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Cd and Zn Concentrations in Soil and Silage Maize following the Addition of P Fertilizer

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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, the Zn uptake 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 uptake, while the Zn level decreased to 50% of the initial uptake. 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 uptake and its relation to Zn under field conditions at early and mature stages.

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

1. Introduction

Cd is a heavy metal with high mobility and bioaccumulation capacity, representing a potential hazard for crop production [1]. In plants, Cd can generate oxidative stress and disturb enzyme activity, photosynthesis, and nutrient uptake, thus affecting plant metabolism and development [2]. 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 [3,4]. 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 [5].

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



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). 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 [6]. In Germany, the arable land used for silage maize increased from 2.2 million ha in 2018 to 2.7 million ha in 2020 [7,8]; 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 [9,10].

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 [11–13]. 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 [14]; however, a high P concentration could also enhance biomass production in plants, leading to a Zn concentration dilution effect [15].

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 uptake by assessing the bioavailable metal fraction than the aqua regia method, which extracts the (pseudo) total metal concentration in soils [16]. A common technique for determining the exchangeable fraction (readily bioavailable) in soils is the single-extraction method using 1 M NH₄NO₃ solution, e.g., to monitor and prevent high Cd bioaccumulation in staple crops in Germany [17]. This method has been used to characterize the Cd concentration found in grain wheat [18].

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, 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 uptake by silage 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 uptake by silage maize, according to the growth stage.

2. Materials and Methods

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 monthly precipitation was 0.018 mm. The coldest month was May, with a registered monthly temperature of 10.8 °C. July was the warmest month, with a monthly temperature of 35.8 °C. The highest monthly precipitation was 0.026 mm in June, and the lowest monthly precipitation was recorded in September with a mean of 0.009 mm.

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 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 and 58.80 ± 3.80 mg Zn kg⁻¹.

P treatment	Phosphorus	Fertilizer	Nitrogen	Fertilizer	Potassium	Fertilizer	
Units	kg (P_2O_5) ha ⁻¹	kg ha ⁻¹ (DAP)	${ m kg}{ m N}{ m ha}^{-1}$	kg ha $^{-1}$ (urea)	kg K ₂ O ha $^{-1}$	kg ha ⁻¹ (Patentkali 30% K ₂ O)	
Low-placed Low-broad	114	248		294			
High-placed High-broad	171	372	180	245	368	1127	
Čontrol	No P applied	No P applied		391			

Table 1. Description of the experimental design for silage maize.

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

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 (Figure 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.



Figure 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).

2.3. Extraction Methods and Analysis

Reagent-grade chemicals were used, and solutions were prepared with H_2O (electrical resistance < 18.2 M Ω cm⁻¹). All glass materials and containers were rinsed with concentrated HNO₃ and H₂O 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 CaCl₂ 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 [19].

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% HNO₃, 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 NH₄NO₃ extraction, 20 g of soil and 50 mL of 1M NH₄NO₃ solution were mixed for 2 h at 120 rpm in a horizontal shaker (GFL®model 2017), then filtered through a 0.45 μ m SFCA filter (VWR^{TMR}). Subsequently, the soil–extractant solution was stabilized with HNO₃ at 1% of the extraction volume. The samples were stored at 4 ± 1 °C until CFH completed the metal analysis by ICP–MS [20,21].

For the microwave digestion, 0.2–0.3 g of maize was saturated with 1 mL of H_2O and 2.5 mL of HNO₃, followed by digestion for 1.5 h in a Milestone UltraCLAVE III microwave heated digestion reactor (MLS GmbH, Leutkirch, Germany) [22]. After digestion, H_2O was added to each sample solution to reach 10 mL volume for further analytical measurement by ICP–MS.

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.

3. Results

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 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 2).

Variable	Soil pH	Cd _{soil}	$Cd_{NH_4NO_3}$	Zn _{Soil}	$Zn_{\rm NH_4NO_3}$
Units		$(mg kg^{-1})$	$(mg kg^{-1})$	$(\mathrm{mg}\mathrm{kg}^{-1})$	$(mg kg^{-1})$
Block	***	***	***	***	*
Ι	5.32 ± 0.03 ^a	0.166 ± 0.003 $^{\rm a}$	$0.005 \pm 0.003 \; ^{\rm a}$	66.7 ± 0.57 ^b	0.125 ± 0.010 a
II	5.46 ± 0.03 ^b	$0.149 \pm 0.003 \ ^{\mathrm{b}}$	$0.004 \pm 0.003 \ ^{\mathrm{b}}$	65.3 ± 0.56 ^{a,b}	$0.114 \pm 0.010~^{\mathrm{a,b}}$
III	5.54 ± 0.03 ^b	0.145 ± 0.003 ^b	$0.003 \pm 0.003 \ { m b}$	64.2 ± 0.59 ^b	$0.094 \pm 0.010~^{\mathrm{a,b}}$
IV	$5.54\pm0.03~^{\rm b}$	0.162 ± 0.003 a	$0.004 \pm 0.003 \ ^{\rm b}$	$64.2\pm0.59^{\text{ b}}$	$0.082 \pm 0.010 \ ^{\rm b}$
Time	***	***	ns	***	ns
Sowing	5.28 ± 0.02 a	0.145 ± 0.002 a	0.004 ± 0.002	63.8 ± 0.45 a	0.101 ± 0.007
Harvest	$5.65\pm0.02~^{\rm b}$	$0.166 \pm 0.002 \ ^{\rm b}$	0.004 ± 0.002	$66.3\pm0.44^{\text{ b}}$	0.107 ± 0.007
Rate	ns	ns	ns	ns	ns
Place	ns	ns	ns	ns	ns

Table 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, $p \ge 0.05$. Different letters indicate significant differences at the p < 0.05 level (Tukey's test).

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 2).

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 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 (Figure 2a).



Figure 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).

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.

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 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.

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 3).

Table 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	

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 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 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.

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 4).

P Treatment/Plant O	rgan 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		

Table 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).

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 R² 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). 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, the Cd uptake was low. Contrarily, low pH and high Cd levels in soil resulted in higher Cd accumulation in silage maize at maturity.

Table 5. Linear regressions for the exchangeable Cd fraction in soil and the total Cd concentration in silage maize.

Stage		Equation	R ²
Leaf	With Zn	$\begin{split} Cd_{NH_4NO_3} &= -0.0011 + 0.0178Cd_{Soil} + 0.0275Zn_{NH_4NO_3} \\ Cd_{Maize} &= 0.2273 - 0.0922pH + 0.0041Zn_{Soil} + 0.0044Zn_{Maize} \end{split}$	0.90 0.42
development	Without Zn	$Cd_{NH_4NO_3} = 0.0320 - 0.0053 pH$	0.34
Ripening		$\begin{array}{l} Cd_{NH_4NO_3} = 0.0299 - 0.0050 pH + 0.0159 Cd_{Soil} \\ Cd_{Maize} = 0.1099 - 0.0184 pH + 0.0605 Cd_{Soil} \\ Cd_{Maize} = -0.4988 + 3.7540 Cd_{Soil} + 0.0888 pH - 0.6504 pH:Cd_{Soil} \end{array}$	0.66 0.38 0.46

4. Discussion

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) [23]. During the growing season, P, in the form of DAP–(NH₄)₂HPO₄–could have temporarily increased the soil pH around fertilizer granules, owing to the HPO₄^{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 [24]. 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.

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) [25]. 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) [26]. 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 [27]. 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 [23,28]. 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) [29].

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 [1]. 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 [18,30].

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 [31].

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 [32]. 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 [33]. 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 [34]. 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 [35,36]. 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 [15,37].

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 [38,39]. The depressant effect of P on Zn uptake agrees with the results of Drissi et al. [14], 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) [28]. Compared to the study of Imran et al. [40], 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) [4].

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 [41].

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 [42,43]. 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 [3]. The extraction method with 1 M NH₄NO₃ might not be optimal to characterize the actual Zn uptake by silage maize, including the grain. Other techniques, such as DTPA extraction, may be more appropriate than this extraction method [28].

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. For silage maize, Cd uptake was explained by soil pH and the total Zn concentration in both soil and silage maize at the leaf development stage. Nonetheless, the Cd accumulation in mature plants was, instead, described by the soil pH and total Cd concentration in the soil. At the early development stage, the mobility and uptake of Cd and Zn were closely correlated. After the growing period, the Cd uptake and bioavailability were instead correlated to the soil pH and soil Cd concentration.

The low R^2 for Cd uptake by 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 NH₄NO₃ [44]. Still, our results agree with those of Hou et al. [43], who obtained an R^2 lower than 0.50 for a multilinear regression for Cd uptake by 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 [45,46]; 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 uptake by 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 [25,42]; 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. 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 uptake 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 uptake were closely correlated. At maturity, however, the Cd uptake 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.

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