Assessing bioavailable fraction and bioconcentration factors of Cd and Zn in young silage maize under different P fertilization and crop rotation

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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 NH₄NO₃ 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.

**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 [1,2]. Phosphorus is a limiting element in crop development due to its low availability in arable soils and its high crop demand [3]. Thus, phosphate-sufficient conditions, usually achieved by P fertilization, are necessary to reach optimal silage maize yields. However, P fertilization can decrease the plant uptake of Zn, an essential micronutrient, and it might incorporate toxic impurities such as Cd in arable land [4,5].

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 [6,7]. 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 [8,9]. 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 [3,10].

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 [6,11]. 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 [8]. The single extraction methods simulate the plant uptake using chelating agents or neutral salts. Specifically, the extraction with 1 M NH₄NO₃ 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 [12,13].

In soil-plant systems, one of the control mechanisms in metal plant assimilation is the diffusive transport of metals [14,15]. 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 [16–19]. 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 [20,21]. The BCF for maize has been derived previously, yet its calculation usually focuses on maize grain at mature stages or in polluted sites [22].
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 NH₄NO₃), 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.

Materials and methods

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 P₂O₅. 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 P₂O₅. Due to DAP containing N, the application of N as urea was adjusted to adequate levels, depending on the P treatment (Table 1). The Cd level in both P fertilizers was close to the limit of 60 mg Cd kg⁻¹ established by the European Union [23].

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) (Figure 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 H₂O (electrical resistance <18.2 MΩ cm⁻¹) and oven-dried at 75 ± 5°C for 48 h (Heraeus UT 6760,

Table 1. Phosphorus, nitrogen, and potassium requirements for silage maize, field season 2020; a after winter pea cultivation.

| Treatment | Phosphorus | Nitrogen | Potassium |
|-----------|------------|----------|-----------|
| Units     | kg (P₂O₅) ha⁻¹ | kg ha⁻¹ | kg K₂O ha⁻¹ | kg ha⁻¹ (Patentkali 30% K₂O) |
| Low band  | 114        | 248      | 230        | 318          |
| Low broad | 171        | 372      | 190        | 145          |
| High band | 230        | 372      | 190        | 145          |
| High broad| 230        | 372      | 190        | 145          |
| Control   | No P applied | No P applied | No P applied | No P applied |

Figure 1. Experimental and sampling design for field season 2020.
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.

Sample pretreatment and analytical measurements

The CaCl₂ 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 H₂O with an electrical resistance lower than 18.2 MΩ cm⁻¹ by the Core Facility of the University of Hohenheim (CFH), Stuttgart, Germany [24].

For the aqua regia extraction, soil samples (3 g) were moistened with C₆H₅O in 250 ml digestion tubes. The soil samples were saturated with aqua regia solution (78% HCl and 22% HNO₃, 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 H₂O (1 ml) and HNO₃ (2.5 ml) and digested for 1.5 h by a Milestone UltraCLAVE III microwave heated digestion reactor (MLS GmbH, Leutkirch, Germany) [25]. After digestion, H₂O was added to each sample solution to reach 10 ml for further analytical analysis via ICP-MS.

Metal bioavailability

For the methods assessing the bioavailable fraction, H₂O 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 HNO₃ and H₂O. The first method was the single extraction with 1 M NH₄NO₃ solution [13,26]. In an Erlenmeyer flask, each soil sample (20 g) was moistened with 1 M NH₄NO₃ 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 (VWRTM). After filtration, the solution was stabilized with HNO₃ 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 [16–18]. 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 cm² according to the data provided by DGT Research Ltd. (Lancaster, UK). Before deployment, each soil sample (50 g) was saturated with H₂O 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 H₂O, 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 HNO₃ solution (1 ml) for 24 h [27]. 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.

Equations

The time-averaged concentrations of Cd and Zn by the DGT technique were estimated with Equation (1) [28]:

\[
Metal_{DGT} = \frac{M \Delta g}{D^{diff} A_p t}
\]  

where \(\Delta g\) is the thickness of the diffusive layer (0.92 mm), \(D^{diff}\) is the diffusion coefficient of Cd and Zn in the diffusive layer (6.09 x 10⁻⁶ and 6.08 x 10⁻⁶ cm²/s at 25°C, correspondingly), \(A_p\) represents the surface area of the DGT device (2.54 cm²), and \(t\) is the deployment time (24 h). The accumulated mass (M) of Cd and Zn was estimated using Equation (2):

\[
M = \frac{C_e (V_e + V_e)}{f_e}
\]

where \(C_e\) is the metal concentration measured in the eluent (µg l⁻¹), \(V_e\) is the volume of the binding gel (0.20 ml), \(V_e\) 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. [29].

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 Equation (3) [20,21]:

\[
BCF_{roots or shoots} = \frac{\text{total metal concentration in roots or shoots (mg kg}⁻¹\text{ dry weight)}}{\text{total metal concentration in soil (mg kg}⁻¹)}
\]

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.

Results

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 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.

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 2).

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 2). The high-banded treatment showed the highest bioavailable Cd concentration, and the lowest was found in the low-banded fertilization (Figure 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 \( \text{NH}_4\text{NO}_3 \) (Table 2).

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 \( \text{NH}_4\text{NO}_3 \), no interaction between placement and application rate was observable (Table 2).

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 3).

Table 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         | pH  | Cd<sub>total</sub> | Zn<sub>total</sub> | Cd<sub>NH<sub>4</sub>NO<sub>3</sub></sub> | Cd<sub>DGT</sub> | Zn<sub>Mrianski</sub> | Zn<sub>DGT</sub> |
|------------------|-----|-------------------|-------------------|------------------------|-----------------|-----------------------|-----------------|
| Units            | ns  | ns                | ns                | ns                     | ns              | ns                    | ns              |
| 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±7.55 | 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±4.24 | 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±5.62 | 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±7.55 | 9.25±3.28 |
| Broad            | 5.78±0.50 | 0.18±0.03 | 60.39±2.22 | 2.52±1.52 | 0.27±0.18 | 73.28±4.95 | 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±5.32 | 8.90±2.96 |
| Rate: Place      | ns  | ns                | ns                | ns                     | ns              | ns                    | ns              |
| Block            | *** | ***               | ***               | ***                    | ***             | ***                   | ***             |
| I                | 5.41±0.22 | 0.17±0.02 | 58.66±1.01 | 3.85±1.77 | 0.33±0.10 | 105.49±46.38 | 10.59±1.98 |
| II               | 5.58±0.16 | 0.19±0.02 | 60.17±0.76 | 2.79±1.00 | 0.33±0.21 | 86.48±29.58 | 9.22±4.33 |
| III              | 5.70±0.10 | 0.23±0.03 | 62.68±0.77 | 3.43±1.27 | 0.34±0.13 | 85.53±26.43 | 9.37±3.33 |
| IV               | 6.47±0.37 | 0.18±0.03 | 61.85±1.52 | 0.59±0.43 | 0.12±0.04 | 10.15±11.28 | 5.98±1.43 |
| Prev. Crop       | ns  | ns                | ns                | ns                     | ns              | ns                    | ns              |
| Wheat            | 5.90±0.56 | 0.19±0.04 | 60.91±1.92 | 2.55±1.85 | 0.24±0.11 | 64.77±44.46 | 8.57±2.63 |
| Winter pea       | 5.68±0.34 | 0.19±0.03 | 60.78±1.86 | 2.78±1.64 | 0.32±0.19 | 79.05±50.51 | 9.01±4.01 |
**Figure 2.** Interaction effect between P placement and P rate for bioavailable Cd concentration by (a) Extraction with 1 M NH₄NO₃ solution and (b) DGT technique.

**Table 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      | Cdₗoretos | Cdₗoots | Znₗoots  | Znₗoots  |
|---------------|-----------|---------|----------|----------|
| Rate          | ns        | ns      | ns       | ns       |
| Place         | *         | ns      | **       | ns       |
| Control       | 0.36 ± 0.15* | 0.14 ± 0.06 | 416.25 ± 143.00* | 39.01 ± 9.17* |
| Broad         | 0.45 ± 0.20* | 0.14 ± 0.04 | 318.00 ± 151.98** | 41.23 ± 9.97** |
| Band          | 0.38 ± 0.14* | 0.12 ± 0.03 | 275.06 ± 95.40** | 32.73 ± 7.26** |
| Rate:Place    | ns        | ns      | ns       | ns       |
| Block         | ***       | ***     | ns       | **       |
| I             | 0.45 ± 0.21* | 0.17 ± 0.05* | 326.90 ± 159.87 | 42.25 ± 8.59* |
| II            | 0.44 ± 0.16* | 0.14 ± 0.03* | 354.70 ± 108.73 | 33.95 ± 8.72** |
| III           | 0.42 ± 0.13* | 0.14 ± 0.04* | 338.60 ± 161.01 | 37.62 ± 8.10** |
| IV            | 0.27 ± 0.08b | 0.09 ± 0.02b | 261.70 ± 100.95 | 33.95 ± 7.31b |
| Prev. Crop    | ***       | ***     | ns       | ***      |
| Wheat         | 0.30 ± 0.07a | 0.15 ± 0.04a | 379.25 ± 152.14* | 41.45 ± 7.57* |
| Winter pea    | 0.52 ± 0.16b | 0.11 ± 0.03b | 261.70 ± 83.14b | 32.44 ± 6.72b |
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 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.

**Pearson correlations**

The correlation between the Cd concentration in shoots and the Cd concentration determined by NH$_4$NO$_3$ 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$_4$NO$_3$ 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$_4$NO$_3$ was higher and more significant than the Cd concentration estimated by DGT (Figure 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 (Figure 3).

**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). 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).

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.

**Discussion**

**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 [30]. 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 [31]. 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 [22,32]. 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. [33].

**Total metal concentration in soil**

Although the 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 $0.145 \pm 0.002$ 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 [34,35]. 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) [10,36].

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 [6]. 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 [7]. 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\(_4\)NO\(_3\) 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. [37].

**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 [38]. 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. Römken [34] demonstrated that P fertilizer containing >40 mg Cd kg⁻¹ P₂O₅ 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. [35] 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 intake 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) [5]. 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 [11]. Furthermore, the soybean crop and its following substitution with another legume could have decreased soil pH [22], leading to a higher Cd bioavailability in soil. Interestingly, this crop rotation effect was not visible in the Cd extracted using NH₄ NO₃, a method usually more dependent on soil pH [12].

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 [39]. 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 [10]. However, there was no significant increase in the total Zn concentration in the soil after TSP fertilization.

**Silage maize**

The Cd concentration in shoots was relatively high but below the limit for feed materials of vegetable origin (1 mg kg⁻¹) [40]. The control treatment had the highest total Cd concentration in shoots, probably due to the lack of P fertilization and dilution effect [41]. 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 [30]. 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 [42].

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 [6,10]. 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 [4,43].

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 [44]. 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 [5,34].

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**Table 4. BCF of Cd and Zn for young silage maize by field variable (n = 40); significance code for p-value: ***p<0.001, **p<0.01, *p<0.05; different letters indicate significant differences at p < 0.05 level (Tukey test).**

| Variable/Rate | Metal | BCFroots | BCFshoots | BCFroots | BCFshoots |
|---------------|-------|----------|-----------|----------|-----------|
|               | Cd    |          |           | Zn       |           |
| Control       | ns    | ns       | a         | ns       | a         |
| Low           | 2.23 ± 0.88 | 0.68 ± 0.22 | 5.15 ± 2.49b | 0.60 ± 0.11 | n         |
| High          | 2.23 ± 1.25 | 0.71 ± 0.28 | 4.63 ± 1.46b | 0.59 ± 0.15 | a         |
| Place         | ns    | **      |           |         | ns        |
| Control       | 1.99 ± 0.02ab | 0.79 ± 0.37 | 6.80 ± 2.47b | 0.67 ± 0.17 | a         |
| Broad         | 2.54 ± 1.29a | 0.76 ± 0.28a | 5.26 ± 2.40a | 0.65 ± 0.13b | a         |
| Band          | 1.92 ± 0.69ab | 0.62 ± 0.19a | 4.52 ± 1.56a | 0.54 ± 0.12a | a         |
| Rate/Place    | ns    | ***     |           |         | ns        |
| Block         | ns    | ***     |           |         | ***       |
| Wheat         | 2.80 ± 1.10b | 0.62 ± 2.3b | 4.31 ± 1.40b | 0.53 ± 0.11b | a         |
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 NH₄NO₃ under acidic soil conditions or due to plant limiting uptake mechanisms rather than diffusion [12,15]. The correlation between the Cd concentration in shoots and the bioavailable Cd fraction estimated by DGT indicated lower values than other studies [16,17,19]. 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 [45]. 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 [14].

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. [46], where the Zn concentration in bean shoots highly correlated with the Zn extracted by NH₄NO₃. The 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 [47]. In addition, the DGT method has been proved unsuitable as a surrogate of Zn content in other cereal crops, such as wheat [18].

BCF

The BCFroots 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 BCFroots 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 BCFroots 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, BCFshoots of Cd was lower than 1.0, indicating a weak response of aerial biomass to the metal concentration in the soil. Meanwhile, the BCFroots 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 [9,48]. The lowest BCFshoots of Cd, similar to the BCFroots of Cd, occurred in the banded fertilization, possibly resulting from a dilution effect [41]; or by coupled uptake with Zn that also had the lowest BCF in the banded fertilization [10]. In general, the BCFshoots of Zn were low, and the BCFroots 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. [4] already discarded this hypothesis and attributed the allocation to a dilution effect in silage maize.

The BCFshoots of Cd suggested that the previous summer wheat enabled a higher Cd transfer from the soil to the maize shoots. The BCFshoots of Zn was also higher in maize following wheat, suggesting a synergistic behavior between Cd and Zn [10]. Still, the BCFroots 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 [11], 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 [33].

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 NH₄NO₃ 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.

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