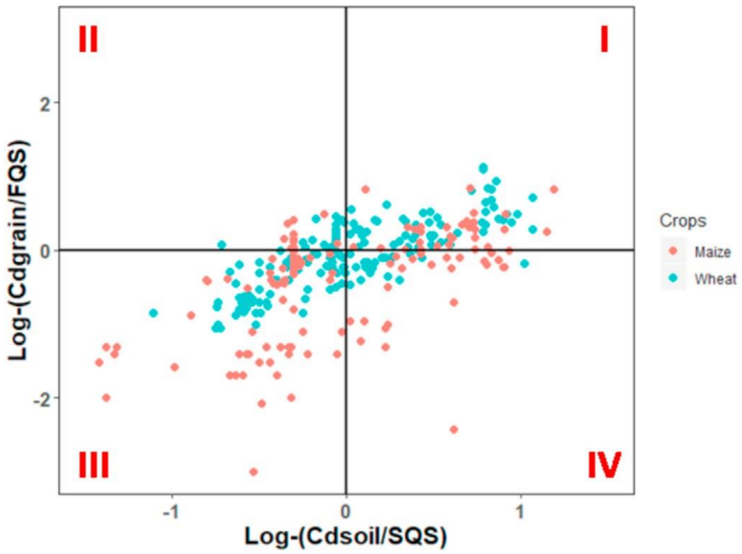


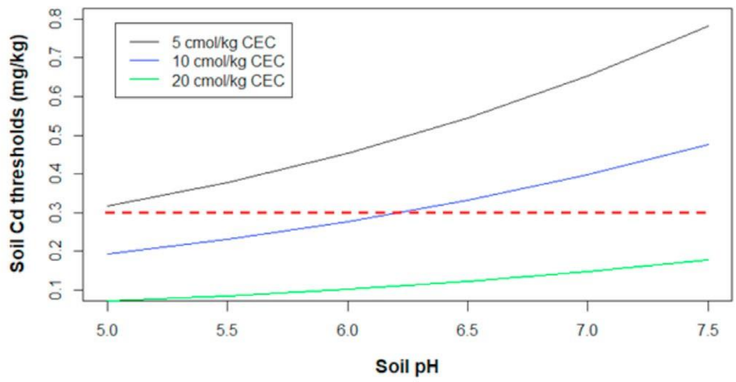

Fig. 3.5 Validity assessment of current soil Cd threshold (FQS: Food Quality Standard; SQS: Soil Quality Standard).

On basis of the Chinese national food quality standard (NHFC & S.F.A.D.A., 2017), 0.1 mg/kg is the maximum limit value for Cd in wheat and maize grains; soil Cd threshold were computed to compare the present soil quality standard (GB 15618 — 2018). In this study, the derived soil Cd thresholds were mainly focused on the group in which the average grain Cd content is around the threshold (0.1 mg/kg). For wheat, according to the results showed in Table 3.2, group (1), (3) and (4) were selected to compute the Cd thresholds. The three groups corresponding to two soil conditions, in which the MLR models of grain Cd levels were significantly different. Fig. 3.6 (Wheat) presents the derived Cd thresholds by using Eqs. (2) and (4), respectively, combining the maximum permissible Cd concentration in wheat grain (0.1 mg/kg). In addition, soil CEC (5, 10 and 20 cmol/kg represent the low, moderate, and high soil CEC contents) as the control variable was used to compute the Cd thresholds, respectively. Fig. 3.6 wheat (a) illustrates that when soil pHs are lower or equal to 7.27, the derived soil Cd threshold ranged from 0.32 to 0.72 mg/kg in low CEC soil (5 cmol/ kg). In moderate

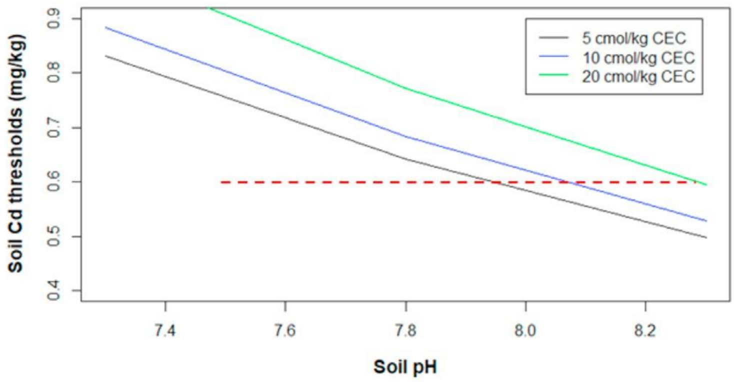
(10 cmol/kg) and high (20 cmol/kg) CEC soil, the derived soil Cd thresholds are 0.19 — 0.44 mg/kg and 0.07 — 0.16 mg/ kg, respectively. Meanwhile, the derived soil Cd threshold (mg/kg) are 0.45 — 0.75, 0.48 — 0.80 and 0.54 — 0.90 (Fig. 3.6, wheat b) in alkaline soil conditions (soil pH is > 7.27). In acidic soil conditions, the derived Cd threshold is directly proportional to the pH and inversely proportional to the CEC (Fig. 3.6, wheat a). As stated above in strong acidic soil, the higher CEC content in soil, the less metal cations were leached. And the higher Cd-competing metals may be released from soil particles to soil solution, which may delay Cd uptake by plants (Khaliq et al., 2019; Sarwar et al., 2010). However, in alkaline soil conditions, soil CEC may delay the migration of Cd due to its ability for binding cations (Ding et al., 2013), and the high pH condition may prevent the cations release from soil particles.

Subsequently, the derived soil Cd threshold for maize was computed from Eq. (8) showed in Table 3.3. Fig. 3.6 (Maize) presents that the derived Cd thresholds range from 0.89 to 1.85 mg/kg, when soil pH values are between 4.5 and 8.5. As compared to wheat, the derived soil Cd thresholds of maize are much higher. This could be explained by the weak impact of soil pH on Cd uptake by maize, and by total soil Cd content, which predominantly controls the grain Cd level. Moreover, the comparative low bioaccumulation factor of Cd for maize could decrease the Cd accumulation in maize grain, and therefore improves the Cd threshold.

**Wheat (a)**




**Wheat (b)**




**Maize**

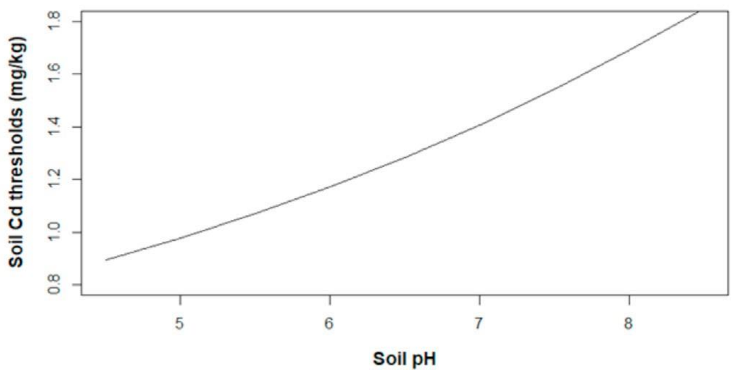



Fig. 3.6 Derived soil Cd thresholds for wheat and maize (red dash lines indicate the present Chinese soil quality standard GB 15618—2018; when pH ≤ 7.5, the threshold for Cd is 0.3 mg/kg; when pH > 7.5, the threshold for Cd is 0.6 mg/kg). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The current national Cd thresholds of China are only related to soil pH and thus may be not completely suitable to the diverse soil conditions. It was illustrated by Fig. 3.6 (Wheat a) that in acidic soil, the current Cd threshold may be conservative when soil CEC content is low (5 cmol/kg) and be overestimated when soil CEC content is high (20 cmol/kg); in moderate CEC (10 cmol/kg) soil, the current soil Cd threshold may be cautious when soil pH is lower than 6.2 and be overestimated for wheat when soil pH is greater than 6.2. However, in high soil pH conditions (≥ 7.3), the critical soil pH values are 7.9, 8.1 and 8.3 corresponding to low, moderate and high CEC content soil, respectively, and the current soil Cd threshold may be overestimated or conservative for wheat, when soil pHs are lower or greater than these critical values (Fig. 3.6 Wheat b). Meanwhile, the current Cd thresholds for maize may be excessively conservative comparing to the derived values (Fig. 3.6 Maize).

There are also some uncertainties of derived soil Cd thresholds. The minimal values present in strong acidic and high CEC soil condition as well as the positive effects of soil pH at alkaline soil conditions, should be further studied. Nevertheless, the results of derived Cd thresholds reveal that if only the effect of soil pH is considered, the current soil Cd threshold is not suitable for providing an accurate benchmark to Cd at different soil conditions. Therefore, more precise Cd thresholds for cultivated soils should be framed for different plants/crops in view of the interaction of soil properties (including soil pH, SOM and CEC).

## 3.4 Conclusion

The present study of soil properties and Cd contents in wheat and maize soils of China is significantly diverse. There is a considerable amount of Cd content in soil and grain samples above their corresponding Chinese standards, in part because of the publication bias, meaning more studies conducted on contaminated soil have been published. Soil pH and soil Cd content were the two critical factors on Cd transfer from soil to wheat or maize grain. It is worth noting that analysis of structural mathematic equation indicates that the interactive effects of soil properties (including soil pH and CEC) contributed more to Cd transfer from soil to wheat grain, compared to soil total Cd content. Meanwhile, the results of maize were statistically divergent, and it should be studied further. CITs-MLR models indicated the influential factors soil pH and soil total Cd content may mask the effects of other environmental variables on Cd transfer. The current national soil quality standard, which is only correlated to soil pH may be not completely suitable for reflecting the real soil contamination status. The interaction of soil properties as well as the type of plants should be taken into consideration to obtain more precise soil Cd thresholds.

### 3.5 Author statement

Zhong Zhuang: Writing — original draft, Visualization, Investigation, Methodology. Andrea Giovanna Niño-Savala: Writing — review & editing. Zi-dong Mi: Data curation. Ya-nan Wan: Writing — review & editing. De-chun Su: Formal analysis. Hua-fen Li: Writing — review & editing, Funding acquisition, Conceptualization, Supervision. Andreas Fangmeier: Writing — review & editing.

### 3.6 Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### 3.7 Acknowledgement

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## 3.8 Appendix A. Supplementary data

## Supplementary

Table S1. The classified average Cd contents in wheat and maize grains

| Crops | Groups | Cd content in grain (mg/kg) |
| --- | --- | --- |
| Wheat | $Cd_{soil} \leq 1.82$; $pH \leq 7.27$ (1) | 0.17 |
| | $pH > 7.27$; $Cd_{soil} \leq 0.23$ (2) | 0.03 |
| | $pH > 7.27$; $0.23 < Cd_{soil} \leq 0.50$ (3) | 0.09 |
| | $pH > 7.27$; $0.50 < Cd_{soil} \leq 1.82$ (4) | 0.13 |
| | $Cd_{soil} > 1.82$; $pH \leq 6.69$ (5) | 0.86 |
| | $Cd_{soil} > 1.82$; $pH > 6.69$ (6) | 0.29 |
| Maize | $Cd_{soil} \leq 0.26$; $pH \leq 7.89$ (7) | 0.03 |
| | $Cd_{soil} \leq 0.26$; $pH > 7.89$ (8) | 0.008 |
| | $0.26 < Cd_{soil} \leq 3.029$ (9) | 0.10 |
| | $Cd_{soil} > 3.029$ ()10 | 0.40 |

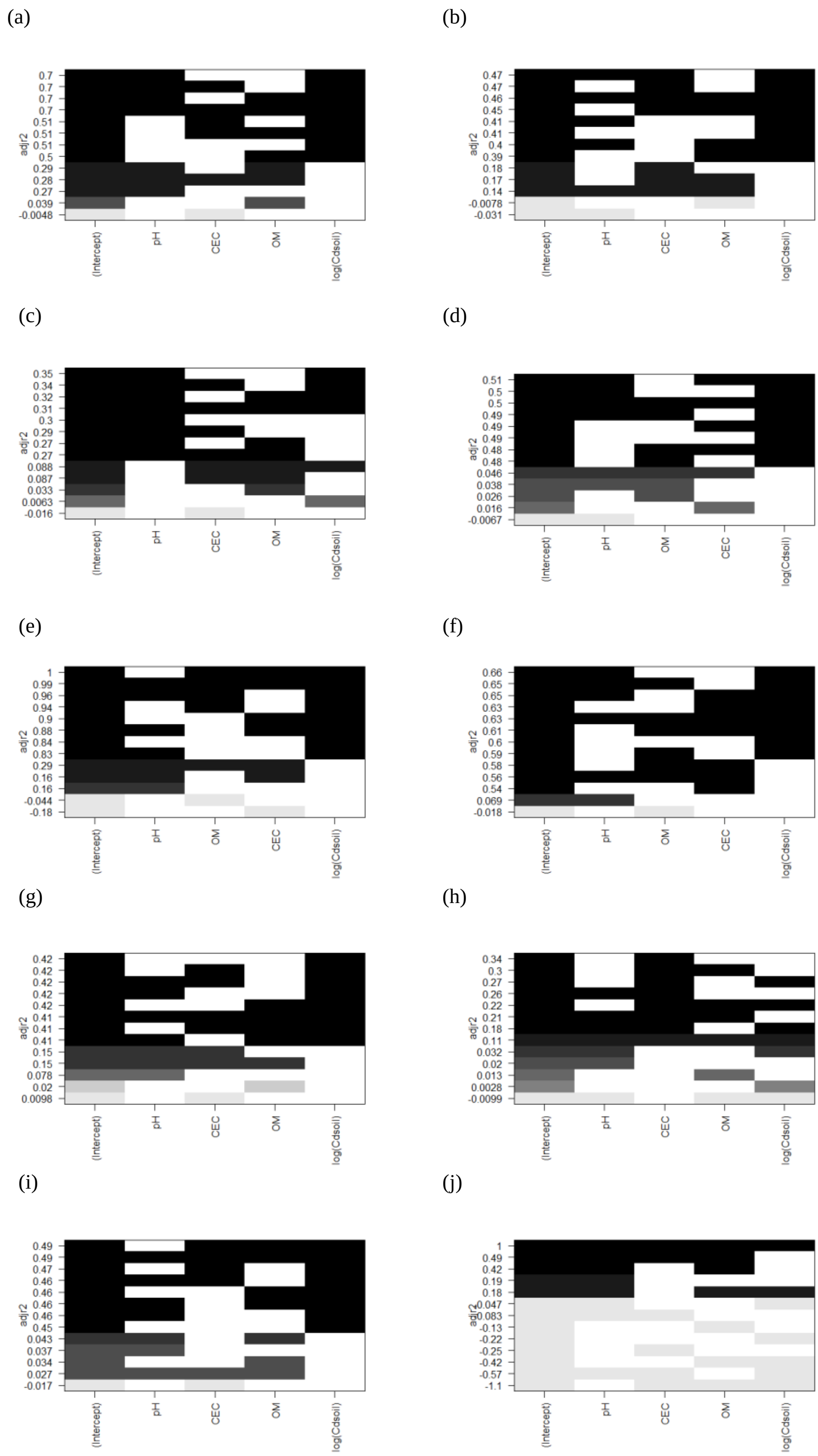


Fig. S1. Subset regressions of wheat and maize (x: variables; y: adjusted $R^2$; a: total; b: wheat, $Cd_{soil} \leq 1.82$; $pH \leq 7.27$; c: wheat, $pH > 7.27$; $Cd_{soil} \leq 0.23$; d: wheat, $pH > 7.27$; $0.23 < Cd_{soil} \leq 1.82$; e: wheat, $Cd_{soil} > 1.82$; $pH \leq 6.69$; f: wheat, $Cd_{soil} > 1.82$; $pH > 6.69$; g: maize, total; h: maize, $Cd_{soil} \leq 0.26$; i: maize, $0.26 < Cd_{soil} \leq 3.03$; j: maize, $Cd_{soil} > 3.029$ )

# 4. Assessing bioavailable fraction and bioconcentration factors of Cd and Zn in young silage maize under different P fertilization and crop rotation [c]

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**Abstract**

The bioconcentration factors and two methods for estimating the bioavailable fraction of Cd and Zn were evaluated to their concentrations in young silage maize under different phosphate and crop management. The DGT technique and the extraction method with $NH_4NO_3$ indicated a moderate correlation to Cd levels in maize. After the first crop rotation, Cd bioavailability increased under high-banded P fertilization, indicating a potential accumulation of labile Cd in arable soil in a short period of time. This effect was not visible in the Cd uptake by the following maize crop. P placement strongly affected Zn concentration in maize. A previous legume crop enhanced Cd bioavailability and Cd uptake compared with a wheat crop rotation. Particular attention should be paid to interactions between essential and toxic elements (P, Zn, and Cd), P overfertilization, and high Cd contents in P fertilizers even in the short term to prevent accumulation of labile Cd in soil-maize systems.

**Keywords:** Cd bioavailability; DGT; bioconcentration factor; silage maize; arable soil; P fertilization

## 4.1 Introduction

Silage maize is one of the main crops in Germany, with 2.3 million ha of cultivable land in 2020, and approximately 35% of the harvested maize for biogas production and 65% for livestock feed (FNR, 2019; Statistisches Bundesamt [Destatis], 2020). Phosphorus is a limiting element in crop development due to its low availability in arable soils and its high crop demand (Niño-Savala et al., 2019)

Zn is essential for plant development and survival. It is involved in enzyme activity, gene regulation, and stress tolerance. In agricultural production, cereal crops are often prone to Zn deficiency, reducing the nutrient quality of derived food and feed (Alloway, 2009; Gupta et al., 2016). Conversely, Cd is a hazardous heavy metal without any known metabolic function for living organisms. Its toxicity is enhanced by its high mobility and bioaccumulation potential.

Consequently, high Cd concentrations in crops can decrease leaf size, fresh weight, root and shoot development, leaf photosynthesis, and nutrient absorption, decreasing harvested production (Bashir et al., 2021; Khan et al., 2017) . In general, soil properties (pH, clay content, organic matter), plant characteristics (species and genotype), crop and fertilizer management (P fertilizer type, P application rate, P placement, and crop rotation) influence the concentration of Cd and Zn in soils and crops (Kabata-Pendias & Szteke, 2015; Niño-Savala et al., 2019).

Soil measurements, such as the total metal content and pH, can be a helpful tool to assess potential Zn deficiency or hazardous Cd levels for crop uptake (Alloway, 2009; Zhuang et al., 2021). Still, not all metal found in soil is available for plants, and its uptake depends mainly on chemical speciation. Evaluating the bioavailable metal forms in soil could be advantageous over measuring only the total metal content (Khan et al., 2017). The single extraction methods simulate the plant uptake using chelating agents or neutral salts. Specifically, the extraction with 1 M $NH_4NO_3$ solution is an environmentally friendly method employed to determine the readily bioavailable metal fraction in soils and estimate the metal uptake by crops in Germany (Adamo et al., 2018; Deutsches Institut für Normung e.V., 2009).

In soil-plant systems, one of the control mechanisms in metal plant assimilation is the diffusive transport of metals (Degryse et al., 2009; Li et al., 2019). The diffusion gradients in thin films (DGT) technique uses this principle to estimate the bioavailable metal concentration in soils. Several studies have revealed high correlations between the DGT-measured metal levels and the metal concentration in plants (Grüter et al., 2019; Luo et al., 2021; Pérez & Anderson, 2009; Yao et al., 2016). Compared to traditional single extraction methods, the DGT devices are user-friendly, with a low amount of lab material and reagents.

Another tool for estimating pollutant exposure and crop uptake is the bioconcentration factor (BCF). The BCF indicates the plant capacity to accumulate a metal into its biomass in comparison to the metal concentration found in soil (Ramana et al., 2021; Retamal-Salgado et al., 2017). The BCF for maize has been derived previously, yet its calculation usually focuses on maize grain at mature stages or in polluted sites (Wang et al., 2017).

Thus, the aims of this study were: i) to assess the bioavailable fraction of Cd and Zn via two different methods (DGT and conventional extraction with 1 M $NH_4NO_3$), ii) to analyze the levels of Cd and Zn in silage maize at the leaf development stage and its relation to their bioavailable fraction, and iii) to calculate the BCFs, all under different crop rotations and different P placements and application rates.

## 4.2 Materials and methods

### 4.2.1 Study design

The study was performed in the framework of a field experiment studying the P fertilizer use efficiency under different P and crop management, with silage maize as the main focus. In 2019, the field experiment was established in Hirrlingen, Baden-Württemberg, Germany (48° 24' N 8° 53' E). The experimental design was a randomized complete block design with 120 plots in total. It included three different crops (silage maize, wheat, and soybean), four replicates (or blocks), two levels of P application rate (low and high) combined with two levels of P placement (broadcasted and banded fertilization), and a control treatment without P fertilization. The low application rate corresponded to 100% of the P required by the crop, and the high application rate was equal to 150% of the P required.

In 2019, 40 out of the 120 plots were sown with soybean (*Glycine max* cv. Sirelia, n=20) and summer wheat (*Triticum aestivum* cv. Quintus, n=20) as crop rotation. According to the nutrition requirements and the specified P treatments, soybean and summer wheat were fertilized with triple superphosphate (TSP), which had a Cd concentration of 27 mg $kg^{-1}$, equivalent to 58.69 mg Cd $kg^{-1}$ $P_2O_5$. However, in May 2019, soybean could not recover after hail damage, and winter pea (*Pisum sativum* cv. James) replaced it in autumn without additional fertilization. For the field season 2020, the following crop rotation, silage maize (*Zea mays* cv. Ronaldinho) was fertilized with diammonium phosphate (DAP), with a Cd concentration of 22 mg $kg^{-1}$, corresponding to 47.82 mg Cd $kg^{-1}$ $P_2O_5$. Due to DAP containing N, the application of N as urea was adjusted to adequate levels, depending on the P treatment (Table 4.1). The Cd level in both P fertilizers was close to the limit of 60 mg Cd $kg^{-1}$ established by the European Union (Ulrich, 2019).

Table 4.1 Phosphorus, nitrogen, and potassium requirements for silage maize, field season 2020; [a] after winter pea cultivation.

<table>
<tr><th>Treatment</th><th>Phosphorus</th><th>Fertilizer</th><th>Nitrogen</th><th>Fertilizer</th><th>Potassium</th><th>Fertilizer</th></tr>
<tr><td>Units</td><td>kg ($P_2O_5$) $ha^{-1}$</td><td>kg $ha^{-1}$ (DAP)</td><td>kg N $ha^{-1}$</td><td>kg $ha^{-1}$ (Urea)</td><td>kg $K_2O$ $ha^{-1}$</td><td>kg $ha^{-1}$ (Patentkali 30% $K_2O$)</td></tr>
<tr><td>Low band<br>Low broad</td><td>114</td><td>248</td><td rowspan="3">230<br>190[a]</td><td>185<br>145[a]</td><td rowspan="3">318</td><td rowspan="3">1060</td></tr>
<tr><td>High band<br>High broad</td><td>171</td><td>372</td><td>163<br>123[a]</td></tr>
<tr><td>Control</td><td>No P applied</td><td>No P applied</td><td>230<br>190[a]</td></tr>
</table>

### 4.2.2 Soil and maize samples

In 2020, soil and plant samples were collected from the plots where summer wheat and soybean had previously been cultivated (n=40) (Fig. 4.1). Before P fertilization and sowing of silage maize, the soil samples were collected, consisting of six randomized core subsamples from each plot at 0 to 30 cm depth. The soil subsamples were mixed, air-dried, and sieved (2 mm) to obtain a homogenous sample of each plot.

Shoots and roots of silage maize (n=5) were collected from each of the 40 plots at the leaf development stage (BBCH 17). Subsequently, the material was rinsed with $H_2O$ (electrical resistance <18.2 MΩ cm$^{-1}$) and oven-dried at 75±5°C for 48 h (Heraeus UT 6760, Thermo Scientific, Hanau, Germany). The plant samples were pulverized in a mixer mill MM 400 Retsch (Verder Scientific, Haan, Germany), operated with a metal-free jar at 29 Hz for 1.5 min.

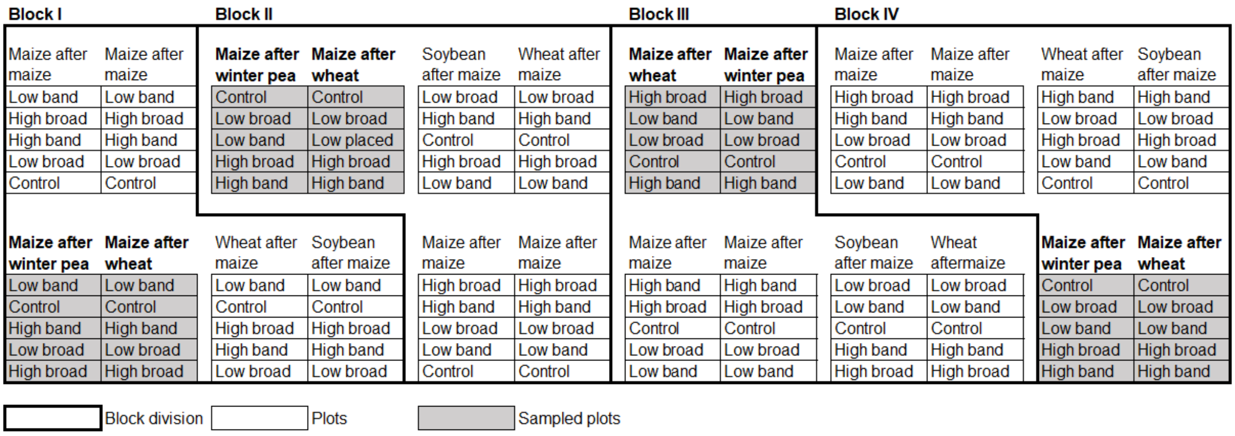


Fig. 4.1 Experimental and sampling design for field season 2020

### 4.2.3 Sample pretreatment and analytical measurements

The $CaCl_2$ method for soil pH measurement, the *aqua regia* extraction for (pseudo) total metal concentration in soil samples, the pretreatment with microwave digestion for total metal concentration in maize samples, and the analytical technique with Inductively Coupled Plasma combined with Mass Spectrometry (ICP-MS) were all performed following standardized methods, using chemical reagents of analytical grade and $H_2O$ with an electrical resistance lower than 18.2 MΩ cm$^{-1}$ by the Core Facility of the University of Hohenheim (CFH), Stuttgart, Germany (Deutsches Institut für Normung e.V., 1997).

For the *aqua regia* extraction, soil samples (3 g) were moistened with $C_8H_{18}O$ in 250 ml digestion tubes. The soil samples were saturated with a*qua regia* solution (78% HCl and 22% $HNO_3$, 50 ml). Next, the suspension was digested at 140°C for 3 h in a reflux condenser and, later, filtered through a folded filter MN 280 1/4 into 100 ml vessels. The heavy metal concentrations were measured using an ICP-MS NexION 300x (PerkinElmer, Rodgau, Germany).

For the pretreatment with microwave digestion, the plant samples (0.2 g) were saturated with $H_2O$ (1 ml) and $HNO_3$ (2.5 ml) and digested for 1.5 h by a Milestone UltraCLAVE III microwave heated digestion reactor (MLS GmbH, Leutkirch, Germany) (VDLUFA, 2011). After digestion, $H_2O$ was added to each sample solution to reach 10 ml for further analytical analysis via ICP-MS.

### 4.2.4 Metal bioavailability

For the methods assessing the bioavailable fraction, $H_2O$ had an electrical resistance lower than 18.2 MΩ $cm^{-1}$, all chemical reagents were of analytical grade, and all material was previously rinsed with $HNO_3$ and $H_2O$. The first method was the single extraction with 1 M $NH_4NO_3$ solution (Bashir et al., 2018; Deutsches Institut für Normung e.V., 2009). In an Erlenmeyer flask, each soil sample (20 g) was moistened with 1 M $NH_4NO_3$ solution (50 ml). The soil-extractant solution was homogenized for 2 h at 120 rpm in a horizontal shaker (GFL® model 2017) and transferred to plastic containers through a 0.45 µm SFCA filter (VWR$^{TMR}$). After filtration, the solution was stabilized with $HNO_3$ at 1% of the extraction volume. The samples were stored at 4±1°C until the metal analysis via ICP-MS was completed by the CFH.

The DGT technique was the second procedure to estimate the bioavailable metal fraction (Grüter et al., 2019; Pérez & Anderson, 2009; Yao et al., 2016). Each DGT device consisted of a) one filter membrane made of polyethersulphone, b) one binding layer of Chelex gel made of iminodiacetate with a thickness of 0.40 mm, and c) one diffusive gel made of agarose crosslinked polyacrylamide with a thickness of 0.78 mm and exposure area of 2.54 $cm^2$ according to the data provided by DGT Research Ltd. (Lancaster, UK).

Before deployment, each soil sample (50 g) was saturated with $H_2O$ in a glass container and covered with Parafilm®. After 24 h at room temperature (25°C), the DGT device was pressed onto the saturated paste, with a deployment time of 24 h. Next, the DGT devices were rinsed with $H_2O$, and after removing the Chelex gel from the DGT device with metal-free tweezers, the Cd attached to it was extracted by immersing the gel into a clean tube with 1 M $HNO_3$ solution (1ml) for 24 h (Yao et al., 2017). Subsequently, the gel was removed from the solution, and the samples were stored at 4±1°C until the CFH completed the metal analysis via ICP-MS.

### 4.2.5 Equations

The time-averaged concentrations of Cd and Zn by the DGT technique were estimated with Eq. (1) (DGT research Ltd, 2019):

$$(1)\ Metal_{DGT} = \frac{M\Delta g}{D^{mdl}A_p t}$$

where $\Delta g$ is the thickness of the diffusive layer (0.92 mm), $D^{mdl}$ is the diffusion coefficient of Cd and Zn in the diffusive layer (6.09 x $10^{-6}$ and 6.08 x $10^{-6}$ $cm^2/s$ at 25°C, correspondingly), $A_p$ represents the surface area of the DGT device (2.54 $cm^2$), and $t$ is the deployment time (24 h). The accumulated mass (M) of Cd and Zn was estimated using Eq. (2).

$$(2)\ M = \frac{C_e(V_g+Ve)}{f_e}$$

where $C_e$ is the metal concentration measured in the eluent (µg $l^{-1}$), $V_g$ is the volume of the binding gel (0.20 ml), $Ve$ is the volume of the eluent (1 ml), and $f_e$ corresponds to the elution factor with the value of 0.85, according to Devillers et al. (2017).

For the BCF, the total concentrations of Cd and Zn in soil and silage maize were employed. The BCF values for roots and shoots for Cd and Zn were calculated with Eq.

(3) (Ramana et al., 2021; Retamal-Salgado et al., 2017):

$$(3)\ BCF_{roots\ or\ shoots} = \frac{total\ metal\ concentration\ in\ roots\ or\ shoots\ (mg\ kg^{-1}\ dry\ weight)}{total\ metal\ concentration\ in\ soil\ (mg\ kg^{-1})}$$

### 4.2.6 Data analysis

The normality of the data distribution, analysis of variance (ANOVA), post-hoc test at 0.05 level of significance, and the Pearson correlations were conducted using the software RStudio® version 1.3.1093. The linear model included the soil measurements, the metal concentration in silage maize, and the BCF as numerically dependent variables. In contrast, the independent variables were block division, previous crop rotation, P rate, and P placement. Although the soil sampling was done before P fertilization, P placement and P rate were part of the model as the previous crop rotation had the same treatments. Furthermore, the interaction between P placement and P rate was considered for the modeling.

## 4.3 Results

### 4.3.1 Soil pH

Soil pH was on average 5.79 units before the maize growing season, indicating slightly acidic soil conditions. The ANOVA results indicated that the block division and the previous crop rotation affected the soil pH response at a $p < 0.01$ (Table 4.2). There was a gradient from block I to block IV, with a difference of one pH unit between the first and the last block. The soil where winter pea was previously grown had a significantly lower pH than the soil where summer wheat was grown.

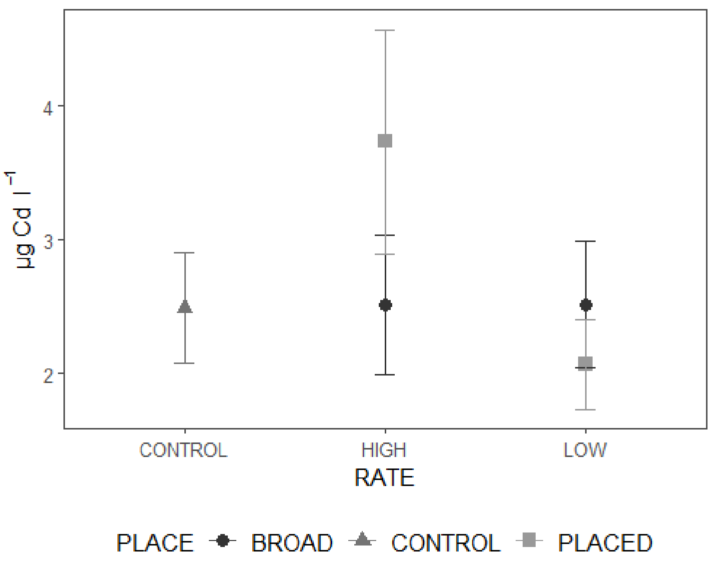
### 4.3.2 Total metal concentration in soil

The P treatments did not reveal any influence on the total Cd concentration in soil. However, the banded fertilization had a higher Cd level than soil from the other P placements ($p$ = 0.06). Neither the P treatments nor the previous crop rotation significantly influenced the total Zn concentration in soil. The only parameter affecting the total Zn concentration in soil was the block division (Table 4.2).

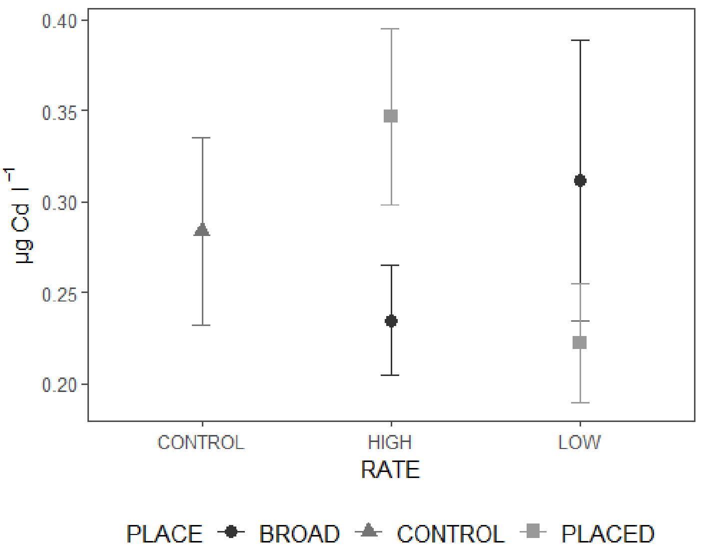
### 4.3.3 Bioavailable fraction in soil

Regardless of P placement and P rate not affecting the bioavailable Cd fraction, there was a significant interaction between these at a $p < 0.05$ for both methods (Table 4.2). The high-banded treatment showed the highest bioavailable Cd concentration, and the lowest was found in the low-banded fertilization (Fig. 4.2). However, the application rate did not indicate a clear tendency for the broadcasted fertilization and depended mainly on the method used. Concerning the block division, block IV had a significantly lower bioavailable Cd concentration than the rest of the blocks.

Moreover, the identity of previously cultivated crops affected the bioavailable Cd fraction. The soil where wheat was grown had a significantly lower Cd concentration as estimated by means of DGT than soil where winter pea was cultivated. However, this difference was not visible in the Cd amount extracted by $NH_4NO_3$ (Table 4.2).




a)




b)

Fig. 4.2 Interaction effect between P placement and P rate for bioavailable Cd concentration by a) extraction with 1 M $NH_4NO_3$ solution and b) DGT technique.

Neither P placement nor P rate impacted the bioavailable Zn concentration in soil. However, the P rate had a probable effect at $p < 0.1$, with the highest bioavailable Zn concentration determined by DGT in the high application rate. According to the DGT technique, the highest bioavailable Zn concentration occurred in the high application rate and banded fertilization treatment. In contrast, the high-broad treatment had the lowest Zn concentration measured by DGT. For the bioavailable Zn concentration estimated via $NH_4NO_3$, no interaction between placement and application rate was observable (Table 4.2).

Table 4.2 Soil measurements by variable (n=40); significance code for *p*-value: ***<0.001, **<0.01, *<0.05, ns >0.05; different letters indicate significant differences at $p < 0.05$ level (Tukey test).

| **Variable** *Units* | **pH** | **$Cd_{Total}$** *(mg kg$^{-1}$)* | **$Zn_{Total}$** *(mg kg$^{-1}$)* | **$Cd_{NH4NO3}$** *(µg l$^{-1}$)* | **$Cd_{DGT}$** *(µg l$^{-1}$)* | **$Zn_{NH4NO3}$** *(µg l$^{-1}$)* | **$Zn_{DGT}$** *(µg l$^{-1}$)* |
|---|---|---|---|---|---|---|---|
| Rate | ns | ns | ns | ns | ns | ns | ns |
| Control | 5.78±0.45 | 0.19±0.03 | 60.34±1.51 | 2.49±1.31 | 0.28±0.16 | 66.15±37.55 | 9.25±3.28 |
| Low | 5.82±0.43 | 0.19±0.04 | 60.91±1.96 | 2.29±1.28 | 0.27±0.19 | 64.17±42.48 | 8.71±3.71 |
| High | 5.77±0.54 | 0.19±0.03 | 60.52±1.99 | 3.12±2.23 | 0.29±0.14 | 82.54±56.62 | 8.64±3.21 |
| Place | ns | ns | ns | ns | ns | ns | ns |
| Control | 5.78±0.45 | 0.19±0.03 | 61.34±1.51 | 2.49±1.31 | 0.28±0.16 | 66.15±37.55 | 9.25±3.28 |
| Broad | 5.78±0.50 | 0.18±0.03 | 60.59±2.22 | 2.52±1.52 | 0.27±0.18 | 73.28±49.52 | 8.45±3.90 |
| Band | 5.80±0.47 | 0.20±0.03 | 60.84±1.71 | 2.90±2.13 | 0.28±0.14 | 73.47±52.33 | 8.90±2.96 |
| Rate: Place | ns | ns | ns | * | * | ns | * |
| Block | *** | *** | *** | *** | *** | *** | ** |
| I | 5.41±0.22$^{c}$ | 0.17±0.02$^{b}$ | 58.66±1.01$^{c}$ | 3.85±1.77$^{a}$ | 0.33±0.10$^{a}$ | 105.49±46.38$^{a}$ | 10.59±1.98$^{a}$ |
| II | 5.58±0.16$^{bc}$ | 0.19±0.02$^{b}$ | 60.17±0.76$^{b}$ | 2.79±1.00$^{a}$ | 0.33±0.21$^{a}$ | 86.48±29.58$^{a}$ | 9.22±4.33$^{ab}$ |
| III | 5.70± 0.10$^{b}$ | 0.23±0.03$^{a}$ | 62.68±0.77$^{a}$ | 3.43±1.27$^{a}$ | 0.34±0.13$^{a}$ | 85.53±26.43$^{a}$ | 9.37±3.33$^{ab}$ |
| IV | 6.47±0.37$^{a}$ | 0.18±0.03$^{b}$ | 61.85±1.52$^{a}$ | 0.59±0.43$^{b}$ | 0.12±0.04$^{b}$ | 10.15±11.28$^{b}$ | 5.98±1.43$^{b}$ |
| Prev. Crop | ** | ns | ns | ns | * | ns | ns |
| Wheat | 5.90± 0.56$^{a}$ | 0.19±0.04 | 60.91±1.92 | 2.55±1.85 | 0.24±0.11$^{b}$ | 64.77±44.46 | 8.57±2.63 |
| Winter pea | 5.68±0.34$^{b}$ | 0.19±0.03 | 60.78±1.86 | 2.78±1.64 | 0.32±0.19$^{a}$ | 79.05±50.51 | 9.01±4.01 |

### 4.3.4 Silage maize

For the total Cd concentration in the roots, the fertilizer placement had a significant influence at $p = 0.05$. Although a higher Cd concentration in roots occurred in the broadcasted fertilization than in the other treatments, the post-hoc test did not reveal significant differences between the P placements. For the total Cd concentration in shoots, the differences between the P placements were not significant and marginal (Table 4.3).

Similar to Cd, the total Zn concentration in silage maize was analyzed in roots and shoots. The P placement led to lower Zn concentration both in roots and shoots in the banded fertilization than in the other treatments. The highest Zn concentration in roots was found in the control treatment, while the highest Zn concentration in shoots was found in the broad placement (Table 4.3).

The crop cultivated before silage maize affected the total Zn concentration in roots and shoots ($p < 0.01$), with a higher value in maize following summer wheat than in maize following winter pea for both plant fractions. Regarding the block division, block IV had a significantly lower Cd concentration in roots and shoots and a lower Zn concentration in roots than the other blocks.

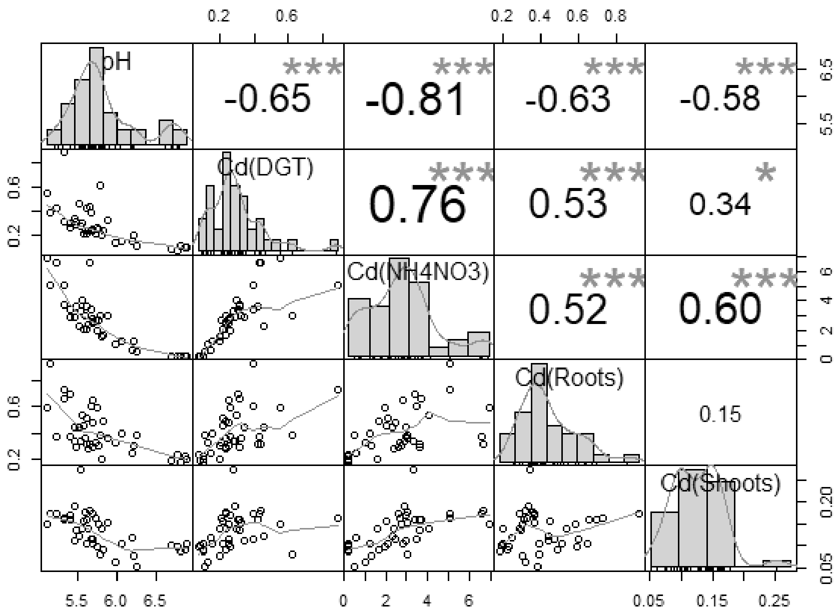
Table 4.3 Amounts of Cd and Zn in young silage maize (mg $kg^{-1}$ dry weight) by variable (n=40); significance code for *p*-value: ***<0.001, **<0.01, *<0.05, ns >0.05; different letters indicate significant differences at $p < 0.05$ level (Tukey test).

| **Variable** | **$Cd_{roots}$** | **$Cd_{shoots}$** | **$Zn_{roots}$** | **$Zn_{Shoots}$** |
|---|---|---|---|---|
| Rate | ns | ns | ns | ns |
| Place | * | ns | * | ** |
| Control | $0.36±0.15^{a}$ | 0.14±0.06 | $416.25±143.00^{a}$ | $39.01±9.17^{a}$ |
| Broad | $0.45±0.20^{a}$ | 0.14±0.04 | $318.00±151.98^{ab}$ | $41.23±9.97^{a}$ |
| Band | $0.38±0.14^{a}$ | 0.12±0.03 | $275.06±95.40^{b}$ | $32.73±7.26^{b}$ |
| Rate:Place | ns | ns | ns | ns |
| Block | *** | *** | ns | ** |
| I | $0.45±0.21^{a}$ | $0.17±0.05^{a}$ | 326.90±159.87 | $42.25±8.59^{a}$ |
| II | $0.44±0.16^{a}$ | $0.14±0.03^{a}$ | 354.70±108.73 | $33.95±7.87^{ab}$ |
| III | $0.42±0.13^{a}$ | $0.14±0.04^{a}$ | 338.60±161.01 | $37.62±8.10^{b}$ |
| IV | $0.27±0.08^{b}$ | $0.09±0.02^{b}$ | 261.70±100.95 | $33.95±7.31^{b}$ |
| Prev. Crop | *** | *** | ** | *** |
| Wheat | $0.30±0.07^{a}$ | $0.15±0.04^{a}$ | $379.25±152.14^{a}$ | $41.45±7.57^{a}$ |
| Winter pea | $0.52±0.16^{b}$ | $0.11±0.03^{b}$ | $261.70±83.14^{b}$ | $32.44±6.72^{b}$ |

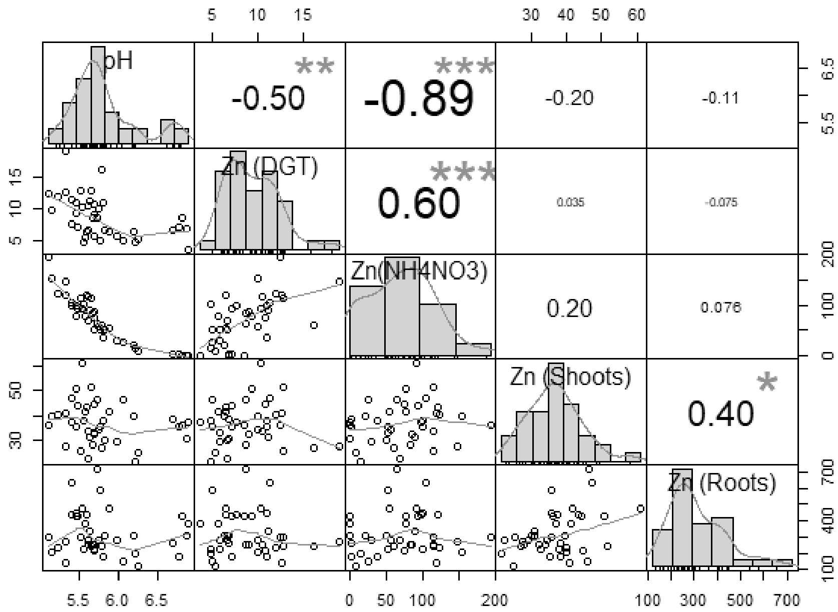
### 4.3.5 Pearson correlations

The correlation between the Cd concentration in shoots and the Cd concentration determined by $NH_4NO_3$ was higher than the correlation between the Cd concentration found in shoots and the Cd concentration extracted by the DGT technique. Both methods were highly correlated to soil pH. However, the correlation between soil pH and the Cd concentration estimated by $NH_4NO_3$ was stronger than that of soil pH and the Cd concentration determined by DGT. The correlation between the Cd concentration in roots and both extraction methods was significant and similar. The Cd concentration estimated by DGT correlated better with the Cd concentration in roots than with the Cd found in shoots. For the Cd concentration in shoots, the correlation with the Cd concentration using $NH_4NO_3$ was higher and more significant than the Cd concentration estimated by DGT (Fig. 3.3). Regarding Cd concentration in silage maize, there was no correlation between the Cd found in roots and shoots. Still, both were significantly associated with soil pH.

The Zn concentration determined by DGT indicated a high correlation with the Zn concentration measured by the traditional method. However, the correlation of both methods with the Zn found in silage maize was low and insignificant. Similar to Cd, both methods significantly correlated to soil pH (Fig. 4.3).




a)




b)

Fig. 4.3 Correlation matrix between metal concentration in soil and metal concentration in silage maize of a) Cd and b) Zn (n=40), significant correlation at *p*-value: ***<0.001, **<0.01, *<0.05.

### 4.3.6 BCF

The values for both metals indicated a $BCF_{roots}$>1. The highest $BCF_{roots}$ of Cd was found in the broadcasted fertilization, while the lowest $BCF_{roots}$ of Cd was visible in the banded fertilization (Table 4.4). The banded fertilization revealed significantly low $BCF_{shoots}$ of Cd in comparison with the other treatments. The P rate affected the $BCF_{shoots}$ of Cd at $p < 0.05$, yet after running the post hoc test, the differences between the application rates were not significant.

The control treatment had the highest $BCF_{roots}$ of Zn, while the high application rate treatment had a significantly lower $BCF_{roots}$ of Zn than the other treatments. Despite no significant differences between the P placements for the $BCF_{roots}$ of Zn, the banded fertilization had a lower $BCF_{roots}$ value than the other treatments. The $BCF_{shoots}$ of Zn was significantly lower in the banded fertilization than in the control and the broadcasted treatment (Table 4.4).

For the $BCF_{roots}$, the crop rotation had a significant effect ($p < 0.001$): maize following winter pea had a higher $BCF_{roots}$ of Cd than maize following wheat. The $BCF_{roots}$ of Zn indicated the opposite behavior, with a higher value in maize following wheat than maize following winter pea. However, for the $BCF_{shoots}$, higher values for Cd and Zn were found in maize following wheat than in maize following winter pea.

Table 4.4 BCF of Cd and Zn for young silage maize by field variable (n=40); significance code for *p*-value: ***<0.001, **<0.01, *<0.05, ns >0.05; different letters indicate significant differences at $p < 0.05$ level (Tukey test).

| **Variable / Metal** | **Cd** | | **Zn** | |
|---|---|---|---|---|
| | $BCF_{roots}$ | $BCF_{shoots}$ | $BCF_{roots}$ | $BCF_{shoots}$ |
| Rate | ns | ns | * | ns |
| Control | 1.99±0.92 | 0.79±0.37 | 6.80±2.47$^{a}$ | 0.67±0.17 |
| Low | 2.23±0.88 | 0.68±0.22 | 5.15±2.49$^{ab}$ | 0.60±0.11 |
| High | 2.23±1.25 | 0.71±0.28 | 4.63±1.46$^{b}$ | 0.59±0.15 |
| Place | * | * | ns | ** |
| Control | 1.99±0.92$^{ab}$ | 0.79±0.37$^{a}$ | 6.80±2.47 | 0.67±0.17$^{a}$ |
| Broad | 2.54±1.29$^{a}$ | 0.76±0.28$^{a}$ | 5.26±2.40 | 0.65±0.13$^{a}$ |
| Band | 1.92±0.69$^{b}$ | 0.62±0.19$^{a}$ | 4.52±1.56 | 0.54±0.12$^{b}$ |
| Rate:Place | ns | ns | ns | ns |
| Block | *** | ** | ns | *** |
| I | 2.83±1.45$^{a}$ | 0.99±0.29$^{a}$ | 5.57±2.72 | 0.72±0.15$^{a}$ |
| II | 2.42±0.83$^{ab}$ | 0.76±0.17$^{b}$ | 5.89±1.76 | 0.56±0.13$^{b}$ |
| III | 1.98±0.65$^{bc}$ | 0.61±0.19$^{bc}$ | 5.40±2.56 | 0.60±0.13$^{b}$ |
| IV | 1.49±0.51$^{c}$ | 0.49±0.13$^{c}$ | 4.24±1.65 | 0.55±0.12$^{b}$ |
| Prev. Crop | *** | ** | ** | *** |
| Wheat | 1.57±0.42$^{a}$ | 0.81±0.28$^{a}$ | 6.23±2.51$^{a}$ | 0.69±0.13$^{a}$ |
| Winter pea | 2.80±1.10$^{b}$ | 0.62±0.23$^{b}$ | 4.31±1.40$^{b}$ | 0.53±0.11$^{b}$ |

## 4.4 Discussion

### 4.4.1 Soil pH

The ANOVA results indicated that the previous P fertilization did not impact soil pH, likely due to the lack of influence of TSP in soil pH compared to other P fertilizers containing N or under polluted soil conditions (Sarwar et al., 2010). An increasing soil pH gradient was visible from block I to block IV. This last block had the highest soil pH, the lowest bioavailable Cd fraction, and the lowest Cd concentration in roots and shoots, indicating a significant heterogeneity between blocks (Soriano-Disla et al., 2014). A higher clay or organic matter content in the soil of block IV could explain the high pH and the low Cd mobilization in this soil (Malik et al., 2021; Wang et al., 2017). However, the significant differences, especially between block IV and the rest of the blocks, could have masked P fertilization's effects on soil pH and the other variables of interest.

The crop rotation caused a lower soil pH when winter pea was previously grown than when wheat was cultivated before. The soybean cultivated in spring and the field pea cultivated in winter 2019 could have decreased soil pH by the nitrification process, causing a higher Cd mobilization and uptake by maize following the legume cultivation, as has also been shown by Yan et al. (1996) .

### 4.4.2 Total metal concentration in soil

Although P placement did not strongly affect the total Cd concentration in soil, the banded treatment had the highest total Cd concentration. In 2019, the background concentration of total Cd in soil was 0.145±0.002 mg $kg^{-1}$, with a visible increase in 2020. The increase in Cd level could result from P fertilization in plots where the previous crops were fertilized with relatively high Cd-TSP (Molina-Roco et al., 2018; Römkens et al., 2018). However, the total Cd concentration remained in the range of unpolluted soils (0.06 to 1.10 mg $kg^{-1}$) and below the limit specified by German standards (0.40 mg Cd $kg^{-1}$ at pH<6) (Kabata-Pendias & Szteke, 2015; LUFA, 1999).

The average total Zn concentration in soil was 60.84±1.87 mg $kg^{-1}$, in accordance with the average value for loamy-silty soils in Germany (Alloway, 2009). Like for Cd, neither the P treatments nor the previous crop rotation affected the total Zn concentration in soil. Although the mobile fraction exhibited significant differences between the P treatments, this Zn fraction is usually low compared to the total Zn concentration (Gupta et al., 2016). In this study, the bioavailable Zn fraction represented only 0.30%, explaining the lack of impact of P fertilization on the total Zn concentration in soil. The same explanation could be valid for the total Cd concentration in soil since the bioavailable Cd concentration identified by $NH_4NO_3$ represented only 3.5% of the total Cd concentration, a low percentage compared to the mobile Cd fraction (45.2%) reported by Liu et al. (2013).

### 4.4.3 Bioavailable fraction in soil

Before the field trial, the bioavailable Cd concentration measured by the conventional extraction method was 1.50±0.66 µg $l^{-1}$ in 2019. The values obtained after the first crop rotation were higher than the background bioavailable Cd concentration. The increase indicated a change in the total and bioavailable Cd levels in the control treatment, possibly resulting from atmospheric Cd deposition (Ilyin et al., 2020). Still, the high rate combined with the banded fertilization led to the highest bioavailable Cd in soil with a $p < 0.05$, independently of the assessment method. Römkens (2018) demonstrated that P fertilizer containing > 40 mg Cd $kg^{-1}$ $P_2O_5$ could trigger Cd accumulation in arable land; still, this increase usually takes longer, and changes in the bioavailable fraction are undetectable after one year of cultivation under greenhouse conditions. In contrast, Molina-Roco et al. (2018) revealed that the labile Cd found in soil after fertilization corresponds to the labile Cd found in TSP. Additionally, freshly applied Cd derived from TSP fertilization can experience an immediate low uptake by the crop (summer wheat and winter pea), and Cd might stay mobile and potentially bioavailable in the soil for the follow-up crops (silage maize) (Bracher et al., 2021). Thus, the Cd derived from P fertilizer, combined with the excess of fertilization in the high banded treatment, could explain the fast change in the bioavailable Cd fraction in soil.

The low Cd concentration estimated by DGT in soils where summer wheat was grown compared to winter pea could result from wheat taking up more Cd than other crops (Zhuang et al., 2021). Furthermore, the soybean crop and its following substitution with another legume could have decreased soil pH (Wang et al., 2017), leading to a higher Cd bioavailability in soil. Interestingly, this crop rotation effect was not visible in the Cd extracted using $NH_4NO_3$, a method usually more dependent on soil pH (Adamo et al., 2018).

The bioavailable Zn fraction obtained by DGT was high under high-banded and low-broad fertilization. The antagonistic behavior between P and Zn in nutrient uptake by previous crops could lead to an accumulation of bioavailable Zn in the P fertilized plots (Mousavi, 2011). Additionally, the Zn concentration was around 600 mg $kg^{-1}$ in the TSP applied to soybean and wheat, explaining the high bioavailable Zn concentration estimated by DGT in the high-banded fertilization (Kabata-Pendias & Szteke, 2015). However, there was no significant increase in the total Zn concentration in the soil after TSP fertilization.

### 4.4.4 Silage maize

The Cd concentration in shoots was relatively high but below the limit for feed materials of vegetable origin (1 mg $kg^{-1}$) (EC, 2013). The control treatment had the highest total Cd concentration in shoots, probably due to the lack of P fertilization and dilution effect (Chien & Menon, 1996). The low Cd concentration in shoots in the banded fertilization could result from high P uptake, with a consequently higher biomass, diluting the Cd concentration in the plant (Sarwar et al., 2010). Nevertheless, a relatively high Cd concentration in roots was visible in the same treatment. This high Cd level in roots could be due to the relatively high total and bioavailable Cd concentration in soil detected in the banded fertilization (Lux et al., 2011).

The Zn concentration in roots was high compared to the Zn concentration in shoots. However, the Zn level in shoots was in the upper range of the optimum Zn concentration in plants (12 to 47 mg $kg^{-1}$). This distribution might result from maize sensitivity to Zn and accumulating most of it in the roots (Alloway, 2009; Kabata-Pendias & Szteke, 2015). Although the Zn level in soil and young maize shoots was adequate, the banded fertilization effect on the Zn uptake by roots and shoots of silage maize was strongly evident. Furthermore, the bioavailable Zn concentration estimated by DGT was higher under this type of fertilization. The P fertilization could cause this difference between the bioavailable Zn fraction and the Zn concentration in maize by diluting this micronutrient within the plant or depressing its uptake (Bogdanovic et al., 1999; Drissi et al., 2015).

Regarding the crop rotation, a lower Zn concentration in maize roots and shoots following winter pea than in maize following wheat could be due to winter pea taking up high Zn quantities, causing a lower Zn concentration in the follow-up crop (Płaza et al., 2019). However, none of the methods assessing the bioavailable Zn fraction indicated lower values after the legume cropping.

The interaction between P application rate and P placement influenced the bioavailable Cd fraction obtained by both methods, suggesting significant changes in the soil after only one crop cultivation under moderate Cd-P fertilizer. Nevertheless, this interaction effect was not yet visible in the maize roots and shoots. The interactions between Cd, Zn, and P in the uptake process and the lack of significant changes for Cd uptake by the crop after one application of P fertilizer could explain the differences between the bioavailable fraction and the maize measurements (Bracher et al., 2021; Römkens et al., 2018).

### 4.4.5 Pearson correlations

The traditional method indicated a significantly stronger correlation with the Cd concentration in shoots than the DGT technique, probably caused by a more complete extraction with $NH_4NO_3$ under acidic soil conditions or due to plant limiting uptake mechanisms rather than diffusion (Adamo et al., 2018; Li et al., 2019). The correlation between the Cd concentration in shoots and the bioavailable Cd fraction estimated by DGT indicated lower values than other studies (Luo et al., 2021; Pérez & Anderson, 2009; Yao et al., 2016). Still, the DGT technique is independent of soil pH resulting in a better correlation with maize uptake under alkaline and neutral soils than the traditional method (Dai et al., 2017). Furthermore, the bioavailable Cd concentration measured by DGT had a higher correlation with the Cd concentration in roots than with the Cd found in shoots. This finding agrees with the DGT principle: the diffusive transportation to the plant roots is the regulating mechanism in metal uptake (Degryse et al., 2009).

Since the correlation coefficients were low, none of the bioavailability methods seemed suitable to predict the Zn uptake by silage maize. Our results contradicted the study from Meer et al. (2007), where the Zn concentration in bean shoots highly correlated with the Zn extracted by $NH_4NO_3$. P fertilization was performed between the collection of soil samples and the collection of plant samples, explaining the low correlation between the bioavailable Zn fraction and the Zn concentration in silage maize. However, the methods assessing bioavailability might not simulate the suppressant effect of P in Zn uptake by plants (Sönmez et al., 2016). In addition, the DGT method has been proved unsuitable as a surrogate of Zn content in other cereal crops, such as wheat (Grüter et al., 2019).

### 4.4.6 BCF

The $BCF_{roots}$ from both metals indicated a relatively high accumulation of Cd and Zn in relation to the total metal concentration in the soil with values >1.0. The broad fertilization showed the highest $BCF_{roots}$ of Cd, probably by the combination of relatively low biomass production and the uptake of Cd, derived from P fertilizer. The P fertilization rate significantly affected the $BCF_{roots}$ of Zn, exhibiting lower values in the high application rate. This significant effect of the P rate was not visible in the soil and plant analyses, providing valuable information compared with the soil and maize measurements.

In general, $BCF_{shoots}$ of Cd was lower than 1.0, indicating a weak response of aerial biomass to the metal concentration in the soil. Meanwhile, the $BCF_{roots}$ of Cd was >1.0, suggesting a higher response of the maize roots to the Cd concentration in soil but a low

Cd translocation from roots to shoots (Bashir et al., 2021; Mirecki et al., 2015). The lowest $BCF_{shoots}$ of Cd, similar to the $BCF_{roots}$ of Cd, occurred in the banded fertilization, possibly resulting from a dilution effect (Chien & Menon, 1996); or by coupled uptake with Zn that also had the lowest BCF in the banded fertilization (Kabata-Pendias & Szteke, 2015). In general, the $BCF_{shoots}$ of Zn were low, and the $BCF_{roots}$ of Zn were high. This behavior might be due to competition between P and Zn in the translocation process from roots to stems. However, Drissi et al. (Drissi et al., 2015) already discarded this hypothesis and attributed the allocation to a dilution effect in silage maize.

The $BCF_{shoots}$ of Cd suggested that the previous summer wheat enabled a higher Cd transfer from the soil to the maize shoots. The $BCF_{shoots}$ of Zn was also higher in maize following wheat, suggesting a synergistic behavior between Cd and Zn (Kabata-Pendias & Szteke, 2015). Still, the $BCF_{roots}$ of Cd, the bioavailable Cd fraction, and the Cd uptake by roots were lower in the wheat rotation. The Cd distribution after wheat cultivation could be explained by wheat taking up more Cd than other crops (Zhuang et al., 2021), leading to lower Cd bioavailability and lower root uptake for the next crop, e.g., silage maize. At the same time, soil pH was lower under the winter pea rotation, likely by the nitrification process, leading to a higher Cd bioavailability in soil and uptake in maize roots following the cultivation of legumes (Yan et al., 1996).

## 4.5 Conclusion

The DGT technique and the traditional method indicated a moderately positive correlation with the Cd concentration found in young maize roots. Still, the extraction employing $NH_4NO_3$ might be more suitable for predicting Cd levels in young maize shoots. None of the methods indicated a strong correlation between the bioavailable Zn fraction and maize Zn levels.

The P placement strongly affected Zn concentration in maize roots and shoots. Assessing the BCF of Zn provided helpful information about the effects of the P application rate that were not evident by assessing only the metal concentration in silage maize.

The previous crop rotation, the interaction between Cd and Zn, and the P fertilization effect on Zn uptake seem to be critical drivers for Cd uptake by young silage maize. The methods assessing the bioavailable metal fraction might overlook some of these interactions. Still, both methods demonstrated the potential accumulation of labile Cd in arable soil by overfertilization in a relatively short time. Further investigation is needed to understand the interactions between Cd, Zn, and P in soil-maize systems. Special attention should be paid to P fertilizer and crop management and the Cd contents in P fertilizers to avoid labile Cd accumulation in non-polluted soils and maintain adequate Zn levels in silage maize.

### 4.6 Disclosure statement

No potential conflict of interest was reported by the authors.

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# 5. Cd and Zn Concentrations in Soil and Silage Maize following the Addition of P Fertilizer [d]

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**Abstract:**

Studies of soil Cd and Zn are often performed on sites that are contaminated or have deficient Zn conditions. Soil characteristics and crop management could impact the soil mobility and uptake of Cd and Zn, even when considering unpolluted Cd soils and adequate soil Zn levels. The concentrations of these two metals were assessed in soil and silage maize under five P fertilization treatments at two growth stages under low Cd- and sufficient Zn conditions. Pearson correlation coefficients and stepwise linear regressions were calculated to investigate the soil characteristics influencing the bioavailable metal fraction in soil and the metal concentration in silage maize. P treatments did not impact Cd accumulation in maize; however, Zn level in silage maize was affected by P placement at the leaf development stage. From early development to maturity, the Cd level in maize decreased to 10% of the initial concentration, while the Zn level decreased to 50% of the initial concentration. This reduction in both metals may be attributed to a dilution effect derived from high biomass production. Silage maize could alleviate the initial Cd uptake while diminishing the depressant effect of P fertilizer on Zn concentration. Further research is required to understand the effect of P fertilizer on Cd levels in maize and its relation to Zn under field conditions at early and mature stages.

**Keywords:** Cd pollution; Zn uptake; arable land; maize crop; P fertilization.

## 5.1 Introduction

Cd is a heavy metal with high mobility and bioaccumulation capacity, representing a potential hazard for crop production (Niño-Savala et al., 2019). In plants, Cd can generate oxidative stress and disturb enzyme activity, photosynthesis, and nutrient uptake, thus affecting plant metabolism and development (Haider et al., 2021). Contrary to Cd, Zn is an essential element for plants, animals, and humans, which is crucial for enzyme and metabolic activities. In plants, Zn is part of many enzymes and, organic complexes, being involved in N metabolism and growth processes. A total Zn content between 15 and 20 mg $kg^{-1}$ dry weight (DW) is considered sufficient for the nutrition of most crops. Lower Zn concentrations can cause leaf chlorosis, low growth, and poor harvest quality (Balafrej et al., 2020; Nieder et al., 2018). For livestock, Zn is also essential for enzyme activity, and has been implicated in growth development, adequate performance, immune response, and disease resistance. In farm animals, Zn deficiency due to low-quality feed can lead to several problems, such as reduced growth, lesions in the skin, and poor reproduction (Hill & Shannon, 2019).

Among feeding crops, silage maize (*Zea mays* spp.) is one of the most relevant crops around the globe. In general, maize is cultivated under several conditions with numerous purposes, such as food, feed, and bioenergy. In developing countries, maize is one of the essential grain crops for daily calorie intake, while its use in Europe is often limited to livestock feed and bioenergy production. In 2019, the worldwide production was 1.14 billion metric tons, while the production of silage maize was around 8.3 million metric tons (FAO, 2021). In Germany, the arable land used for silage maize increased from 2.2 million ha in 2018 to 2.7 million ha in 2020 (Deutsches Maiskomitee e.v [DMK], 2020; Statistisches Bundesamt & Deutschland, 2019); however, to achieve profitable high yields, high fertilization rates (including phosphorus) are required. In this respect, the P supply to arable land is a concern, owing to its expected scarcity in the future and the addition of potentially harmful metals (e.g., Cd) to uncontaminated agricultural soil (Bigalke et al., 2017; Rosemarin & Ekane, 2016).

Cd accumulation in arable soils generally derives from air pollution, irrigation, mineral and organic P fertilizers contaminated with Cd, and fertilization management. In addition, certain soil characteristics, such as soil pH, metal speciation, organic matter content, and the presence of other metals, influence the mobility of Cd (Guan et al., 2018; Kirkham, 2006; Schipper et al., 2011). P fertilization can further inhibit Zn uptake and translocation from feeding crops, due to the antagonistic interaction between P and Zn, resulting in low-Zn feed (Drissi et al., 2015); however, a high P concentration could also enhance biomass production in plants, leading to a Zn concentration dilution effect (Mousavi, 2011).

Measuring the total metal concentration in soils can be valuable for evaluating potential metal accumulation or deficits in crops. However, single-extraction methods have higher accuracy in estimating the metal levels in plants by assessing the bioavailable metal fraction than the aqua regia method, which extracts the (pseudo) total metal concentration in soils (Liu et al., 2019). A common technique for determining the exchangeable fraction (readily bioavailable) in soils is the single-extraction method using 1 M $NH_4NO_3$ solution, e.g., to monitor and prevent high Cd bioaccumulation in staple crops in Germany (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, 2015). This method has been used to characterize the Cd concentration found in grain wheat (LWK Niedersachsen, 2015).

Cd and Zn levels in arable soils and silage maize might be affected by P fertilization management, even under low Cd conditions and sufficient Zn levels; however, field experiments studying the risk of Cd bioaccumulation and Zn deficiency in maize crops are often performed in conditions with Cd-polluted soil or deficient soil Zn levels. Soil characteristics such as the total and exchangeable metal fractions and soil pH have been assessed for metal uptake by plants, considering several crops and edible plants. Still, few studies have investigated its relationship to metal uptake by silage maize at different growth stages.

This study provides an understanding of the effect of P fertilizer on Cd and Zn levels in maize under low Cd- and sufficient Zn conditions in arable soils. Additionally, the effects of the exchangeable metal fraction, total metal concentration, and soil pH on Cd and Zn levels in silage maize were investigated, employing Pearson correlations and stepwise linear regressions. The results are expected to contribute to a better understanding of Cd accumulation in silage maize, according to the growth stage.

## 5.2 Materials and Methods

### 5.2.1 Field Design

The study was performed in a field experiment investigating P efficiency in maize crop systems located in Hirrlingen, Baden-Württemberg, Germany (48.420368° N, 8.886270° E). The study area has not experienced intense atmospheric deposition in the past from industrial emissions and has low soil Cd and Zn concentrations. During the growing season (May–October 2019), the weather and precipitation conditions were recorded at a nearby weather station. The monthly temperature was 15.5 °C, and the coldest month was May, with a registered monthly temperature of 10.8 °C. July was the warmest month, with a monthly temperature of 19.4 °C. The precipitation was close to the long-term average in Baden-Württemberg with ca. 5% less precipitation (LUBW, 2020).

The experiment consisted of a randomized complete block design (RCBD), divided into four blocks with 30 plots each block, yielding a total of 120 plots. The main cultivated crop was silage maize (*Zea mays* cv. "Ricardinho"). Each plot was 6 × 11 m with eight maize rows and a row spacing of 0.75 m. P fertilizer was applied at two different rates — high (150% of required P) and low (100% of required P) — in combination with two different placements—placed and broadcast— along with the control treatment (no P application), generating a total of five treatments (Table 5.1). As stated before, this field experiment was designed to investigate the P use efficiency in soil — maize systems. The band-placed fertilization was included in the experimental design due to its standard practice in Germany. To the contrary, the broadcasted fertilization was included as a variable for scientific purposes, assuming that the P placement affects the P use efficiency. The treatment with 100% of required P was based on P offtake by silage maize, which considered background P levels in the soil field and the optimal P level for the expected maize yield. The treatment with 150% of required P (high-rate application) was included to observe the effect of a high P input on P uptake and harvested biomass. Diammonium phosphate (DAP) was the P fertilizer applied to silage maize, containing 1.99 ± 0.08 mg Cd $kg^{-1}$ (4.34 ± 0.18 mg Cd $kg^{-1}$ $P_2O_5$) and 58.80 ± 3.80 mg Zn $kg^{-1}$ (126.99 ± 8.27 mg Zn $kg^{-1}$ $P_2O_5$).

Table 5.1 Description of the experimental design for silage maize.

| P treatment | Phosphorus | Fertilizer | Nitrogen | Fertilizer | Potassium | Fertilizer |
|---|---|---|---|---|---|---|
| Units | kg ($P_2O_5$) $ha^{-1}$ | kg $ha^{-1}$ (DAP) | kg N $ha^{-1}$ | kg $ha^{-1}$ (urea) | kg $K_2O$ $ha^{-1}$ | kg $ha^{-1}$ (Patentkali 30% $K_2O$) |
| Low-placed<br>Low-broad | 114 | 248 | 180 | 294 | 368 | 1127 |
| High-placed<br>High-broad | 171 | 372 | | 245 | | |
| Control | No P applied | No P applied | | 391 | | |

Two crop rotations were initially considered for silage maize cultivation: monocropping and intercropping with under-sown clover. The clover emergence failed due to weed growth; thus, the intercropping effect was excluded from this study.

### 5.2.2 Sample Collection and Pretreatment

In 2019, soil samples were collected from 40 plots belonging to the silage maize rotation, that is, ten plots of each block (Fig. 5.1). One sample collection was performed before sowing, while the second took place after the final harvest. The soil collection consisted of five subsamples from each plot at 0 to 30 cm depth (plough layer). The subsamples were mixed, air-dried, and sieved (2 mm) in order to obtain a composite sample of each plot.

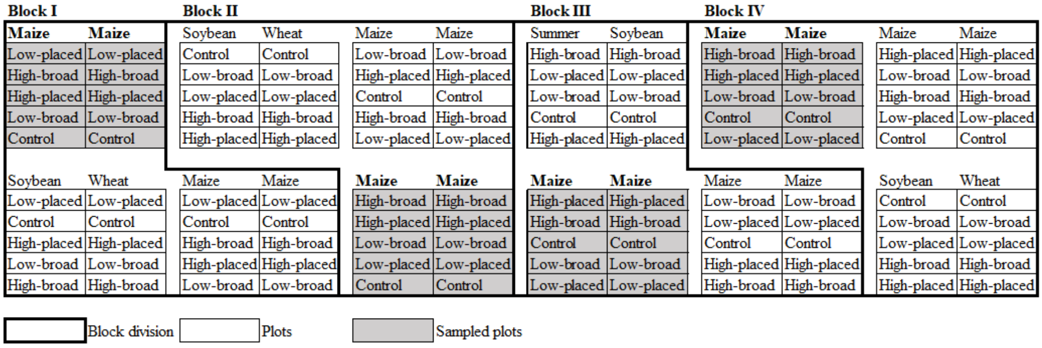



Fig. 5.1 Experimental design and sample collection in the field season of 2019.

Aboveground biomass of silage maize was collected at two different growth stages: the leaf development stage (around BBCH 15) and the ripening stage (around BBCH 85). At the leaf development stage, leaves and stems were analyzed collectively as shoot biomass. In contrast, maize samples were divided into leaves, stems, cobs, and grains, for metal analysis at the ripening stage.

For the metal analysis, maize samples were oven-dried at 75±5°C for a minimum of 56 h (Heraeus UT 6760, Thermo Scientific, Hanau, Germany). Plant milling was performed in several mills, due to the different organ structures and tissue roughness. At the leaf development stage, maize shoots were milled in a MM 400 Retsch, operated with a metal-free jar at 29 Hz for 1.5 min (Verder Scientific, Haan, Germany). At the ripening stage, maize leaves were ground in a metal-free MM 400 (Retsch, Haan, Germany). Stems and cobs were pre-milled in an SM 1 cutting mill (Retsch, Haan, Germany), followed by milling grains, stems, and cobs in a Pulverissete 14 classic (Fritsch, Idar-Oberstein, Germany).

### 5.2.3 Extraction Methods and Analysis

Reagent-grade chemicals were used, and solutions were prepared with $H_2O$ (electrical resistance <18.2 MΩ $cm^{-1}$). All glass materials and containers were rinsed with concentrated $HNO_3$ and $H_2O$ prior to analyses. The Core Facility of the University Hohenheim (CFH) performed the aqua regia extraction for the (pseudo) total metal concentration in soil; the measurement of soil pH was carried out by the $CaCl_2$ method; microwave digestion was used to determine the total metal concentration in silage maize; the analytical method was inductively coupled plasma mass spectrometry (ICP–MS) following standardized methods (Deutsches Institut für Normung e.V., 1997).

To determine the total metal concentration, 3 g of soil were saturated with $C_8H_{18}O$ in 250 mL digestion tubes. Next, 50 mL of aqua regia solution, consisting of 78% HCl and 22% $HNO_3$, was added to each soil sample. The suspension was digested at 140 °C for 3 h in a reflux condenser and filtered through a folded filter MN 280 1/4 into vessels. The digested suspension was used for analyzing the metal concentration in soil samples using a NexION300X ICP–MS (PerkinElmer, Rodgau, Germany).

For the exchangeable fraction by $NH_4NO_3$ extraction, 20 g of soil and 50 mL of 1M $NH_4NO_3$ solution were mixed for 2 h at 120 rpm in a horizontal shaker (GFL® model 2017), then filtered through a 0.45 µm SFCA filter (VWR$^{TMR}$). Subsequently, the soil–extractant solution was stabilized with $HNO_3$ at 1% of the extraction volume. The samples were stored at 4 ± 1 °C until CFH completed the metal analysis by ICP–MS (Deutsches Institut für Normung e.V., 2009; Traub & Scharf, 2001).

For the microwave digestion, 0.2–0.3 g of maize was saturated with 1 mL of $H_2O$ and 2.5 mL of $HNO_3$, followed by digestion for 1.5 h in a Milestone UltraCLAVE III microwave heated digestion reactor (MLS GmbH, Leutkirch, Germany) (VDLUFA, 2011).

After digestion, $H_2O$ was added to each sample solution to reach 10 mL volume for further analytical measurement by ICP–MS.

### 5.2.4 Statistics

In the field design —namely, the RCBD model— one sample of each treatment per block was assumed. However, the failure of planned intercropping with a legume crop (n = 20) provided two samples of each treatment per block. Thus, a linear mixed-effect model was employed to comply with the assumptions of the RCBD design. The model for the variables of interest included sampling time, block division, and the different P treatments as fixed effects. In contrast, the subsample variable was categorized as a random effect. Pearson correlation tests and linear regressions were performed to evaluate the single and combined effects of soil pH, total metal concentration in soil, and exchangeable metal fraction on the metal concentration in silage maize. Testing for normal distribution, analyses of variance (ANOVA) of the linear mixed-effect model, Tukey's test to check for treatment differences at $p < 0.05$, Pearson correlations, and stepwise linear regressions were all conducted using the RStudio® version 1.4.1717 software.

## 5.3 Results

### 5.3.1 Soil pH

Neither the P application rate nor P placement significantly influenced the soil pH response. However, this variable showed significant variation with sampling time and block division (Table 4.2). The mean soil pH before sowing was 5.28 ± 0.02. After the final harvest, the average soil pH reached 5.65 ± 0.02, indicating a significant increase by 0.37 pH units during the growing season. According to Tukey's test, block I had significantly lower soil pH than the other blocks (Table 4.2).

### 5.3.2 Total Metal Concentration in Soil

The ANOVA results revealed that the sampling time and block division strongly influenced the total Cd concentration in soil. A significant increase of 0.021 mg $kg^{-1}$ during the growing season was observed. Significant differences between blocks were observed for the total Cd concentration in soil (Table 5.2).

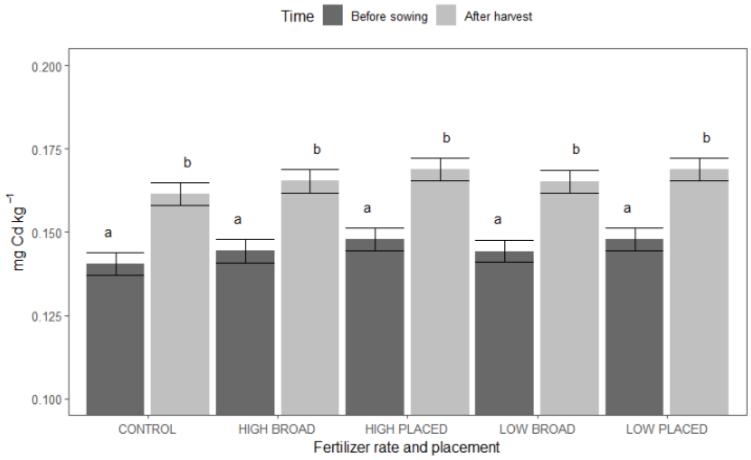
Table 5.2 Significant effect of field variables on the concentrations of Cd and Zn in soil and silage maize (n=80). Significance levels for $p$-value: ***, $p$ <0.001; ; *, $p$ <0.05; ns , $p \geq 0.05$. Different letters indicate significant differences at the $p < 0.05$ level (Tukey's test).

| **Variable** | **Soil pH** | **$Cd_{soil}$** | **$Cd_{NH4NO3}$** | **$Zn_{Soil}$** | **$Zn_{NH4NO3}$** |
|---|---|---|---|---|---|
| Units | | (mg $kg^{-1}$) | (mg $kg^{-1}$) | (mg $kg^{-1}$) | (mg $kg^{-1}$) |
| Block | *** | *** | *** | *** | * |
| I | $5.32 \pm 0.03^{a}$ | $0.166 \pm 0.003^{a}$ | $0.005 \pm 0.003^{a}$ | $66.7 \pm 0.57^{b}$ | $0.125 \pm 0.010^{a}$ |
| II | $5.46 \pm 0.03^{\mathbf{b}}$ | $0.149 \pm 0.003^{b}$ | $0.004 \pm 0.003^{b}$ | $65.3 \pm 0.56^{a,b}$ | $0.114 \pm 0.010^{a,b}$ |
| III | $5.54 \pm 0.03^{b}$ | $0.145 \pm 0.003^{b}$ | $0.003 \pm 0.003^{b}$ | $64.2 \pm 0.59^{b}$ | $0.094 \pm 0.010^{a,b}$ |
| IV | $5.54 \pm 0.03^{b}$ | $0.162 \pm 0.003^{a}$ | $0.004 \pm 0.003^{b}$ | $64.2 \pm 0.59^{b}$ | $0.082 \pm 0.010^{b}$ |
| Time | *** | *** | ns | *** | ns |
| Sowing | $5.28 \pm 0.02^{a}$ | $0.145 \pm 0.002^{a}$ | $0.004 \pm 0.002$ | $63.8 \pm 0.45^{a}$ | $0.101 \pm 0.007$ |
| Harvest | $5.65 \pm 0.02^{b}$ | $0.166 \pm 0.002^{b}$ | $0.004 \pm 0.002$ | $66.3 \pm 0.44^{b}$ | $0.107 \pm 0.007$ |
| Rate | Ns | ns | ns | ns | ns |
| Place | Ns | ns | ns | ns | ns |

None of the P treatments significantly influenced the Cd level in soil, despite Cd being applied through P fertilization at 742.8 mg $ha^{-1}$ in the high application rate and 495.2 mg $ha^{-1}$ in the low application rate (Table 4.2). As expected, the highest total Cd concentration in soil was found in the high application treatment, while the control treatment had the lowest total Cd concentration (Fig. 5.2a).

The total Zn concentration in soil significantly increased throughout the growing season with the highest increases in the control, high–broad, and low–broad treatments, from $63.16 \pm 0.93$ to $66.59 \pm 0.87$ mg $kg^{-1}$, $63.82 \pm 0.81$ to $66.18 \pm 0.81$ mg $kg^{-1}$, and $63.45 \pm 0.78$ to $65.82 \pm 0.78$ mg $kg^{-1}$, respectively. Considering the block division, a significant increase in the total Zn concentration in soil was visible after harvest only in blocks I and II: from $66.01 \pm 0.79$ to $67.32 \pm 0.75$ mg Zn $kg^{-1}$, and from $64.29 \pm 0.75$ to $66.30 \pm 0.79$ mg Zn $kg^{-1}$, respectively.

(**a**)




(**b**)

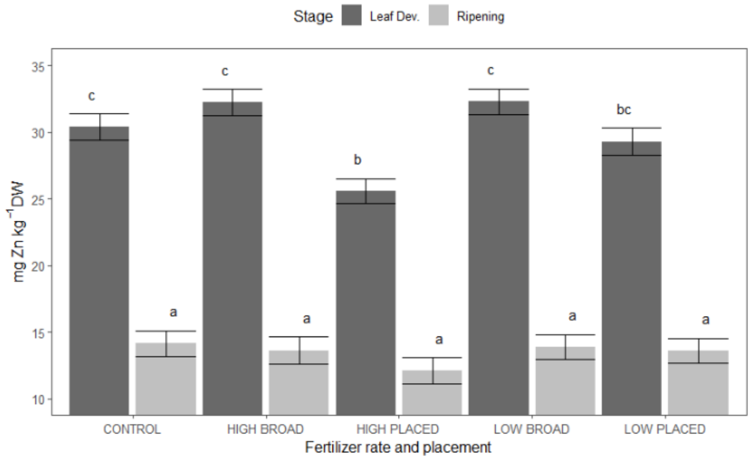



Figure 5.2 Means (bar plot) and standard error (error bar) for P treatments: (a) total Cd concentration in soil and (b) total Zn concentration in maize (n = 80). Different letters indicate significant differences at $p < 0.05$ (Tukey's test).

### 5.3.3 Exchangeable Metal Fraction in soil

According to the ANOVA results, neither the sampling time nor the P treatments significantly affected the exchangeable Cd fraction in soil; however, the block division differed, with a higher exchangeable Cd concentration in block I than in the other blocks (Table 5.2). Similar to Cd, the exchangeable Zn fraction did not significantly change during the growing season, with an average of 0.105 ± 0.008 mg Zn $kg^{-1}$. The block division was the only variable influencing the exchangeable Zn fraction, with the highest concentration in the soil belonging to block I and the lowest in block IV, regardless of the sampling time.

### 5.3.4 Total Metal Concentration in Silage Maize

Unlike the soil fractions, the total Cd concentration in maize shoots indicated a non-normal distribution due to the significant differences between the Cd levels at the two growth stages. Thus, the stages were analyzed separately.

None of the P treatments influenced the total Cd concentration in maize significantly, regardless of the stage. At the leaf development stage, the mean total Cd concentration in maize shoots was 0.133±0.004 mg $kg^{-1}$ DW. At the ripening stage, the average total Cd concentration in the entire plant was reduced to 0.016 ± 0.004 mg $kg^{-1}$ DW, indicating ten times lower Cd concentrations than early development. The metal analysis was performed in different maize organs at the ripening stage, and the results showed that Cd accumulated mainly in leaves, followed by stems. In contrast, the Cd level was below the detection limit (<0.02 mg $kg^{-1}$ DW) in grain and almost all cob samples. Similar to the other variables of interest, the P treatments did not affect Cd accumulation in the different maize organs (Table 5.3).

The total Zn concentration in maize shoots was analyzed separately for each growth stage. Contrary to the other variables of interest, the P placement significantly influenced the total Zn concentration in maize shoots at the leaf development stage, indicating lower Zn concentration in the placed fertilization groups (Figure 5.2b).

The high-placed fertilization group had the lowest Zn concentration in maize and was significantly different from the other P treatments, except the low-placed fertilization group; however, this difference disappeared at the ripening stage. The block division influenced the total Zn concentration in maize shoots at the leaf development stage, with a significantly lower Zn concentration from block I than in maize from the other blocks (see Table 5.2). This statistical variation between the blocks disappeared at the ripening stage. Furthermore, maize from block I had a lower Zn concentration than that from the other blocks. Contrary to Zn levels in maize, block I had the highest exchangeable and total Zn concentration in soil.

Table 5.3 Means of Cd concentration (mg $kg^{-1}$ DW) and standard error (SE) at the ripening stage as a function of P treatment and maize organs (n = 160). Different letters indicate significant differences at the $p < 0.05$ level (Tukey's test).

| **P Treatment/Plant Organ** | **Leaf** | **Stem** | **Grain** | **Cob** | **Total** | **SE** |
|---|---|---|---|---|---|---|
| Control | 0.0531 $^{a}$ | 0.0449 $^{a,b}$ | <0.020 | <0.020 | 0.0189 $^{d}$ | 0.0028 |
| High-broad | 0.0525 $^{a}$ | 0.0443 $^{a,b}$ | <0.020 | 0.0230 $^{c}$ | 0.0182 $^{d}$ | 0.0028 |
| High-placed | 0.0483 $^{a,b}$ | 0.0401 $^{a,b}$ | <0.020 | 0.0350$^{c}$ | 0.0141 $^{d}$ | 0.0028 |
| Low-broad | 0.0449 $^{a,b}$ | 0.0367 $^{b}$ | <0.020 | <0.020 | 0.0182 $^{d}$ | 0.0028 |
| Low-placed | 0.0530 $^{a}$ | 0.0448 $^{a,b}$ | <0.020 | <0.020 | 0.0188 $^{d}$ | 0.0028 |
| Total | 0.0504 $^{a}$ | 0.0422 $^{b}$ | <0.020 | 0.0140 $^{c}$ | 0.0161 $^{d}$ | |
| SE | 0.0017 | 0.0017 | <0.020 | 0.0017 | 0.0017 | |

At the ripening stage, Zn accumulated mainly in leaves, followed by grains, cobs, and stems. The low-placed fertilization group showed a higher Zn concentration in the different organs than the other treatments; however, this difference was not statistically significant (Table 5.4).

Table 5.4 Means of Zn concentration (mg $kg^{-1}$ DW) and standard error (SE) at the ripening stage as a function of P treatments and maize parts (n = 160), different letters indicate significant differences at the $p < 0.05$ level (Tukey's test).

| **P Treatment/Plant Organ** | **Leaf** | **Stem** | **Grain** | **Cob** | **Total** | **SE** |
|---|---|---|---|---|---|---|
| Control | 24.55 $^{d}$ | 7.65 $^{a}$ | 15.37 $^{c}$ | 11.04 $^{b}$ | 13.70 $^{c}$ | 0.63 |
| High-broad | 23.70 $^{d}$ | 6.80 $^{a}$ | 14.51 $^{c}$ | 10.19 $^{b}$ | 12.84 $^{c}$ | 0.63 |
| High-placed | 24.03 $^{d}$ | 7.13 $^{a}$ | 14.85 $^{c}$ | 10.52 $^{b}$ | 13.18 $^{c}$ | 0.63 |
| Low-broad | 24.10 $^{d}$ | 7.20 $^{a}$ | 14.92 $^{c}$ | 10.59 $^{b}$ | 13.25 $^{c}$ | 0.63 |
| Low-placed | 25.22 $^{d}$ | 8.32 $^{a}$ | 16.03 $^{c}$ | 11.71 $^{b}$ | 14.36 $^{c}$ | 0.63 |
| Total | 24.32 $^{d}$ | 7.42 $^{a}$ | 15.14 $^{c}$ | 10.81 $^{b}$ | 13.47 $^{c}$ | |
| SE | 0.47 | 0.47 | 0.47 | 0.48 | 0.47 | |

### 5.3.5 Pearson Correlations and Linear Regressions

The Pearson correlations between soil pH, exchangeable metal fraction, and total metal concentration in soil and maize were calculated. Using the data before sowing and after harvest, the exchangeable Cd concentration was significantly correlated with soil pH (r = –0.46, $p < 0.001$) and with the total Cd concentration in soil (r = 0.25, $p < 0.05$). A stronger correlation occurred between the exchangeable Cd fraction and the corresponding Zn fraction (r = 0.81, $p < 0.001$).

At the leaf development stage, the total Cd concentration in maize correlated significantly and positively with the exchangeable Cd concentration (r = 0.40, $p < 0.05$) and was significantly and negatively associated with the soil pH (r = –0.42, $p < 0.01$). In contrast, the correlation between the total Cd concentration in maize and the total Cd concentration in soil was low and insignificant (r = 0.06, $p > 0.05$). This correlation increased at the ripening stage but remained insignificant (r = 0.28, $p > 0.05$). At this later stage, the total Cd concentration in maize had a higher correlation with the exchangeable Cd fraction (r = 0.51, $p < 0.001$) and soil pH (r = –0.62, $p < 0.001$) than at the leaf development stage.

Before sowing, the soil pH was not significantly associated with the exchangeable Zn concentration (r = –0.28, $p > 0.05$), but this association became highly significant and negative after harvest (r = –0.78, $p < 0.001$). Regarding the total Zn concentration in maize, the correlation with the total Zn concentration in soil was negative and insignificant at the leaf development stage (r = –0.29, $p > 0.05$). At this stage, the total Zn concentration in maize had a positive but insignificant correlation with both the soil pH (r = 0.05, $p > 0.05$) and the exchangeable Zn concentration (r = 0.19, $p > 0.05$). At the ripening stage, these correlations did not become more substantial. The total Zn concentration in grains correlated poorly to the exchangeable Zn concentration at the ripening stage (r = 0.03, $p > 0.05$).

Stepwise linear regressions were employed to evaluate the single and combined effects of soil measurements on the exchangeable Cd fraction and the total Cd concentration in silage maize. Linear regressions with the combined effect of soil pH and the exchangeable Cd concentration were not considered for the total Cd concentration in maize, as the likelihood of multicollinearity between these two variables was extremely high.

Regarding Zn, the Pearson correlation between the exchangeable Zn fraction and the total Zn concentration in silage maize was low and insignificant, regardless of the growth stage. Thus, no stepwise linear regression for the exchangeable Zn fraction was performed. For the total Zn concentration in silage maize, all linear regressions had extremely low $R^2$ values (data not shown).

Considering the Zn measurements, the stepwise regression indicated that 90% of the exchangeable Cd concentration could be explained by the total Cd concentration and the corresponding exchangeable Zn concentration in soil. The total Cd concentration in silage maize had a lower $R^2$ value, having a positive correlation with the total Zn concentration in soil and silage maize and a negative correlation with soil pH. By removing the Zn measurements, the soil pH could explain 34% of the exchangeable Cd fraction.

At the ripening stage, the exchangeable Cd fraction had an $R^2$ of 0.66, regardless of the Zn measurements (Table 5.5). After stepwise regression, the soil pH explained 36% of the total Cd concentration in silage maize; when combined with the total Cd concentration in soil, the coefficient of determination rose to 0.38, and even to 0.46 when the interaction between these variables was considered. This combination showed that, under low Cd conditions and high soil pH, Cd level in maize was low. Contrarily, low pH and high Cd levels in soil resulted in higher Cd accumulation in silage maize at maturity.

Table 5.5 Linear regressions for the exchangeable Cd fraction in soil and the total Cd concentration in silage maize.

| **Stage** | | **Equation** | **$R^2$** |
|---|---|---|---|
| Leaf development | With Zn | $Cd_{NH4NO3} = -0.0011 + 0.0178Cd_{Soil} + 0.0275Zn_{NH4NO3}$ | 0.90 |
| | | $Cd_{Maize} = 0.2273 - 0.0922pH + 0.0041Zn_{Soil} + 0.0044Zn_{Maize}$ | 0.42 |
| | Without Zn | $Cd_{NH4NO3} = 0.0320 - 0.0053pH$ | 0.34 |
| Ripening | | $Cd_{NH4NO3} = 0.0299 - 0.0050pH + 0.0159Cd_{Soil}$ | 0.66 |
| | | $Cd_{Maize} = 0.1099 - 0.0184pH + 0.0605Cd_{Soil}$ | 0.38 |
| | | $Cd_{Maize} = -0.4988 + 3.7540Cd_{Soil} + 0.0888pH - 0.6504pH{:}Cd_{Soil}$ | 0.46 |

## 5.4 Discussion

### 5.4.1 Soil pH

Several measurements in soil and silage maize were performed to identify the possible effects of P fertilization on Cd and Zn concentrations in soil–maize systems. Before sowing, the soil pH was above the critical range for Cd mobilization (4.0–4.5 pH units) (Kabata-Pendias & Pendias, 2001). During the growing season, P, in the form of DAP — $(NH_4)_2HPO_4$— could have temporarily increased the soil pH around fertilizer granules, owing to the $HPO_4^{2-}$ anion taking up $H^+$ under acidic conditions (pH < 7.2). An increase in soil pH after the harvest was also visible in the control plots. If the added urea (N fertilization) in the control treatment was nitrified and taken up with $H^+$, or if maize roots excreted $OH^-$, the soil pH could have increased in the rhizosphere (Hinsinger et al., 2003). Still, a heavy rainfall event (17.28 mm in one hour) occurred on 19 June, resulting in a possible mobilization of soil ions and, thus, increased the soil pH.

### 5.4.2 Total Metal Concentration in Soil

Regardless of the sampling time, the total Cd concentration in soil was in the range for unpolluted soils (0.06 – 1.10 mg $kg^{-1}$) (Kabata-Pendias & Szteke, 2015). After the growing season, an increase in the total Cd concentration in the soil was visible. However, the Cd level stayed below the precautionary limit specified by German standards (0.40 mg Cd $kg^{-1}$ at pH < 6) (LUFA, 1999). The P treatments did not significantly affect the total Cd concentration in soil after the cultivation of maize, likely due to the low Cd concentration in the fertilizer used. As an increase of the total Cd concentration in soil was observed under all P treatments (including the controls) another Cd input is likely. The range of Cd deposition in southwest Germany is approximately 0.15–0.20 g $ha^{-1}$ $y^{-1}$, explaining the increase in total Cd concentration in the soil (Ilyin et al., 2020).

Nonetheless, the Cd input by P fertilizer was higher than the possible atmospheric deposition: 0.50 g Cd $ha^{-1}$ $y^{-1}$ in the low application rate and 0.74 g Cd $ha^{-1}$ $y^{-1}$ in the high application rate.

Generally, the total Zn concentration in soil was slightly higher than the typical range in loamy and clay soil from Germany (40–50 mg $kg^{-1}$), indicating sufficient Zn concentration levels in the field soil (Alloway, 2009; Kabata-Pendias & Pendias, 2001). The total Zn concentration in soil increased after the growing season under all P treatments, including the control plots without any P fertilization. A higher Zn concentration in the field soil might stem from atmospheric deposition in the southwest areas (around 40 g $ha^{-1}$ $y^{-1}$) (Schaap et al., 2018).

### 5.4.3 Exchangeable Metal Fraction

The exchangeable Cd did not significantly change during the growing season, and it was not affected by any P treatment, possibly due to the low Cd concentration in P fertilizer and soil. However, the soil pH and total Cd concentration in soil increased during the growing season. Immobilization by the increased soil pH and Cd uptake by the maize crop could result in the insignificant variation observed in the exchangeable Cd concentration (Niño-Savala et al., 2019). Before sowing and after harvest, the exchangeable Cd fraction did not surpass the limit of 0.04 mg $kg^{-1}$ for agricultural soil, indicating low Cd mobility in soil despite the relatively low soil pH values (BBodSchV, 1999; LWK Niedersachsen, 2015).

Most of the total Zn in soils is often unavailable for plants (<90%), with only 0.1–2.0 mg $kg^{-1}$ belonging to the bioavailable fraction. The exchangeable Zn concentration was in the range of sufficient Zn, explaining the recovery from the negative effect of P fertilizer at maturity (Gupta et al., 2016).

### 5.4.4 Total Metal Concentration in Silage Maize

The P treatments did not affect the total Cd concentration in maize shoots, probably owing to the Cd concentration in the P fertilizer used, which was low compared to the Cd levels found in other P fertilizers used in Germany (Weissengruber et al., 2018). From the leaf development stage to maturity, the Cd accumulation in silage maize decreased by a factor of 10 times. Thus, the Cd limit for feed (1 mg $kg^{-1}$) was not surpassed at the final harvest, presenting no or only a low potential hazard for animal consumption (EC, 2013). The reduction in Cd concentration in silage maize during the growing season was observed under all P treatments, and a dilution effect could explain this phenomenon. This dilution effect might result from nutrient —and coupled Cd— uptake occurring in early development, rather than in the maturity stages of silage maize (Molina et al., 2013). Furthermore, the P levels in soil could have been sufficient for silage maize, leading to high biomass production in all P treatments, including the control plots.

The low grain Cd level could have resulted from an efficient maize defense system to actively exclude translocation from roots and shoots to the grain (Akram et al., 2021; Kato et al., 2020). Cd accumulation occurred mainly in the leaves and stems, rather than in reproductive organs. Cd can enter the plant roots using other metal channels (often enzymatic cofactors for photosynthesis such as Zn). From there, Cd can be translocated to the aerial biomass —especially to leaves, where photosynthesis primarily occurs— explaining the higher Cd concentration found in these rather than in other maize organs, such as the grain (Mousavi, 2011; Song et al., 2017).

The total Zn concentration in maize was significantly lower in the high-placed fertilization group than in other treatments at the leaf development stage, likely due to the high macronutrient uptake at the early stage and the antagonist behavior between P and Zn in the plant uptake process (Gao & Grant, 2012; Grant et al., 2002). The depressant effect of P on Zn levels in maize agrees with the results of Drissi et al. (2015), in which harvested silage maize had a lower Zn concentration when more P was applied. Nonetheless, our results indicated that the negative effect of P disappeared at the ripening stage. At this later stage, the total Zn concentration in leaves, stems, cobs, and grains was not affected by any of the P treatments.

In contrast to Cd, the Zn concentrations in grains were above the detection limit but relatively low (< 20 mg $kg^{-1}$) (Alloway, 2009). Compared to the study of Imran *et al*. (Imran et al., 2016), where the Zn concentration in maize grains was above 20 mg $kg^{-1}$, regardless of the addition or lack of P (in the form of DAP), the total Zn concentration in maize grain was low, regardless of the P treatment. Meanwhile, the Zn concentration in leaves was, on average, 24.49 mg $kg^{-1}$ DW, indicating a sufficient Zn supply to this organ (15–30 mg $kg^{-1}$ DW) (Nieder et al., 2018).

A block division was suitable for the field experiment due to the high heterogeneity in the field soil. Blocks I and IV had a significantly high total and bioavailable Cd concentration in soil compared with blocks II and III. This variation might be due to slight topographic differences between blocks, with the plots within blocks II and II located at a slightly higher elevation than those within blocks I and IV. Thus, the randomized completed block design may have been somewhat biased by the spatial variability and the local differences in soil characteristics, possibly masking the effects of P fertilization in the metal content in soil and silage maize (Jones et al., 2015).

### 5.4.5 Pearson Correlations and Linear Regressions

The exchangeable Cd fraction was moderately correlated to soil pH and total Cd concentration in soil, and strongly to the presence of Zn (Hou, Zheng, Tang, Ji, & Li, 2019; Roberts, 2014). At both stages, the correlation between the total Zn concentration in maize and the exchangeable Zn concentration was low, possibly owing to several factors (e.g., P application, root exudates, soil pH, organic matter content, and microbial communities) controlling the bioavailability and the plant uptake of Zn (Balafrej et al., 2020). The extraction method with 1 M $NH_4NO_3$ might not be optimal to characterize the actual Zn level in silage maize, including the grain. Other techniques, such as DTPA extraction, may be more appropriate than this extraction method (Alloway, 2009).

Before sowing, the exchangeable Zn fraction and the total Cd content in soil explained most of the exchangeable Cd concentration; however, after the harvest, the coefficient of determination of the exchangeable Cd fraction decreased to 0.66, which was explained by soil pH and the total Cd concentration in soil rather than by Zn. In silage maize, Cd level was explained by soil pH and the total Zn concentration in both soil and silage maize at the leaf development stage. Nonetheless, the Cd concentration in mature plants was, instead, described by the soil pH and total Cd concentration in the soil. At the early development stage, the mobile concentrations and plant levels of Cd and Zn were closely correlated. After the growing period, Cd concentration in silage maize and bioavailable Cd level in the soil were instead correlated to the soil pH and soil Cd concentration.

The low $R^2$ for Cd concentration in silage maize, compared to the coefficient of determination of the exchangeable Cd fraction, could be explained by other factors influencing Cd uptake by silage maize, which were not imitated by the extraction with $NH_4NO_3$ (Ning et al., 2019). Still, our results agree with those of Hou *et al*. (Hou, Zheng, Tang, Ji, & Li, 2019), who obtained an $R^2$ lower than 0.50 for a multilinear regression for Cd concentration in different maize organs employing the bioavailable Cd fraction, the organic matter content, and soil pH. Other studies have found higher Pearson correlations and better fitting regressions for Cd concentration in maize when employing the total or bioavailable Cd concentration (Chen et al., 2021; Zhuang et al., 2021); however, these calculations were often performed under Cd-polluted conditions and for maize grains rather than for the entire maize plant.

Soil pH was significant for Cd concentration in silage maize in the present study. Higher Cd levels in silage maize were observed when the soil pH was lower, regardless of the growth stage (Kabata-Pendias & Szteke, 2015; Roberts, 2014); however, the effect of Zn on the bioavailability and mobility of Cd at the early growth stage, which disappeared at maturity, requires further research. At the ripening stage, the interaction between the soil pH and soil Cd concentration was significant for Cd accumulation in silage maize under unpolluted soils. This interaction could have been easily overlooked, as the total Cd concentration in soil appeared insignificant for the plant uptake.

## 5.5 Conclusions

Under unpolluted field conditions, the Cd levels in soil and silage maize were not affected by the application rate or placement of P, regardless of the development stage. Due to high biomass production, the Cd level in silage maize was diluted to 10% of its initial level at the ripening stage, representing no harm for animal consumption. During the growing season, silage maize could recover from the adverse effects of P fertilization on Zn concentration observed at the leaf development stage, leading to relatively adequate Zn levels at maturity.

At the leaf development stage, Cd and Zn bioavailability and their accumulation in silage maize were closely correlated. At maturity, however, Cd concentration was instead related to the soil Cd content and soil pH. Further research is needed to understand Cd uptake and its relation to Zn through the different growth stages in silage maize.

## 5.6 Author Contributions

Conceptualization, A.F. and A.G.N.S.; methodology, A.G.N.S. and B.W.; data analysis, A.G.N.S. and B.W.; sample preparation, A.G.N.S. and B.W.; writing—original draft preparation, A.G.N.S and B.W.; writing—review and editing, J.F., X.L., and A.F.;

supervision, X.L. and A.F. All authors have read and agree to the published version of the manuscript.

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### 5.8 Institutional Review Board Statement

Not applicable.

### 5.9 Informed Consent Statement

Not applicable

### 5.10 Data Availability Statement

The data presented in this study are available from the corresponding author upon request. The data are not publicly available due to being part of an ongoing doctoral thesis.

### 5.11 Acknowledgments

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### 5.12 Conflicts of Interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

# 6. Unpublished data

In 2020, soil and maize samples were collected and analyzed for heavy metal levels at harvest. These results are not part of any publication mentioned in this dissertation, and the results of the statistical analysis are included in Table 6.1. For the soil measurements, the results indicated that the mean labile Cd fraction by the DGT technique, 0.14± 0.03 mg $kg^{-1}$, and the average total Cd concentration in soil, 0.98± 0.53 µg $l^{-1}$, decreased during the second growing season of maize. Despite the reduction of the labile Cd concentration, the soil data indicated that the P application rate significantly influenced the labile Cd fraction at $p < 0.5$. The highest labile Cd concentration was found in the high application rate (P fertilization at 150%), while the lowest Cd level was found in the control treatment (Table 6.1). However, the statistical analysis revealed that the P treatments did not influence the Cd concentration in any maize organ at maturity ( $p >$ 0.05, Table 6.1), with the Cd level in the cobs and grains being under the detection limit. Additionally, the effect of the crop rotation was investigated for soil and maize samples at maturity. Soil pH was still significantly lower where the legume was previously grown even after the mature silage maize was harvested. The labile Cd fraction calculated by the DGT method indicated the same trend, with a significantly higher concentration in the soil where legume was grown than in soil with previously grown wheat.

Table 6.1 Soil pH, total and labile Cd concentration in soil, and total Cd concentration in maize organs at ripening stage, field season 2020 (n=120). Significance levels: p-value: *** <0.001, ** <0.01, * <0.05, ns ≥ 0.05. Different letters indicate significant differences at the p<0.05 level (Tukey test).

| **Variable** | **soil pH** | **$Cd_{soil}$** | **$Cd_{DGT}$** | **$Cd_{Leaf}$** | **$Cd_{Stem}$** |
|---|---|---|---|---|---|
| ***Units*** | | *(mg kg$^{-1}$)* | *(µg l$^{-1}$)* | *(mg kg$^{-1}$ DW)* | *(mg kg$^{-1}$ DW)* |
| Block | *** | *** | *** | *** | *** |
| I | 5.47±0.24$^{b}$ | 0.1041±0.0092$^{c}$ | 1.2582±0.5331$^{a}$ | 0.0538±0.0140$^{a}$ | 0.0449±0.116$^{a}$ |
| II | 5.51±0.12$^{b}$ | 0.1286±0.0374$^{bc}$ | 1.1822±0.3867$^{a}$ | 0.0406± 0.0090$^{ab}$ | 0.0417±0.0139$^{a}$ |
| III | 5.67±0.09$^{b}$ | 0.1698±0.0177$^{ab}$ | 1.1866±0.2818$^{a}$ | 0.0458±0.0076$^{b}$ | 0.0399±0.0138$^{a}$ |
| IV | 6.53±0.39$^{a}$ | 0.1485±0.0166$^{a}$ | 0.3386 ±0.2459$^{b}$ | 0.0353±0.0067$^{b}$ | 0.0282±0.0075a |
| Rate | ns | ns | * | ns | ns |
| Control | 5.79±0.42 | 0.1230±0.0252 | 0.6887±0.3531$^{b}$ | 0.0454±0.0115 | 0.0416±0.0130 |
| Low | 5.79±0.47 | 0.1443±0.0357 | 0.9996±0.5028$^{ab}$ | 0.0458±0.0138 | 0.0402±0.0140 |
| High | 5.79±0.57 | 0.1386±0.0329 | 1.0931±0.6018$^{a}$ | 0.0413±0.0093 | 0.0357±0.0125 |
| Place | ns | ns | ns | ns | ns |
| Place: Rate | ns | ns | ns | ns | ns |
| Prev. Crop | *** | ns | ** | ** | *** |
| Wheat | 5.93±0.59$^{a}$ | 0.1435±0.0318$^{a}$ | 0.7901±0.5298$^{b}$ | 0.0481±0.0140$^{a}$ | 0.0299±0.0065$^{a}$ |
| Winter Pea | 5.66±0.35$^{b}$ | 0.1320±0.0338$^{a}$ | 1.1684±0.4709$^{a}$ | 0.0397±0.0067$^{b}$ | 0.0474±0.0123$^{b}$ |

# 7. General discussion

## 7.1 Contribution of publications

Due to mining and industrial activities, polluted irrigation water, and P fertilizers application, Cd accumulation in arable soils has been studied for a long time. In the first publication, the literature review of derived Cd from P fertilizers allowed the scientific community from diverse disciplines to understand the impact of Cd accumulation in crop-soil systems. Different inputs and outputs of Cd in soil, long-term studies, and Cd content in P fertilizers worldwide were summarized in this publication. Furthermore, this publication provided an overview of the soil characteristics and the plant mechanisms influencing Cd accumulation and mobilization in arable land and crops.

The second publication consisted of a meta-analysis of the available data related to the Cd accumulation in wheat and maize grain in China. The soil Cd content and the soil pH explained 44% of the analyzed data for Cd concentration in maize grain. The soil Cd content, soil pH, and CEC explained 70% of the accumulated Cd found in wheat grain.

The interactive effect of soil properties could influence the Cd accumulation in wheat and maize grain; however, this effect might be masked by the high soil Cd content and soil pH. Furthermore, almost half of the analyzed data of wheat and maize grain surpassed the limit established by the Chinese authorities.

The third publication indicated that a P overfertilization could enhance the accumulation of labile Cd in the short term. Additionally, this publication compared two bioavailability methods on the actual uptake of Cd and Zn by silage maize. Both methods were positively correlated to the Cd concentration in silage maize; however, none of the bioavailability methods appeared to predict the Zn uptake by silage maize, probably by not simulating the suppressant effect of P on Zn concentration in this crop.

In the fourth publication, under field conditions, the effect of different placements and application rates of P fertilizer was insignificant for the Cd uptake by silage maize, regardless of the growth stage. Still, the soil Cd content increased during the growing season. In the case of Zn content, the placed fertilization significantly reduced Zn concentration in young silage maize; however, at maturity, the negative effect of P fertilization disappeared, and silage maize recovered, with no significant difference between the broadcasted and placed fertilization. The high biomass of silage maize very likely diluted Cd and Zn concentration at the ripening stage.

## 7.2 P fertilization and heavy metal concentrations in maize and soil

Regarding soil Cd concentrations, arable land in Germany and Europe is relatively unpolluted, with mean concentrations of 0.31 and 0.28 mg Cd $kg^{-1}$ soil, respectively (Smolders & Six, 2013). The low degree of pollution agrees with the findings of the third and fourth publications, where the soil Cd levels were in the range of unpolluted soils, despite the arable soil being previously used for crop cultivation. On the other hand, the meta-analysis performed in the second publication confirmed that Cd pollution in Chinese arable soils is still a concern for agricultural activities (Zhang et al., 2014; Zhuang et al., 2022).

In the fourth publication, silage maize was fertilized with a fertilizer (diammonium phosphate, DAP) containing low Cd concentration. After one field season, the soil presented changes in pH and concentrations of Cd and Zn. Nevertheless, the P treatments did not impact the total Cd concentration in silage maize, regardless of the developmental stage.

As mentioned in the third publication, a high rate application (150% of required P) and placed fertilization led to a higher bioavailable Cd accumulation in this fertilizer placement. Even though the P fertilizer (DAP) applied to silage maize had a relatively high Cd level, and significant changes in the labile fraction were observed, the total Cd concentration in maize at an early growth stage did not indicate any difference between the different placements or application rates of P fertilizer. The same trend was observed at maturity (*unpublished data*): no effect of P fertilization was observed. These results were similar to those described in the fourth publication, where a P fertilizer with low Cd was applied.

In another field experiment from RS 1.1 located in the Heidfeldhof station at the University of Hohenheim, maize grain intended for feeding was analyzed in 2019. Although the P fertilizer applied to the field contained relatively high Cd levels (23± 0.21 mg $kg^{-1}$ equal to 44.44 mg $kg^{-1}$ $P_2O_5$), the metal analysis indicated that Cd concentration in maize grain was under the detection limit. These results are similar to those in the fourth publication, where silage maize did not accumulate high Cd quantities in reproductive organs such as grain; instead, Cd was accumulated primarily in leaves, followed by stems.

Therefore, silage maize is likely unaffected by Cd derived from P fertilization, resulting from the high forage production during the growing period. It is worth noting that this behavior was independent of P fertilizer containing low or high Cd levels and might be only applicable under uncontaminated conditions.

The low soil Cd levels could explain the low Cd accumulation in maize grain in these field experiments. In maize, Cd accumulates mainly in the roots, with a low percentage being translocated to aboveground biomass (Haider et al., 2021; Rizwan, Ali, & Qayyum et al., 2017). The low Cd percentage that can be transferred to the aboveground biomass concentrates in non-reproductive organs, with a low transfer to grain resulting from its high biomass production (Wang et al., 2014). Furthermore, the uptake and translocation of Cd might differ in maize cultivars according to their genetic variation (Rizwan, Ali, & Qayyum et al., 2017).

Concerning the micronutrient Zn, the P placement significantly affected the Zn concentration in maize at the leaf development stage. A lower Zn concentration in young silage maize was visible under placed P fertilization than under the control or the broadcasted fertilization. The high biomass produced by placed P fertilization could have diluted Zn at the leaf development stage; however, an adverse effect of placed fertilization on the Zn uptake cannot be discarded. In the third publication, the effect of placed fertilization was also visible in the aboveground and root biomass in young silage maize. For the aboveground biomass, the dilution of Zn due to high biomass production can be considered; however, the significantly lower Zn accumulation in the maize roots could indeed be the result of a direct depressant effect of P in the Zn uptake process (Bogdanovic et al., 1999; Suganya et al., 2020; Zhang et al., 2017). This antagonist behavior between P and Zn has been seen in other vegetables and crops, including chia, wheat, and rice (Da Su et al., 2018; Korkmaz et al., 2021; Ryan et al., 2008; Zhang et al., 2017). In these studies, the application rate of P affected the Zn accumulation in crops: a higher P application triggered lower Zn levels in the plant. Contrary to these results, the results of the third and fourth publication indicated that the placement of the P fertilizer rather than the application rate significantly affected Zn levels in silage maize.

### 7.3 Soil characteristics and Cd uptake

Soil pH was the main driver of Cd uptake by silage maize rather than total and bioavailable Cd concentration in the soil in field seasons 2019 and 2020, possibly by the low Cd conditions in the soil. Still, soil pH was at optimum for mobilization and uptake of Cd in both seasons. At the leaf development stage, the Cd concentration in silage maize was relatively higher than the limit for leafy vegetables and cereals (0.1 mg $kg^{-1}$), regardless of the Cd content in the P fertilizers and the field season (European Commission, 2006). At maturity, the results from the fourth publication showed that silage maize diluted Cd to safe levels for animal consumption (<1 mg $kg^{-1}$) (European Commission, 2002). Furthermore, the assessed soil characteristics in the third and fourth publication seemed insufficient to predict Cd uptake by silage maize, considering the results of both publications.

In the second publication, the soil Cd concentration rather than soil pH appeared to be the main driver for Cd accumulation in wheat and maize grain in China; however, this meta-analysis was performed with data from polluted sites as most of the current research for

Cd uptake by crops is done in polluted soils, leading to a possible bias. The regression for grain maize in the second publication was relatively moderate and similar to that for silage maize at maturity in the fourth publication. Thus, the regressions employing soil measurements for Cd uptake by grain and silage maize had a relatively low coefficient of determination, regardless of the degree of pollution in soil and the geographical location.

Contrary to maize, the regressions for Cd accumulation in wheat grain had a high coefficient of determination in the second publication. This phenomenon could stem from the differences between wheat and maize, such as the crop species and type of cultivars (Rizwan et al., 2016; Rizwan, Ali, & Qayyum et al., 2017). The wheat crop might accumulate less Cd than maize; still wheat may transport higher Cd quantities to the grain as a result of its low biomass production compared to the maize crop, which is a fodder crop (Wang et al., 2014). Additionally, it has been proved that maize and wheat slightly influence the Cd chemical speciation. For instance, Cd allocates in a more plant-available fraction in soil when wheat is grown than when maize is cultivated. The Cd concentration might also be higher in wheat than in maize due to a better defense mechanism by maize or the release of organic acids, enhancing the Cd bioavailability and uptake by wheat (Yang et al., 2014). Still, the fourth publication results indicated that the Cd accumulation in maize requires further research to assess the processes and the variables influencing Cd uptake by maize under unpolluted conditions.

## 7.4 Relationship between Cd and Zn in soil accumulation and uptake

Pearson correlations employing soil pH, soil Cd concentration, and bioavailable Cd fraction were calculated in the third publication. In the same publication, the relationship between the uptake of Cd and Zn was studied utilizing the bioconcentration factors and the correspondent coefficient of correlation. The results indicated a high correlation between the Cd uptake and the Zn accumulation in maize.

For the fourth publication, stepwise linear regression employing soil measurements was employed to estimate the exchangeable Cd fraction and the Cd uptake at two different development stages. The linear regressions for the total Cd concentration in silage maize had a moderated $R^2$, regardless of the growth stage. However, before sowing, the exchangeable Cd fraction was highly correlated to the exchangeable Zn concentration in soil ($R^2$=0.90). Furthermore, the total Zn concentration in the soil and silage maize could partly explain the Cd uptake at the leaf development stage, with a positive correlation. At maturity, the best-fitting model for Cd uptake by silage maize did not include Zn levels in the soil or silage maize.

In the third and fourth publication, the relationship between the uptake of Cd and Zn by young silage maize was positive in unpolluted conditions. These results contradict several studies in which Zn decreases the Cd accumulation in edible plants, such as rice, Pak Choi, and wheat (Choudhary et al., 1995; Rafiq et al., 2014; Römkens et al., 2009). The differences between these studies and those presented in this dissertation might be attributed to the specific mechanisms and processes involved in Cd uptake by maize crops. The positive correlation between Cd and Zn in the soil and the silage maize could stem from Cd using Zn transporters (Haider et al., 2021). Moreover, plants that absorb high quantities of Zn, such as maize, can also take up more Cd under Cd stress or Zn deficiency conditions (Hussain et al., 2019).

Another interesting finding was the performance of the bioavailability methods for Zn and the actual Zn uptake by silage maize. In the third publication, it was observed that neither the traditional method with ammonium nitrate extraction nor the DGT technique had a significantly high correlation with the actual uptake by silage maize at the leaf development stage; instead, a moderate correlation was found between the Zn concentration in the roots and the shoots. Only the ammonium nitrate extraction method was employed in the fourth publication, indicating the same trend as in the third publication: no significant correlation was found between the bioavailable Zn fraction and the Zn levels in maize shoots regardless of the development stage. Other single extraction methods for Zn bioavailability assessment, such as $CaCl_2$, EDTA, DTPA, and ammonium acetate, might be more suitable as a surrogate for Zn uptake (Adamo et al., 2018; Feng et al., 2005; Rehman et al., 2018). Still, these results might originate from the suppressive effect of P fertilizer in the Zn uptake by silage maize which might not be simulated by the bioavailability methods (Sönmez et al., 2016).

## 7.5 Crop management and Cd uptake

In 2020, silage maize was cultivated after two different crop rotations: a legume and a cereal. In 2019, these crops were fertilized with P fertilizers containing relatively high Cd concentrations. The mobile Cd fraction increased after only one growing season of the legume and the cereal. Silage maize cultivated after the legume crop accumulated more Cd than the silage maize after wheat cultivation (Liu et al., 2013; Rizwan, Ali, & Qayyum et al., 2017). At maturity, Cd accumulation appeared to be still higher in maize following legume than in maize following wheat, which could be related to the high nutrient uptake and consequent heavy metal uptake, when maize is cultivated in combination with legume crops (Uzoh et al., 2019; Zhu et al., 2016). Furthermore, the soil pH of the legume-maize rotation before the maize growing season and after the maize harvest was significantly lower than the soil pH where summer wheat followed by silage maize was grown, enhancing the Cd mobility in soil (Lambert et al., 2007; Yan et al., 1996). However, the effect of legume crop on the soil pH was still present after the legume-maize crop rotation (*unpublished data*), enhancing the bioavailability of Cd in the field soil.

### 7.6 P fertilizers and their potential risk for Cd pollution in arable soils

Cd limits in P fertilizers and arable soil were not surpassed during the field seasons reported in the third and fourth publication, and thus, labile Cd content in soils was not yet concerning (European Parliament, 2019b; LWK Niedersachsen, 2015). The data analysis for silage maize at maturity in 2020 (*unpublished data*) supported the results of the third publication: overfertilization with polluted P fertilizers causes significant changes in total and labile Cd concentration in soil, despite the plant uptake of Cd. Furthermore, the decrease in labile and total recoverable Cd fraction after harvest in 2020 confirms that Cd derived from the first crop rotation, e.g., cereal and legume, was available for the next crop rotation, e.g., silage maize (Bracher et al., 2021). A high Cd input by P fertilizers could lead to high Cd concentrations in the field soil when crop uptake outputs are lower than Cd inputs (Pérez & Anderson, 2009).

The results of the third publication also showed that even when P fertilizer contained a Cd concentration lower but close to the limit established by European Parliament (60 mg Cd $kg^{-1}$ $P_2O_5$) (European Parliament, 2019b), there was still Cd mobilization after P fertilization, and Cd was bioavailable for the following crop. The Cd limit for P fertilizers might be permissive in the new regulation (EU) No. 2019/1009. In the upcoming years, stricter Cd levels in mineral P fertilizers should be considered to avoid further Cd inputs and mobilization in arable soils derived from these P fertilizers.

According to Dharma-Wardana (2018), the increase in the bioavailable Cd fraction after P fertilization could derive from changes in the soil pH and microbiota due to fertilization action rather than from Cd derived from P fertilizers, refuting the results of the third publication. Nevertheless, the results from the fourth publication showed that when P fertilizers contained low Cd levels (2 mg $kg^{-1}$ equivalent to 4.2 mg Cd $kg^{-1}$ $P_2O_5$), there were no significant changes in the bioavailable fraction after P fertilization, despite soil pH and total Cd concentration increasing after the growing season.

After stopping Cd input via polluted P fertilizers, Cd accumulation might decrease via plant uptake (especially with phytoremediation techniques) (Haider et al., 2021). Nevertheless, a recent study from Gray et al. (2020) revealed that even after stopping the input of Cd via P fertilizers in grasslands for 22 years, soil Cd concentration did not decrease via leaching or plant uptake. Instead, Cd was retained in the soil even under acidic soils conditions, indicating a low Cd bioaccumulation potential in pastures. Still, arable soils can stay polluted years after the Cd addition via P fertilizers.

Since the first publication (mini-review), several studies and reviews regarding Cd accumulation in soil and crop systems have been published (Abedi & Mojiri, 2020; Haider et al., 2021; Oliva et al., 2020; Zhao & Wang, 2020). It is worth noting that in general, the Cd studies are becoming increasingly specialized in plant breeding and new techniques using microorganisms with the aim of increasing the Cd tolerance or decreasing the Cd uptake in staple crops (Anjum et al., 2015; Haider et al., 2021; Zafar-ul-Hye et al., 2020). This specialized research is beneficial for arable soils highly polluted with Cd. Nevertheless, recycled fertilizers and soil amendments, such as struvite and biochar, which naturally contain low Cd concentrations, might be better to avoid further Cd accumulation in arable soils (Bindraban et al., 2020; Das et al., 2020; You et al., 2021). Furthermore, this new fertilizer generation will still produce high field yields comparable to mineral fertilizers, facilitating the closure of the P cycle and decreasing other environmental issues, such as eutrophication (Childers et al., 2011). Additionally, maize breeding and other crops breeding focusing on higher nutrient efficiency, independently of soil P content, could decrease the dependency on external inputs, including mineral P fertilizers, without compromising high field yields (Weiß et al., 2021). Still, Cd accumulation in arable soils remains a challenge in agricultural production, resulting from a high dependency on polluted raw phosphate rock in Germany or due to the increasing anthropogenic activities in countries like China.

## 8. Summary

The problem of polluted agricultural lands with heavy metals due to anthropogenic activities, including applying phosphorous (P) fertilizers polluted with cadmium (Cd) and other metal such as uranium, has been extensively studied. Several reviews, including the one in the present dissertation, have elaborated this issue with often the same results: the application of P fertilizers with high Cd levels is strongly correlated to Cd accumulation in arable soil, which could imply environmental risks as well as health risks for humans and animals through the food chain. Therefore, these reviews have often the same conclusion: the application of low Cd-P fertilizers, either mineral, organic or recycled, is diminishing the risks of Cd pollution at the soil, crop and consumption level. However, globalization, trade politics, economy, dependency on Morocco mineral P fertilizers, and the finite stock in the raw material have challenged this possibility, especially in the European Union. Meanwhile, in China, polluted arable soils are related to other anthropogenic activities and type of fertilizers rather than Cd-polluted phosphate rock and mineral P fertilizers.

At the farm level, other options to diminish Cd pollution in soil and crops, besides low Cd-P fertilizers, could consist of different fertilizer and crop management. These options were studied in this dissertation. A different P management, including different rate applications and placements, did not influence the total Cd concentration in silage maize grown in Germany, regardless of the developmental stage of the crop and the Cd levels in P fertilizer. Silage maize might take up Cd derived from P fertilizers under unpolluted soils, without high risks due to its high biomass production. However, significant changes in the labile Cd fraction were already visible after applying Cd-polluted P fertilizers at 150% of the required amount to the soil after only two growing seasons. Further research should be done to understand the correlations between the bioavailable metal fraction and

the actual Cd uptake by silage maize, especially in unpolluted soils. This recommendation also follows the meta- analysis results presented in the second publication, which indicated a possible bias as most of the studies are performed under polluted conditions.

Considering the results of the third and fourth publication, the Cd uptake by silage maize was strongly correlated to labile Zn in the soil and the Zn uptake at the early development stage after two field seasons. Placed P fertilizer had a significant and negative effect on the Zn uptake by young silage maize. Further research is needed to understand the behavior of Cd and Zn in the uptake process by maize under P fertilization in unpolluted soils.

According to three of the four publications presented in this dissertation, the soil pH was the main soil characteristic influencing the bioavailability and the plant uptake of Cd under unpolluted conditions, regardless of the P treatment, the development stage, and the maize's intended use. However, the total Cd concentration in the soil was the dominant variable for the Cd concentration in maize grain when the soil was polluted with high Cd levels, which was the case in several experiments analyzed in the second publication.

P fertilizers with average Cd contamination might enhance labile Cd accumulation in arable land and crops when applied to low biomass crops, such as wheat and legume crops. In this regard, crop management such as crop rotation in the central field experiment indicated that the wheat rotation induced a lower Cd accumulation in maize-soil systems, owing to wheat likely accumulating Cd at higher levels than other crops. The results presented in the second publication also indicated high Cd accumulation by the wheat crop: the wheat grain accumulated more Cd than the maize grain. Thus, potential hazards related to Cd accumulation in wheat grain should also be considered in wheat-maize systems.

In conclusion, suitable crop rotations considering the crop-specific potential of Cd accumulation, efficient P management including soil P levels and nutrient use efficiency, and low Cd-P fertilizers remain the most viable options and the main challenge to avoid Cd accumulation in arable soils.

## 9. Zusammenfassung

Das Problem der Belastung landwirtschaftlicher Flächen mit Schwermetallen aufgrund anthropogener Aktivitäten, einschließlich des Einsatzes von Phosphor-(P)-Düngemitteln, die mit Cadmium (Cd) und anderen Metallen wie Uran verunreinigt sind, wurde bereits ausführlich untersucht. Mehrere Reviews, einschließlich des Reviews in der vorliegenden Dissertation, haben sich mit diesem Thema befasst und sind häufig zu dem gleichen Ergebnis gekommen: die Anwendung von P-Düngemitteln mit hohem Cd-Gehalt korreliert stark mit der Cd-Anreicherung in Ackerböden, was wiederum sowohl Umweltrisiken als auch Gesundheitsrisiken für Menschen und Tiere über die Nahrungskette zur Folge haben könnte.

Daher haben diese Reviews oftmals dieselbe Schlussfolgerung: die Anwendung von mineralischen, organischen oder recycelten P-Düngemitteln mit geringen Cd-Anteilen reduziert die Cd-Belastung auf den Ebenen Boden, Pflanze und Konsum. Die Globalisierung, die Handelspolitik, die Wirtschaft, die Abhängigkeit von P-Mineraldüngern aus Marokko und die Endlichkeit des Rohstoffs erschweren diese Möglichkeit, insbesondere in der Europäischen Union. Währenddessen stehen in China verunreinigte Ackerböden im Zusammenhang mit anderen anthropogenen Aktivitäten und anderen Arten von Düngemitteln als mit Cd-verunreinigtem Phosphatgestein und mineralischen P- Düngemitteln.

Andere Möglichkeiten neben P-Düngemitteln mit geringen Cd-Anteilen, um Cd-Verschmutzung auf Betriebsebene in Böden und Pflanzen zu verringern, könnten in einem anderen Dünge- und Pflanzenmanagement bestehen. Diese Möglichkeiten wurden in der vorliegenden Dissertation untersucht. Ein anderes P-Management, einschließlich verschiedener Anwendungsmengen und Platzierungen, hatte keinen Einfluss auf die Gesamtkonzentration an Cd bei Silomais, welcher in Deutschland angebaut wurde, unabhängig vom Entwicklungsstadium der Pflanzen und der Cd-Anteile in den P-Düngemitteln. Silomais könnte auf unbelasteten Böden Cd aus P-Düngemitteln aufnehmen, ohne dass wegen der hohe Biomasseproduktion ein hohes Risiko besteht. Signifikante Veränderungen des labilen Cd-Anteils im Boden waren bereits nach der Ausbringung von Cd-verunreinigten P-Düngemitteln in Höhe von 150% der erforderlichen Menge nach nur zwei Vegetationsperioden zu erkennen. Weitere Untersuchungen sind nötig, um die Zusammenhänge zwischen der bioverfügbaren Metallfraktion und der tatsächlichen Cd- Aufnahme von Silomais zu verstehen, insbesondere in unbelasteten Böden. Diese Empfehlung folgt den Ergebnissen der Metaanalyse in der zweiten Publikation, welche eine mögliche Verzerrung zeigt, da die meisten Studien unter belasteten Bedingungen durchgeführt wurden.

Unter Berücksichtigung der Ergebnisse der dritten und vierten Veröffentlichung, korrelierte die Cd-Aufnahme von Silomais mit dem Anteil an labilem Zink (Zn) im Boden und mit der Zn-Aufnahme im frühen Entwicklungsstadium innerhalb von zwei Vegetationsperioden. Platzierte P-Düngung hatte einen signifikanten und negativen Einfluss auf die Zn-Aufnahme durch jungen Silomais. Weitere Untersuchungen sind notwendig, um das Verhalten von Cd und Zn bei der Aufnahme von Mais mit P-Düngemitteln in unbelasteten Böden zu verstehen.

In drei der vier Publikationen der vorliegenden Dissertation war der Boden-pH die wichtigste Bodeneigenschaft mit Einfluss auf die Bioverfügbarkeit und die Pflanzenaufnahme von Cd unter unbelasteten Bedingungen, unabhängig von der P-Anwendung, vom Entwicklungsstadium und der beabsichtigten Maisnutzung. Hingegen war die Gesamt-Cd- Konzentration die dominante Variable für die Cd-Konzentration in den Maiskörnern, wenn der Boden mit hohen Cd-Leveln verunreinigt war, was in einigen Experimenten der Fall war, die in der zweiten Publikation analysiert wurden.

P-Düngemittel mit durchschnittlicher Cd-Kontamination könnten die labile Cd-Anreicherung in Ackerböden und Pflanzen verbessern, wenn sie auf Pflanzen mit geringer Biomasse, wie Weizen und Leguminosen, ausgebracht werden. In dieser Hinsicht wiesen Pflanzenbaumanagementmaßnahmen wie die Fruchtfolge im Hauptfeldexperiment darauf hin, dass eine Fruchtfolge mit Weizen zu einer geringeren Cd-Akkumulation in Maisanbausystemen führt, was darauf zurückzuführen ist, dass Weizen wahrscheinlich höhere Cd-Konzentrationen anreichert als andere Kulturen. Die Ergebnisse in der zweiten Publikation deuteten ebenfalls auf eine Cd-Akkumulation der Weizenpflanze hin: Die Weizenkörner reicherten mehr Cadmium an als die Maiskörner. Daher sollte das Gefahrenpotential in Weizenkörnern auch bei Weizen-Mais-Fruchtfolgen betrachtet werden.

Zusammenfassend lässt sich sagen, dass geeignete Fruchtfolgen unter Berücksichtigung des pflanzenspezifischen Potentials der Cd-Anreicherung, eines effizienten P-Managements, das den P-Gehalt im Boden und der Effizienz der Nährstoffnutzung einschließt, sowie P- Düngemittel mit niedrigem Cd-Gehalt nach wie vor die praktikabelsten Optionen und die Hauptherausforderung zur Vermeidung von Cd-Anreicherung in Ackerböden bleiben.

## 10. References


Abedi, T.; Mojiri, A. (2020). Cadmium Uptake by Wheat (Triticum aestivum L.): An Overview. *Plants (Basel, Switzerland)*, *9*(4). https://doi.org/10.3390/plants9040500

Adamo, P.; Agrelli, D.; Zampella, M. (2018). Chemical speciation to assess bioavailability, bioaccessibility and geochemical forms of potentially toxic metals (PTMs) in polluted soils. In B. de Vivo, H. E. Belkin, & A. Lima (Eds.), *Environmental Geochemistry: Site Characterization, Data Analysis and Case Histories* (2nd ed., pp. 153–194). Elsevier. https://doi.org/10.1016/B978-0-444-63763-5.00010-0

Adams, M.; Zhao, F.; McGrath, S.; Nicholson, F.; Chambers, B. (2004). Predicting cadmium concentrations in wheat and barley grain using soil properties. *Journal of Environmental Quality*, *33*(2), 532–541. https://doi.org/10.2134/jeq2004.5320

Akram, M.; Wahid, A.; Abrar, M.; Manan, A.; Naeem, S.; Zahid, M.; Gilani, M.; Paudyal, R.; Gong, H.; Ran, J.; Deng, J. (2021). Comparative study of six maize (Zea mays L.) cultivars concerning cadmium uptake, partitioning and tolerance. *Applied Ecology and Environmental Research*, *19*(3), 2305–2331. https://doi.org/10.15666/aeer/1903_23052331

Alloway, B. (2009). Soil factors associated with zinc deficiency in crops and humans. *Environmental Geochemistry and Health*, *31*(5), 537–548. https://doi.org/10.1007/s10653-009-9255-4

Andjelkovic, M.; Buha Djordjevic, A.; Antonijevic, E.; Antonijevic, B.; Stanic, M.; Kotur-Stevuljevic, J.; Spasojevic-Kalimanovska, V.; Jovanovic, M.; Boricic, N.; Wallace, D.; Bulat, Z. (2019). Toxic Effect of Acute Cadmium and Lead Exposure in Rat Blood, Liver, and Kidney. *International Journal of Environmental Research and Public Health*, *16*(2). https://doi.org/10.3390/ijerph16020274

Anjum, S.; Tanveer, M.; Hussain, S.; Bao, M.; Wang, L.; Khan, I.; Ullah, E.; Tung, S.; Samad, R.; Shahzad, B. (2015). Cadmium toxicity in Maize (Zea mays L.): consequences on antioxidative systems, reactive oxygen species and cadmium accumulation. *Environmental Science and Pollution Research International*, *22*(21), 17022–17030. https://doi.org/10.1007/s11356-015-4882-z

Aydin, I.; Aydin, F.; Saydut, A.; Bakirdere, E.; Hamamci, C. (2010). Hazardous metal geochemistry of sedimentary phosphate rock used for fertilizer. *Microchemical Journal*, *96*(2), 247–251. https://doi.org/10.1016/j.microc.2010.03.006

Balafrej, H.; Bogusz, D.; Triqui, Z.-E.; Guedira, A.; Bendaou, N.; Smouni, A.; Fahr, M. (2020). Zinc Hyperaccumulation in Plants: A Review. *Plants (Basel, Switzerland)*, *9*(5). https://doi.org/10.3390/plants9050562

Bashir, S.; Bakhsh Gulshan, A.; Iqbal, J.; Husain, A.; Alwahibi, M.; Alkahtani, J.; Dwiningsih, Y.; Bakhsh, A.; Ahmed, N.; Jamal Khan, M.; Ibrahim, M.; Diao, Z.-H. (2021). Comparative role of animal manure and vegetable waste induced compost for polluted soil restoration and maize growth. *Saudi Journal of Biological Sciences*, *28*(4), 2534–2539. https://doi.org/10.1016/j.sjbs.2021.01.057

Bashir, S.; Salam, A.; Chhajro, M.; Fu, Q.; Khan, M.; Zhu, J.; Shaaban, M.; Kubar, K.; Ali, U.; Hu, H. (2018). Comparative efficiency of rice husk-derived biochar (RHB) and steel slag (SS) on cadmium (Cd) mobility and its uptake by Chinese cabbage in highly contaminated soil. *International Journal of Phytoremediation*, *20*(12), 1221–1228. https://doi.org/10.1080/15226514.2018.1448364

BBodSchV. (1999, July 12). *Bundes-Bodenschutz- und Altlastenverordnung vom 12. Juli 1999 (BGBl. I S. 1554), die zuletzt durch Artikel 3 Absatz 4 der Verordnung vom 27.*

*September 2017 (BGBl. I S. 3465) geändert worden ist"*. Germany. BBodSchV. https://www.gesetze-im-internet.de/bbodschv/BBodSchV.pdf

Beaumelle, L.; Vile, D.; Lamy, I.; Vandenbulcke, F.; Gimbert, F.; Hedde, M. (2016). A structural equation model of soil metal bioavailability to earthworms: Confronting causal theory and observations using a laboratory exposure to field-contaminated soils. *The Science of the Total Environment*, *569-570*, 961–972. https://doi.org/10.1016/j.scitotenv.2016.06.023

Bengtsson, H.; Alvenäs, G.; Nilsson, S.; Hultman, B.; Öborn, I. (2006). Cadmium, copper and zinc leaching and surface run-off losses at the Öjebyn farm in Northern Sweden—Temporal and spatial variation. *Agriculture, Ecosystems & Environment*, *113*(1-4), 120–138. https://doi.org/10.1016/j.agee.2005.09.001

Bešter, P.; Lobnik, F.; Eržen, I.; Kastelec, D.; Zupan, M. (2013). Prediction of cadmium concentration in selected home-produced vegetables. *Ecotoxicology and Environmental Safety*, *96*, 182–190. https://doi.org/10.1016/j.ecoenv.2013.06.011

Bigalke, M.; Ulrich, A.; Rehmus, A.; Keller, A. (2017). Accumulation of cadmium and uranium in arable soils in Switzerland. *Environmental Pollution*, *221*, 85–93. https://doi.org/10.1016/j.envpol.2016.11.035

Bindraban, P.; Dimkpa, C.; Pandey, R. (2020). Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. *Biology and Fertility of Soils*, *56*(3), 299–317. https://doi.org/10.1007/s00374-019-01430-2

Bogdanovic, D.; Ubavic, M.; Cuvardic, M. (1999). Effect of phosphorus fertilization on Zn and Cd contents in soil and corn plants. *Nutrient Cycling in Agroecosystems*, *54*(1), 49–56. https://doi.org/10.1023/A:1009779415919

Bolan, N.; Hedley, M.; White, R. (1991). Processes of soil acidification during nitrogen cycling with emphasis on legume based pastures. *Plant and Soil*, *134*(1), 53–63.

Bošković-Rakočević, L.; Pavlović, R.; Đurić, M. (2017). Effect of phosphorus fertilizers on yield and cadmium content of potato tubers. *Acta Agriculturae Serbica*, *22*(44), 37–46. https://doi.org/10.5937/AASer1743037B

Bracher, C.; Frossard, E.; Bigalke, M.; Imseng, M.; Mayer, J.; Wiggenhauser, M. (2021). Tracing the fate of phosphorus fertilizer derived cadmium in soil-fertilizer-wheat systems using enriched stable isotope labeling. *Environmental Pollution*, *287*, 117314. https://doi.org/10.1016/j.envpol.2021.117314

Broadley, M.; White, P.; Hammond, J.; Zelko, I.; Lux, A. (2007). Zinc in plants. *The New Phytologist*, *173*(4), 677–702. https://doi.org/10.1111/j.1469-8137.2007.01996.x

Changling, L.; Guosen, Z.; Hongbo, R.; Jing, Z. (2005). Distribution of heavy metals and nutrients in rainwater in coastal regions between the southern Yellow Sea and East China Sea. *Chinese Journal of Oceanology and Limnology*, *23*(2), 230–237. https://doi.org/10.1007/BF02894244

Chaudhary, D. (2017). Nutritional and nutraceutical/functional properties of maize. In D. Watson (Ed.), *Burleigh Dodds Series in Agricultural Science. Achieving sustainable cultivation of maize Volume 1* (pp. 177–188). Burleigh Dodds Science Publishing. https://doi.org/10.19103/AS.2016.0001.12

Chaudri, A.; McGrath, S.; Gibbs, P.; Chambers, B.; Carlton-Smith, C.; Godley, A.; Bacon, J.; Campbell, C.; Aitken, M. (2007). Cadmium availability to wheat grain in soils treated with sewage sludge or metal salts. *Chemosphere*, *66*(8), 1415–1423. https://doi.org/10.1016/j.chemosphere.2006.09.068

Chen, H.; Teng, Y.; Lu, S.; Wang, Y.; Wang, J. (2015). Contamination features and health risk of soil heavy metals in China. *The Science of the Total Environment*, *512-513*, 143–153.

https://doi.org/10.1016/j.scitotenv.2015.01.025

Chen, R.; Gao, T.; Cheng, N.; Ding, G.; Wang, Q.; Shi, R.; Hu, G.; Cai, X. (2021). Application of DGT/DIFS to assess bioavailable Cd to maize and its release in agricultural soils. *Journal of Hazardous Materials*, *411*, 124837. https://doi.org/10.1016/j.jhazmat.2020.124837

Chien, S.; Menon, R. (1996). Dilution effect of plant biomass on plant cadmium concentration as induced by application of phosphate fertilizers. In C. Rodriguez-Barrueco (Ed.), *Fertilizers and Environment* (pp. 437–442). Springer Netherlands. https://doi.org/10.1007/978-94-009-1586-2_74

Childers, D.; Corman, J.; Edwards, M.; Elser, J. (2011). Sustainability Challenges of Phosphorus and Food: Solutions from Closing the Human Phosphorus Cycle. *BioScience*, *61*(2), 117–124. https://doi.org/10.1525/bio.2011.61.2.6

Choudhary, M.; Bailey, L.; Grant, C.; Leisle, D. (1995). Effect of Zn on the concentration of Cd and Zn in plant tissue of two durum wheat lines. *Canadian Journal of Plant Science*, *75*(2), 445–448. https://doi.org/10.4141/cjps95-074

Cupit, M.; Larsson, O.; Meeûs, C. de; Eduljee, G.; Hutton, M. (2002). Assessment and management of risks arising from exposure to cadmium in fertilisers — II. *Science of the Total Environment*, *291*(1-3), 167–187. https://doi.org/10.1016/S0048-9697(01)01098-1

Da Su; Zhou, L.; Zhao, Q.; Pan, G.; Cheng, F. (2018). Different Phosphorus Supplies Altered the Accumulations and Quantitative Distributions of Phytic Acid, Zinc, and Iron in Rice (Oryza sativa L.) Grains. *Journal of Agricultural and Food Chemistry*, *66*(7), 1601–1611. https://doi.org/10.1021/acs.jafc.7b04883

Dai, Y.; Nasir, M.; Zhang, Y.; Wu, H.; Guo, H.; Lv, J. (2017). Comparison of DGT with traditional methods for assessing cadmium bioavailability to Brassica chinensis in different soils. *Scientific Reports*, *7*(1), 14206. https://doi.org/10.1038/s41598-017-13820-3

Das, S.; Ghosh, G.; Avasthe, R. (2020). Application of biochar in agriculture and environment, and its safety issues. *Biomass Conversion and Biorefinery.* Advance online publication. https://doi.org/10.1007/s13399-020-01013-4

Degryse, F.; Smolders, E.; Zhang, H.; Davison, W. (2009). Predicting availability of mineral elements to plants with the DGT technique: a review of experimental data and interpretation by modelling. *Environmental Chemistry*, *6*(3), 198. https://doi.org/10.1071/EN09010

Delvaoux, M.; Brahy, V. *Mineral Soils conditioned by a Wet (Sub)Tropical Climate*. Belgium. Food and Agriculture Organizations of the United Nations (FAO). http://www.fao.org/3/y1899e/y1899e08a.htm

Deng, C.; Sun, G.; Yang, W.; Li, Z.; Zhang, L.; Ding, J.; Fu, A. (2012). Analysis of the deposition flux and source of heavy metal elements in atmospheric dustfall in Gannan county, Heilongjiang province. *Earth Environ*, *40*(3), 342–348.

Desmidt, E.; Ghyselbrecht, K.; Zhang, Y.; Pinoy, L.; van der Bruggen, B.; Verstraete, W.; Rabaey, K.; Meesschaert, B. (2015). Global Phosphorus Scarcity and Full-Scale P-Recovery Techniques: A Review. *Critical Reviews in Environmental Science and Technology*, *45*(4), 336–384. https://doi.org/10.1080/10643389.2013.866531

Deutsches Institut für Normung e.V. DIN ISO 11466:1997-06 Bodenbeschaffenheit - Extraktion in Königswasser löslicher Spuren elemente, 1997.

Deutsches Institut für Normung e.V. DIN ISO 19730 Bodenbeschaffenheit – Extraktion von Spurenelementen aus Böden mit Ammoniumnitratlösung (ISO 19730:2008), 2009.

Deutsches Maiskomitee e.v. (2020). *Maize- A key crop for the implementation of the arable farming strategy 2035*. Bonn. Deutsches Maiskomitee e.v. (DMK). www.maiskomitee.de

Devillers, D.; Buzier, R.; Charriau, A.; Guibaud, G. (2017). Improving elution strategies for Chelex®-DGT passive samplers. *Analytical and Bioanalytical Chemistry, 409*(30), 7183–7189. https://doi.org/10.1007/s00216-017-0680-4

DGT research Ltd. (2019). *LSNM-NP Loaded DGT device for metals (A) in solution*. Lancaster, Lancashire. DGT research Ltd. https://www.dgtresearch.com/wp-content/uploads/2019/11/DGT-method-LSNM-Nov-2019.pdf

Dharma-Wardana, M. (2018). Fertilizer usage and cadmium in soils, crops and food. *Environmental Geochemistry and Health*, *40*(6), 2739–2759. https://doi.org/10.1007/s10653-018-0140-x

Dikilitas, M.; Karakas, S.; Ahmad, P. (2016). Chapter 13: Effect of Lead on Plant and Human DNA Damages and Its Impact on the Environment. In P. Ahmad (Ed.), *Plant metal interaction: Emerging remediation techniques* (pp. 41–67). Elsevier. https://doi.org/10.1016/B978-0-12-803158-2.00003-5

Ding, C.; Zhang, T.; Wang, X.; Zhou, F.; Yang, Y.; Huang, G. (2013). Prediction model for cadmium transfer from soil to carrot (Daucus carota L.) and its application to derive soil thresholds for food safety. *Journal of Agricultural and Food Chemistry*, *61*(43), 10273–10282. https://doi.org/10.1021/jf4029859

Dittrich, B.; Klose, R. (2008). *Bestimmung und Bewertung von Schwermetallen in Düngemitteln, Bodenhilfsstoffen und Kultursubstraten.* Sächsische Landesanstalt für Landwirtschaft. https://repository.publisso.de/resource/frl:3974607 https://doi.org/10.4126/98-003974607

Drissi, S.; Houssa, A.; Bamouh, A.; Coquant, J.-M.; Benbella, M. (2015). Effect of zinc-phosphorus interaction on corn silage grown on sandy soil. *Agriculture*, *5*(4), 1047–1059. https://doi.org/10.3390/agriculture5041047

EC. Regulation (EC) No. 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, Oficial Journal of the European Union (2013). https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex:32004R0882

European Commission. DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 7 May 2002 on undesirable substances in animal feed, Official Journal of the European Union (2002 & rev. 28.11.2019). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002L0032&qid=1638464437958&from=EN

European Commission. Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, Official Journal of the European Union (2006 & rev. 01.01.2020). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32006R1881

European Commission. (2016, March 17). *Limits for cadmium in phosphate fertilisers: Proposal for a Regulation of the European Parliament and of the Council laying down rules on the making available on the market of CE marked fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009*. Brussels. https://ec.europa.eu/transparency/regdoc/rep/10102/2016/EN/SWD-2016-64-F1-EN-MAIN-PART-2.PDF

European Parliament. Maximum cadmium levels in fertilisers, 2019a. http://www.europarl.europa.eu/doceo/document/E-8-2019-000004_EN.html

European Parliament. Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003, 62 Official Journal of the European Union 1 (2019b). http://data.europa.eu/eli/reg/2019/1009/oj

FAO. (2021). *Crops*. Food and Agriculture Organizations of the United Nations (FAO). http://www.fao.org/faostat/en/#data/QC

FAO. (2022). *Crops and livestock products.* Food and Agriculture Organizations of the United Nations (FAO). https://www.fao.org/faostat/en/#data/QCL

Feng, M.-H.; Shan, X.-Q.; Zhang, S.; Wen, B. (2005). A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl2, and NaNO3 extraction methods for prediction of bioavailability of metals in soil to barley. *Environmental Pollution*, *137*(2), 231–240. https://doi.org/10.1016/j.envpol.2005.02.003

Fijalkowski, K.; Rorat, A.; Grobelak, A.; Kacprzak, M. (2017). The presence of contaminations in sewage sludge - The current situation. *Journal of Environmental Management*, *203*(Pt 3), 1126–1136. https://doi.org/10.1016/j.jenvman.2017.05.068

FNR. (2019). *Bioenergy in Germany Fact and Figures 2020*. Gülzow-Prüzen. FNR. https://www.fnr.de/fileadmin/allgemein/pdf/broschueren/broschuere_basisdaten_bioenergie_2020_engl_web.pdf

Food and Agriculture Organizations of the United Nations. (2019). *What is a black soil?* Food and Agriculture Organizations of the United Nations (FAO). http://www.fao.org/global-soil-partnership/intergovernmental-technical-panel-soils/gsoc17-implementation/internationalnetworkblacksoils/more-on-black-soils/what-is-a-black-soil/en/

Gao, X.; Grant, C. (2012). Cadmium and Zinc Concentration in Grain of Durum Wheat in Relation to Phosphorus Fertilization, Crop Sequence and Tillage Management. *Applied and Environmental Soil Science*, *2012*(1), 1–10. https://doi.org/10.1155/2012/817107

Ge, Y.; Murray, P.; Hendershot, W. (2000). Trace metal speciation and bioavailability in urban soils. *Environmental Pollution*, *107*(1), 137–144. https://doi.org/10.1016/s0269-7491(99)00119-0

Genchi, G.; Sinicropi, M.; Lauria, G.; Carocci, A.; Catalano, A. (2020). The Effects of Cadmium Toxicity. *International Journal of Environmental Research and Public Health*, *17*(11). https://doi.org/10.3390/ijerph17113782

Godt, J.; Scheidig, F.; Grosse-Siestrup, C.; Esche, V.; Brandenburg, P.; Reich, A.; Groneberg, D. (2006). The toxicity of cadmium and resulting hazards for human health. *Journal of Occupational Medicine and Toxicology (London, England)*, *1*, 22. https://doi.org/10.1186/1745-6673-1-22

Grace, J.; Anderson, T.; Olff, H.; Scheiner, S. (2010). On the specification of structural equation models for ecological systems. *Ecological Monographs*, *80*(1), 67–87. https://doi.org/10.1890/09-0464.1

Grant, C.; Bailey, L.; Harapiak, J.; Flore, N. (2002). Effect of phosphate source, rate and cadmium content and use ofPenicillium bilaii on phosphorus, zinc and cadmium concentration in durum wheat grain. *Journal of the Science of Food and Agriculture*, *82*(3), 301–308. https://doi.org/10.1002/jsfa.1034

Grant, C.; Buckley, W.; Bailey, L.; Selles, F. (1998). Cadmium accumulation in crops. *Canadian Journal of Plant Science*, *78*(1), 1–17. https://doi.org/10.4141/P96-100

Grant, C.; Sheppard, S. (2008). Fertilizer Impacts on Cadmium Availability in Agricultural Soils and Crops. *Human and Ecological Risk Assessment: An International Journal*, *14*(2), 210–228. https://doi.org/10.1080/10807030801934895

Gray, C.; McDowell, R.; Condron, L.; McLenaghen, R. (2020). Changes in soil cadmium concentrations with time following cessation of phosphorus fertilizer inputs. *Journal of Environmental Quality*, *49*(4), 1054–1061. https://doi.org/10.1002/jeq2.20086

Gray, C.; McLaren, R.; Roberts, A. (2003). Cadmium leaching from some New Zealand pasture soils. *European Journal of Soil Science*, *54*(1), 159–166. https://doi.org/10.1046/j.1365-2389.2003.00495.x

Gray, C.; McLaren, R.; Roberts, A.; Condron, L. (1999a). The effect of long-term phosphatic fertiliser applications on the amounts and forms of cadmium in soils under pasture in New Zealand. *Nutrient Cycling in Agroecosystems*, *54*(3), 267–277. https://doi.org/10.1023/A:1009883010490

Gray, C.; McLaren, R.; Roberts, A.; Condron, L. (1999b). Effect of soil pH on cadmium phytoavailability in some New Zealand soils. *New Zealand Journal of Crop and Horticultural Science*, *27*(2), 169–179. https://doi.org/10.1080/01140671.1999.9514093

Grüter, R.; Costerousse, B.; Mayer, J.; Mäder, P.; Thonar, C.; Frossard, E.; Schulin, R.; Tandy, S. (2019). Long-term organic matter application reduces cadmium but not zinc concentrations in wheat. *The Science of the Total Environment*, *669*, 608–620. https://doi.org/10.1016/j.scitotenv.2019.03.112

Guan, D.; Sun, F.; Yu, G.; Polizzotto, M.; Liu, Y. (2018). Total and available metal concentrations in soils from six long-term fertilization sites across China. *Environmental Science and Pollution Research International*, *25*, 31666–31678. https://doi.org/10.1007/s11356-018-3143-3

Guo, X.; Wei, Z.; We, Q.; Qiu, J.; Zhou, J. (2011). Cadmium and Zinc Accumulation in Maize Grain as Affected by Cultivars and Chemical Fixation Amendments. *Pedosphere*, *21*(5), 650–656. https://doi.org/10.1016/S1002-0160(11)60167-7

Gupta, D.; Chatterjee, S.; Datta, S.; Veer, V.; Walther, C. (2014). Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals. *Chemosphere*, *108*, 134–144. https://doi.org/10.1016/j.chemosphere.2014.01.030

Gupta, N.; Ram, H.; Kumar, B. (2016). Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. *Reviews in Environmental Science and Bio/Technology*, *15*(1), 89–109. https://doi.org/10.1007/s11157-016-9390-1

Haider, F.; Liqun, C.; Coulter, J.; Cheema, S.; Wu, J.; Zhang, R.; Wenjun, M.; Farooq, M. (2021). Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicology and Environmental Safety*, *211*, 111887. https://doi.org/10.1016/j.ecoenv.2020.111887

Hameed, A.; Rasool, S.; Azooz, M.; Hossain, M.; Ahanger, M.; Ahmad, P. (2016). Heavy Metal Stress: Responses and signaling. In P. Ahmad (Ed.), *Plant metal interaction: Emerging remediation techniques* (pp. 557–583). Elsevier. https://doi.org/10.1016/B978-0-12-803158-2.00024-2

Hatch, D.; Jones, L.; Burau, R. (1988). The effect of pH on the uptake of cadmium by four plant species grown in flowing solution culture. *Plant and Soil*, *105*(1), 121–126. https://doi.org/10.1007/BF02371149

He, S.; Yang, X.; He, Z.; Baligar, V. (2017). Morphological and physiological responses of plants to cadmium toxicity: A review. *Pedosphere*, *27*(3), 421–438. https://doi.org/10.1016/S1002-0160(17)60339-4

He, Z.; Xu, H.; Zhu, Y.; Yang, X.; Chen, G. (2005). Adsorption-Desorption Characteristics of Cadmium in Variable Charge Soils. *Journal of Environmental Science and Health, Part a*, *40*(4), 805–822. https://doi.org/10.1081/ESE-200048273

Hill, G.; Shannon, M. (2019). Copper and Zinc Nutritional Issues for Agricultural Animal Production. *Biological Trace Element Research*, *188*(1), 148–159. https://doi.org/10.1007/s12011-018-1578-5

Hinsinger, P.; Plassard, C.; Tang, C.; Jaillard, B. (2003). Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. *Plant and Soil*, *248(1/2), 43-59*, *248*(1), 43–59. http://www.jstor.org/stable/24129566

Hou, Q.; Yang, Z.; Ji, J.; Yu, T.; Chen, G.; Li, J.; Xia, X.; Zhang, M.; Yuan, X. (2014). Annual net input fluxes of heavy metals of the agro-ecosystem in the Yangtze River delta, China. *Journal of Geochemical Exploration*, *139*, 68–84. https://doi.org/10.1016/j.gexplo.2013.08.007

Hou, S.; Zheng, N.; Tang, L.; Ji, X.; Li, Y. (2019). Effect of soil pH and organic matter content on heavy metals availability in maize (Zea mays L.) rhizospheric soil of non-ferrous metals smelting area. *Environmental Monitoring and Assessment*, *191*(10), 634. https://doi.org/10.1007/s10661-019-7793-5

Hou, S.; Zheng, N.; Tang, L.; Ji, X.; Li, Y.; Hua, X. (2019). Pollution characteristics, sources, and health risk assessment of human exposure to Cu, Zn, Cd and Pb pollution in urban street dust across China between 2009 and 2018. *Environment International*, *128*, 430–437. https://doi.org/10.1016/j.envint.2019.04.046

Houben, D.; Evrard, L.; Sonnet, P. (2013). Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. *Chemosphere*, *92*(11), 1450–1457. https://doi.org/10.1016/j.chemosphere.2013.03.055

Hough, R.; Young, S.; Crout, N. (2003). Modelling of Cd, Cu, Ni, Pb and Zn uptake, by winter wheat and forage maize, from a sewage disposal farm. *Soil Use and Management*, *19*(1), 19–27. https://doi.org/10.1111/j.1475-2743.2003.tb00275.x

Hu, Y.; Ge, Y.; Zhang, C.; Ju, T.; Cheng, W. (2009). Cadmium toxicity and translocation in rice seedlings are reduced by hydrogen peroxide pretreatment. *Plant Growth Regulation*, *59*(1), 51–61. https://doi.org/10.1007/s10725-009-9387-7

Hu, Y.; Cheng, H.; Tao, S. (2016). The Challenges and Solutions for Cadmium-contaminated Rice in China: A Critical Review. *Environment International*, *92-93*, 515–532. https://doi.org/10.1016/j.envint.2016.04.042

Huang, J.; Guo, S.; Zeng, G.-M.; Li, F.; Gu, Y.; Shi, Y.; Shi, L.; Liu, W.; Peng, S. (2018). A new exploration of health risk assessment quantification from sources of soil heavy metals under different land use. *Environmental Pollution (Barking, Essex : 1987)*, *243*(Pt A), 49–58. https://doi.org/10.1016/j.envpol.2018.08.038

Huang, Q.; Yu, Y.; Wan, Y.; Wang, Q.; Luo, Z.; Qiao, Y.; Su, D.; Li, H. (2018). Effects of continuous fertilization on bioavailability and fractionation of cadmium in soil and its uptake by rice (Oryza sativa L.). *Journal of Environmental Management*, *215*, 13–21. https://doi.org/10.1016/j.jenvman.2018.03.036

Huang, Y.; Wang, L.; Wang, W.; Li, T.; He, Z.; Yang, X. (2019). Current status of agricultural soil pollution by heavy metals in China: A meta-analysis. *The Science of the Total Environment*, *651*(Pt 2), 3034–3042. https://doi.org/10.1016/j.scitotenv.2018.10.185

Hussain, S.; Khan, A.; Rengel, Z. (2019). Zinc-biofortified wheat accumulates more cadmium in grains than standard wheat when grown on cadmium-contaminated soil regardless of soil and foliar zinc application. *The Science of the Total Environment*, *654*, 402–408. https://doi.org/10.1016/j.scitotenv.2018.11.097

Ilyin, I.; Travnikov, O.; Schütze, G.; Feigenspan, S.; Uhse, K. (2020). *Country-scale assessment of heavy metal pollution: A case study for Germany.: Technical Report 1/2020*. http://en.msceast.org/reports/1_2020.pdf

Imran, M.; Rehim, A.; Sarwar, N.; Hussain, S. (2016). Zinc bioavailability in maize grains in response of phosphorous-zinc interaction. *Journal of Plant Nutrition and Soil Science*, *179*(1), 60–66. https://doi.org/10.1002/jpln.201500441

International Fertilizer Industry Association. *IFAData*. http://ifadata.fertilizer.org/ucSearch.aspx

Jafarnejadi, A.; Homaee, M.; Sayyad, G.; Bybordi, M. (2011). Large Scale Spatial Variability of Accumulated Cadmium in the Wheat Farm Grains. *Soil and Sediment Contamination: An International Journal*, *20*(1), 98–113. https://doi.org/10.1080/15320383.2011.528472

Jasinski, S. (2019). *Phosphate Rock: Mineral Commodities Summaries.* U.S. Geological Survey. https://prd-wret.s3-us-west-2.amazonaws.com/assets/palladium/production/atoms/files/mcs-2019-phosp.pdf

Jiang, W.; Hou, Q.; Yang, Z.; Yu, T.; Zhong, C.; Yang, Y.; Fu, Y. (2014). Annual input fluxes of heavy metals in agricultural soil of Hainan Island, China. *Environmental Science and Pollution Research International*, *21*(13), 7876–7885. https://doi.org/10.1007/s11356-014-2679-0

Jiao, W.; Chen, W.; Chang, A.; Page, A. (2012). Environmental risks of trace elements associated with long-term phosphate fertilizers applications: a review. *Environmental Pollution (Barking, Essex : 1987)*, *168*, 44–53. https://doi.org/10.1016/j.envpol.2012.03.052

Jinqiu, S.; Chuchen, L.; Xiulan, Y.; Linsheng, Y. (2019). Cadmium distribution characteristics and environmental risk assessment in typical sewage irrigation area of Hebei Province. *Acta Scientiae Circumstantiae*, *39*(3), 917–927.

Jones, M.; Woodward, R.; Stoller, J. (2015). Increasing Precision in Agronomic Field Trials Using Latin Square Designs. *Agronomy Journal*, *107*(1), 20–24. https://doi.org/10.2134/agronj14.0232

Kabata-Pendias, A.; Pendias, H. (2001). *Trace elements in soils and plants* (3rd ed.). CRC Press.

Kabata-Pendias, A.; Szteke, B. (2015). *Trace elements in abiotic and biotic environments: Cadmium [Cd, 48]; Zinc [Zn, 30]*. CRC Press.

Kassir, L.; Darwish, T.; Shaban, A.; Olivier, G.; Ouaini, N. (2012). Mobility and bioavailability of selected trace elements in Mediterranean red soil amended with phosphate fertilizers: Experimental study. *Geoderma*, *189-190*, 357–368. https://doi.org/10.1016/j.geoderma.2012.05.017

Kato, F.; Carvalho, M.; Gaziola, S.; Azevedo, R. (2020). Maize plants have different strategies to protect their developing seeds against cadmium toxicity. *Theoretical and Experimental Plant Physiology*, *32*(3), 203–211. https://doi.org/10.1007/s40626-020-00179-6

Keltjens, W.; van Beusichem, M. (1998). Phytochelatins as biomarkers for heavy metal stress in maize (Zea mays L.) and wheat (Triticum aestivum L.): combined effects of copper and cadmium. *Plant and Soil*, *203*(1), 119–126. https://doi.org/10.1023/A:1004373700581

Khaliq, M.; James, B.; Chen, Y.; Ahmed Saqib, H.; Li, H.; Jayasuriya, P.; Guo, W. (2019). Uptake, translocation, and accumulation of Cd and its interaction with mineral nutrients (Fe, Zn, Ni, Ca, Mg) in upland rice. *Chemosphere*, *215*, 916–924. https://doi.org/10.1016/j.chemosphere.2018.10.077

Khan, M.; Khan, S.; Khan, A.; Alam, M. (2017). Soil contamination with cadmium, consequences and remediation using organic amendments. *The Science of the Total Environment*, *601-602*, 1591–1605. https://doi.org/10.1016/j.scitotenv.2017.06.030

Kim, H.; Seo, B.-H.; Owens, G.; Kim, Y.-N.; Lee, J.-H.; Lee, M.; Kim, K.-R. (2020). Phytoavailability-based threshold values for cadmium in soil for safer crop production. *Ecotoxicology and Environmental Safety*, *201*, 110866. https://doi.org/10.1016/j.ecoenv.2020.110866

Kirkham, M. (2006). Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments. *Geoderma*, *137*(1-2), 19–32. https://doi.org/10.1016/j.geoderma.2006.08.024

Korkmaz, K.; Akgün, M.; Özcan, M.; Özkutlu, F.; Kara, Ş. (2021). Interaction effects of phosphorus (P) and zinc (Zn) on dry matter, concentration and uptake of P and Zn in chia. *Journal of Plant Nutrition*, *44*(5), 755–764. https://doi.org/10.1080/01904167.2020.1845373

Kratz, S.; Schick, J.; Schnug, E. (2016). Trace elements in rock phosphates and P containing mineral and organo-mineral fertilizers sold in Germany. *The Science of the Total Environment*, *542*(Pt B), 1013–1019. https://doi.org/10.1016/j.scitotenv.2015.08.046

Kumar, R.; Mishra, R.; Mishra, V.; Qidwai, A.; Pandey, A.; Shukla, S.; Pandey, M.; Pathak, A.; Dikshit, A. (2016). Detoxification and Tolerance of Heavy Metals in Plants. In P. Ahmad (Ed.), *Plant metal interaction: Emerging remediation techniques* (pp. 335–359). Elsevier. https://doi.org/10.1016/B978-0-12-803158-2.00013-8

Lambert, R.; Grant, C.; Sauvé, S. (2007). Cadmium and zinc in soil solution extracts following the application of phosphate fertilizers. *The Science of the Total Environment*, *378*(3), 293–305. https://doi.org/10.1016/j.scitotenv.2007.02.008

Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen. (2015). *Cadmium-Monitoring zu Böden und Nahrungspflanzen.*

Lee, H.; Owens, V.; Park, S.; Kim, J.; Hong, C. (2018). Adsorption and precipitation of cadmium affected by chemical form and addition rate of phosphate in soils having different levels of cadmium. *Chemosphere*, *206*, 369–375. https://doi.org/10.1016/j.chemosphere.2018.04.176

Legind, C.; Rein, A.; Serre, J.; Brochier, V.; Haudin, C.-S.; Cambier, P.; Houot, S.; Trapp, S. (2012). Simultaneous simulations of uptake in plants and leaching to groundwater of cadmium and lead for arable land amended with compost or farmyard manure. *PloS One*, *7*(10), e47002. https://doi.org/10.1371/journal.pone.0047002

Li, C.; Ding, S.; Yang, L.; Wang, Y.; Ren, M.; Chen, M.; Fan, X.; Lichtfouse, E. (2019). Diffusive gradients in thin films: devices, materials and applications. *Environmental Chemistry Letters*, *17*(2), 801–831. https://doi.org/10.1007/s10311-018-00839-9

Li, F.; Okazaki, M.; Zhou, Q. (2003). Evaluation of Cd uptake by plants estimated from total soil Cd, pH, and organic matter. *Bulletin of Environmental Contamination and Toxicology*, *71*(4), 714–721. https://doi.org/10.1007/s00128-003-0191-1

Li, H.; Luo, N.; Li, Y.; Cai, Q.; Li, H.; Mo, C.; Wong, M. (2017). Cadmium in rice: Transport mechanisms, influencing factors, and minimizing measures. *Environmental Pollution (Barking, Essex : 1987)*, *224*, 622–630. https://doi.org/10.1016/j.envpol.2017.01.087

Li, H.; Yang, Z.; Dai, M.; Diao, X.; Dai, S.; Fang, T.; Dong, X. (2020). Input of Cd from agriculture phosphate fertilizer application in China during 2006-2016. *The Science of the Total Environment*, *698*, 134149. https://doi.org/10.1016/j.scitotenv.2019.134149

Li, L.; Wu, H.; van Gestel, C. A. M.; Peijnenburg, W. J. G. M.; Allen, H. E. (2014). Soil acidification increases metal extractability and bioavailability in old orchard soils of Northeast Jiaodong Peninsula in China. *Environmental Pollution (Barking, Essex : 1987)*, *188*, 144–152. https://doi.org/10.1016/j.envpol.2014.02.003

Li, Y.; Xiong, X.; Lin, C.; Zhang, F.; Wei, L.; Wei, H. (2010). Cadmium in animal production and its potential hazard on Beijing and Fuxin farmlands. *Journal of Hazardous Materials*, *177*(1-3), 475–480. https://doi.org/10.1016/j.jhazmat.2009.12.057

Li, Y.; Wang, L.; Yang, L.; Li, H. (2014). Dynamics of rhizosphere properties and antioxidative responses in wheat (Triticum aestivum L.) under cadmium stress. *Ecotoxicology and Environmental Safety*, *102*, 55–61. https://doi.org/10.1016/j.ecoenv.2014.01.004

Liang, J.; Feng, C.; Zeng, G.; Zhong, M.; Gao, X.; Li, X.; He, X.; Li, X.; Fang, Y.; Mo, D. (2017). Atmospheric deposition of mercury and cadmium impacts on topsoil in a typical coal mine city, Lianyuan, China. *Chemosphere*, *189*, 198–205. https://doi.org/10.1016/j.chemosphere.2017.09.046

Linderholm, K.; Tillman, A.; Mattsson, J. (2012). Life cycle assessment of phosphorus alternatives for Swedish agriculture. *Resources, Conservation and Recycling*, *66*, 27–39. https://doi.org/10.1016/j.resconrec.2012.04.006

Liu, B.; Mo, C.-H.; Zhang, Y. (2019). Using cadmium bioavailability to simultaneously predict its accumulation in crop grains and the bioaccessibility in soils. *The Science of the Total Environment*, *665*, 246–252. https://doi.org/10.1016/j.scitotenv.2019.01.427

Liu, C.; Chang, C.; Fei, Y.; Li, F.; Wang, Q.; Zhai, G.; Lei, J. (2018). Cadmium accumulation in edible flowering cabbages in the Pearl River Delta, China: Critical soil factors and enrichment models. *Environmental Pollution (Barking, Essex : 1987)*, *233*, 880–888. https://doi.org/10.1016/j.envpol.2017.08.092

Liu, G.; Tao, L.; Liu, X.; Hou, J.; Wang, A.; Li, R. (2013). Heavy metal speciation and pollution of agricultural soils along Jishui River in non-ferrous metal mine area in Jiangxi Province, China. *Journal of Geochemical Exploration*, *132*, 156–163. https://doi.org/10.1016/j.gexplo.2013.06.017

Liu, K.; Lv, J.; He, W.; Zhang, H.; Cao, Y.; Dai, Y. (2015). Major factors influencing cadmium uptake from the soil into wheat plants. *Ecotoxicology and Environmental Safety*, *113*, 207–213. https://doi.org/10.1016/j.ecoenv.2014.12.005

Loganathan, P.; Vigneswaran, S.; Kandasamy, J.; Naidu, R. (2012). Cadmium Sorption and Desorption in Soils: A Review. *Critical Reviews in Environmental Science and Technology*, *42*(5), 489–533. https://doi.org/10.1080/10643389.2010.520234

Louekari, K. (2000). *Cadmium in fertilizers risks to human health and the environment*. *Publications: 4/2000*. Ministry of Agriculture and Forestry.

Lu, J.; Yang, X.; Meng, X.; Wang, G.; Lin, Y.; Wang, Y.; Zhao, F. (2017). Predicting Cadmium Safety Thresholds in Soils Based on Cadmium Uptake by Chinese Cabbage. *Pedosphere*, *27*(3), 475–481. https://doi.org/10.1016/S1002-0160(17)60343-6

LUBW. (2020). *Wieder außergewöhnlich warm und heiß, mit Nachwirkungen des Trockenjahrs 2018: Eine klimatische Einordnung des Jahres 2019 für Baden-Württemberg*. Karlsruhe, Baden-Württemberg. LUBW Landesanstalt für Umwelt Baden-Württemberg. https://pudi.lubw.de/detailseite/-/publication/10102

LUFA. (1999). *Vorsorge-, Prüf-und Maßnahmenwerte für Boden gemäß Bundes-Bodenschutz- und Altlastenverordnung (BBodSchV).* Landwirtschaftskammer Nordrhein-Westfalen. https://www.landwirtschaftskammer.de/lufa/download/fachinfo/bodengarten/richtwerte-bbodschv.pdf

Lugon-Moulin, N.; Ryan, L.; Donini, P.; Rossi, L. (2006). Cadmium content of phosphate fertilizers used for tobacco production. *Agronomy for Sustainable Development*, *26*(3), 151–155. https://doi.org/10.1051/agro:2006010

Luo, H.; Du, P.; Shi, J.; Yang, B.; Liang, T.; Wang, P.; Chen, J.; Zhang, Y.; He, Y.; Jia, X.; Duan, G.; Li, F. (2021). Dgt methodology is more sensitive than conventional extraction strategies in assessing amendment-induced soil cadmium availability to rice.

*The Science of the Total Environment*, *760*, 143949. https://doi.org/10.1016/j.scitotenv.2020.143949

Luo, L.; Ma, Y.; Zhang, S.; Wei, D.; Zhu, Y.-G. (2009). An inventory of trace element inputs to agricultural soils in China. *Journal of Environmental Management*, *90*(8), 2524–2530. https://doi.org/10.1016/j.jenvman.2009.01.011

Lux, A.; Lackovič, A.; van Staden, J.; Lišková, D.; Kohanová, J.; Martinka, M. (2015). Cadmium translocation by contractile roots differs from that in regular, non-contractile roots. *Annals of Botany*, *115*(7), 1149–1154. https://doi.org/10.1093/aob/mcv051

Lux, A.; Martinka, M.; Vaculík, M.; White, P. (2011). Root responses to cadmium in the rhizosphere: A review. *Journal of Experimental Botany*, *62*(1), 21–37. https://doi.org/10.1093/jxb/erq281

Lv, J.; Liu, Y. (2019). An integrated approach to identify quantitative sources and hazardous areas of heavy metals in soils. *The Science of the Total Environment*, *646*, 19–28. https://doi.org/10.1016/j.scitotenv.2018.07.257

LWK Niedersachsen. (2015). *Anbauempfehlungen für schwermetallbelastete Böden zur Gewährleistung der Lebensmittel- und Futtermittelqualität*. Oldenburg, Germany. Landwirtschaftskammer Niedersachsen. https://www.lwk-niedersachsen.de/index.cfm/portal/6/nav/196/article/16643.html

Malik, K.; Khan, K.; Rukh, S.; Khan, A.; Akbar, S.; Billah, M.; Bashir, S.; Danish, S.; Alwahibi, M.; Elshikh, M.; Al-Ghamdi, A.; Mustafa, A. (2021). Immobilization of Cd, Pb and Zn through Organic Amendments in Wastewater Irrigated Soils. *Sustainability*, *13*(4), 2392. https://doi.org/10.3390/su13042392

Mar, S.; Okazaki, M. (2012). Investigation of Cd contents in several phosphate rocks used for the production of fertilizer. *Microchemical Journal*, *104*, 17–21. https://doi.org/10.1016/j.microc.2012.03.020

Mclaughlin, M.; Tiller, K.; Naidu, R.; Stevens, D. (1996). Review: the behaviour and environmental impact of contaminants in fertilizers. *Australian Journal of Soil Research*, *34*(1), 1. https://doi.org/10.1071/SR9960001

Mee, C. (2018). *Soil Environmental Quality: Risk Control Standard for Soil Contamination of Agricultural Soil*. GB15618-2018.

Meers, E.; Samson, R.; Tack, F.; Ruttens, A.; Vandegehuchte, M.; Vangronsveld, J.; Verloo, M. (2007). Phytoavailability assessment of heavy metals in soils by single extractions and accumulation by Phaseolus vulgaris. *Environmental and Experimental Botany*, *60*(3), 385–396. https://doi.org/10.1016/j.envexpbot.2006.12.010

MEP, M. (2014). *The Ministry of Environmental Protection; the Ministry of Land and Resources Report on the National Soil Contamination Survey.*

Mirecki, N.; Agič, R.; Ljubomir; Šunić; Milenković, L.; Ilić, Z. (2015). Transfer factor as indicator of heavy metals content in plants. *Fresenius Environmental Bulletin*, *24*, 4212–4219.

Molina, M.; Escudey, M.; Chang, A.; Chen, W.; Arancibia-Miranda, N. (2013). Trace element uptake dynamics for maize (*Zea mays* L.) grown under field conditions. *Plant and Soil*, *370*(1-2), 471–483. https://doi.org/10.1007/s11104-013-1628-x

Molina-Roco, M.; Escudey, M.; Antilén, M.; Arancibia-Miranda, N.; Manquián-Cerda, K. (2018). Distribution of contaminant trace metals inadvertently provided by phosphorus fertilisers: movement, chemical fractions and mass balances in contrasting acidic soils. *Environmental Geochemistry and Health.* Advance online publication. https://doi.org/10.1007/s10653-018-0115-y

Mousavi, S. (2011). Zinc in crop production and interaction with phosphorus. *Australian Journal of Basic and Applied Sciences*, *5*(9), 1503–1509. https://pdfs.semanticscholar.org/253f/57be26413f093583b06f10ef8c1f688f8ee8.pdf

NHFC; S.F.A.D.A. (2017). *National Health and Family Commission and State Food and Drug Adminstation: National Standard for Food Safety and Limit of Contaminants in Food.*

Nieder, R.; Benbi, D.; Reichl, F. (2018). Microelements and Their Role in Human Health. In R. Nieder, D. K. Benbi, & F. X. Reichl (Eds.), *Soil Components and Human Health* (Vol. 47, pp. 317–374). Springer Netherlands. https://doi.org/10.1007/978-94-024-1222-2_7

Ning, Y.; Zhang, X.; Li, B.; Wang, Y.; Guo, J. (2019). Distribution of Cd and Cu Fractions in Chinese Soils and Their Relationships with Soil pH: A Meta-Analysis. *Sustainability*, *11*(2), 337. https://doi.org/10.3390/su11020337

Niño-Savala, A.; Zhuang, Z.; Ma, X.; Fangmeier, A.; Li, H.; Tang, A.; Liu, X. (2019). Cadmium pollution from phosphate fertilizers in arable soils and crops: an overview. *Frontiers of Agricultural Science and Engineering*, *6*(4), 419. https://doi.org/10.15302/J-FASE-2019273

Novotná, M.; Mikeš, O.; Komprdová, K. (2015). Development and comparison of regression models for the uptake of metals into various field crops. *Environmental Pollution (Barking, Essex : 1987)*, *207*, 357–364. https://doi.org/10.1016/j.envpol.2015.09.043

Nziguheba, G.; Smolders, E. (2008). Inputs of trace elements in agricultural soils via phosphate fertilizers in European countries. *The Science of the Total Environment*, *390*(1), 53–57. https://doi.org/10.1016/j.scitotenv.2007.09.031

Oliva, M.; Rubio, K.; Epquin, M.; Marlo, G.; Leiva, S. (2020). Cadmium Uptake in Native Cacao Trees in Agricultural Lands of Bagua, Peru. *Agronomy*, *10*(10), 1551. https://doi.org/10.3390/agronomy10101551

Pan, Y.; Wang, Y. (2015). Atmospheric wet and dry deposition of trace elements at 10 sites in Northern China. *Atmospheric Chemistry and Physics*, *15*(2), 951–972. https://doi.org/10.5194/acp-15-951-2015

Pérez, A.; Anderson, K. (2009). Dgt estimates cadmium accumulation in wheat and potato from phosphate fertilizer applications. *The Science of the Total Environment*, *407*(18), 5096–5103. https://doi.org/10.1016/j.scitotenv.2009.05.045

Phillips, C. J. C [C. J. C.], & Prankel, S. H. (Eds.). (2011). Cadmium and the Welfare of Animals [Special issue]. *Encyclopedia of Environmental Health*, 5. Elsevier.

Płaza, A.; Gąsiorowska, B.; Rzążewska, E. (2019). Heavy metal content in the green fodder of field pea/oat mixtures destined for cattle feed. *Environmental Monitoring and Assessment*, *191*(11), 680. https://doi.org/10.1007/s10661-019-7874-5

Qian, X.; Wang, Z.; Shen, G.; Chen, X.; Tang, Z.; Guo, C.; Gu, H.; Fu, K. (2018). Heavy metals accumulation in soil after 4 years of continuous land application of swine manure: A field-scale monitoring and modeling estimation. *Chemosphere*, *210*, 1029–1034. https://doi.org/10.1016/j.chemosphere.2018.07.107

Qingqing, H.; Xing, L.; Qian, Z. (2016). Evaluating the environmental risk and the bioavailability of Cd in phosphorus fertilizers (in Chinese). *Environmental Science & Technology*, *39*(2), 156–161.

Qiu, M.; Li, T.; Gao, X.; Yin, G.; Zhou, J. (2020). Effects of urbanization on Cd accumulation in agricultural soils: From the perspective of accessibility gradient. *The Science of the Total Environment*, *701*, 134799. https://doi.org/10.1016/j.scitotenv.2019.134799

Qu, X.; Xu, W.; Ren, J.; Zhao, X.; Li, Y.; Gu, X. (2020). A field study to predict Cd bioaccumulation in a soil-wheat system: Application of a geochemical model. *Journal* *of Hazardous Materials*, *400*, 123135. https://doi.org/10.1016/j.jhazmat.2020.123135

Rafiq, M.; Aziz, R.; Yang, X.; Xiao, W.; Stoffella, P.; Saghir, A.; Azam, M.; Li, T. (2014). Phytoavailability of cadmium (Cd) to Pak choi (Brassica chinensis L.) grown in Chinese soils: A model to evaluate the impact of soil Cd pollution on potential dietary toxicity. *PloS One*, *9*(11), e111461. https://doi.org/10.1371/journal.pone.0111461

Ramana, S.; Tripathi, A.; Kumar, A.; Dey, P.; Saha, J.; Patra, A. (2021). Evaluation of Furcraea foetida (L.)Haw. For phytoremediation of cadmium contaminated soils. *Environmental Science and Pollution Research International*, *28*(11), 14177–14181. https://doi.org/10.1007/s11356-021-12534-4

Ran, J.; Wang, D.; Wang, C.; Zhang, G.; Zhang, H. (2016). Heavy metal contents, distribution, and prediction in a regional soil-wheat system. *The Science of the Total Environment*, *544*, 422–431. https://doi.org/10.1016/j.scitotenv.2015.11.105

Rehman, A.; Farooq, M.; Ozturk, L.; Asif, M.; Siddique, K. (2018). Zinc nutrition in wheat-based cropping systems. *Plant and Soil*, *422*(1-2), 283–315. https://doi.org/10.1007/s11104-017-3507-3

Rehman, A.; Jingdong, L. (2017). An econometric analysis of major Chinese food crops: An empirical study. *Cogent Economics & Finance*, *5*(1), 1323372. https://doi.org/10.1080/23322039.2017.1323372

Rehman, H.; Aziz, T.; Farooq, M.; Wakeel, A.; Rengel, Z. (2012). Zinc nutrition in rice production systems: a review. *Plant and Soil*, *361*(1-2), 203–226. https://doi.org/10.1007/s11104-012-1346-9

Retamal-Salgado, J.; Hirzel, J.; Walter, I.; Matus, I. (2017). Bioabsorption and bioaccumulation of cadmium in the straw and grain of maize (Zea mays L.) in growing soils contaminated with cadmium in different environment. *International Journal of Environmental Research and Public Health*, *14*(11). https://doi.org/10.3390/ijerph14111399

Rezapour, S.; Atashpaz, B.; Moghaddam, S.; Kalavrouziotis, I.; Damalas, C. (2019). Cadmium accumulation, translocation factor, and health risk potential in a wastewater-irrigated soil-wheat (Triticum aestivum L.) system. *Chemosphere*, *231*, 579–587. https://doi.org/10.1016/j.chemosphere.2019.05.095

Rizwan, M.; Ali, S.; Abbas, T.; Zia-Ur-Rehman, M.; Hannan, F.; Keller, C.; Al-Wabel, M.; Ok, Y. (2016). Cadmium minimization in wheat: A critical review. *Ecotoxicology and Environmental Safety*, *130*, 43–53. https://doi.org/10.1016/j.ecoenv.2016.04.001

Rizwan, M.; Ali, S.; Adrees, M.; Ibrahim, M.; Tsang, D.; Zia-Ur-Rehman, M.; Zahir, Z.; Rinklebe, J.; Tack, F.; Ok, Y. (2017). A critical review on effects, tolerance mechanisms and management of cadmium in vegetables. *Chemosphere*, *182*, 90–105. https://doi.org/10.1016/j.chemosphere.2017.05.013

Rizwan, M.; Ali, S.; Qayyum, M.; Ok, Y.; Zia-Ur-Rehman, M.; Abbas, Z.; Hannan, F. (2017). Use of Maize (Zea mays L.) for phytomanagement of Cd-contaminated soils: A critical review. *Environmental Geochemistry and Health*, *39*(2), 259–277. https://doi.org/10.1007/s10653-016-9826-0

Roberts, T. (2014). Cadmium and Phosphorous Fertilizers: The Issues and the Science. *Procedia Engineering*, *83*, 52–59. https://doi.org/10.1016/j.proeng.2014.09.012

Rochayati, S.; Verloo, M.; Du Laing, G. (2010). Availability of Cadmium and Zinc as Affected by the Use of Reactive Phosphate Rock, Lime, and Chicken Manure on an Indonesian Acidic Upland Soil under Field Conditions. *Communications in Soil Science and Plant Analysis*, *41*(16), 1986–2003. https://doi.org/10.1080/00103624.2010.495808

Römkens, P.; Guo, H.; Chu, C.; Liu, T.; Chiang, C.; Koopmans, G. (2009). Prediction of

Cadmium uptake by brown rice and derivation of soil-plant transfer models to improve soil protection guidelines. *Environmental Pollution (Barking, Essex : 1987)*, *157*(8-9), 2435–2444. https://doi.org/10.1016/j.envpol.2009.03.009

Römkens, P.; Salomons, W. (1998). Cd, Cu and Zn solubility in arable forest soils: consequences of land use changes for metal mobility and risk assessment. *Soil Science*, *163*, 859–871.

Römkens, P.; Rietra, R.; Kros, H.; Voogd, J.; Vries, W. de. (2018). *Impact of cadmium levels in fertilisers on cadmium accumulation in soil and uptake by food crops: Report 2883*. Wageningen. Wageningen Environmental Research. https://edepot.wur.nl/451353

Rosemarin, A.; Ekane, N. (2016). The governance gap surrounding phosphorus. *Nutrient Cycling in Agroecosystems*, *104*(3), 265–279. https://doi.org/10.1007/s10705-015-9747-9

Roskosch, A.; Heidecke, P. (2019). *Sewage sludge disposal in the Federal Republic of Germany*. Dessau-Roßlau, Germany. German Environmental agency. https://www.umweltbundesamt.de/en/publications

Ryan, M.; McInerney, J.; Record, I.; Angus, J. (2008). Zinc bioavailability in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. *Journal of the Science of Food and Agriculture*, *88*(7), 1208–1216. https://doi.org/10.1002/jsfa.3200

Sanderson, D.-V.; Voutchkov, M.; Benkeblia, N. (2019). Bioaccumulation of cadmium in potato tuber grown on naturally high levels cadmium soils in Jamaica. *The Science of the Total Environment*, *649*, 909–915. https://doi.org/10.1016/j.scitotenv.2018.08.220

Sarwar, N.; Malhi, S.; Zia, M.; Naeem, A.; Bibi, S.; Farid, G. (2010). Role of mineral nutrition in minimizing cadmium accumulation by plants. *Journal of the Science of Food and Agriculture*, *90*(6), 925–937. https://doi.org/10.1002/jsfa.3916

Schaap, M.; Hendriks, C.; Jonkers, S.; Builtjes, P. (2018). *Impacts of Heavy Metal Emission on Air Quality and Ecosystems across Germany – Sources, Transport, Deposition and potential Hazards: Part 1: Assessment of the atmospheric heavy metal deposition to terrestrial ecosystems in Germany.* Umweltbundesamt. http://www.umweltbundesamt.de/publikationen

Schipper, L.; Sparling, G.; Fisk, L.; Dodd, M.; Power, I.; Littler, R. (2011). Rates of accumulation of cadmium and uranium in a New Zealand hill farm soil as a result of long-term use of phosphate fertilizer. *Agriculture, Ecosystems & Environment*, *144*(1), 95–101. https://doi.org/10.1016/j.agee.2011.08.002

Schütze, G [Gudrun], Becker, R., Däammgen, U., Nagel, H.-D., Schlutow, A., & Weigel, H.-J. (Eds.). (2003). Risikoabschätzung der Cadmium-Belastung für Mensch und Umwelt infolge derAnwendung von cadmiumhaltigen Düngemitteln [Special issue]. *Landbauforschung Völkenrode: FAL Agricultural Research*, *2/3*(53), 109. OpenAgrar.

Sebastian, A.; Prasad, M. (2015). Iron- and manganese-assisted cadmium tolerance in Oryza sativa L.: Lowering of rhizotoxicity next to functional photosynthesis. *Planta*, *241*(6), 1519–1528. https://doi.org/10.1007/s00425-015-2276-6

Selim, H. M. (Ed.). (2015). *Advances in trace elements in the environment*. *Phosphate in soils: Interaction with micronutrients, radionuclides and heavy metals*. CRC Press Taylor and Francis Group.

Shahbandeh, M. (2020). *Distribution of global corn production in 2019/2020, by country*. Statista. https://www.statista.com/statistics/254294/distribution-of-global-corn-production-by-country-2012/

Shi, T.; Ma, J.; Wu, F.; Ju, T.; Gong, Y.; Zhang, Y.; Wu, X.; Hou, H.; Zhao, L.; Shi, H. (2019). Mass balance-based inventory of heavy metals inputs to and outputs from agricultural soils in Zhejiang Province, China. *The Science of the Total Environment*, *649*, 1269–1280. https://doi.org/10.1016/j.scitotenv.2018.08.414

Shi, T.; Ma, J.; Wu, X.; Ju, T.; Lin, X.; Zhang, Y.; Li, X.; Gong, Y.; Hou, H.; Zhao, L.; Wu, F. (2018). Inventories of heavy metal inputs and outputs to and from agricultural soils: A review. *Ecotoxicology and Environmental Safety*, *164*, 118–124. https://doi.org/10.1016/j.ecoenv.2018.08.016

Six, L.; Smolders, E. (2014). Future trends in soil cadmium concentration under current cadmium fluxes to European agricultural soils. *The Science of the Total Environment*, *485-486*, 319–328. https://doi.org/10.1016/j.scitotenv.2014.03.109

Smith, J.; Gardner, C.; Costich, D. (2017). Ensuring the genetic diversity of maize and its wild relatives. In D. Watson (Ed.), *Burleigh Dodds Series in Agricultural Science. Achieving sustainable cultivation of maize Volume 1* (pp. 3–50). Burleigh Dodds Science Publishing. https://doi.org/10.19103/AS.2016.0001.02

Smolders, E.; Six, L. (2013). *Revisiting and updating the effect of Revisiting and updating the effect of phosphate fertilizers to cadmium accumulation in European agricultural soil.* Scientific Committee on Health and Environmental Risks (European Comission). http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_168_rd_en.pdf

Song, Y.; Jin, L.; Wang, X. (2017). Cadmium absorption and transportation pathways in plants. *International Journal of Phytoremediation*, *19*(2), 133–141. https://doi.org/10.1080/15226514.2016.1207598

Sönmez, O.; Pierzynski, G.; Kaya, C.; Aydemir, S. (2016). The effects of phosphorus addition on phytoavailability of zinc by diffusive gradients in thin films (DGT). *Turkish Journal of Agriculture and Forestry*, *40*, 379–385. https://doi.org/10.3906/tar-1511-18

Soriano-Disla, J.; Gómez, I.; Navarro-Pedreño, J.; Jordán, M. (2014). The transfer of heavy metals to barley plants from soils amended with sewage sludge with different heavy metal burdens. *Journal of Soils and Sediments*, *14*(4), 687–696. https://doi.org/10.1007/s11368-013-0773-4

Statistisches Bundesamt. (August 2020). *Ackerland nach Hauptnutzungsarten und Kulturarten: Feldfrüchte und Grünland*. Germany. Statistisches Bundesamt (Destatis). https://www.destatis.de/DE/Themen/Branchen-Unternehmen/Landwirtschaft-Forstwirtschaft-Fischerei/Feldfruechte-Gruenland/Tabellen/ackerland-hauptnutzungsarten-kulturarten.html#fussnote-3-123084

Statistisches Bundesamt. (2022). *Feldfrüchte und Grünland.* Statistisches Bundesamt (Destatis). https://www.destatis.de/DE/Themen/Branchen-Unternehmen/Landwirtschaft-Forstwirtschaft-Fischerei/Feldfruechte-Gruenland/Tabellen/gtz-zeitreihe-erntemengen-augewaehlter-anbaukulturen.html

Statistisches Bundesamt; Deutschland. (2019). *Statistisches Jahrbuch Deutschland 2019* (1., Auflage). Statistisches Bundesamt.

Sterckeman, T.; Gossiaux, L.; Guimont, S.; Sirguey, C.; Lin, Z. (2018). Cadmium mass balance in French soils under annual crops: Scenarios for the next century. *The Science of the Total Environment*, *639*, 1440–1452. https://doi.org/10.1016/j.scitotenv.2018.05.225

Suganya, A.; Saravanan, A.; Manivannan, N. (2020). Role of Zinc Nutrition for Increasing Zinc Availability, Uptake, Yield, and Quality of Maize (Zea Mays L.) Grains: An Overview. *Communications in Soil Science and Plant Analysis*, *51*(15), 2001–2021. https://doi.org/10.1080/00103624.2020.1820030

Tan, W.; Li, Z.; Qiu, J.; Zou, B.; Li, N.; Zhuang, P.; Wang, G. (2011). Lime and Phosphate Could Reduce Cadmium Uptake by Five Vegetables Commonly Grown in South China.

*Pedosphere*, *21*(2), 223–229. https://doi.org/10.1016/S1002-0160(11)60121-5

Taylor, M.; Kim, N.; Smidt, G.; Busby, C.; McNally, S.; Robinson, B.; Kratz, S.; Schnug, E. (2016). Trace Element Contaminants and Radioactivity from Phosphate Fertiliser. In E. Schnug & L. J. de Kok (Eds.), *Phosphorus in agriculture: 100% zero* (Vol. 22, pp. 231–266). Springer Berlin Heidelberg. https://doi.org/10.1007/978-94-017-7612-7_12

Tian, K.; Hu, W.; Xing, Z.; Huang, B.; Jia, M.; Wan, M. (2016). Determination and evaluation of heavy metals in soils under two different greenhouse vegetable production systems in eastern China. *Chemosphere*, *165*, 555–563. https://doi.org/10.1016/j.chemosphere.2016.09.012

Tlustos, P.; Szakova, J.; Korinek, K.; Pavlikova, D.; Hanc, A.; Balik, J. (2006). The effect of liming on cadmium, lead, and zinc uptake reduction by spring wheat grown in contaminated soil. *Plant, Soil and Environment*, *52*, 16–24. https://www.agriculturejournals.cz/publicFiles/50512.pdf

Traub, H.; Scharf, H. (2001). NH4NO3 extractable trace element contents of soil samples prepared for proficiency testing - a stability study. *Fresenius' Journal of Analytical Chemistry*, *370*(2-3), 270–274. https://doi.org/10.1007/s002160100748

Tudoreanu, L.; Phillips, C. (2004). Empirical models of cadmium accumulation in maize, rye grass and soya bean plants. *Journal of the Science of Food and Agriculture*, *84*(8), 845–852. https://doi.org/10.1002/jsfa.1730

Ulrich, A. (2019). Cadmium governance in Europe's phosphate fertilizers: Not so fast? *The Science of the Total Environment*, *650*(Pt 1), 541–545. https://doi.org/10.1016/j.scitotenv.2018.09.014

US Geological Survey. (1996, 2000, 2006, 2011, 2016). *Phosphate Rock: Mineral Commodity Summaries*. https://minerals.usgs.gov/minerals/pubs/commodity/phosphate_rock/

US Geological Survey. (1996, 2001, 2006, 2012, 2016). *Phosphate Rock: Mineral Yearbook*. https://minerals.usgs.gov/minerals/pubs/commodity/phosphate_rock/

Uzoh, I.; Igwe, C.; Okebalama, C.; Babalola, O. (2019). Legume-maize rotation effect on maize productivity and soil fertility parameters under selected agronomic practices in a sandy loam soil. *Scientific Reports*, *9*(1), 8539. https://doi.org/10.1038/s41598-019-43679-5

Vatehová, Z.; Malovíková, A.; Kollárová, K.; Kučerová, D.; Lišková, D. (2016). Impact of cadmium stress on two maize hybrids. *Plant Physiology and Biochemistry : PPB*, *108*, 90–98. https://doi.org/10.1016/j.plaphy.2016.06.035

VDLUFA. (2011). *Methodenbuch VII Umweltanalytik* (4th ed.). VDLUFA-Verl.

Viala, Y.; Laurette, J.; Denaix, L.; Gourdain, E.; Méléard, B.; Nguyen, C.; Schneider, A.; Sappin-Didier, V. (2017). Predictive statistical modelling of cadmium content in durum wheat grain based on soil parameters. *Environmental Science and Pollution Research International*, *24*(25), 20641–20654. https://doi.org/10.1007/s11356-017-9712-z

Vieira da Silva, F.; Araújo do Nascimento, C.; Muniz Araújo, P. (2017). Environmental risk of trace elements in P-containing fertilizers marketed in Brazil. *Journal of Soil Science and Plant Nutrition*, *17*(3), 635–647. https://doi.org/10.4067/S0718-95162017000300007

Vig, K.; Megharaj, M.; Sethunathan, N.; Naidu, R. (2003). Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. *Advances in Environmental Research*, *8*(1), 121–135. https://doi.org/10.1016/S1093-0191(02)00135-1

Wajid, K.; Ahmad, K.; Khan, Z.; Nadeem, M.; Bashir, H.; Chen, F.; Ugulu, I. (2020). Effect of

Organic Manure and Mineral Fertilizers on Bioaccumulation and Translocation of Trace Metals in Maize. *Bulletin of Environmental Contamination and Toxicology*, *104*(5), 649–657. https://doi.org/10.1007/s00128-020-02841-w

Wan, D.; Song, L.; Mao, X.; Yang, J.; Jin, Z.; Yang, H. (2019). One-century sediment records of heavy metal pollution on the southeast Mongolian Plateau: Implications for air pollution trend in China. *Chemosphere*, *220*, 539–545. https://doi.org/10.1016/j.chemosphere.2018.12.151

Wang, H.; Dong, Y.; Yang, Y.; Toor, G.; Zhang, X. (2013). Changes in heavy metal contents in animal feeds and manures in an intensive animal production region of China. *Journal of Environmental Sciences*, *25*(12), 2435–2442. https://doi.org/10.1016/S1001-0742(13)60473-8

Wang, K.; Qiao, Y.; Li, H.; Zhang, H.; Yue, S.; Ji, X.; Liu, L. (2018). Structural equation model of the relationship between metals in contaminated soil and in earthworm (Metaphire californica) in Hunan Province, subtropical China. *Ecotoxicology and Environmental Safety*, *156*, 443–451. https://doi.org/10.1016/j.ecoenv.2018.03.038

Wang, Q.; Zhang, J.; Zhao, B.; Xin, X.; Zhang, C.; Zhang, H. (2014). The influence of long-term fertilization on cadmium (Cd) accumulation in soil and its uptake by crops. *Environmental Science and Pollution Research International*, *21*(17), 10377–10385. https://doi.org/10.1007/s11356-014-2939-z

Wang, S.; Wu, W.; Liu, F.; Liao, R.; Hu, Y. (2017). Accumulation of heavy metals in soil-crop systems: A review for wheat and corn. *Environmental Science and Pollution Research International*, *24*(18), 15209–15225. https://doi.org/10.1007/s11356-017-8909-5

Weiß, T.; Leiser, W.; Reineke, A.-J.; Li, D.; Liu, W.; Hahn, V.; Würschum, T. (2021). Optimizing the P balance: How do modern maize hybrids react to different starter fertilizers? *PloS One*, *16*(4), e0250496. https://doi.org/10.1371/journal.pone.0250496

Weissengruber, L.; Möller, K.; Puschenreiter, M.; Friedel, J. (2018). Long-term soil accumulation of potentially toxic elements and selected organic pollutants through application of recycled phosphorus fertilizers for organic farming conditions. *Nutrient Cycling in Agroecosystems*, *110*(3), 427–449. https://doi.org/10.1007/s10705-018-9907-9

Wiechmann, B.; Dienemann, C.; Kabbe, C.; Brandt, S.; Vogel, I.; Roskosch, A. (2015). *Sewage sludge management in Germany*. Germany. Umweltbundesamt. https://www.umweltbundesamt.de/en/publikationen/sewage-sludge-management-in-germany

World Health Organization / Food and Agriculture Organization of the United Nations. JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (2010). https://www.who.int/foodsafety/publications/chem/summary73.pdf

Wu, L.; Tan, C.; Liu, L.; Zhu, P.; Peng, C.; Luo, Y.; Christie, P. (2012). Cadmium bioavailability in surface soils receiving long-term applications of inorganic fertilizers and pig manure. *Geoderma*, *173-174*, 224–230. https://doi.org/10.1016/j.geoderma.2011.12.003

Wu, Y.; Zhang, J.; Ni, Z.; Liu, S.; Jiang, Z.; Huang, X. (2018). Atmospheric deposition of trace elements to Daya Bay, South China Sea: Fluxes and sources. *Marine Pollution Bulletin*, *127*, 672–683. https://doi.org/10.1016/j.marpolbul.2017.12.046

Wyszkowska, J.; Kucharski, J.; Kucharski, M.; Borowik, A. (2012). Effect of cadmium, copper and zinc on plants, soil microorganisms and soil enzymes. *Journal of Elementology*. Advance online publication. https://doi.org/10.5601/jelem.2013.18.4.455

Xia, X.; Yang, Z.; Cui, Y.; Li, Y.; Hou, Q.; Yu, T. (2014). Soil heavy metal concentrations and their typical input and output fluxes on the southern Song-nen Plain, Heilongjiang Province, China. *Journal of Geochemical Exploration*, *139*, 85–96. https://doi.org/10.1016/j.gexplo.2013.06.008

Xiao, D.; Liu, D.; Wang, B.; Feng, P.; Bai, H.; Tang, J. (2020). Climate change impact on yields and water use of wheat and maize in the North China Plain under future climate change scenarios. *Agricultural Water Management*, *238*, 106238. https://doi.org/10.1016/j.agwat.2020.106238

Xing, J.; Song, J.; Yuan, H.; Wang, Q.; Li, X.; Li, N.; Duan, L.; Qu, B. (2017). Atmospheric wet deposition of dissolved trace elements to Jiaozhou Bay, North China: Fluxes, sources and potential effects on aquatic environments. *Chemosphere*, *174*, 428–436. https://doi.org/10.1016/j.chemosphere.2017.02.004

Xu, Y.; Yu, W.; Q., M.; H., Z. (2015). Potential risk of cadmium in a soil-plant system as a result of long-term (10 years) pig manure application. *Plant, Soil and Environment*, *61*(8), 352–357. https://doi.org/10.17221/100/2015-PSE

Xu, Y.; Liang, X.; Xu, Y.; Qin, X.; Huang, Q.; Wang, L.; Sun, Y. (2017). Remediation of Heavy Metal-Polluted Agricultural Soils Using Clay Minerals: A Review. *Pedosphere*, *27*(2), 193–204. https://doi.org/10.1016/S1002-0160(17)60310-2

Xue, P.; Zhao, Q.; Sun, H.; Geng, L.; Yang, Z.; Liu, W. (2019). Characteristics of heavy metals in soils and grains of wheat and maize from farmland irrigated with sewage. *Environmental Science and Pollution Research International*, *26*(6), 5554–5563. https://doi.org/10.1007/s11356-018-3997-4

Yan, F.; Schubert, S.; Mengel, K. (1996). Soil pH changes during legume growth and application of plant material. *Biology and Fertility of Soils*, *23*(3), 236–242. https://doi.org/10.1007/BF00335950

Yang, H.; Zhang, G.; Fu, P.; Li, Z.; Ma, W. (2020). The evaluation of in-site remediation feasibility of Cd-contaminated soils with the addition of typical silicate wastes. *Environmental Pollution (Barking, Essex : 1987)*, *265*(Pt B), 114865. https://doi.org/10.1016/j.envpol.2020.114865

Yang, X.; Li, Q.; Tang, Z.; Zhang, W.; Yu, G.; Shen, Q.; Zhao, F.-J. (2017). Heavy metal concentrations and arsenic speciation in animal manure composts in China. *Waste Management*, *64*, 333–339. https://doi.org/10.1016/j.wasman.2017.03.015

Yang, Y.; Chen, W.; Wang, M.; Peng, C. (2016). Regional accumulation characteristics of cadmium in vegetables: Influencing factors, transfer model and indication of soil threshold content. *Environmental Pollution (Barking, Essex : 1987)*, *219*, 1036–1043. https://doi.org/10.1016/j.envpol.2016.09.003

Yang, Y.; Li, Y.; Chen, W.; Wang, M.; Wang, T.; Dai, Y. (2020). Dynamic interactions between soil cadmium and zinc affect cadmium phytoavailability to rice and wheat: Regional investigation and risk modeling. *Environmental Pollution (Barking, Essex : 1987)*, *267*, 115613. https://doi.org/10.1016/j.envpol.2020.115613

Yang, Y.; Nan, Z.; Zhao, Z. (2014). Bioaccumulation and translocation of cadmium in wheat (Triticum aestivum L.) and maize (Zea mays L.) from the polluted oasis soil of Northwestern China. *Chemical Speciation & Bioavailability*, *26*(1), 43–51. https://doi.org/10.3184/095422914X13888342841789

Yao, Y.; Sun, Q.; Wang, C.; Wang, P.-F.; Ding, S.-M. (2017). Evaluation of organic amendment on the effect of cadmium bioavailability in contaminated soils using the DGT technique and traditional methods. *Environmental Science and Pollution Research International*, *24*(9), 7959–7968. https://doi.org/10.1007/s11356-015-5218-8

Yao, Y.; Sun, Q.; Wang, C.; Wang, P.-F.; Miao, L.-Z.; Ding, S.-M. (2016). The Combination of

DGT Technique and Traditional Chemical Methods for Evaluation of Cadmium Bioavailability in Contaminated Soils with Organic Amendment. *International Journal of Environmental Research and Public Health*, *13*(6). https://doi.org/10.3390/ijerph13060595

Yi, K.; Fan, W.; Chen, J.; Jiang, S.; Huang, S.; Peng, L.; Zeng, Q.; Luo, S. (2018). Annual input and output fluxes of heavy metals to paddy fields in four types of contaminated areas in Hunan Province, China. *The Science of the Total Environment*, *634*, 67–76. https://doi.org/10.1016/j.scitotenv.2018.03.294

You, Y.; Klein, J.; Hartmann, T.; Nkebiwe, P.; Yang, H.; Zhang, W.; Chen, X.; Müller, T. (2021). Producing Superphosphate with Sewage Sludge Ash: Assessment of Phosphorus Availability and Potential Toxic Element Contamination. *Agronomy*, *11*(8), 1506. https://doi.org/10.3390/agronomy11081506

Yu, G.; Zheng, W.; Wang, W.; Dai, F.; Zhang, Z.; Yuan, Y.; Wang, Q. (2017). Health risk assessment of Chinese consumers to Cadmium via dietary intake. *Journal of Trace Elements in Medicine and Biology: Organ of the Society for Minerals and Trace Elements (GMS)*, *44*, 137–145. https://doi.org/10.1016/j.jtemb.2017.07.003

Yu, H.-Y.; Liu, C.; Zhu, J.; Li, F.; Deng, D.-M.; Wang, Q.; Liu, C. (2016). Cadmium availability in rice paddy fields from a mining area: The effects of soil properties highlighting iron fractions and pH value. *Environmental Pollution (Barking, Essex : 1987)*, *209*, 38–45. https://doi.org/10.1016/j.envpol.2015.11.021

Zafar-ul-Hye, M.; Naeem, M.; Danish, S.; Fahad, S.; Datta, R.; Abbas, M.; Rahi, A.; Brtnicky, M.; Holátko, J.; Tarar, Z.; Nasir, M. (2020). Alleviation of Cadmium Adverse Effects by Improving Nutrients Uptake in Bitter Gourd through Cadmium Tolerant Rhizobacteria. *Environments*, *7*(8), 54. https://doi.org/10.3390/environments7080054

Zeng, F.; Ali, S.; Zhang, H.; Ouyang, Y.; Qiu, B.; Wu, F.; Zhang, G. (2011). The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environmental Pollution (Barking, Essex : 1987)*, *159*(1), 84–91. https://doi.org/10.1016/j.envpol.2010.09.019

Zethner, G.; Goodchild, R. (2000). *A risk assessment for cadmium in Austria based on the recommendations of ERM.* Austrian Federal Environment Agency.

Zhang, H.; Luo, Y.; Song, J.; Zhang, H.; Xia, J.; Zhao, Q. (2011). Predicting As, Cd and Pb uptake by rice and vegetables using field data from China. *Journal of Environmental Sciences*, *23*(1), 70–78. https://doi.org/10.1016/S1001-0742(10)60375-0

Zhang, S.; Song, J.; Gao, H.; Zhang, Q.; Lv, M.-C.; Wang, S.; Liu, G.; Pan, Y.-Y.; Christie, P.; Sun, W. (2016). Improving prediction of metal uptake by Chinese cabbage (Brassica pekinensis L.) based on a soil-plant stepwise analysis. *The Science of the Total Environment*, *569-570*, 1595–1605. https://doi.org/10.1016/j.scitotenv.2016.07.007

Zhang, W.; Liu, D.; Liu, Y.; Chen, X.; Zou, C. (2017). Overuse of Phosphorus Fertilizer Reduces the Grain and Flour Protein Contents and Zinc Bioavailability of Winter Wheat (Triticum aestivum L.). *Journal of Agricultural and Food Chemistry*, *65*(8), 1473–1482. https://doi.org/10.1021/acs.jafc.6b04778

Zhang, X.; Chen, D.; Zhong, T.; Zhang, X.; Cheng, M.; Li, X. (2014). Assessment of cadmium (Cd) concentration in arable soil in China. *Environmental Science and Pollution Research International*, *22*(7), 4932–4941. https://doi.org/10.1007/s11356-014-3892-6

Zhang, Y.; Zhang, S.; Zhu, F.; Wang, A.; Dai, H.; Cheng, S.; Wang, J.; Tang, L. (2018). Atmospheric heavy metal deposition in agro-ecosystems in China. *Environmental Science and Pollution Research International*, *25*(6), 5822–5831. https://doi.org/10.1007/s11356-017-0892-3

Zhao, F.; Jiang, R.; Dunham, S.; McGrath, S. (2006). Cadmium uptake, translocation and tolerance in the hyperaccumulator Arabidopsis halleri. *The New Phytologist*, *172*(4), 646–654. https://doi.org/10.1111/j.1469-8137.2006.01867.x

Zhao, F.-J.; Wang, P. (2020). Arsenic and cadmium accumulation in rice and mitigation strategies. *Plant and Soil*, *446*(1-2), 1–21. https://doi.org/10.1007/s11104-019-04374-6

Zhao, K.; Zhang, L.; Dong, J.; Wu, J.; Ye, Z.; Zhao, W.; Ding, L.; Fu, W. (2020). Risk assessment, spatial patterns and source apportionment of soil heavy metals in a typical Chinese hickory plantation region of southeastern China. *Geoderma*, *360*, 114011. https://doi.org/10.1016/j.geoderma.2019.114011

Zhao, X.; Jiang, T.; Du, B. (2014). Effect of organic matter and calcium carbonate on behaviors of cadmium adsorption-desorption on/from purple paddy soils. *Chemosphere*, *99*, 41–48. https://doi.org/10.1016/j.chemosphere.2013.09.030

Zhen, H.; Jia, L.; Huang, C.; Qiao, Y.; Li, J.; Li, H.; Chen, Q.; Wan, Y. (2020). Long-term effects of intensive application of manure on heavy metal pollution risk in protected-field vegetable production. *Environmental Pollution (Barking, Essex : 1987)*, *263*(Pt A), 114552. https://doi.org/10.1016/j.envpol.2020.114552

Zheng, S.; Wang, Q.; Yu, H.; Huang, X.; Li, F. (2020). Interactive effects of multiple heavy metal(loid)s on their bioavailability in cocontaminated paddy soils in a large region. *The Science of the Total Environment*, *708*, 135126. https://doi.org/10.1016/j.scitotenv.2019.135126

Zhou, S.; Liu, J.; Xu, M.; Lv, J.; Sun, N. (2015). Accumulation, availability, and uptake of heavy metals in a red soil after 22-year fertilization and cropping. *Environmental Science and Pollution Research International*, *22*(19), 15154–15163. https://doi.org/10.1007/s11356-015-4745-7

Zhu, S.; Ma, X.; Guo, R.; Ai, S.; Liu, B.; Zhang, W.; Zhang, Y. (2016). A field study on heavy metals phytoattenuation potential of monocropping and intercropping of maize and/or legumes in weakly alkaline soils. *International Journal of Phytoremediation*, *18*(10), 1014–1021. https://doi.org/10.1080/15226514.2016.1183570

Zhuang, Z.; Niño-Savala, A.; Mi, Z.; Wan, Y.; Su, D.; Li, H.; Fangmeier, A. (2021). Cadmium accumulation in wheat and maize grains from China: Interaction of soil properties, novel enrichment models and soil thresholds. *Environmental Pollution*, *275*, 116623. https://doi.org/10.1016/j.envpol.2021.116623

Zhuang, Z.; Wang, Q.; Huang, S.; Niño Savala, A.; Wan, Y.; Li, H.; Schweiger, A.; Fangmeier, A.; Franzaring, J. (2022). Source-specific risk assessment for cadmium in wheat and maize: Towards an enrichment model for China. *Journal of Environmental Sciences.* Advance online publication. https://doi.org/10.1016/j.jes.2022.02.024

Zwolak, I. (2020). The Role of Selenium in Arsenic and Cadmium Toxicity: An Updated Review of Scientific Literature. *Biological Trace Element Research*, *193*(1), 44–63. https://doi.org/10.1007/s12011-019-01691-w

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# Curriculum Vitae

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**Personal data**

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Bad Kreuznach, 16$^{th}$ April 2022

**Annex 3**

**Declaration in lieu of an oath on independent work**

**according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences**

1. The dissertation submitted on the topic

Heavy metals from phosphate fertilizers in maize-based food-feed-energy systems

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is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Bad Kreuznach, 18th April 2022 | Andrea Giovanna Niño Savala

Place, Date | Signature