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Effects of Zinc Chemistry on Phytoextraction in *Thlaspi caerulescens*–Vermiculite System

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Zinc phytoextraction capability of the hyperaccumulator is usually limited by zinc content in the soil. To investigate the effects of different zinc fractions (water–soluble, exchangeable, and non–exchangeable) on phytoextraction of *Thlaspi caerulescens* from medium of vermiculite, pot experiments were performed for various zinc application treatments (0~2000 mg kg^{-1}). Relationships between zinc quantity accumulated in the plants and zinc content in the medium were investigated. Zinc content accumulated in the shoot of *T. caerulescens* increased and approached a constant value with zinc content in vermiculite. The analysis of zinc balance in vermiculite before and after the growth of *T. caerulescens* revealed that more than 40% of the zinc content accumulated by *T. caerulescens* is contributed by exchangeable zinc and that a high linear correlation exists between the zinc content in shoot of *T. caerulescens* and the initial exchangeable zinc in vermiculite. Moreover, 17.73–32.94% of the total zinc uptake by *T. careulescens* was from non–exchangeable fraction. Thus, it was suggested that exchangeable zinc predominantly influenced the zinc uptake by plants and that *T. caerulescens* could contribute to the mobilization of non–labile zinc fraction in vermiculite.

INTRODUCTION

Phytoextraction relies on the hyperaccumulator plants with exceptional metal–accumulating capacities for mobilizing, uptaking, transporting and accumulating the contaminants in harvestable–plant tissues. The phytoextraction has been used to extract heavy metals from contaminated soil recent years (Reeves and Baker, 2000). Zinc (Zn) is an essential micronutrient for plant growth and development but is toxic at high concentrations. A zinc hyperaccumulator plant, *Thlaspi caerulescens*, has attracted considerable interest because of its remarkable ability to accumulate high contents of metals in its above–ground biomass (Ebbs *et al.*, 1997). The constitutively high expression of zinc transporter genes may play an essential role in zinc hyperaccumulation (Pence *et al.*, 2000; Assunção *et al.*, 2001).

However, the phytoextraction capability of the hyperaccumulator will be limited by zinc content in the soil. The existing type of zinc in soil can be divided into several fractions: (a) in soil solution as free metal ions and soluble metal complexes; (b) adsorbed to inorganic constituent at ion exchange sites; (c) bound to soil organic matter; (d) precipitated such as hydroxides and carbonates; and (e) embedded in crystal lattices of primary minerals (Zhu and Alva, 1993). These different fractions are all in dynamic

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equilibrium with each other. Among the zinc fractions, the plant availability differs. The zinc fractions of (a) and (b) mentioned above is available more easily for plant compared with others (Iyengar *et al.*, 1981). Moreover, zinc can be more transferable to the root surface according to convective and dispersion flow, which are controlled by the soil water movement and adsorption/desorption reactions between the bulk soil and the soil solution (Whiting *et al.*, 2003).

Therefore, to successfully manipulate and optimize future phytoextraction technologies, many researches have concerned the chemistry and the plant availability of zinc in soil as well as the mechanism of zinc uptake by *T. caerulescens*. Brown *et al.* (1994) concluded that decrease in soil pH could increase the zinc uptake by *T. caerulescens*. However, McGrath *et al.* (1997) indicated that *T. caerulescens* did not cause significant decrease in the pH of rhizosphere. They also found that the decrease zinc of the mobilizing fraction (NH_4NO_3–extractable) in soil after *T. caerulescens* growth accounted for less than 10% of that accumulated in the plant shoot. Similarly, Knight *et al.* (1997) reported that the decrease of zinc in soil solution accounted for 1% of the total zinc uptake by *T. caerulescens*, suggesting that *Thlaspi caerulescens* has the ability to mobilize zinc from less–available pools in the soil. But to date, the process by which *T. caerulescens* mobilizes and takes up zinc has not been identified.

In this study, the distribution of zinc in a *T. caerulescens*–vermiculite system was investigated to find the relationship between zinc content in medium and zinc uptake by *T. caerulescens*. The actual amount of the metal available to the plant could differ from that of soil existing in a metal–contaminated site. Vermiculite is a layer–lattice mineral with a mica structure and has characteristic properties such as high cation exchange capacity and high water holding capacity (Wild and Keay, 1964). Therefore, by using vermiculite, zinc content in medium can be analyzed even at relatively high content. This experiment may also be helpful to define the causes of zinc phytotoxicity.

MATERIALS AND METHODS

Pot culture

Some seedlings of *T. caerulescens* were planted into pots containing 250 g pot^{-1} of vermiculite. Basal fertilizers applied were 100 mg N kg^{-1} dry vermiculite as NH_4NO_3, 80 mg P kg^{-1}, and 100 mg K kg^{-1} as KH_2PO_4. Zinc was added as $ZnSO_4$ solution. Zinc treatments were 0, 100, 200, 500, 1000, and 2000 mg kg^{-1}vermiculite. Water was supplied to the pots everyday to maintain the moisture content of vermiculite about 1.5 g g^{-1} (water/vermiculite). Each treatment was brought to two pH levels of 6.00 and 6.50 by using 0.1 N HCl and 0.1 N NaOH, because the plant could not grow on low pH (<5.5) condition and almost all of zinc in vermiculite would be insoluble at high pH value (>7.0). The plants were grown for three months inside the greenhouse, in which the temperature was maintained at 25 °C in the daytime and 20 °C at night, and the relative humidity was set at 70%. To investigate the changes of pH values inside and outside rhizosphere, cylindrical rhizobags (12 cm in height and 8 cm in diameter) made of polyester were used to separate the vermiculite as inside and outside rhizosphere parts. The rhizobags were placed in the center of the pots, seedlings of *T. caerulescens* were planted inside the rhizobags. Water and solutes can pass through the rhizobag while the root of the plant can only grow inside the rhozobag.

Measurement of zinc concentration

Zinc contents of plant and vermiculite samples were determined using a fluorescent X–ray (FXR) analyzer (EDX–800, Shimadzu) at the Center of Advanced Instrumental Analysis, Kyushu University (Japan).

Test of zinc accumulation in plants

After the plant harvesting, shoots and roots were separated, washed and dried at 80 °C for 24 hours. Dried plant materials were ground using a ball mill and zinc concentrations were determined.

Analysis of vermiculite samples

Drying and sieving vermiculite samples may change the number of exchange sites in contact with the extracting solution, and properties of the investigated vermiculite may therefore not be in agreement with the vermiculite in pots (Grimshaw, 1989). Hence, moist sub–samples of vermiculite from the pots were collected for the experiments below, but the data were corrected for dry weight (oven–dried at 105 °C for 24 hours).

Adsorption isotherms determination

Adsorption isotherms were determined by equilibrating initial pot vermiculite samples with deionized water at a vermiculite/water ratio of 1:8 (g mL^{-1}) in 50–mL centrifuge tubes. Samples were equilibrated for 24 hours at room temperature. One–hour shaking (110 oscillations per min) on a horizontal shaker was carried out every 6 hours. Samples were centrifuged and filtered after equilibration. Zinc concentrations in the filtrate were analyzed. The difference between the initial zinc concentration and the equilibrium concentration was used to calculate the adsorbed zinc of vermiculite.

Measurements of water–soluble zinc concentrations and pH values of vermiculite

Before and after the growth of plants, vermiculite samples inside and outside rhizobags were collected respectively. The extractions of water–soluble zinc in vermiculite was extracted and shaken by the same procedure mentioned above. After the pH values in the vermiculite–water suspension were measured with a pH meter (HM 16–S), the suspension water was centrifuged and filtered. Zinc concentrations of filtrate were determined, and water–soluble zinc concentrations in vermiculite were calculated.

Determination of bioavailable (exchangeable + water–soluble) zinc

Vermiculite samples inside and outside rhizobags were collected respectively before and after the growth of plants. Bioavailable zinc was extracted using $CaCl_2$ (0.01 M) solution. The vermiculite/solution ratio was 1:8 (g mL^{-1}). The vermiculite samples and $CaCl_2$ (0.01 M) solution were mixed in 50–mL centrifuge tubes. Samples were shaken, centrifuged and filtered as mentioned above. The filtrate zinc concentrations were measured, and bioavailable zinc concentrations of vermiculite samples were calculated.

RESULTS

Adsorption isotherms

Figure 1 shows the relationship between the different quantities of zinc adsorbed per unit mass of vermiculite and the equilibrium concentrations of solution zinc.

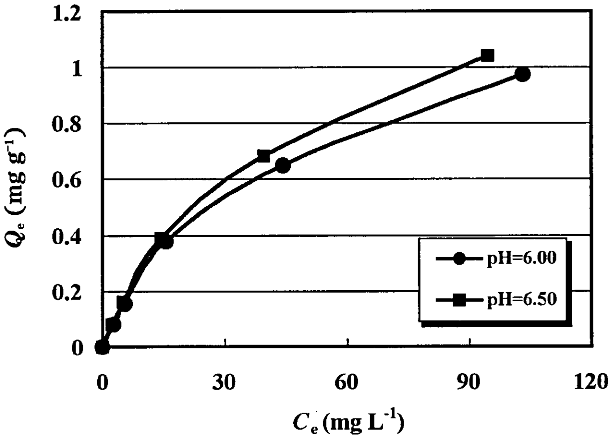

Fig. 1. Adsorption isotherms of zinc on vermiculite.

The Langmuir adsorption isotherm is represented by the following equation:

$$\frac{C_e}{Q_e} = \frac{1}{K_l d} + \frac{C_e}{d}$$

where C_e is the equilibrium zinc concentration in vermiculite (mg L^{-1}), Q_e is the amount of zinc adsorbed by vermiculite at equilibrium time (mg g^{-1}), d and K_l are Langmuir constants related to the adsorption capacity and energy of adsorption, respectively. The plots of C_e/Q_e versus C_e are linear, showing that zinc adsorption conforms to Langmuir model (Fig. 2).

The Freundlich adsorption isotherm is represented by the following equation:

$$\log Q_e = \log K_f + n \log C_e$$

where Q_e is the amount of zinc adsorbed by vermiculite at equilibrium time (mg g^{-1}), C_e is the equilibrium zinc concentration in vermiculite (mg L^{-1}), and K_f and n are Freundlich constants. Linear plots of log Q_e versus log C_e show that zinc adsorption by vermiculite also follows Freundlich model (Fig. 3). Values of Langmuir and Freundlich constants are shown in Table 1.

Zinc uptake in *Thlaspi caerulescens*

In this experiment, the maximum zinc concentration accumulated in the shoot of *T. caerulescens* was 23.69 mg g^{-1} (dry weight). For each treatment, zinc concentration of

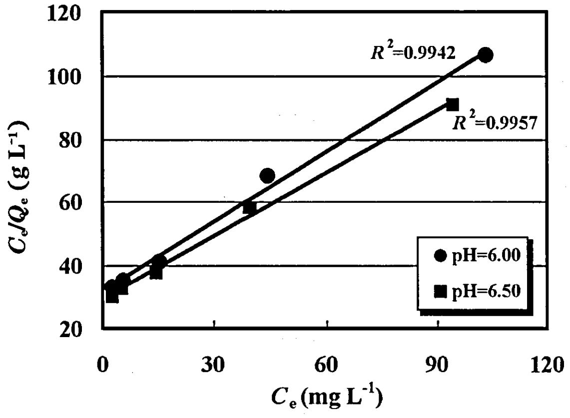

Fig. 2. Langmuir isotherms for zinc adsorption by vermiculite.

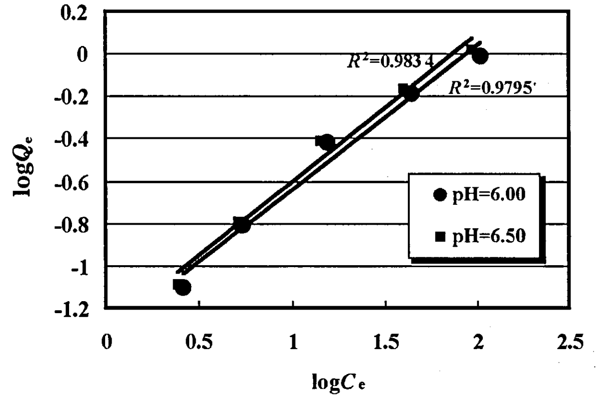

Fig. 3. Freundlich isotherms for zinc adsorption by vermiculite.

Table 1. Langmuir and Freundlich constants for zinc adsorption.

pH	Langmuir Adsorption			Freundlich Adsorption		
	K_l	d	R^2	K_f	n	R^2
6.00	0.023	1.36	0.9943	0.047	0.68	0.9795
6.50	0.022	1.52	0.9957	0.049	0.70	0.9834

the plant shoot was higher at pH6.00 than that at pH6.50. But the differences were not significantly large (Fig. 4).

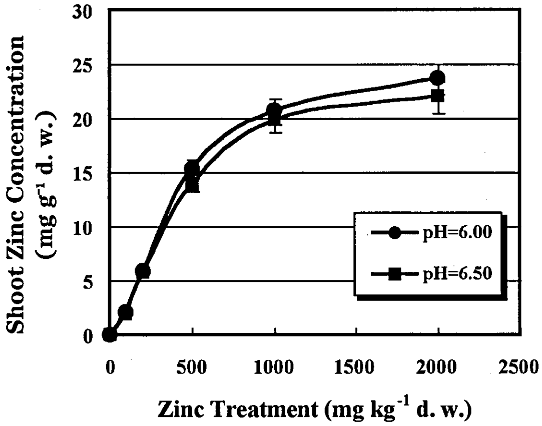

Fig. 4. Concentrations of zinc accumulated in the shoots of *Thlaspi caerulescens*.

In this paper, the difference between $CaCl_2$–extractable and water–extractable zinc was regarded as exchangeable zinc, and non–exchangeable zinc was obtained from the difference between zinc added to vermiculite and $CaCl_2$–extractable zinc. Raising initial zinc concentrations in vermiculite resulted in the increase in zinc accumulation in shoots of *T. caerulescens*. However, zinc accumulated in shoots of *T. caerulescens* indicated high linear–correlation with zinc in exchangeable fraction compared with that in other fractions of vermiculite. The relationships between shoot zinc concentrations of *T. caerulescens* and the initial zinc concentrations in different fractions of vermiculite are shown in Figure 5. The values of coefficient of determination R^2 are presented in Table 2.

Changes of pH values in vermiculite

Table 3 shows that pH values of vermiculite did not differ significantly between inside and outside rhizobags after the growth of plants. This results conformed with McGrath *et al.* (1997) and Knight *et al.* (1997), suggesting that the rhizosphere acidification was not the main mechanism for zinc uptake by *T. caerulescens*.

Comparison between zinc uptake by plants and the loss of zinc in vermiculite

The average decrease in zinc for different fractions of vermiculite and the average amount of zinc accumulated by *T. caerulescens* of each treatment are shown in Figure 6. For each treatment, the amount of zinc accumulated by the plant in each pot is larger than the decrease in $CaCl_2$–extractable zinc. This means that zinc accumulated by *T. careulescens* is not only from water–soluble and exchangeable fractions, but also from non–exchangeable pool. The further calculation revealed that 67.06–82.27% of the total zinc uptake by *T. careulescens* was contributed by $CaCl_2$–extrctable zinc, and the remaining 17.73–32.94% was from non–exchangeable fraction. With the enhancement of zinc concentration in vermiculite, the contribution of water–extractable zinc to the total plant uptake rose from 24.62–41.41% while the contribution of exchangeable zinc decreased

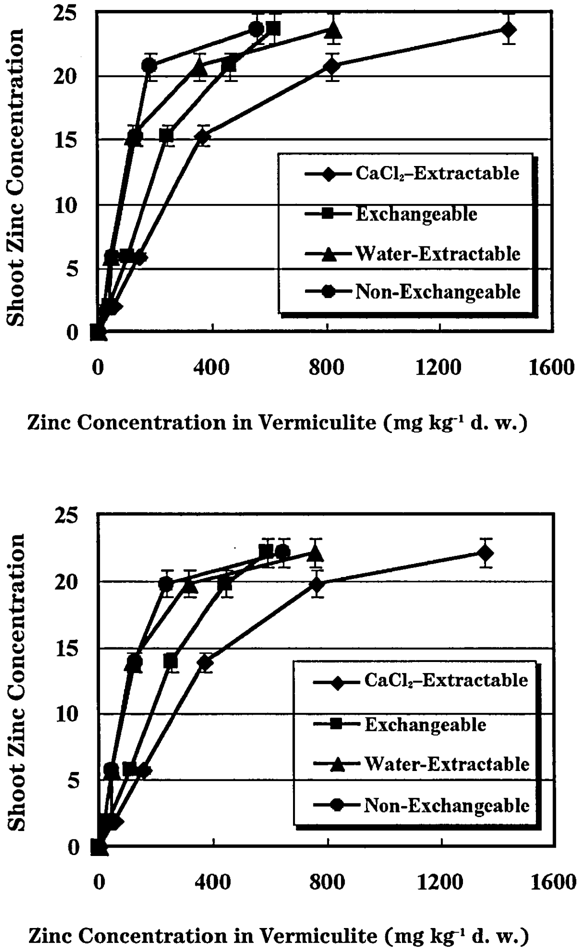

Fig. 5. Relationships between zinc accumulated in the shoots of *Thlaspi caerulescens* and different initial zinc fractions in vermiculite. (A): pH=6.00. (B): pH=6.50.

Table 2. Values of coefficient of determination R^2 for linear-correlation between zinc in shoot and vermiculite.

Fraction	pH=6.00	pH=6.50
$CaCl_2$-Extractable	0.8437	0.8540
Exchangeable	0.9483	0.9616
Water-Extractable	0.7253	0.7242
Non-Exchangeable	0.6836	0.7002

Table 3. pH values of vermiculite after the growth of plants.

Treatment (mg kg^{-1})	Final pH Values			
	Initial pH=6.00		Initial pH=6.50	
	Inside Rhizobag	Outside Rhizobag	Inside Rhizobag	Outside Rhizobag
0	6.03	6.01	6.54	6.51
100	6.06	6.01	6.54	6.50
200	6.05	6.02	6.57	6.51
500	6.08	6.00	6.52	6.50
1000	6.05	6.02	6.53	6.51
2000	6.04	6.00	6.54	6.50

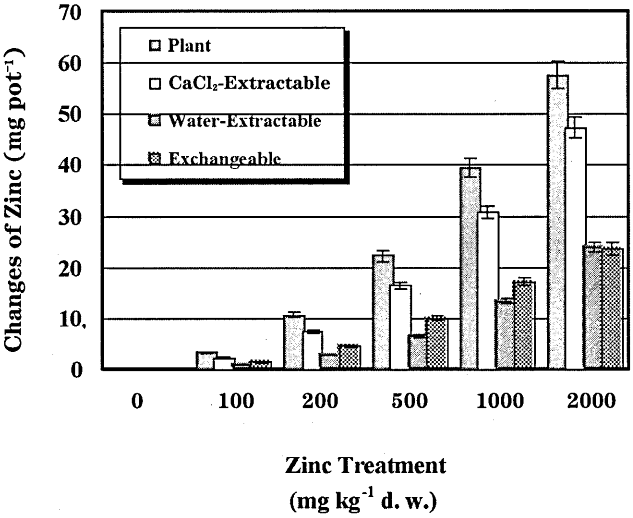

Fig. 6. Comparison between zinc accumulated by *Thlaspi caerulescens* and the decrease of zinc in vermiculite.

from 42.44–40.86%.

DISCUSSION

As a hyperaccumulator, *T. caerulescens* accumulated large quantity of zinc in its above-ground biomass after the growth in high zinc concentration vermiculite (Fig. 4). This can be considered to be resulted from its genetic advantages, which have been investigated by many researchers (Pence *et al.*, 2000; Assunção *et al.*, 2001).

Generally, when water-soluble zinc cations from ZnSO$_4$ are added to vermiculite, they can migrate from the water–soluble fraction to exchangeable and non–exchangeable fractions. A zinc dynamic equilibrium will occur among different fractions. The pH value is widely acknowledged to be the principal factor governing the plant availability because of its influence on zinc solubility and adsorption (Singh and Abrol, 1985). However, in this research, the results of shoot zinc uptake by *T. caerulescens* obtained at pH6.00 and pH6.50 did not show significant pH dependence (Fig. 4). One of the reasons may be that the zinc concentration in vermiculite does not change with pH due to high permanent charge on vermiculite surface. In addition, $ZnSO_4$ added to the soil can be completely dissolved at both pH6.00 and pH6.50, and by comparison, the concentrations of less–soluble zinc species such as zinc carbonate and hydroxide are very low in this experiment. Therefore, the adsorption equilibrium and zinc distribution for different fractions were predominantly controlled by concentrations of zinc added to vermiculite (Fig. 1, Fig. 2, and Fig. 3). This leads to a concentration dependent result rather than a pH dependent one.

Although increasing the amount of zinc added to vermiculite enhanced the shoot zinc uptake by *T. caerulescens*, the linear correlations between shoot zinc content and the initial zinc concentrations of various fractions in vermiculite are quite different (Fig. 5 and Table 2). Generally, the growing roots of plants are thought to be exposed to the solution phase. The zinc concentration in the solution phase seems to be a predominant soil condition affecting zinc uptake by plant roots. However, the high rate of zinc uptake into roots of *T. caerulescens* results in very high demands for zinc at the root surface. This may cause the zinc depletion in the solution near the root surface. The mass flow can only contribute a small amount of zinc to the root even at relatively high soil concentrations (Whiting *et al.*, 2003). Hence, a concentration gradient will develop and diffusion will occur. On the other hand, zinc uptake by plant disturbs the equilibrium of zinc between solution phase and solid phase near the zone of zinc depletion. The soluble zinc will be replenished by exchangeable pool as the plant removes it. Thus, the supply of zinc to the root surface conforms to a circulation process involving zinc adsorption, desorption, and resorption to the medium. As zinc movement from non–exchangeable fraction to exchangeable pool is much more difficult than zinc releasing to water–soluble fraction from exchangeable pool, exchangeable pool could play a key role in the zinc supply in vermiculite. Therefore, a high linear–correlation existed between the shoot zinc of *T. caerulescens* and the initial exchangeable zinc (Fig. 5 and Table 2). In the further investigation, it was discovered that more than 40% of the zinc accumulated by *T. caerulescens* was contributed by exchangeable zinc. Only for initial zinc concentration of 2000 mg kg^{-1}, zinc supplied by water–soluble pool accounted for more than 40% of the total zinc accumulated by the plant (Fig. 6). Apparently, exchangeable zinc predominantly influenced the zinc uptake by plants, especially when zinc concentration in vermiculite is relatively lower.

Some authors (Knight *et al.*, 1997; McGrath *et al.*, 1997) reported that *T. caerulescens* had remarkable ability to mobilize zinc in non–labile fraction of the soil, which contributed more than 90% of the total zinc uptake by plants. Such strong zinc mobilizing results were not obtained in our experiment, but still there are 17.73–32.94% of the total zinc uptake by the plants can be considered from non–exchangeable fraction. The lower the zinc concentration, the more the zinc acquired by the plant from non–exchangeable fraction. The explanation is that, in the case of high zinc concentration, plant roots are easy to uptake more zinc from soil solution and exchangeable pool. In agreement with the authors above, by determining the pH changes in vermiculite before and after the growth of *T. caerulescens* (Table 3), we concluded that the acidification of solution phase could not be the method by which *T. caerulescens* increase zinc availability. In other words, the acquirement of extremely insoluble zinc from vermiculite by *T. caerulescens* was not due to the acidification. Small amount of zinc may reverse from non–exchangeable fraction to exchangeable part in the dynamic equilibrium of zinc in vermiculite. But the rate of this process is very slow. Moreover, it is impossible to supply large amount of available zinc to the plant. Thus, an active zinc–mobilizing mechanism by *T. caerulescens* may exist, especially when the concentrations of water–soluble and exchangeable zinc are relatively lower. It has been proved by Schwartz *et al.* (1999) that the root growth of *T. caerulescens* responded positively to zinc in soil. The roots of *T. caerulescens* exhibited high affinity for patches containing zinc with high concentrations. As the root of *T. caerulescens* cannot dissolve zinc by the acidification or secrete specific zinc–mobilizing compounds (Zhao *et al.*, 2001), the solid phase–root–uptake may be a possible way for *T. caerulescens* to mobilize zinc, which can be explained by the contact exchange theory (Jenny and Overstreet, 1939). If the solid phase–root–uptake was one of the mechanisms involved in zinc mobilization, it would also support the predominant effect of exchangeable pool on zinc uptake by *T. caerulescens*. However, the contact exchange theory has not been established as an essential mechanism. Therefore, it is necessary to confirm that in what case the solid phase–root–uptake will occur. And surface characteristics of the plant root and the solid phase must be seriously investigated. Moreover, mechanisms of interactions between zinc and the plant root surface and the movement of zinc in the solid phase should be well understood.

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