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ZINC PRIMING OF MAIZE SEED ENHANCES ROOT TO SHOOT Zn TRANSLOCATION BUT NOT OF ANALOGOUS HEAVY METALS

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ABSTRACT

Soil contaminated with heavy metals is considered a leading environmental concern as they are translocated to harvestable part of plant and ultimately influence animals and human health. Pre-germination metabolic processes stimulated by seed priming with mineral nutrient may facilitates the availability of that particular nutrient under adverse soil conditions. Seed priming with zinc (Zn) impact on Zn and heavy metals, for instance, cadmium (Cd) and nickel (Ni), uptake and their translocation within plant was evaluated in this research study. Seeds of maize were hydro-primed and Zn-primed ($ZnSO_4$ solution) before sowing. Soil was amended with heavy metals, namely Cd and Ni and seedling was harvested after twenty days of sowing. There was no considerable treatment effect found in the various plant morphological and physiological attributes. However, interestingly, on the one hand, seed priming with Zn enhanced its uptake and distribution within plant; on the other hand, reduction in root-to-shoot translocation of Cd and Ni was observed. As a result, seed priming with Zn is not only an advantageous approach to improve Zn nutrition but also valuable to hinder the translocation of heavy metals and ultimately it can suppress inclusive deleterious impacts on human health.

Key words: Contaminated Soils; Heavy Metals; Seed Priming; *Zea mays*; Zinc

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INTRODUCTION

Seed priming with mineral nutrients is an approach to improve the seed nutrient contents in conjunction with the priming effect per se obviously to increase the seed quality for better germination and the seedling establishment. The process of seed priming makes a seed more resilient prior to an abiotic stress exposure. In detail, pre-germination, metabolic processes of seed are stimulated, accordingly makes it ready for radicle protrusion and eventually improve seed potency in the course of germination and emergence. It helps the repair of membranes and also boosts the activity of the antioxidant system (Paparella *et al*., 2015; Qayyum *et al*., 2018). Furthermore, Zn priming of seed may accelerate Zn transportation and eventually improve its uptake from soils, which ultimately strengthen plant growth, yield and Zn contents in the grains (Mondal and Bose, 2019; Rashid *et al*., 2019).

Zinc performs a substantial role in energy transfer reactions in plants, photosynthesis, bio-membrane stability, structural activities, and catalytic protein synthesis (Alloway, 2008; Hajiboland and Amirazad, 2010). It can also detoxify reactive oxygen species (ROS) to mitigate the oxidative stress (Feigl *et al*., 2015). Furthermore, it involves as a cofactor for the stimulation of more than 300 enzymes, accomplish a substantial part in water transport and uptake by reducing effect of abiotic stresses like heat and salt stresses antagonistically (Kasim, 2007; Sbartai *et al*., 2011). Zinc deficiency ranks 5th amongst the reasons of deaths and illnesses in human, especially in developing countries (WHO, 2009). Likewise, a substantial number of people in Pakistan especially women and children are Zn deficient, according to our most recent survey more than 30% of the investigated Faisalabad region of Pakistan found Zn deficient in the soil, wheat grain along with humans (unpublished data).

Heavy metals are toxic metals and considerable reduce plant growth and yield even at low concentration. The plant uptake them from contaminated soils, translocate them from root-to-shoot and ultimately become the part of the harvestable portion of the plant. In contaminated soils, Cd has appeared as one of the critical hazards not only to environmental stability as well as to global food security; as once it amasses in the soil remains there for a longer period of time. Therefore, it can certainly become the part of the food chain when existing in agricultural soils (Simmons *et al*., 2003). Reduced uptake of other essential plant nutrients, for example, N, P, K along with Ca and Mg was found and it also suppresses their translocation from root to shoot (Wang *et al*., 2014; Yourtchi and Bayat, 2013), consequently, adversely affects crop growth and yield (de Souza Guilherme *et al*., 2015; Chen *et al*., 2017; Shiyu *et al*., 2020). Furthermore, Ni toxicity is also becoming an emerging concern as its availability in soils are increasing especially in urban areas as a result of continuous application of polluted sewage water. Same like Cd, higher concentration of Ni can also reduce the availability of other plant nutrients, for instance, substitute iron (Fe) and Zn in soil solution (Sabir *et al*., 2011) or nitrogen (N) and phosphorus (P) (Santos *et al*., 2019).

The Cd translocation in plants can be suppressed if the sufficient amount of Zn is applied to Cd contaminated soils (Ishfaq *et al*., 2018; Ma *et al*., 2020) Due to their competitive interaction of Cd and Zn uptake via plant roots, they are transported by the same carrier proteins of plasma membrane (Köleli *et al*., 2004) or expression regulation specific ZIP genes (De Oliveira *et al*., 2020; Martha-Paz *et al*., 2019; Palusińska *et al*., 2020). Most recently, it is revealed that Zn efficient plants can reduce Cd uptake, its root to shoot translocation and finally accumulation in grains (Sarwar *et al*., 2010; Qaswar *et al*., 2017;) or just suppress its accumulation in edible part of the plant (Ishfaq *et al*., 2018). Nevertheless, little is known about the impact seed priming with Zn in the uptake and translocation of heavy metals, for instance, Cd and Ni from root-to-shoot (Murtaza *et al*., 2017).

As maize plants are able to store Cd and Ni, become adaptable for metals and show the reduction in growth (Gallego *et al*., 2012). It is evident that such heavy metals may share Zn uptake systems of plant cells. Therefore, this research study was planned with the objective to evaluate the consequence of Zn-primed maize seed in the uptake and its root to shoot translocation for Zn along with Cd and Ni. The ultimate objectives of the intended study were to explore: i) the beneficial aspect of Zn seed priming on Zn uptake from soil and its distribution within plant ii) the impact of seed priming with Zn on uptake and root to shoot translocation of heavy metals, namely Cd and Ni from the contaminated soil.

MATERIALS AND METHODS

Experimental layout: A greenhouse experiments was carried out in this research study in pots with one kg soil per pot placed at the Botanic garden, University of Agriculture, Faisalabad-Pakistan to evaluate the consequence of seed priming with Zn in the form of $ZnSO_4.7H_2O$ (analytical grade) in Zn and heavy metals, for instance, Cd and Ni uptake from the soil and their root to shoot translocation. Basic analysis of soil used in experiment, for instance, ECe, pH, texture, Zn and Cd concentration is presented in Table 1. Seeds of Maize (*Zea mays*) variety Malka-2016 was obtained from the Ayub Agricultural Research Institute, Faisalabad, Pakistan for assessment of treatments.

Table 1. Physicochemical characteristics of the soil used in the experiment.

Parameter	Unit	Value
pH[a]	---	7.16
EC_e[a]	dS m^{-1}	1.38
Sand, Silt, Clay[b]	%	53, 24, 23
Textural class[c]	---	Sandy clay loam
Saturation percentage[d]	%	33
Organic matter[e]	g kg^{-1}	7.2
Zn[f]	mg kg^{-1}	0.91
Cd[f]	mg kg^{-1}	0.14

a. Measured in 1:1 soil to water
b. Hydrometer method (Page *et al*., 1982)
c. USDA classification
d. Determined by soil saturated paste and oven drying method
e. Walkley-Black method (De Vos *et al*., 2007)
f. AB-DTPA extractable (Soltanpour, 1985)

There were two sets of treatments, first with Zn primed and second with water primed seeds. Each of the sets had control (without soil application of Zn, Cd and Ni and mentioned as CK for water primed or for Zn primed seeds), soil Zn application (10 mg kg^{-1} soil mentioned as Zn), soil Cd application (17 mg kg^{-1} soil mentioned as Cd) and Ni (9 mg kg^{-1} soil mentioned as Ni). The application rate of Zn was as per recommended dose whereas Cd and Ni were applied equivalent to that of the Zn application rate. The experiment was laid out as stated by two-way factorial design and each treatment was replicated four times, in this fashion total thirty-two experimental units.

Seed priming was done by soaking the maize seeds for 16 hours in distilled water and 0.5 mM $ZnSO_4.7H_2O$ (144 mg L^{-1} DW) solution separately. After soaking, six primed seeds were sown in each pot reserved in a control greenhouse. A basal dose of NPK fertilizers was applied in each pot equivalent to 200, 120 and 80 kg ha^{-1}, respectively using urea, diammonium phosphate and potassium sulfate fertilizers. Soil was contaminated with heavy metal as stated above one week before the time of sowing. After contaminating soil thorough mixing was done to attain uniform distribution of nutrients and heavy metals.

Crop husbandry and harvesting: After the establishment, three seedlings as biological replicates were maintained in each pot. Irrigation of pots were done on water requirements of 60% water holding capacity of soil. Maize seedling was harvested after twenty days of sowing to test treatment effect. Plant growth traits i.e. dry and fresh shoot and root weight was measured with digital weighing balance. Plant physiological attributes, namely transpiration rate, photosynthesis rate, stomatal conductance and sub-stomatal CO_2 concentration were recorded before harvesting by using digital Infrared gas analyzer. SPAD 502 P meter was used to measure spectral plant analysis diagnostic (SPAD) value which reflect the chlorophyll contents. For determination of physiological attributes, the youngest fully expanded leaf was analyzed three times. The process was carried out for all three plants in each pot and averages from each pot was deliberated as technical replicates.

Determination of concentration and translocation of Zn, Cd and Ni: Root and shoot of maize seedling were processed further to analyze the concentrations of Zn, Cd and Ni according to the prescribed protocol (Hseu, 2004). In brief, fine ground root and shoot samples (oven dried at 70$\pm$5 ºC till constant weight) were digested in the di-acid mixture with a ratio of 2:1 (HNO_3:$HClO_4$) at 250±5 °C before mixing it for one night. Dense fumes (whitish) of perchloric acid were observed in tubes and this digestion process was continued for further 30 minutes. After that samples were diluted with distilled water up to 25 mL volume and filtered using Whatman 42 filter paper. Zn, Cd and Ni concentrations were determined in samples using the atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) and drawing calibration curve with working standards. The translocation factor for Zn, Cd and Ni translocation from root-to-shoot was calculated by using following formula:

$$\text{Translocation factor} = \frac{(\text{Shoot dry Wt.} \times \text{Zn, Cd or Ni Conc. in shoot})}{(\text{Root dry Wt.} \times \text{Zn, Cd or Ni Conc. in root})}$$

Statistical Analysis: All collected data from each experimental unit was analyzed by statistical technique (Johnson and Bhattacharyya, 2019) by using Statistix 9® for Windows (Analytical Software, Tallahassee, USA). Two-way analysis of variance (ANOVA) subsequently the LSD test was used at $P \leq 0.05$ for the evaluation of treatment effect.

RESULTS

***Effect of seed priming on morphological attributes*:** There was no considerable treatment effect was found in fresh root weight of primed seed, however dry root weight improved with Zn priming. Maximum dry root weight was found in Zn primed CK (2.55 g) and is significantly ($P \leq$ 0.05) reduced in distilled water primed Cd contaminated condition (1.04 g). There was no statistically significant difference found for fresh and dry shoot weight of Zn primed treatments ($P \leq 0.05$); however, maximum fresh shoot weight was 3.42 g and dry shoot weight of 0.51 g was found in water primed Ni contaminated treatment (Table 2).

***Effect of seed priming on physiological traits*:** The collected data regarding physiological traits was found in the irregular pattern with seed priming and contamination of soil with heavy metals. Maximum photosynthesis rate was noticed under water primed CK treatment (6.17 µmol CO_2 m^{-2} S^{-1}) and it was found the minimum water primed Cd contaminated treatment (1.99 µmol CO_2 m^{-2} S^{-1}). In case of Zn primed, the rate of photosynthesis was found higher in Zn primed Ni contaminated soil (4.40 µmol CO_2 m^{-2} S^{-1}). The maximum rate of transpiration was observed in the leaves of water primed Ni contaminated treatment (1.51 mmol H_2O m^{-2} S^{-1}), while the minimum was detected in Zn primed CK (0.68 mmol H_2O m^{-2} S^{-1}). In Zn primed Cd contaminated treatment stomatal conductance was noticed higher (0.04 mmol H_2O m^{-2} S^{-1}) relatively other treatments while it was found the minimum in Zn primed Ni contaminated treatment and water primed Zn applied soil. Sub-stomatal CO_2 concentration was also affected by priming; it was observed significantly ($P \leq 0.05$) higher in Zn primed CK (472.3 µmol mol^{-1}) as compared to all other treatments (Figure 1). The data regarding SPAD value was also found non-significant in different treatments and seed priming conditions ($P \leq 0.05$) (Table 2).

***Effect of Zn seed priming on uptake and translocation of Zn, Cd and Ni*:** After analysis of plant root and shoot samples, there was the considerable treatment effect was found in the pattern of Zn, Cd and Ni uptake and translocation within plant (Figure 2 & 3). Maximum Zn concentration (16.90 mg kg^{-1} DW) was found in the shoot of Zn primed seeds under Ni contaminated soil and it was found minimum (2.58 mg kg^{-1} DW) in Cd contaminated soil where water primed seed were sown. In shoot, overall Zn concentration improved 48.80, 82.62 and 80.94 % with Zn, Cd and Ni application in Zn primed seed as compare to the water primed (Figure 2A). In root Zn concentration increased (7.29 %) in Cd contaminated Zn primed treatment as compare with Cd contaminated water primed seed. Non-significant effect of treatments was found @ P $\leq$ 0.05 in other treatments (Figure 2D).

In contrast to Zn, Cd concentration found higher in water primed treatments. In shoot maximum Cd concentration (36.20 µgkg^{-1} DW) was found in Cd contaminated water primed treatment and with Zn priming it was significantly reduced (6.86 %). In other treatments, there was no significant variation was found ($P \leq 0.05$) (Figure 2B). In root, Cd concentration also increased (40.35 µg kg^{-1} DW) with Cd contamination, however, in Zn primed seed it was significantly reduced (5.32%) as compared to water primed. In other treatments, the pattern was found statistically non-significant ($P \leq 0.05$) (Figure 2E).

Like Cd, Ni concentration reduced in the shoot of maize seedling with Zn priming as compared to water primed seed. Ni concentration was found higher in Ni contaminated treatment; this pattern was found higher in water primed seed; however, it was reduced with Zn priming. Maximum Ni concentration (94.36 µg kg^{-1} DW) was found in the shoot of Ni contaminated water primed treatment and with Zn priming it was reduced 80.60, 106 and 33.61 % in CK, Zn application and Ni contamination, respectively (Figure 2C). In roots Ni concentration also found the maximum in Ni contaminated water primed treatment (58.52 µg kg^{-1} DW) and it was reduced to 21.44 µg kg^{-1} with Zn priming. In other treatment reduction was found non-significant (Figure 2F).

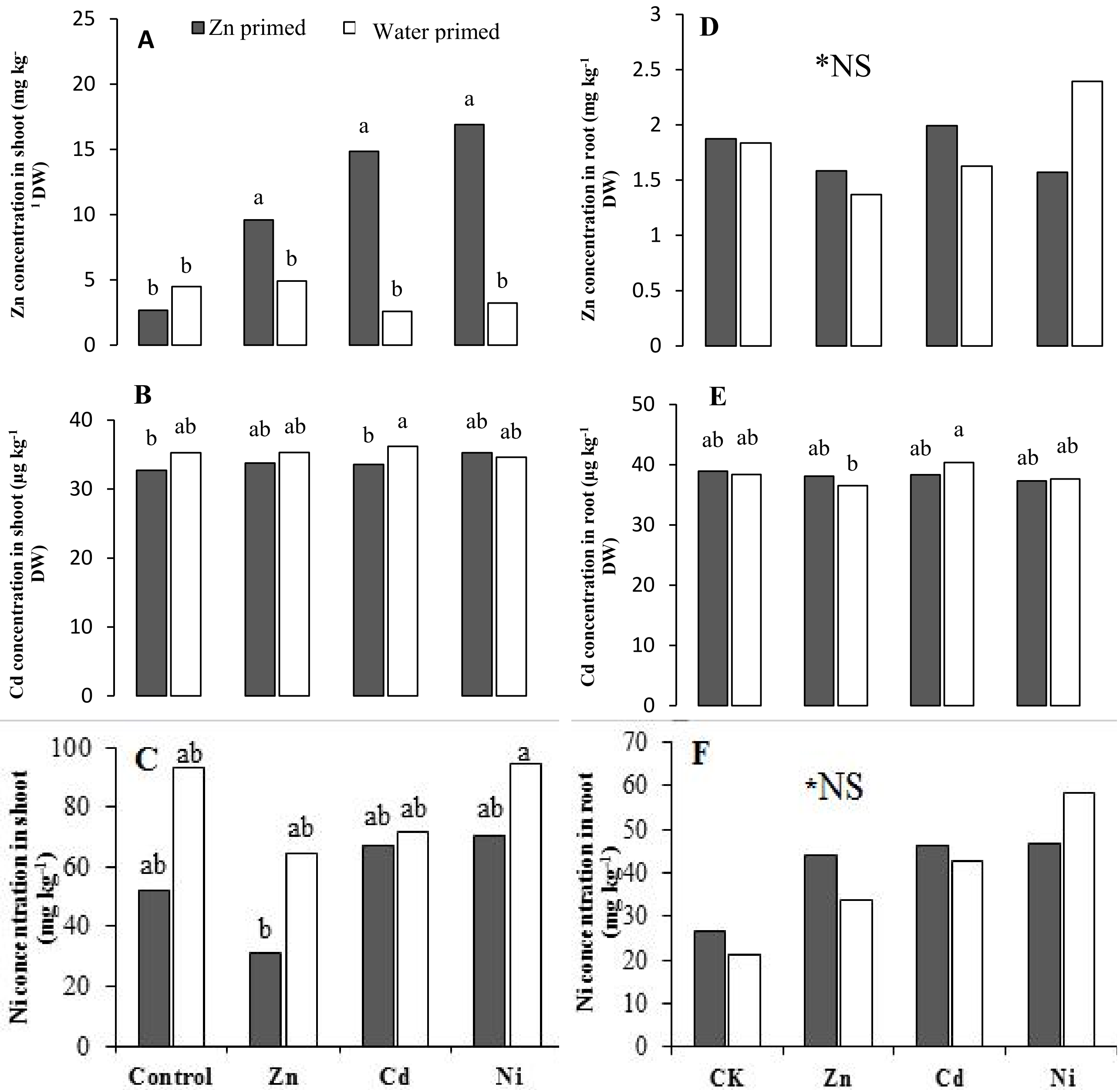

Figure 2. The effect of Zn and water seed priming on shoot and root Zn and heavy metals, namely Cd and Ni uptake and translocation via maize (*Zea mays*) seedling in contaminated soil. Zn concentration in shoot (A), Cd concentration in shoot (B), Ni concentration in shoot (C), Zn concentration in root (D), Cd concentration in root (E), and Ni concentration in root (F). Column shows mean of four replicates while bars shows standard deviation (±). The mean sharing similar letter (s) do not differ significantly ($P \leq 0.05$) according to LSD (Zn 2.78, 1.59; Cd 1.31, 1.67 and Ni 24.65, 18.44 for shoot and root, respectively). *NS= No significant treatment effect was detected

DISCUSSION

The impact of seed priming with Zn was evaluated in this study with reference to its uptake and distribution within plant along with its influence on the root to shoot translocation of heavy metals (Cd and Ni) in contaminated soils. Considering plant growth mentioned as shoot and root weights, asymmetrical treatment effects were found among various treatments. Maximum root dry weight in Zn primed CK treatment was recorded as compared with soil Zn applied treatment. Maximum fresh shoot and dry shoot weight were recorded in the Zn applied Ni contaminated soils. As the low doses of these metals were applied, therefore a symmetrical difference between different treatments were not expected for plant growth attributes. However, similar finding is revealed by a number of researchers in various crop species (Aremu and Meshitsuka, 2005; Sabir *et al*., 2011; Syam *et al*., 2016; Chen *et al*., 2017). Data regarding physiological traits showed that photosynthesis rate was less in water primed Cd contaminated soil and Zn priming might reduce the deleterious impact of Ni contamination on photosynthesis rate. Such a reduced rate of photosynthesis in contaminated soil under water primed seed might be due to the accretion of heavy metals primarily in photosynthetically active (transpiring) leaves. Similarly, improvement in sub-stomatal CO_2 concentration and stomatal conductance with Zn priming in contaminated soil was observed (Figure 2). Afzal *et al*. (2013, 2015) previously reported positive effect of Zn priming on physiological performance of maize.

Improved shoot Zn contents 48.80, 82.62 and 80.94 % due to Zn seed priming under soil Zn, Cd and Ni treatments may be attributed to better stand establishment and root architecture as revealed (Mohsin *et al*., 2014). Such improvement in Zn concentration in the plant with Zn application has also been reported by other researchers as well (Ishfaq *et al*., 2018; Joy *et al*., 2015; Cioccio *et al*., 2017; Rizwan *et al*., 2019). In addition, root Zn concentration increased (7.29 %) in Cd contaminated Zn primed treatment (Figure 2E) showing that Zn transporters family accelerated by Zn priming and restrict the Cd translocation within plants due to the availability of Zn in the soil solution (Ishfaq *et al*., 2018) or up-regulation of root specific ZIP genes involved in its root to shoot translocation (Palusińska *et al*., 2020). Reduced translocation due to Zn priming was also observed (Table 2). For instance, the Cd uptake by plant root can be minimized if sufficient level of Zn is applied to Cd contaminated soils (Venkatachalam *et al*., 2017; Ishfaq *et al*., 2018; Rizwan *et al*., 2019), due to their competitive interaction in uptake via roots because they are transported by the same carrier membrane proteins (Köleli *et al*., 2004; Moustakas *et al*., 2011; Sarwar *et al*., 2010). Murtaza *et al*. (2017) also observed antagonistic effect of Zn as reduction in Cd concentration in cereal plants tissues with a significant increase in Zn concentration.

Being an essential element plants can take up Ni easily if it is present in the soil and its beneficial impact can also be observed in plants. However, in highly contaminated soils it may have toxic effects on plant growth (Santos *et al*., 2019). In present study, low level of Ni was used to understand the impact of Zn priming on Ni uptake. Zn priming decreased the Ni content in shoot; however, it was not significant (Figure 2), therefore further studies with higher Ni contamination are still remains open. However, it can be assumed that unlike Cd transport, Ni uptake is not directly dependent on the Zn uptake system and may have its own system which can even work efficiently in the presence of Zn (Mohseni *et al*., 2019; Yusuf *et al*., 2011), though specific underlying mechanisms of Ni uptake and transportation in plant still remains open to uncover. Nevertheless, increased Zn uptake, its competing trend with Cd for binding to critical cell constituents and translocation in Cd contaminated soils also indicate Zn specific uptake system (Köleli *et al*., 2004; Palusińska *et al*., 2020).

The schematic mechanism of our research findings for the uptake and root to shoot translocation of Cd and Ni mediated by Zn seed priming is demonstrated in below given diagram (Figure 3) adapted from Banakar *et al*., (2017) and DalCorso *et al*. (2019). Banakar *et al*., (2017) and DalCorso *et al*. (2019). However, various underlying molecular mechanisms, selective redistribution of heavy metals, their uptake, translocation within plant via specific transport systems, and other intermediated signaling pathways need to be further elucidated for biofortification of plants (Page and Feller, 2015). The most recent advances in the omics approaches, for instance, transcriptomics, proteomics, and ionomics ultimately required to significantly improve heavy metal tolerance in crop plants and can also be beneficial for revealing the underlying molecular regulatory systems in the translocation of heavy metals mediated by Zn seed priming (Singh *et al*., 2016).

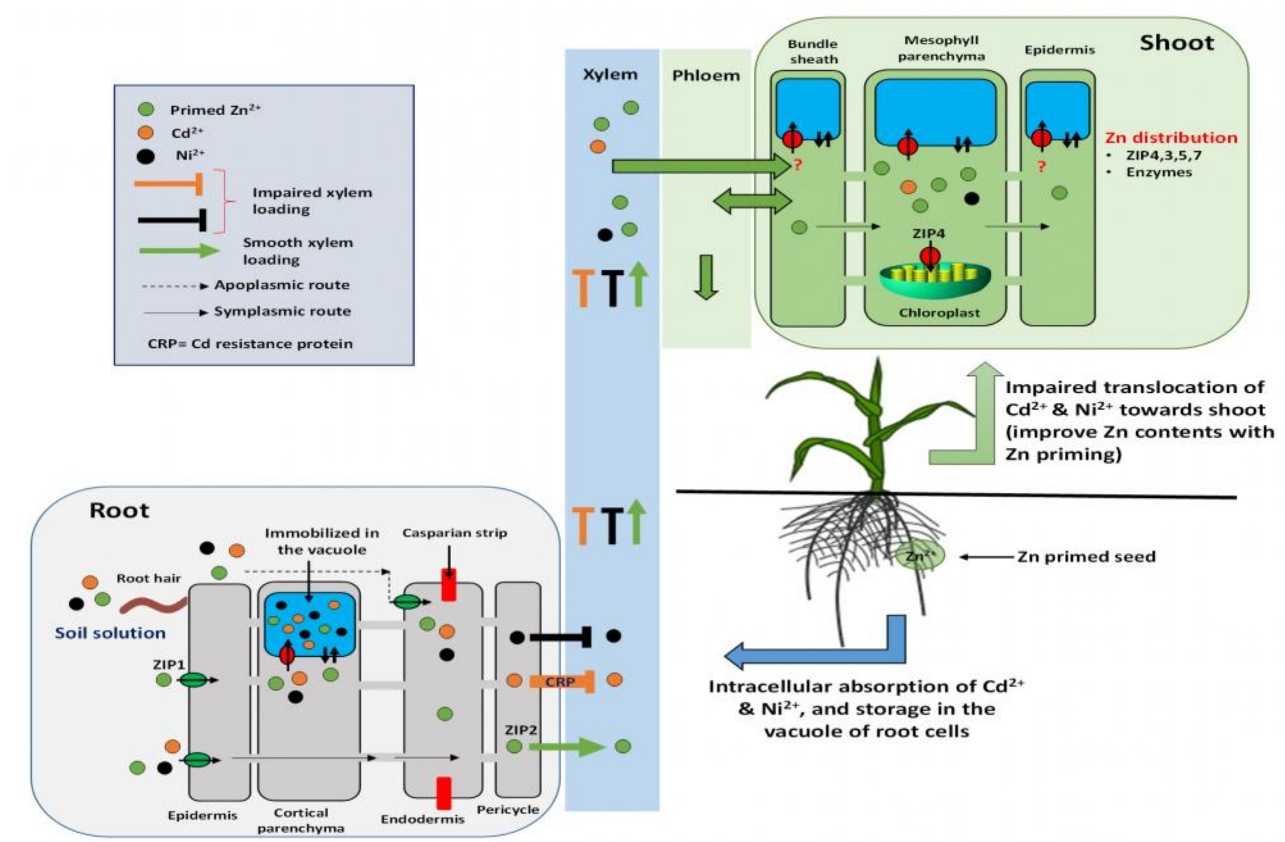

Figure 3. A schematic diagram of uptake and translocation of Zn, Cd and Ni from root-to-shoot mediated by Zn priming. Plant uptake them in the form of Zn^{2+}, Cd^{2+} and Ni^{2+} from soil solution; due to their equivalent valence charge they can share common transport systems and/or translocate via specific transporter per se. In the root cells, a considerable portion of Cd and Ni can be absorbed in the intracellular or immobilize in the vacuole by means of Zn availability. Furthermore, Zn priming can impair the xylem loading of Cd and Ni owing to its antagonistic affect or other factors, i.e. plant Cd resistance protein. Hence, Zn priming improves Zn concentration in shoot but not analogous heavy metals, for instance, Cd and Ni translocation from root-to-shoot.

Table 2. Maize seedling morphological attributes and SPAD value mediated by Zn and water primed seeds along with heavy metals (Cd and Ni) contamination.

Seed priming	Treatments	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	SPAD value	Zn translocation factor	Cd translocation factor	Ni translocation factor
Zn primed	CK	6.54 ± 1.2^{a}	2.55 ± 0.6^{a}	3.20 ± 0.3^{ab}	0.47 ± 0.00^{abc}	32.67 ± 3.8^{a}	0.45 ± 0.09^{c}	0.17 ± 0.05^{b}	0.41 ± 0.30^{bc}
	Zn	4.22 ± 1.3^{a}	1.15 ± 0.2^{b}	2.81 ± 0.2^{ab}	0.38 ± 0.01^{cd}	32.67 ± 2.1^{a}	1.94 ± 0.06^{a}	0.26 ± 0.05^{b}	0.16 ± 0.08^{c}
	Cd	6.45 ± 0.6^{a}	1.77 ± 0.2^{ab}	2.51 ± 0.3^{b}	0.36 ± 0.30^{cd}	32.07 ± 2.5^{a}	1.48 ± 0.31^{ab}	0.21 ± 0.03^{b}	0.24 ± 0.07^{c}
	Ni	6.55 ± 1.0^{a}	$1.94+0.3^{ab}$	3.31 ± 0.3^{ab}	0.45 ± 0.03^{abcd}	29.27 ± 1.9^{a}	1.43 ± 0.19^{ab}	0.21 ± 0.02^{b}	0.23 ± 0.01^{c}
Water primed	CK	4.30 ± 1.0^{a}	1.51 ± 0.4^{b}	2.61 ± 0.2^{ab}	0.36 ± 0.03^{d}	28.77 ± 3.1^{a}	0.65 ± 0.23^{c}	0.21 ± 0.06^{ab}	0.87 ± 0.24^{ab}
	Zn	6.14 ± 1.2^{a}	1.95 ± 0.4^{ab}	3.30 ± 0.1^{ab}	0.48 ± 0.01^{ab}	34.35 ± 2.3^{a}	0.87 ± 0.22^{bc}	0.40 ± 0.18^{ab}	0.34 ± 0.10^{bc}
	Cd	3.63 ± 0.1^{a}	1.04 ± 0.0^{b}	2.95 ± 0.2^{ab}	0.38 ± 0.02^{bcd}	31.12 ± 1.4^{a}	0.52 ± 0.25^{c}	0.39 ± 0.04^{ab}	0.81 ± 0.37^{a}

	Ni	4.42±0.8 [a]	2.13±0.5 [ab]	3.42±0.2[a]	0.51±0.04[a]	33.8±1.9[a]	0.25±0.04[c]	0.21±0.04[b] c	0.55±0.10b c

[a]Means followed by similar letter(s) do not differ significantly according to LSD (root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, SPAD, Zn translocation factor, Cd translocation factor and Ni translocation factor value 1.49, 0.66, 0.41, 0.05, 3.57. 0.68, 0.14 and 0.58 respectively) ($P \leq 0.05$)

Conclusion: On behalf of our research findings, conclusively seed priming with Zn is an applicable approach not only to improve Zn nutrition and but also to suppress the translocation of heavy metals, for instance, Cd and Ni in contaminated soils reducing their deleterious effects on plant and human health, ultimately. However, underlying molecular signaling pathways in heavy metal translocation mediated by Zn priming remains open to be further studied.

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