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REGULAR ARTICLE

Reduced root mycorrhizal colonization as affected by phosphorus fertilization is responsible for high cadmium accumulation in wheat

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Abstract

Aims Phosphorus (P) fertilizers are often considered an important source of cadmium (Cd) in crop plants. However, increased plant Cd concentrations are not strictly related to the Cd content of P fertilizers. Considering this, we hypothesized that, alternatively, reduction of arbuscular mycorrhizal colonization by P fertilization enhances Cd accumulation in plants.

Methods Wheat and canola as mycorrhizal and non-mycorrhizal species, respectively, were grown under greenhouse conditions with and without soil sterilization. Phosphorus fertilizers with 0.09, 5, and 28 mg Cd kg^{-1} were applied at different rates with varied zinc (Zn) fertilization.

Results In wheat, all three P fertilizers markedly increased shoot and grain Cd concentrations as P supply was increased, irrespective of the Cd concentration in the fertilizers. These increases were pronounced with soil sterilization or at low zinc supply. Adding mycorrhizal fungi to sterilized soil substantially decreased shoot Cd concentrations. We found a strong negative relationship in wheat between mycorrhizal colonization and shoot Cd concentration, for both high- and low-Cd fertilizers. In contrast to wheat, shoot Cd concentrations in canola showed virtually no response to P supply or soil sterilization. High Zn application also reduced plant Cd concentrations, especially at high P rates.

Conclusion Our findings demonstrate the critical importance of mycorrhizal colonization in reducing Cd accumulation in wheat, and suggest that factors suppressing root mycorrhizal activity including P fertilization, will increase Cd uptake in mycorrhizal plants.

Keywords Cadmium · Canola · Mycorrhizal fungi · Soil sterilization · Phosphorus fertilizers · Wheat · Zinc

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Introduction

Cadmium (Cd) in soil and food crops represents an important scientific question as well as an environmental issue. There are various geogenic and anthropogenic factors affecting Cd abundance in soil and plants. Important anthropogenic Cd sources include mining activities, atmospheric deposition, industrial processes and use of phosphorus (P) fertilizers (McLaughlin et al. 1999, 2021; Kubier et al. 2019). There are several soil and crop management factors that also have a significant impact on solubility of Cd in soil and root uptake such as soil pH, cropping systems, liming, soil salinity, phytoavailability of Zn in soil, the levels of nitrogen and P fertilization and plant genotype (Mench 1998; Sheppard et al. 2009; McLaughlin et al. 2021). It is commonly observed that the soil Cd concentration shows a poor association with the plant Cd concentration in several crops such as wheat, potato and rice (McLaughlin et al. 1997; Li and Zhou 2019; Gray et al. 2019).

Phosphorus fertilizers are often suggested as one of the important sources of Cd in food crops. The Cd concentration of phosphate fertilizers shows large variation, ranging from near zero to 300 mg kg^{-1} depending on the source of the phosphate rock used and its processing procedure (Grant and Sheppard 2008). Increasing application of P fertilizers is usually associated with increases in Cd concentration of cultivated soils and plants (Mench 1998; Grant et al. 2013; Sheppard et al. 2009). The increase in plant Cd concentration through the use of P fertilizers is, however, not necessarily related to the Cd concentration of the applied P fertilizers. This counterintuitive result has been found across several important crop species. For example, in durum wheat grown at different field sites in Canada, increasing application of monoammonium phosphate (MAP) fertilizers containing 0.2, 7.8 and 186 mg Cd kg^{-1} resulted in more or less similar increases in seed Cd concentrations. Even, in the case of the highest P-application rate, the MAP fertilizer with 0.2 mg Cd kg^{-1} tended to increase seed Cd more than the other treatments did (Grant et al. 2002). Even more notably, application of a reagent grade P fertilizer with virtually no Cd also caused marked increases in shoot and seed Cd concentrations (Jiao et al. 2004). In previous field studies conducted on potato in Australia, soil application of P fertilizers widely differing in Cd concentrations (i.e. from 3.6 to 94.5 mg Cd per kg fertilizer) did not differently affect Cd concentration of tubers (McLaughlin et al. 1995). In a long-term P-fertilization experiment using oat plants including 15 cropping seasons at the experimental farm of the Norwegian University of Life Sciences, application of NPK fertilizers containing a wide range of Cd from 1 to 381 mg Cd per kg P increased soil Cd concentrations, but not seed Cd concentrations (Singh et al. 2017).

It is unclear why the P fertilizers differing so much in their Cd concentrations have similar impact on plant Cd concentration. Several possibilities have been discussed in the literature. Generally, it is suggested that P fertilizers increase plant Cd concentration, probably as a result of P fertilization-related i) increase in Cd availability due to soil acidification, ii) reduced Cd sorption in soil due to increased ionic strength of the soil solution, iii) changes in root growth and soil exploration or iv) high residual Cd in soils (McLaughlin et al. 1995; Grant et al. 2002, 2013; Lambert et al. 2007; Gao et al. 2011). In addition, P fertilization at high rates may induce Zn deficiency in plants by reducing root Zn uptake and/or causing an imbalance with Zn within leaf tissues (Loneragan et al. 1982; Cakmak and Marschner 1987; Ova et al. 2015; Zhang et al. 2012; Takagi et al. 2020). Zinc and Cd are chemically similar and compete for the same transporters during root uptake and shoot transport (Cakmak et al. 2000; Hart et al. 2002; Zhao et al. 2006; Chaney 2015). Based on these findings, it has been suggested that if high P application reduces Zn uptake and induces Zn deficiency in plants, this may, in turn, stimulates root Cd uptake due to lack of competitive inhibition of Zn on uptake and transport of Cd (Grant et al. 2002; Grant and Sheppard, 2008). Zinc deficiency might also up-regulate the plant Zn-acquisition system, thus increasing the uptake of Cd that enters the plant via the same system (Baxter et al. 2008; Küpper and Kochian 2010; Clemens et al. 2013; Chaney 2015).

Phosphorus fertilizers may increase plant Cd concentration by suppressing mycorrhizal colonization of roots (Ova et al. 2015; Ryan and Graham 2018; Zhang et al. 2016). Root colonization by mycorrhizal fungi is responsible for 25% to 50% of Zn uptake in

plants (Marschner 2012; Watts-Williams et al. 2015; Coccina et al. 2019). A reduction in root mycorrhizal activity by high P application might therefore enhance Cd uptake due to lack of competition between Zn and Cd for root uptake; alternatively, Zn-acquisition systems might be up-regulated, increasing the capacity for Cd uptake. In accordance, Grant et al. (2010) speculated that a reduced mycorrhizal colonization following P fertilization might enhance Cd uptake. Mycorrhizas also have direct inhibitory effects on root uptake and root-to-shoot transport of Cd. There is greater accumulation and immobilization of Cd in mycorrhizal roots than in non-mycorrhizal roots, and mycorrhizas have a strong ability to phytostabilize Cd in colonized roots and reduce Cd transport from roots to the shoot (Joner et al. 2000; Chen et al. 2018; Rask et al. 2019).

To our knowledge, it is still not unknown how root mycorrhizal activity affects Cd concentrations of plants treated with P fertilizers differing in Cd concentration. Here, wheat plants were grown under a range of P fertilizers differing in Cd concentration to study shoot and seed Cd concentration and root mycorrhizal colonization. To investigate the role of mycorrhizas, we conducted experiments with and without soil sterilization, and with and without mycorrhizal inoculation. In addition to wheat as a mycorrhizal species, we used canola, a non-mycorrhizal plant.

Materials and methods

Soil and fertilizers

A series of pot experiments was conducted in a greenhouse at Sabanci University to study the effect of various P fertilizers on shoot and grain concentrations of Cd in wheat (*Triticum aestivum* L. cv. Tahirova). An additional experiment was carried out on canola (*Brassica napus* L. cv. Suzer). All experiments were performed using a calcareous soil (18% $CaCO_3$) with a pH of 7.8 (in 0.01 mM $CaCl_2$), with a clay loam texture and low organic matter content (1.5%). The total amount of Cd and Zn in the soil were 0.44 mg kg^{-1} and 43 mg kg^{-1}, respectively, while 0.05 M $CaCl_2$-extractable Cd and Zn concentrations were 2.6 µg Cd kg^{-1} soil and 77 µg Zn kg^{-1}, respectively. The extractable soil P concentration was 2.28 mg kg^{-1} (Olsen et al. 1954).

In the experiments, the following P fertilizers were used, differing in Cd concentrations: i) diammonium phosphate (DAP) fertilizer containing 5 mg Cd kg^{-1} designated in this study as low-Cd-containing DAP and originating from the USA, ii) DAP containing 28 mg Cd kg^{-1} fertilizer designated as high-Cd-containing DAP and originating from Turkey, and iii) analytical grade calcium phosphate $Ca(H_2PO_4)_2$ containing a trace amount of Cd (i.e. 0.09 mg kg^{-1}), designated as CaP, which was received from Sigma Aldrich (Steinheim, Germany). The DAP fertilizers with low and high Cd concentrations contained 79 and 426 mg Zn kg^{-1}, respectively, as impurity.

Experiments

In the present study, four separate experiments were conducted. In the 1st experiment, wheat plants were grown until grain maturation during the summer (i.e. June–August) with a cumulative sunshine of 950 h and average night and day temperatures of 23 °C and 28 °C, respectively. Plants received the following three P rates: 20 mg P kg^{-1} (low P), 80 mg P kg^{-1} (medium P) and 240 mg P kg^{-1} (high P). Phosphorus fertilization was delivered as either low-Cd or high-Cd containing DAP fertilizers under marginal (0.2 mg Zn kg^{-1}) and high (5 mg Zn kg^{-1}) Zn treatments in the form of ZnSO4. The 1st experiment also aimed to study the effects of soil sterilization (i.e. elimination of mycorrhizal fungi) on growth and Cd concentration of plants. Therefore, half of the experimental soil was autoclaved at 121 °C for 2 h to eliminate the native mycorrhizal elements. The applied soil sterilization was effective in completely suppressing mycorrhizal fungal activity in the soil (measured by standard plate counting on potato dextrose agar). Pots were filled with 3.1 kg sterilized or unsterilized soil and supplemented with N in the form of NH_4NO_3 in addition to DAP to reach the level of 250 mg N per kg of soil until beginning of heading stage. All pots received 25 mg S per kg of soil in the form of K_2SO_4.

The experiment was arranged as a full factorial completely randomized design with five independent replicates (i.e. pots) per treatment. There were eight plants per pot which were irrigated with deionized

water when required. When the plants were at the beginning of heading stage (i.e. 42 days after sowing) four plants were harvested and the remaining plants were grown until grain maturation. The pots were supplied with additional N at a rate of 200 mg N kg^{-1} in the form of $Ca(NO_3)_2.4H_2O$ after the first harvest. At maturity, spikes were separately harvested, threshed mechanically and subjected to the determination of grain yield and grain concentrations of mineral elements, as described below.

The remaining three experiments were carried out during September and November with a cumulative sunshine of 660 h and average night and day temperatures of 19 °C and 24 °C. In the 2nd experiment, wheat and canola plants were compared in both unsterilized and sterilized soils. The experimental plants were grown with marginal (0.1 mg kg^{-1}) and adequate (2 mg kg^{-1}) Zn in the form of $ZnSO_4$ under a range of P supplies. The P-application rates were: 25, 50, 75, 150 and 300 mg P kg^{-1} soil in the form of analytical grade Ca-phosphate (CaP) containing 0.09 mg Cd kg^{-1}. Whole shoots were harvested when the plants were 56 days old (i.e. 6–7 leaf stages for canola and during head emergence for wheat). The 3rd experiment aimed to measure root mycorrhizal colonization in wheat plants that were grown with increasing P-application rates (i.e. 25, 50, 75, 150 and 300 mg P kg^{-1}) under marginal Zn supply (0.1 mg kg^{-1}). Phosphorus was applied in the form of DAP (containing 28 mg Cd kg^{-1}) and CaP (containing 0.09 mg Cd kg^{-1}). When the plants were 30 and 47 days old, root and shoot samples were collected and subjected to analysis of root mycorrhizal colonization, shoot dry matter content, and shoot mineral element concentrations as described below. In the 2nd and 3rd experiments, the treatments were comprised of four replicates and pots were supplied with 350 mg N per kg of soil.

In the final experiment, the aim was to study the effect of mycorrhizal inoculation on shoot growth and shoot concentration of Cd and nutrients in wheat grown in soils with and without sterilization. In this experiment, wheat was grown until the heading stage with three rates of P fertilization in the form of CaP and high-Cd DAP as follows: 20, 60 and 180 mg P kg^{-1} with five replicates. All pots received 350 mg N per kg of soil. Mycorrhizal inoculum (*Claroideoglomus etunicatum*, BEG 24) propagated on sudangrass [*Sorghum bicolor* (L.) Moench] was used in inoculation of the sterilized soil by applying about 500 spores per pot. Half of the 500 spores was thoroughly mixed in the soil, and the other half was placed as a layer 3 cm below the seeds. At harvest, shoots and spikes were collected separately and analyzed for dry matter production and analysis of Cd and nutrients. Root samples collected at harvest were used for measurement of mycorrhizal colonization as described below.

Analysis

The harvested shoot, spike and grain samples were washed thoroughly in deionized water, oven dried at 65 °C and used to determine dry matter and mineral element concentrations. Dried plant samples were first ground to a fine powder using an agate vibrating cup mill (Pulverisette 9; Fritsch GmbH, Idar-Oberstein, Germany) and digested in a closed-vessel microwave system (MarsExpress; CEM Corp; Matthews, NC, USA) in 2 ml of 30% (v/v) premium-grade H_2O_2 (Merck, EMSURE®, Darmstadt, Germany) and 5 ml of 65% (v/v) premium-grade HNO_3 (Merck, EMSURE®, Darmstadt, Germany). All dilutions were performed using ultra-pure water (18.2 MΩ). Concentrations of Cd, P and Zn and other minerals in the digested plant samples were determined using inductively coupled plasma optical emission spectrometry (ICP-OES; Vista-Pro Axial; Varian Pty Ltd; Mulgrave, Australia). The detection limit for Cd of the ICP-OES was 0.2 to 0.3 µg Cd L^{-1}. The measurements were verified by using certified standard reference material (SRM) obtained from NIST (the National Institute of Standards and Technology; Gaithersburg, MD, USA). The used SRM was 1567a wheat flour that contains 26.0 ± 2.0 µg Cd kg^{-1} and the agreement with this certified value was excellent: 27.7 ± 1.9 µg Cd kg^{-1}. The Cd results were also verified with another ICP-OES in the same lab (i.e. Agilent 5110 Vertical Dual View ICP-OES). Since some of the grain and spike samples contained very low Cd concentrations (i.e. below 30 µg Cd kg^{-1} dry weight), ICP-MS (inductively coupled plasma mass spectrometry; Agilent 7700x) has been used for Cd analyses of the grain and spike samples.

Mycorrhizal root colonization was determined following Giovannetti and Mosse (1980) after staining the roots with trypan blue using the method of Koske and Gemma (1989). The details of the measurement

are presented in Ova et al. https://link.springer.com/article/10.1007/s11104-021-04867-3 (2015).

Statistical analysis

Statistical analyses were performed using "Statistix 10" software. Analysis of variance (ANOVA) was performed for the significance of treatments and their interactions. The treatments showing significant F test ($P < 0.05$) were subjected to Tukey's honestly significant difference (HSD) test for the significance of differences among treatment means.

Results

In the first phase of the experiments, the effects of soil sterilization and two DAP fertilizers with different Cd concentrations were studied in wheat. As shown in Table 1, increasing application of DAP containing low (5 mg Cd kg^{-1}) and high Cd (28 mg Cd kg^{-1}) concentrations resulted in significant increases in shoot dry weight in each treatment, especially in unsterilized soil. Plants grown at medium and high P applications responded more to adequate Zn supply. Dry matter production of plants was greater when the soils were sterilized. There was no consistent difference in shoot growth of plants whether using P fertilizers with low or high Cd concentrations. On unsterilized soil with marginal Zn supply, increasing application of DAP fertilizers enhanced shoot Cd concentration around three-fold, irrespective of their Cd concentration (Table 1). High Zn application strongly reduced shoot Cd concentration. When the soil was autoclaved, shoot Cd concentration increased compared with the unsterilized soil, especially at lower P

Table 1 Effect of increasing application of low-cadmium (Cd) (5 mg kg^{-1}) and high-Cd (28 mg kg^{-1}) containing DAP fertilizers on shoot dry weight (DW) and shoot concentrations of Cd, phosphorus (P) and zinc (Zn) of 42-day-old wheat plants grown with a marginal and high Zn supply in unsterilized and sterilized soils (1st experiment)

					Shoot concentration					
			Shoot DW		Cd		P		Zn	
Soil	Zn supply	P supply	Low-Cd	High-Cd	Low-Cd	High-Cd	Low-Cd	High-Cd	Low-Cd	High-Cd
	(mg kg^{-1})		(g $plant^{-1}$)		(μg kg^{-1})		(g kg^{-1})		(mg kg^{-1})	
Unsterilized	0.2	20	1.62	2.12	76	84	1.89	1.56	16.2	13.4
		80	2.73	2.73	134	148	3.20	2.74	8.1	10.1
		240	2.95	3.27	223	257	4.86	3.65	8.3	9.1
	5	20	1.75	1.36	31	38	2.06	2.03	68.7	66.1
		80	3.17	3.29	41	35	2.68	2.51	44.8	49.7
		240	3.54	4.25	44	68	3.56	3.29	37.1	41.1
Sterilized	0.2	20	2.25	2.41	210	200	1.13	1.16	8.5	9.4
		80	3.75	3.40	216	220	2.18	1.92	7.9	8.4
		240	3.06	3.23	297	308	4.73	3.59	9.3	10.9
	5	20	3.27	2.86	72	104	1.29	1.21	39.6	40.7
		80	4.10	4.21	90	109	2.42	2.34	38.7	39.7
		240	4.69	4.46	86	125	4.07	3.81	35.0	34.0

The amount of Cd applied with the P fertilizers at the rates of 20, 80 and 240 mg P per kg soil were (as μg kg^{-1}): 0.50, 1.99 and 5.97 for the low-Cd-DAP and 2.79, 11.15 and 33.45 for the high-Cd-DAP, respectively. DAP: Diammonium phosphate

HSD0.05 values: A (soil sterilization); B (Zn supply); C (Cd in P source); D (P supply); A×B; A×C; A×D; B×C; B×D; C×D; A×B×C; A×B×D; A×C×D; B×C×D; A×B×C×D

Dry matter: 0.15; 0.15; n.s.; 0.22; 0.29; n.s.; 0.40; n.s.; 0.40; n.s.; n.s.; 0.66; n.s.; 0.64; n.s.

Cd concentration: 10; 10; 10; 14; 19; n.s.; 25; n.s.; 25; n.s., n.s.; 42; n.s.; n.s.; n.s.

P concentration: 0.13; n.s.; 0.13; 0.18; 0.24; n.s.; 0.32; 0.24; 0.32; 0.32; n.s.; 0.54; n.s.; 0.54; n.s.

Zn concentration: 1.84; 1.84; n.s.; 2.70; 3.50; n.s.; 4.72; n.s.; 4.75; n.s.; n.s.; 7.85; n.s.; n.s.; n.s.

rates (Table 1). At the low P and Zn treatments, autoclaving soil enhanced shoot Cd concentration almost three-fold. The large differences in shoot Cd concentration between the low and high P-application rates in unsterilized soil decreased in the autoclaved soil.

Shoot P concentration of plants was significantly affected by P-application rates, P source and soil sterilization (Table 1). As expected, increasing P supply increased shoot P concentration, irrespective of other treatments. When the average of all P concentration values in unsterilized soil was compared with the average of all P concentration values in sterilized soils, we found that soil sterilization tended to reduce the shoot P concentration by about 10%, and this effect was statistically significant. The P fertilizer with the higher Cd concentration significantly reduced shoot P concentrations compared with the low-Cd DAP, probably due to a higher Zn content in high-Cd DAP, as impurity. In unsterilized soil, sufficient Zn supply significantly increased shoot Zn, while increasing P application reduced the shoot Zn concentration. Autoclaving soil markedly reduced the shoot Zn concentration at lower P-application rates but was less effective at the highest P-application rate (Table 1).

At grain maturation, increasing P-application rates enhanced grain yield in almost all treatments (Table 2). Sufficient Zn supply also enhanced grain yield. As found for the shoot, grain Cd concentration strongly increased in response to increasing P application in unsterilized soil, in particular at marginal Zn supply (Table 2). Using P fertilizers with either low or high Cd concentrations did not affect grain Cd concentrations differently in unsterilized soil. A high Zn supply significantly decreased the grain Cd concentration. Also, like in shoot, soil sterilization substantially enhanced grain Cd concentrations, especially at low and medium P-application rates. Grain P concentrations were significantly affected by P and Zn treatments and soil

Table 2 Effect of increasing application of low-cadmium (Cd) (5 mg kg^{-1}) and high-Cd (28 mg kg^{-1}) containing DAP fertilizers on grain yield and grain concentrations of Cd, phosphorus (P) and zinc (Zn) of wheat plants grown with marginal and high Zn supply in native and sterilized soils (1st experiment)

					Grain concentration					
			Grain yield		Cd		P		Zn	
Soil	Zn supply	P supply	Low Cd	High Cd	Low Cd	High Cd	Low Cd	High Cd	Low Cd	High Cd
	(mg kg^{-1})		(g $plant^{-1}$)		(μg kg^{-1})		(g kg^{-1})		(mg kg^{-1})	
Unsterilized	0.2	20	1.12	1.22	40	40	4.45	3.87	42.3	39.0
		80	2.45	2.52	57	63	5.43	5.01	18.5	25.8
		240	2.87	2.68	145	137	5.67	4.93	11.2	12.2
	5	20	1.27	1.11	17	18	4.40	4.47	96.4	101.8
		80	3.21	2.40	15	15	4.47	4.38	63.6	66.1
		240	3.31	3.01	23	32	4.71	4.94	39.8	44.9
Sterilized	0.2	20	1.00	0.94	144	129	2.40	2.80	13.3	23.9
		80	2.08	2.41	132	142	3.38	3.20	9.6	12.3
		240	2.48	3.03	202	222	5.54	4.31	9.1	11.7
	5	20	1.73	1.64	35	43	2.34	2.10	49.1	49.4
		80	3.35	3.39	34	41	3.03	2.91	37.8	38.0
		240	3.47	2.38	37	55	4.80	5.28	34.8	37.7

The amount of Cd applied with the P fertilizers at the rates of 20, 80 and 240 mg P per kg soil were (as μg kg^{-1}): 0.50, 1.99 and 5.97 for low-Cd-DAP and 2.79, 11.15 and 33.45 for the high-Cd-DAP, respectively. DAP: Diammonium phosphate

HSD0.05 values: A (soil sterilization); B (Zn supply); C (Cd in P source); D (P supply); A×B; A×C; A×D; B×C; B×D; C×D; A×B×C; A×B×D; A×C×D; B×C×D; A×B×C×D

Grain yield: n.s., 0.20; n.s.; 0.30; 0.38; n.s.; n.s.; 0.38; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.

Grain Cd: 6; 6; n.s.; 9; 11; n.s.; n.s.; n.s.; 15; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.

Grain P: 0.2; 0.2; n.s.; 0.3; n.s.; n.s.; 0.58; 0.4; n.s.; n.s.; n.s.; 1; n.s.; 1; n.s.

Grain Zn: 3; 3; 3; 4; 5; n.s.;7; n.s.; 7; n.s.; n.s.; 11; n.s.; n.s.; n.s.

sterilization (Table 2). Compared with native soil, autoclaving soil decreased grain P concentrations, mainly under lower P-application rates. A high Zn supply increased the grain Zn concentration, whereas increasing P supply as well as soil sterilization reduced grain Zn concentrations, especially at low and medium P-application rates (Table 2).

In the next experiment, an analytical grade P source [$Ca(H_2PO_4)_2$: CaP], containing only a trace amount of Cd (i.e. 0.09 mg kg^{-1}) was used, and its effect on shoot Cd concentrations was studied in wheat and canola under different Zn supply (Table 3). Increasing application rates of CaP enhanced shoot growth of wheat in both soils and at both Zn treatments, while

Table 3 Effect of increasing phosphorus (P)-application rate in the form of analytical grade CaP (containing 0.09 mg Cd kg^{-1}) on shoot dry weight (DW) and shoot concentrations of cadmium (Cd), phosphorus (P) and zinc (Zn) of 56-day-old wheat and rapeseed plants grown with a marginal and adequate Zn supply in unsterilized and sterilized soils (2^{nd} experiment)

					Shoot concentration					
			Shoot DW		Cd		P		Zn	
Soil	Zn supply	P supply	Wheat	Canola	Wheat	Canola	Wheat	Canola	Wheat	Canola
	(mg kg^{-1})		(g $plant^{-1}$)		(μg kg^{-1})		(g kg^{-1})		(mg kg^{-1})	
Unsterilized	0.1	25	0.69	2.79	64	132	2.96	1.72	10.9	9.2
		50	0.74	2.95	78	152	3.29	3.52	8.9	8.3
		75	0.80	3.05	101	132	4.08	4.47	9.1	7.3
		150	0.88	2.89	119	144	4.38	6.08	8.2	7.6
		300	0.80	2.28	120	158	4.17	9.14	7.8	8.1
	2	25	0.77	3.69	30	69	2.73	1.42	31.5	16.8
		50	0.92	3.59	32	62	3.02	2.53	26.8	14.6
		75	1.01	3.72	34	72	3.27	2.98	23.7	15.4
		150	1.04	3.34	38	75	3.34	3.31	21.5	14.5
		300	1.12	3.52	48	86	3.24	3.87	19.3	14.2
Sterilized	0.1	25	0.88	3.48	134	138	1.55	1.88	6.6	10.9
		50	1.11	3.72	155	132	2.08	2.72	6.5	8.4
		75	1.06	3.75	169	144	2.68	3.27	7.8	8.0
		150	1.04	3.88	186	165	2.62	3.93	7.2	7.9
		300	1.12	3.85	195	162	4.11	5.62	7.9	7.4
	2	25	0.96	3.93	72	67	1.37	1.65	18.6	17.3
		50	1.14	4.24	82	73	2.05	2.43	21.7	14.0
		75	1.10	4.38	76	56	2.17	2.86	20.1	14.5
		150	1.18	4.00	73	57	2.79	3.17	21.3	13.5
		300	1.35	3.85	75	67	3.63	3.60	19.1	13.8

The amount of Cd applied with the CaP fertilization at the rates of 25, 50, 75, 150 and 300 mg P per kg soil were (as μg kg^{-1}): 0.01, 0.02, 0.03, 0.05 and 0.11, respectively. CaP: Calcium phosphate, $Ca(H_2PO_4)_2$

HSD0.05 values: A (soil sterilization); B (Zn supply); C (P supply); A×B; A×C; B×C; A×B×C

Dry weight (wheat): 0.06; 0.06; 0.13; n.s.; n.s.; n.s.; n.s.

Cd concentration (wheat): 5.8; 5.8; 12.9; 10.8; n.s.; 21.3; n.s.

P concentration (wheat): 0.1; 0.1; 0.3; 0.3; 0.3; n.s.; n.s.

Zn concentration (wheat): 0.8; 0.8; 1.8; 1.5; 3.0; 3.0; 4.8

Dry weight (canola): 0.17; 0.17; n.s.; 0.33; n.s.; n.s.; n.s.

Cd concentration (canola): n.s.; 6; 14; 12; n.s.; n.s.; 23

P concentration (canola): 0.3; 0.3; 0.6; 0.5; 1.1; 1.1; 1.7.

Zn concentration (canola): n.s.; 0.4; 0.8; 0.7; 1.4; n.s.; n.s.

the effect of P fertilization on growth was inconsistent in canola (Table 3). Zinc supply generally increased shoot dry weight of wheat and canola in both soils. At a given Zn and P supply, soil sterilization had positive effects on shoot dry weight of wheat and canola.

In unsterilized soil, shoot Cd concentration was higher in canola than in wheat. Increasing CaP application resulted in progressive increases of shoot Cd in wheat but had minimal effects on shoot Cd concentration in canola in unsterilized soil (Table 3). In contrast to the unsterilized soil, wheat and canola cultivated in the sterilized soil exhibited similar shoot Cd concentrations; wheat even contained more Cd than canola at most P-application rates. In unsterilized soil, shoot Zn concentration was significantly reduced by increasing P supply, especially in wheat. However, the reducing effect of P on shoot Zn of wheat was not distinct in sterilized soil (Table 3). The calculated amount of Cd uptake per shoot (i.e., Cd content) was significantly enhanced by CaP application in wheat in both soils (Table S1). Increasing P application remained less effective on shoot Cd accumulation in canola; however, canola plants accumulated much more Cd than wheat (up to tenfold) (Table S1).

A similar experiment as described in Table 3 was conducted to analyze mycorrhizal colonization in wheat roots (Table 4). Plants were grown with increasing P application rates in the form of analytical

Table 4 Effect of increasing phosphorus (P) application in form of analytical grade CaP (containing 0.09 mg Cd kg^{-1}) and DAP (containing 28 mg Cd kg^{-1}) on shoot dry weight (DW) and shoot concentrations of cadmium (Cd), phosphorus (P) and zinc (Zn) of 30- and 47-day-old wheat plants grown with marginal Zn supply (0.1 mg Zn kg^{-1} soil) (3rd experiment)

			Shoot concentration					
	Shoot DW		Cd		P		Zn	
P supply	CaP	DAP	CaP	DAP	CaP	DAP	CaP	DAP
(mg kg^{-1})	(g $plant^{-1}$)		(µg kg^{-1})		(g kg^{-1})		(mg kg^{-1})	
30-days old Plants								
25	0.35	0.37	176	235	1.65	1.92	9.1	9.3
50	0.47	0.40	219	194	2.52	2.79	8.9	8.1
75	0.43	0.46	258	233	3.31	3.32	9.8	8.7
150	0.51	0.50	224	229	4.29	4.68	8.7	8.2
300	0.55	0.47	218	199	5.68	6.83	8.2	8.1
47-days old Plants								
25	0.90	1.04	88	105	1.52	1.70	8.0	8.9
50	1.33	1.24	120	110	1.91	2.26	6.3	6.7
75	1.25	1.35	130	148	1.98	1.98	5.9	5.6
150	1.48	1.36	152	163	2.66	2.94	5.7	5.8
300	1.71	1.30	173	190	4.48	4.31	5.5	6.1

The amount of Cd applied with P fertilization at the rates of 25, 50, 75, 150 and 300 mg P per kg soil were (as µg kg^{-1}): 0.01, 0.02, 0.03, 0.05 and 0.11 for CaP, and 3.48, 6.97, 10.45, 20.91 and 41.82 for DAP, respectively. CaP: Calcium phosphate, $Ca(H_2PO_4)_2$; DAP: Diammonium phosphate

HSD0.05 values: A (P source); B (P supply); A × B

Dry weight (30-days old plants): n.s.; 0.08; n.s.

Cd concentration (30-days old plants): n.s.; n.s.; n.s.

P concentration (30-days old plants): n.s.; 1.0; n.s.

Zn concentration (30-days old plants): n..s.; 1.4; n.s.

Dry weight (47-days old plants): n.s.; 0.24; 0.41

Cd concentration (47-days old plants): n.s.; 26; n.s.

P concentration (47-days old plants): n.s.; 0.6; n.s.

Zn concentration (47-days old plants): n.s.; 1.3; n.s.

grade CaP and high Cd-containing DAP and harvested on days 30 and 47 after sowing. Increasing P supply increased shoot dry weight, irrespective of P source and plant age; however, the effect was more prominent in 47-day-old plants (Table 4). Generally, the plants treated with CaP showed greater shoot dry weight than the DAP-treated plants. Shoot Cd concentration of 30-day-old plants were minimally affected by the treatments. However, as plant age increased (47 days old), increasing P application in both forms significantly enhanced the shoot Cd concertation. As expected, increasing P supply in both forms increased the shoot P concentration irrespective of other treatments (Table 4). Enhancement in P supply was also effective in reducing the shoot Zn concentration especially in 47-day-old plants.

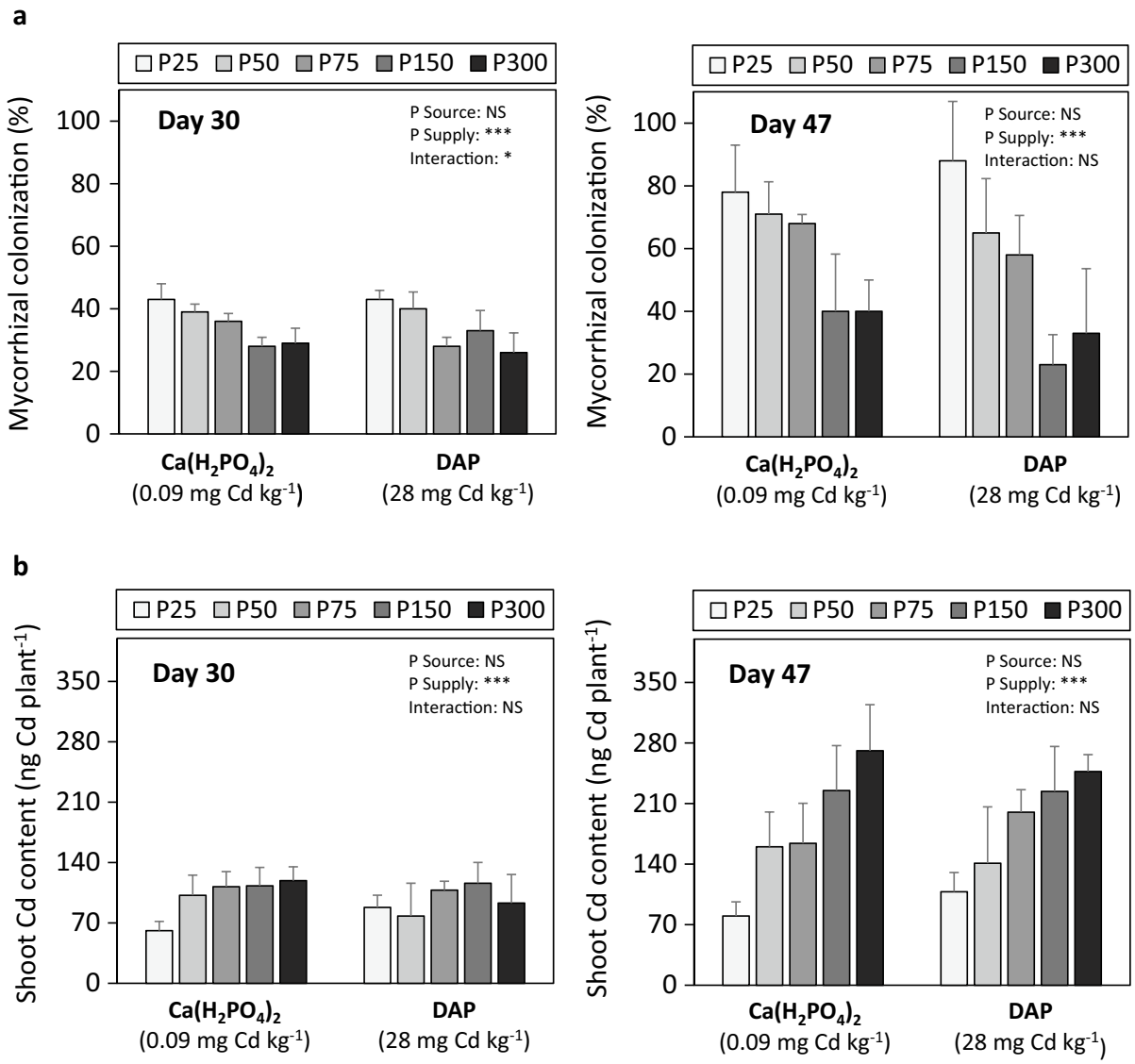

Fig. 1 Effect of phosphorus (P)-application rate in the form of analytical grade CaP (containing 0.09 mg Cd kg^{-1}) and DAP (containing 28 mg Cd kg^{-1}) on mycorrhizal colonization (top; 1a) and shoot cadmium (Cd) content (bottom; 1b) of wheat plants grown with a marginal zinc (Zn) supply (0.1 mg Zn kg^{-1}) for 30 and 47 days after sowing (3rd experiment). CaP: Calcium phosphate, $Ca(H_2PO_4)_2$; DAP: Diammonium phosphate; NS: Non-significant; NS: $P > 0.05$; *: $P < 0.05$; ***: $P < 0.001$

Experimental plants were analyzed for the level of root mycorrhizal colonization. As shown in Fig. 1a, when the plants were 30 days old, mycorrhizal colonization ranged from 27.5% to 42.5% at the highest and lowest P-application rate, respectively (average for both P forms). As the plant age increased from 30 to 47 days, root mycorrhizal colonization was substantially increased, but only in the low-P-supplied plants, while the plants in the higher P treatments (i.e. 150 and 300 mg kg^{-1} soil) showed a reduced mycorrhizal colonization (Fig. 1a). Since the changes in shoot Cd concentrations (Table 4) showed some association with the mycorrhizal colonization (Fig. 1a), the total uptake (content) of Cd per plant was calculated (Fig. 1b). Shoot Cd content per plant was slightly enhanced in 30-day-old plants by increasing P application. In case of the 47-day-old plants, there was a particular increase in shoot Cd content by increasing application of P fertilizers, regardless their Cd levels (Fig. 1b). Root mycorrhizal colonization showed a statistically highly significant negative relationship with the shoot Cd content in the 47-day-old plants, both for the CaP ($R^2 = 0.51***$) and DAP treatments ($R^2 = 0.67***$) (Fig. 2). A similar distinct inverse relationship was also found between shoot Cd concentration and mycorrhizal root colonization for the CaP ($R^2 = 0.51***$) and DAP treatments ($R^2 = 0.63***$) (no data shown).

In the final experiment, the effect of soil sterilization on Cd was studied in wheat plants with and without mycorrhizal inoculation under increasing P-application rates. Plants were harvested at the heading stage, and the spikes and shoots were separately collected and analyzed for dry matter production, root mycorrhizal colonization, mineral nutrients and Cd. In unsterilized soil, increasing P application enhanced dry matter production of shoots and spikes, more clearly with the DAP application (Table 5). When soil was autoclaved, dry matter production of the DAP- and CaP-treated plants showed a significant increase, especially in shoot dry matter (Table 5). Adding mycorrhizal inoculum to the sterilized soil reduced plant dry matter production in almost all treatments. The dry matter production of the plants grown in the sterilized soil with mycorrhizal inoculation was generally similar to that of plants grown in unsterilized soil. Mycorrhizal colonization of roots was significantly reduced by increasing P application, irrespective of other treatments (Fig. 3). When the P application

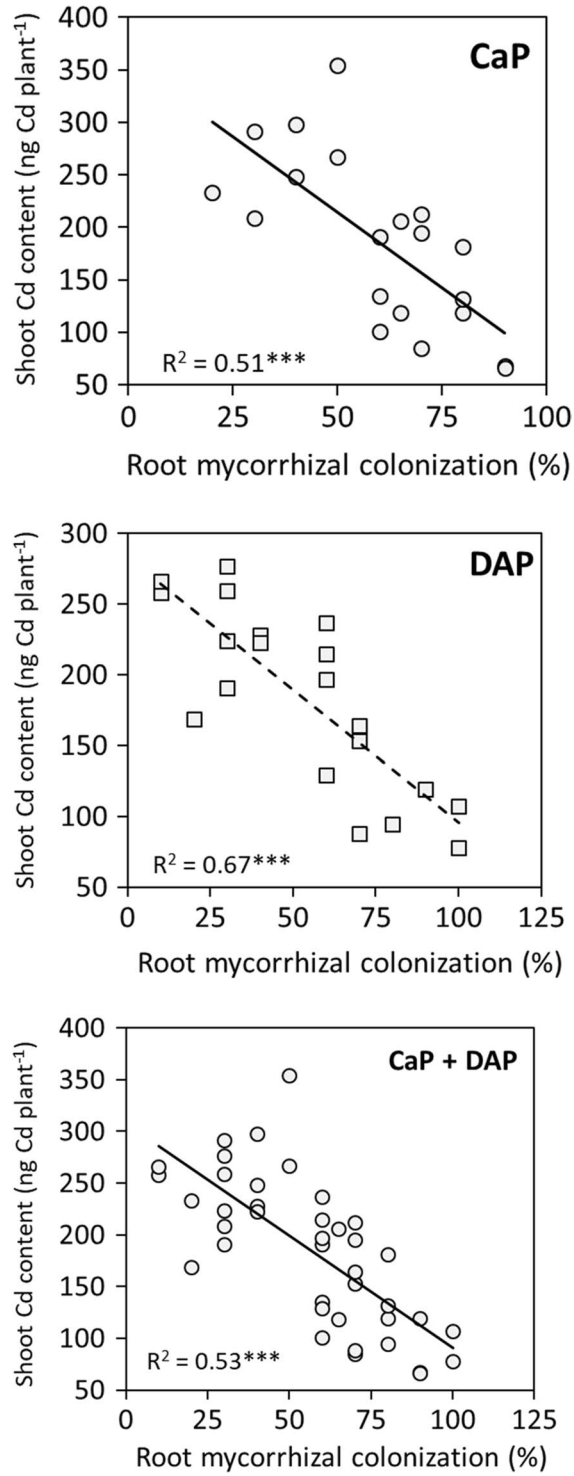

Fig. 2 Correlation between root mycorrhizal colonization and shoot cadmium (Cd) content of 47-day-old wheat plants supplied with increasing rate of phosphorus (P) in the form of CaP and DAP (3rd experiment). CaP: Calcium phosphate, $Ca(H_2PO_4)_2$; DAP: Diammonium phosphate. See Fig. 1 for further details

Table 5 Effect of increasing phosphorus (P) application rate in form of CaP and DAP on shoot and spike dry weight of 61-day-old wheat plants grown with a marginal zinc (Zn) supply (0.1 mg Zn kg^{-1}) in unsterilized and sterilized soils with and without mycorrhizal inoculum (4^{th} experiment)

Soil Treatment	P supply	Dry Weight			
		Shoot		Spike	
		CaP	DAP	CaP	DAP
		(g $plant^{-1}$)			
Unsterilized	20	1.04	1.20	0.37	0.38
	60	1.46	1.48	0.40	0.49
	180	1.49	1.75	0.39	0.53
Sterilized	20	1.35	1.31	0.45	0.40
	60	1.72	1.78	0.56	0.55
	180	1.76	1.92	0.42	0.55
Sterilized + mycorrhizae	20	1.12	1.19	0.36	0.35
	60	1.34	1.41	0.47	0.48
	180	1.56	1.44	0.49	0.41

The amount of Cd applied with P fertilization at the rates of 20, 60, and 180 mg P per kg soil were (as μg kg^{-1}): 0.01, 0.02, and 0.07 for CaP, and 2.79, 8.36 and 25.09, for DAP, respectively. CaP: Calcium phosphate, $Ca(H_2PO_4)_2$; DAP: Diammonium phosphate

HSD0.05 values: A (soil treatment); B (P supply); C (P source); A×B; A×C; B×C; A×B×C

Shoot DW: 0.10; 0.10; 0.07; n.s.; n.s.; n.s.; n.s.

Spike DW: 0.06; n.s.; 0.06; n.s.; n.s.; n.s.; n.s.

was increased from 20 to 180 mg kg^{-1}, root colonization decreased from 64 to 33% in unsterilized soil. As expected, soil sterilization severely suppressed or completely eliminated root mycorrhizal colonization, whereas addition of mycorrhizal inoculum to sterilized soil strongly promoted colonization (up to 96% at the lowest P treatment). However, the suppressive effect of increasing P application on root colonization still persisted in sterilized soil treated with exogenous mycorrhizal inoculum (Fig. 3).

Shoot Cd concentrations were much higher than those found in the spikes, irrespective of treatments (Table 6). Shoot and spike Cd concentrations of wheat grown in unsterilized soil showed progressive increases with increasing rate of P fertilization in both forms (Table 6). Similar to the results in Table 3, soil sterilization resulted in marked increases in Cd concentrations of shoots and spikes with both forms of P applications. When the sterilized soil was inoculated with mycorrhizal fungi, shoot and spike Cd concentrations decreased substantially (Table 6). These decreases were similar in the DAP- and CaP-treated plants, and were pronounced in the spikes.

In unsterilized soil, shoot P and Zn concentrations showed a clear increase and decrease, respectively, by enhancing P-application rates in both forms (Table 7). Soil sterilization significantly reduced shoot P and Zn concentrations at the low and medium P-application rates of both P forms but did not show a clear effect on shoot P and Zn concentrations at the highest P-application rate. When the sterilized soil

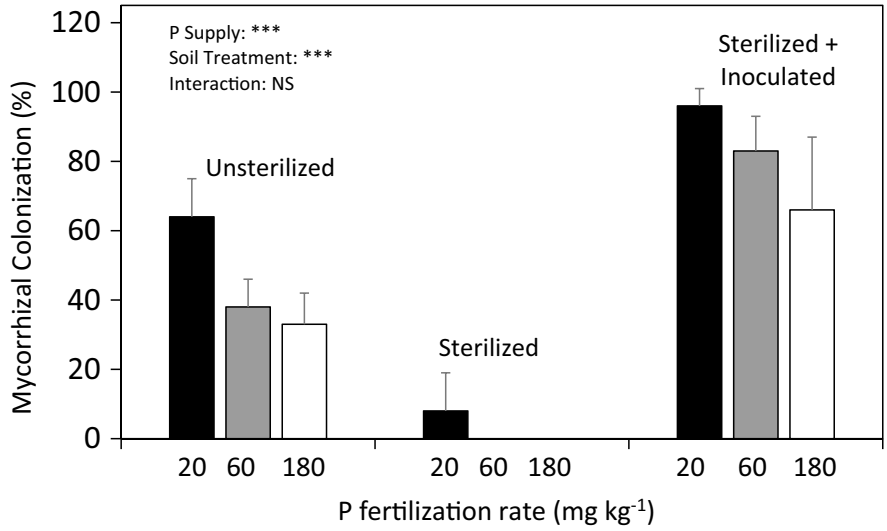

Fig. 3 Effect of increasing phosphorus (P) application rate in the form of DAP on root mycorrhizal colonization of 61-day-old wheat plants grown with a marginal zinc (Zn) supply (0.1 mg Zn kg^{-1}) in an unsterilized and sterilized soil with and without mycorrhizal inoculum (4^{th} experiment). DAP: Diammonium phosphate; NS: Non-significant; NS: $P > 0.05$; ***: $P < 0.001$

Table 6 Effect of increasing phosphorus (P) application in form of CaP and DAP on cadmium (Cd) concentrations of shoot and spikes of 61-day-old wheat plants grown with a marginal zinc (Zn) supply (0.1 mg Zn kg^{-1}) in unsterilized and sterilized soils with and without mycorrhizal inoculum (4^{th} experiment)

Soil Treatment	P supply	Cd concentration			
		Shoot		Spike	
		CaP	DAP	CaP	DAP
		(μg kg^{-1})			
Native	20	78	102	18	22
	60	112	162	38	42
	180	163	202	79	75
Sterilized	20	164	151	48	42
	60	218	210	96	77
	180	212	283	108	119
Sterilized + mycorrhiza	20	69	74	9	10
	60	92	114	26	26
	180	98	162	44	55

The amount of Cd applied with P fertilization at the rates of 20, 60, and 180 mg P per kg soil were (as μg kg^{-1}): 0.01, 0.02, and 0.07 for CaP, and 2.79, 8.36 and 25.09, for DAP, respectively. CaP: Calcium phosphate, $Ca(H_2PO_4)_2$; DAP: Diammonium phosphate

HSD0.05 values: A (soil treatment); B (P supply); C (P source); A × B; A × C; B × C; A × B × C

Shoot Cd: 15; 15; 10; n.s.; n.s.; 26; n.s.

Spike Cd: 6; 6; n.s.; 14; n.s.; n.s.; n.s.

Table 7 Effect of increasing phosphorus (P) application in form CaP and DAP on shoot concentrations of P and zinc (Zn) of 61-day-old wheat plants grown with a marginal Zn supply (0.1 mg Zn kg-^{1}) in unsterilized and sterilized soils with and without mycorrhizal inoculum (4^{th} experiment)

Soil Treatment	P supply	Shoot Concentration			
		P		Zn	
		CaP	DAP	CaP	DAP
		(g kg^{-1})		(mg kg^{-1})	
Unsterilized	20	1.66	1.49	9.71	12.99
	60	3.26	2.16	6.93	8.60
	180	4.20	2.79	5.03	6.79
Sterilized	20	0.64	0.67	4.41	4.77
	60	1.45	1.19	4.90	5.29
	180	3.72	3.31	5.29	6.86
Sterilized + mycorrhiza	20	1.79	1.94	12.02	17.63
	60	2.47	2.68	7.62	11.19
	180	3.38	3.02	5.82	7.69

The amount of Cd applied with P fertilization at the rates of 20, 60, and 180 mg P per kg soil were (as μg kg^{-1}): 0.01, 0.02, and 0.07 for CaP, and 2.79, 8.36 and 25.09, for DAP, respectively. CaP: Calcium phosphate, $Ca(H_2PO_4)_2$; DAP: Diammonium phosphate

HSD0.05 values: A (soil treatment); B (P supply); C (P source); A × B; A × C; B × C; A × B × C

Shoot P: 0.23; 0.23; 0.16; 0.53; 0.40; 0.40; n.s.

Shoot Zn: 0.73; 0.73; 0.50; 1.69; 1.26; n.s.; 2.70

was inoculated with mycorrhizal fungi, shoot P and Zn concentrations rose significantly at the low and medium P-application rates, but were not affected at the highest P-application rate (Table 7). Very similar changes in shoot P and Zn concentrations were also found in the spikes (data not shown). Following the treatments, changes in shoot concentrations of Ca, K, Mg and S, were either not significant or minimal compared with those of P and Zn (Table S2).

Discussion

In the present study, P fertilizers differing in Cd concentrations were studied for their effects on plant Cd concentration. Increasing application rate of P fertilizers to wheat significantly enhanced Cd concentration in shoots and grain, irrespective of their Cd concentration (Tables 1 and 2). Similarly, application of an analytical-reagent grade P source, CaP, containing only trace amount of Cd (i.e. 0.09 mg kg^{-1}) markedly increased Cd concentration of wheat (Tables 3, 4 and 6; Fig. 1).

An increase in plant Cd concentrations by application of P fertilizer, regardless of their Cd content, was also reported for durum wheat and potato grown under field conditions (McLaughlin et al. 1995; Grant et al. 2002, 2013). Similar to the present results, Gao et al. (2011) showed in wheat grown under growth chamber conditions that the shoot Cd concentration and the shoot Cd content were almost the same for all MAP fertilizers containing 3.4, 75.2 and 232 mg Cd kg^{-1}. These findings from greenhouse and field studies clearly indicate that there are factors other than the Cd concentration of P fertilizers that are responsible for enhanced Cd accumulation in plants by P fertilization. Soil acidification and impairment of root Zn uptake by P fertilizers, regardless of their Cd concentration have been discussed as major reasons

promoting Cd phytoavailability and root uptake (Grant et al. 2002, 2013; Jiao et al. 2004; Lambert et al. 2007; Grant and Sheppard 2008). Grant et al. (2010) speculated that suppression of root mycorrhizal colonization by P fertilization might be an additional factor contributing to increases in plant Cd concentrations by P fertilization. The results of the present study support the speculation made by Grant et al. (2010) and highlight a critical role for the reduced root mycorrhizal colonization of plants due to P fertilization, as further discussed below.

Reduction of root mycorrhizal colonization by P fertilization has been reported repeatedly (Smith et al. 2011; Ova et al. 2015; Ryan and Graham 2018; Zhang et al. 2016). In the present study, P fertilization in the form of either $Ca(H_2PO_4)_2$ or DAP also strongly reduced mycorrhizal colonization of wheat roots (Figs. 1 and 3). Mycorrhizas are highly effective in reducing root uptake and shoot accumulation of Cd through various mechanisms including immobilization of Cd in fungal structures and root cell walls. Joner and Leyval (1997) and Joner et al. (2000) reported that mycorrhizal mycelium has a very high Cd sorption capacity, resulting in reduced Cd transfer to shoots. By using synchrotron radiation μX-ray fluorescence imaging, Chen et al. (2018) showed that Cd is localized and immobilized within mycorrhizal roots, and plant cells without fungal structures contained a negligible amount of Cd. Consequently, less Cd is exported to shoots. In a study using six plant species differing in their degree of mycorrhizal colonization, barley plants with the highest root mycorrhizal colonization contained the lowest amount of Cd in shoots (Rask et al. 2019).

The findings referred to above led to our hypothesis that the significant increases in shoot and grain Cd concentrations of wheat at increasing P fertilization in unsterilized soil (Tables 1 and 2) are most related to suppression of root mycorrhizal colonization. When the soils were autoclaved (i.e. elimination of indigenous mycorrhizal fungi), shoot and grain Cd concentrations showed a marked increase, especially under low P supply (Tables 1 and 2). For example, with the low-Cd-DAP supply, the grain Cd concentration was 40 μg kg^{-1} under the low-P treatment (i.e. with high mycorrhizal colonization potential), and this value increased to 144 μg kg^{-1} with soil sterilization (Table 2). Similar increases were found in the case of high-Cd-DAP treatment as well as for the shoot samples. When the plants were treated with high P (i.e. low root mycorrhizal colonization), autoclaving of soil generally showed less effects on shoot or grain Cd concentrations. These results demonstrate that elimination of mycorrhizal colonization of roots either by high P supply or autoclaving soil increases Cd accumulation in wheat. This suggestion is supported by the results showing that addition of mycorrhizal inoculum to sterilized soil resulted in marked increases in mycorrhizal colonization of wheat roots (Fig. 3) and significant decreases in Cd concentrations of shoots and spikes, regardless of the Cd concentration of the applied P fertilizers (Table 6). In agreement with our findings for wheat, mycorrhizal inoculation was also very effective in reducing Cd accumulation in rice grown under aerobic soil conditions (Li et al. 2016; Luo et al. 2017) and maize (Zhang et al. 2019).

Soil sterilization would not only have eliminated mycorrhizal fungi, but also other soil microorganisms, which might also affect Cd uptake and accumulation in plants. However, the changes in plant Cd concentration following soil sterilization in the present study were very much influenced by mycorrhizas. For example, marked increases in Cd concentrations after soil sterilization (i.e. with elimination of root colonization with mycorrhizal fungi; Fig. 3) were reverted, leading to Cd concentrations being significantly lower than the original Cd values after adding mycorrhizal fungi to the sterilized soil (Table 6). This clearly demonstrates a critical role of mycorrhizal fungi in reducing Cd accumulation in shoots. In addition, as discussed further below, in the experiment using canola (Table 3), we found that wheat (as a mycorrhizal species) and canola (as a non-mycorrhizal species) differed in their Cd accumulation in native soil, but behaved very similar when the soil was sterilized. This provides further evidence that changes in shoot Cd concentration following soil sterilization was mainly determined by mycorrhizal fungi.

The experiment presented in Table 4 and Fig. 1 emphasizes the importance of mycorrhizas in reducing Cd uptake and accumulation in wheat treated with P fertilizers with very low and high Cd contents. As shown in Fig. 1, wheat plants with high P supply had a reduced mycorrhizal colonization. However, the differences in root colonization in response to P fertilization became more obvious when the plants were 47 days old (at the beginning of the heading stage).

The results in Fig. 1a agree with results published previously, showing that root mycorrhizal colonization in wheat is generally low during the early growth stages and exhibits a marked increase during heading and flowering (Galvez et al. 2001; Al-Karaki et al. 2004; Brito et al. 2012). When the plants were 30 days old and showed a low mycorrhizal colonization (Fig. 1a), the effect of increasing P supply in the form of CaP or DAP on shoot Cd concentrations was minimal or absent (Table 4). By contrast, when mycorrhizal colonization differed among the P treatments (i.e. in the 47-day-old plant), increasing rates of P fertilization significantly increased shoot Cd concentrations. These increases were very similar for DAP and CaP, but, the relative increases in the 47-day-old plants were even greater for the low-Cd CaP (i.e. 97%) than for the high-Cd DAP (i.e. 81%) plants (Table 4). Similarly, the shoot Cd content (i.e. total amount of Cd per plant) showed particular increases with the diminished mycorrhizal colonization at higher P treatments in the 47-day-old plants (Fig. 1b). There was a very strong negative correlation between shoot Cd content and root mycorrhizal colonization (Fig. 2). Therefore, these results highlight a major role of mycorrhizas in the P-fertilizer-dependent Cd accumulation in wheat.

Both previous and recent experiments, which have used P fertilizers labeled with radioisotope Cd (^{109}Cd) to trace freshly applied Cd from soil in the plant system, showed that plants take up only 1 to 3% of the Cd applied freshly with the P fertilizers (Jensen and Mosbæk 1990; Wiggenhauser et al. 2019; Bracher et al. 2021). These findings highlight that the pre-existing Cd in the soil system represents the predominant source of Cd in shoots. Considering the findings that freshly-applied Cd through P fertilizers has little impact on shoot Cd concentrations, we surmise that the marked increases in grain and shoot Cd concentrations by P fertilization, irrespective of the fertilizer Cd content, most probably results from suppression of mycorrhizal colonization at a high P supply.

Canola belongs to the Brassicaceae family, a typical non-mycorrhizal plant family (Brundrett 2009; Lambers and Teste 2013; Cosme et al. 2018). There are rare exceptions in this family of mycorrhizal species, but canola has been shown to be incapable of forming a functional arbuscular mycorrhizal symbiosis (Glenn et al. 1985; Demars and Boerner 1996; Mozafar et al. 2000). In a different study, we also found that root mycorrhizal colonization is not detectable in the same canola cultivar grown in the same soil (unpublished).The inability of non-mycorrhizal plant species, like canola, to establish a functional symbiosis has been ascribed to the loss of genes that are essential for a mycorrhizal symbiosis during the course of their evolution (Delaux et al. 2014). It was, therefore, interesting to compare this species with wheat regarding their Cd concentration under increasing P application with and without soil sterilization. In contrast to wheat, canola showed a minor or no response to increasing P fertilization and soil sterilization with respect to shoot Cd concentration and content (Tables 3 and S1). Consistently, canola had a higher Cd concentration than wheat in unsterilized soil, but this difference decreased and even disappeared when the soil was sterilized (Tables 3 and S1). For example, at the lowest two P treatments (with higher mycorrhizal colonization potential) wheat had almost half the amount of Cd of canola in unsterilized soil, whereas in sterilized soil without mycorrhizal colonization wheat and canola had almost the same Cd concentrations. In a field experiment in Australia, using various Cd-containing fertilizers (ranging from 5 to 52 mg Cd per kg fertilizer) Brennan and Bolland (2005) showed that, on average, canola had about three times more Cd in the grain than wheat at each level of Cd applied in fertilizer.

Another prominent contribution of mycorrhizas to reducing root Cd uptake is related to their role in root Zn uptake. Mycorrhizas are responsible for about 25–50% of total Zn uptake in various crop species (Marschner 2012; Watts-Williams et al. 2015; Coccina et al. 2019; Ortas 2019). In agreement with this, inhibition of root mycorrhizal colonization by soil sterilization results in significant decreases in plant Zn concentration (Thompson 1990; Ova et al. 2015; Ortas et al. 2018). Similar results were also presented in this study. As shown in Tables 1, 2, 3 and 6, soil sterilization decreased shoot and grain Zn concentrations of wheat, especially at low P treatments. These findings suggest that impairment of mycorrhiza-related root Zn uptake by application of P fertilizers or by soil sterilization promotes root Cd uptake, which is a further reason why plants accumulate Cd when P fertilization is increased. Due to chemical similarities between Zn and Cd and common pathways for Zn and Cd acquisition, there is competition between Zn and Cd during root uptake as well as during xylem and phloem transport (Cakmak et al. 2000;

Jiao et al. 2004; Hart et al. 2005; Zhao et al. 2006). Zinc transporters that are up-regulated under Zn deficiency would also contribute to enhanced root Cd uptake (Clemens et al. 2013; Chaney 2015; Palusinska et al. 2020; Tan et al. 2020). When the Zn supply was increased, Cd concentrations of the plants were strongly reduced, indicating that an adequate Zn fertilization of plants is required to minimize the effects of P fertilization on increasing plant Cd concentrations. Therefore, use of P fertilizers containing Zn would be a useful agronomic practice to curtail increases in Cd uptake by P fertilization. Since plant Zn and P nutritional status and root mycorrhizal colonization are very closely interrelated, it is difficult to disentangle the effects of single factors on Cd uptake.

In conclusion, the present findings demonstrate that the P fertilization- dependent Cd accumulation in plants is similarly affected by P fertilizers varying significantly in Cd concentration (i.e. ranging from 0.09 to 28 mg Cd per kg fertilizer applied). This indicates that P fertilization-dependent Cd accumulation in plants is not simply related to the Cd in the applied P fertilizers. We also show the importance of root mycorrhizal colonization in reducing Cd concentrations in wheat. In addition to P fertilization, several other soil and crop management factors significantly suppress mycorrhizal diversity, richness and root colonization such as intensive soil tillage, crop rotations involving non-mycorrhizal plants, monoculture cropping systems and application of large amounts of N fertilizers (Verbruggen et al. 2013; Bowles et al. 2017; Dietrich et al. 2020; Ma et al. 2021). Under such conditions, an increased Cd accumulation is expected in plants as a result of losing mycorrhizal colonization. Finally, to better understand the source of Cd in food crops and adoption of appropriate management strategies, policy makers should pay particular attention to soil and crop management factors suppressing abundance and activity of mycorrhizal fungi in cropping systems.

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Author contribution M.A.Y. and M. A. designed and conducted all experiments, analyzed the data, drafted the manuscript; Y.T. Analyzed samples for determination of Cd and mineral nutrients; I.O. analyzed root samples for mycorrhizal colonization and contributed to interpretation of data; L.O. conducted statistical analysis and participated in the drafting article; H.L. critically reviewed the manuscript, contributed to intellectual content and the write up; I.C. conceived the idea, conceptualized the study, provided guidelines for conducting experiments, drafted and revised the manuscript. All authors contributed critically to the manuscript and gave final approval for publication.

Data availability Data can be provided upon request.

Code availability Not Applicable.

Declarations

Conflict of interest Authors declare that they have no known conflict of interest.

References

Al-Karaki G, McMichael BZ, Zak J (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263–269

Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot ML, Salt DE (2008) The leaf ionome as a multivariable system to detect a plant's physiological status. Proc Natl Acad Sci USA 105:12081–12086

Bowles TM, Jackson LE, Loeher M, Cavagnaro TR (2017) Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. J Appl Ecol 54:1785–1793

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. Environ Pollut 287:117314

Brennan RF, Bolland MDA (2005) Canola takes up more cadmium and phosphorus from soil than spring wheat. J Plant Nutr 28:931–948

Brito I, Goss MJ, De Carvalho M (2012) Effect of tillage and crop on arbuscular mycorrhiza colonization of winter wheat and triticale under Mediterranean conditions. Soil Use Manag 28:202–208

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77

Cakmak I, Marschner H (1987) Mechanism of phosphorus-induced zinc deficiency in cotton. III. Changes in physiological availability of zinc in plants. Physiol Plant 70:13–20

Cakmak I, Welch RM, Erenoglu B, Römheld V, Norvell WA, Kochian LV (2000) Influence of varied zinc supply on re-translocation of cadmium (^{109}Cd) and rubidium (^{86}Rb) applied on mature leaf of durum wheat seedlings. Plant Soil 219:279–284

Chaney RL (2015) How does contamination of rice soils with Cd and Zn cause high incidence of human Cd disease in subsistence rice farmers. Curr Pollut Rep 1:13–22

Chen B, Nayuki K, Kuga Y, Zhang X, Wu S, Ohtomo R (2018) Uptake and intraradical immobilization of cadmium by arbuscular mycorrhizal fungi as revealed by a stable isotope tracer and synchrotron radiation μX-ray fluorescence analysis. Microbes Environ 33:253–263

Clemens S, Aarts MGM, Thomine S, Verbruggen N (2013) Plant science: the key to preventing slow cadmium poisoning. Trends Plant Sci 18:2

Coccina A, Cavagnaro TR, Pellegrino E, Ercoli L, McLaughlin MJ, Watts-Williams SJ (2019) The mycorrhizal pathway of zinc uptake contributes to zinc accumulation in barley and wheat grain. BMC Plant Biol 19:1–4

Cosme M, Fernández I, Van der Heijden MG, Pieterse CM (2018) Non-mycorrhizal plants: the exceptions that prove the rule. Trends Plant Sci 23:577–587

Delaux PM, Varala K, Edger PP, Coruzzi GM, Pires JC, Ané JM (2014) Comparative phylogenomics uncovers the impact of symbiotic associations on host genome evolution. PLoS Genet 10:e1004487

Demars BG, Boerner RE (1996) Vesicular arbuscular mycorrhizal development in the Brassicaceae in relation to plant life span. Flora 191:179–189

Dietrich P, Roscher C, Clark AT, Eisenhauer N, Schmid B, Wagg C (2020) Diverse plant mixtures sustain a greater arbuscular mycorrhizal fungi spore viability than monocultures after 12 years. J Plant Ecol 13:478–488

Galvez L, Douds DD Jr, Wagoner P (2001) Tillage and farming system affect AM fungus populations, mycorrhizal formation, and nutrient uptake by winter wheat in a high-P soil. Am J Alternative Agr 1:152–160

Gao X, Flaten DN, Tenuta M, Grimmett MG, Gawalko EJ, Grant CA (2011) Soil solution dynamics and plant uptake of cadmium and zinc by durum wheat following phosphate fertilization. Plant Soil 338:423–434

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 1:489–500

Glenn MG, Chew FS, Williams PH (1985) Hyphal penetration of Brassica (Cruciferae) roots by a vesicular–arbuscular mycorrhizal fungus. New Phytol 99:463–472

Grant CA, Sheppard SC (2008) Fertilizer impacts on cadmium availability in agricultural soils and crops. Hum Ecol Risk Assess 14:210–228

Grant C, Bailey L, Flore N, Harapiak J (2002) Effect of phosphate source, rate and cadmium content and use of *Penicillium bilaii* on phosphorus, zinc and cadmium concentration in durum wheat grain. J Sci Food Agric 82:301–308

Grant CA, Monreal MA, Irvine RB, Mohr RM, McLaren DL, Khakbazan M (2010) Preceding crop and phosphorus fertilization affect cadmium and zinc concentration of flaxseed under conventional and reduced tillage. Plant Soil 333:337–350

Grant C, Flaten D, Tenuta M, Malhi S, Akinremi W (2013) The effect of rate and Cd concentration of repeated phosphate fertilizer applications on seed Cd concentration varies with crop type and environment. Plant Soil 372:221–233

Gray CW, Yi Z, Munir K, Lehto NJ, Robinson BH, Cavanagh JA (2019) Cadmium concentrations in New Zealand wheat: effect of cultivar type, soil properties, and crop management. J Environ Qual 48:701–708

Hart JJ, Welch RM, Norvell WA, Kochian LV (2002) Transport interactions between cadmium and zinc in roots of bread and durum wheat seedlings. Physiol Plant 116:73–78

Hart JJ, Welch RM, Norvell WA, Clarke JM, Kochian LV (2005) Zinc effects on cadmium accumulation and partitioning in near-isogenic lines of durum wheat that differ in grain cadmium concentration. New Phytol 167:391–401

Jensen H, Mosbæk H (1990) Relative availability of 200 years old cadmium from soil to lettuce. Chemosphere 20:693–702

Jiao Y, Grant CA, Bailey LD (2004) Effects of phosphorus and zinc fertilizer on cadmium uptake and distribution in flax and durum wheat. J Sci Food Agric 84:777–785

Joner EJ, Leyval C (1997) Uptake of ^{109}Cd by roots and hyphae of a Glomus mosseae/Trifolium subterraneum mycorrhiza from soil amended with high and low concentrations of cadmium. New Phytol 135:353–360

Joner EJ, Briones R, Leyval C (2000) Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil 226:227–234

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res 92:486–488

Kubier A, Wilkin RT, Pichler T (2019) Cadmium in soils and groundwater: A review. App Geochem 108:104338

Küpper H, Kochian LV (2010) Transcriptional regulation of metal transport genes and mineral nutrition during acclimatization to cadmium and zinc in the Cd/Zn hyperaccumulator, *Thlaspi caerulescens* (Ganges population). New Phytol 185:114–129

Lambers H, Teste FP (2013) Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient availability play the same game. Plant Cell Environ 36:1911–1915

Lambert R, Grant C, Sauvé S (2007) Cadmium and zinc in soil solution extracts following the application of phosphate fertilizers. Sci Total Environ 378:293–305

Li X, Zhou D (2019) A meta-analysis on phenotypic variation in cadmium accumulation of rice and wheat: implications for food cadmium risk control. Pedosphere 29:545–553

Li H, Luo N, Zhang LJ, Zhao HM, Li YW, Cai QY, Wong MH, Mo CH (2016) Do arbuscular mycorrhizal fungi affect cadmium uptake kinetics, subcellular distribution and chemical forms in rice? Sci Total Environ 571:1183–1190

Loneragan JF, Grunes DL, Welch RM, Aduayi EA, Tengah A, Lazar VA, Cary EE (1982) Phosphorus accumulation and toxicity in leaves in relation to zinc supply. Soil Sci Soc Am J 46:345–352

Luo N, Li X, Chen AY, Zhang LJ, Zhao HM, Xiang L, Cai QY, Mo CH, Wong MH, Li H (2017) Does arbuscular mycorrhizal fungus affect cadmium uptake and chemical forms in rice at different growth stages? Sci Total Environ 599:1564–1572

Ma X, Geng Q, Zhang H, Bian C, Chen HY, Jiang D, Xu X (2021) Global negative effects of nutrient enrichment on arbuscular mycorrhizal fungi, plant diversity and ecosystem multifunctionality. New Phytol 229:2957–2969

Marschner P (ed) (2012) Marschner's mineral nutrition of higher plants. Academic, London

McLaughlin MJ, Maier NA, Freeman K, Tiller KG, Williams CM, Smart MK (1995) Effect of potassic and phosphatic fertilizer type, fertilizer Cd concentration and zinc rate on cadmium uptake by potatoes. Fert Res 40:63–70

McLaughlin MJ, Maier NA, Rayment GE, Sparrow LA, Berg G, McKay A, Milham P, Merry RH, Smart MK (1997) Cadmium in Australian potato tubers and soils. J Environ Qual 26:1644–1649

McLaughlin MJ, Parker DR, Clarke JM (1999) Metals and micronutrients - food safety issues. Field Crop Res 60:143–163

McLaughlin MJ, Smolders E, Zhao FJ, Grant C, Montalvo D (2021) Chapter One - Managing cadmium in agricultural systems. Adv Agron 166:1–129

Mench MJ (1998) Cadmium availability to plants in relation to major long-term changes in agronomy systems. Agric Ecosyst Environ 67:175–187

Mozafar A, Anken T, Ruh R, Frossard E (2000) Tillage intensity, mycorrhizal and non-mycorrhizal fungi, and nutrient concentrations in maize, wheat, and canola. Agron J 92:1117–1124

Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Depart Agric Circ 939:1–19

Ortas I (2019) Comparison of indigenous and selected mycorrhiza in terms of growth increases and mycorrhizal dependency of sour orange under phosphorus and zinc deficient soils. Europ J Hortic Sci 84:218–225

Ortas I, Demirbas A, Akpinar C (2018) Under sterilized and non-sterilized soil conditions, mycorrhizal dependency in citrus plants depends on phosphorus fertilization rather than zinc application. Europ J Hortic Sci 83:81–87

Ova EA, Kutman UB, Ozturk L, Cakmak I (2015) High phosphorus supply reduced zinc concentration of wheat in native soil but not in autoclaved soil or nutrient solution. Plant Soil 393:147–162

Palusinska M, Barabasz A, Kozak K, Papierniak A, Maslinska K, Antosiewicz DM (2020) Zn/Cd status-dependent accumulation of Zn and Cd in root parts in tobacco is accompanied by specific expression of ZIP genes. BMC Plant Biol 20:37

Rask KA, Johansen JL, Kjøller R, Ekelund F (2019) Differences in arbuscular mycorrhizal colonisation influence cadmium uptake in plants. Environ Exp Bot 162:223–229

Ryan MH, Graham JH (2018) Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. New Phytol 220:1092–1107

Sheppard SC, Grant CA, Sheppard MI, de Jong R, Long J (2009) Risk indicator for agricultural inputs of trace elements to Canadian soils. J Environ Qual 38:919–932

Singh BR, Dependra KC, Åsgeir RA (2017) Long-term effect of phosphate fertilization on cadmium uptake by oat and its accumulation in soil. J Environ Analytic Toxic 7:516

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol 156:1050–1057

Takagi D, Miyagi A, Tazoe Y, Suganami M, Kawai-Yamada M, Ueda A, Suzuki Y, Noguchi K, Hirotsu N, Makino A (2020) Phosphorus toxicity disrupts Rubisco activation and reactive oxygen species defence systems by phytic acid accumulation in leaves. Plant Cell Environ 43:2033–2053

Tan L, Qu M, Zhu Y, Peng C, Wang J, Gao D, Chen C (2020) ZINC TRANSPORTER5 and ZINC TRANSPORTER9 function synergistically in zinc/cadmium uptake. Plant Physiol 183:1235–1249

Thompson JP (1990) Soil sterilization methods to show VA-mycorrhizae aid P and Zn nutrition of wheat in vertisols. Soil Biol Biochem 22:229–240

Verbruggen E, van der Heijden MG, Rillig MC, Kiers ET (2013) Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol 197:1104–1109

Watts-Williams SJ, Smith FA, McLaughlin MJ, Patti AF, Cavagnaro TR (2015) How important is the mycorrhizal pathway for plant Zn uptake? Plant Soil 390:157–166

Wiggenhauser M, Bigalke M, Imseng M, Keller A, Rehkämper M, Wilcke W, Frossard E (2019) Using isotopes to trace freshly applied cadmium through mineral phosphorus fertilization in soil-fertilizer-plant systems. Sci Tot Environ 648:779–786

Zhang YQ, Deng Y, Chen RY, Cui ZL, Chen XP, Yost R, Zhang FS, Zou CQ (2012) The reduction in zinc concentration of wheat grain upon increased phosphorus-fertilization and its mitigation by foliar zinc application. Plant Soil 361:143–215

Zhang W, Liu D, Liu Y, Cui Z, Chen X, Zou C (2016) Zinc uptake and accumulation in winter wheat relative to changes in root morphology and mycorrhizal colonization following varying phosphorus application on calcareous soil. Field Crop Res 197:74–82

Zhang XF, Hu ZH, Yan TX, Lu RR, Peng CL, Li SS, Jing YX (2019) Arbuscular mycorrhizal fungi alleviate Cd phytotoxicity by altering Cd subcellular distribution and chemical forms in Zea mays. Ecotox Environ Safe 171:352–360

Zhao FJ, Jiang RF, Dunham SJ, McGrath SP (2006) Cadmium uptake, translocation and tolerance in the hyperaccumulator *Arabidopsis halleri*. New Phytol 172:646–654