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Vulnerability of Sunflower Germination and Metal Translocation under Heavy Metals Contamination

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Abstract

The germination and metal translocation ability of two sunflower seedlings were compared to identify the cultivar differences towards metal contamination at juvenile stage. The heavy metal treatments include: 0, 50, 100, 150 and 200 mM Ni, Cd and Pb applied in sand filled pots to Hysun-33 and FH-533 sunflower. The highest germination percentage (79%) and vigor index were recorded for Hysun-33 with no heavy metal treatment. Pb and Cd treatments reduced the growth attributes of 20 days old seedlings of both the cultivars. The Ni translocation effectively enhanced the shoot and root biomass of Hysun-33. The high concentration of 150 and 200 mM Cd and Pb drastically reduced Mn and K contents, vigor, length and biomass of two sunflower cultivars. Among three of the heavy metals, Cd was found more toxic than Pb and Ni. Roots of 20 days old seedlings of Hysun-33 were able to hold more Cd metal and stop its translocation to epigenous parts. Although 150 and 200 mM Ni effects the germination and vigor of sunflower cultivars more than 50 and 100 mM Ni, it is found less toxic in comparison to Cd and Pb. The Cd accumulation in roots suggests that it is physiologically most active sink for Cd metal while epigenous parts of sunflower cultivars are sink for Pb and Ni metal as shoot of sunflower cultivars accumulates high contents of Pb and Ni.

Keywords

Translocation, Germination, Heavy Metals, Contamination, Sunflower

1. Introduction

The hyperbolizing amount of heavy metals in soil and water is rapidly depleting the native flora. The well grown and mature plants somehow tolerate these toxic metals by compartmentalization and extraction mechanisms [1] [2]. The germination is very delicate phase of plant life and demands very suitable environmental conditions [3].

The health and vigor at this stage determine the fate of a juvenile plant. But in present era, due to the human invasion, utilization of forest land for agricultural purposes, fertilizer investment and industrialization are killing natural ecosystem and making soil toxic for cultivated crops [4].

Heavy metals such as Cd and Pb are non-essential elements for plant growth [5] although Ni acts as co-factor for various enzymes and this required concentration is very small [6]. As these metals are not part of plant normal metabolism, therefore, their presence in soil hampers the growth and development of crops. The passionate uptake of Cd, Ni and Pb by plant depends upon their concentration in soil or their physicochemical state [7].

Sunflower is known as salt hyper accumulator and tolerant towards heavy metals [8]. It has been used for rhizofileration of Cd metal but it shows low efficiency of Cd translocation towards upper parts of sunflower [9].

Reports are available for using sunflower for phytoremediation of heavy metals but literature is absent that supports the effect of toxic metals on germination of sunflower. The present study was planned to investigate and compare the effect of Cd as well as Ni and Pb at first growth stage (germination) of two sunflower cultivars and translocation of these metals to various parts.

2. Materials and Methods

The study was carried in sand filled pots during Spring season, 2017 at Government College Women University, Madina Town, Faisalabad, Pakistan. Eight seeds of each cultivar, Hysun-33 and FH-533 were subjected to 0, 50, 100, 150 and 200 mM of Ni, Pb and Cd contamination. One hundred twenty (120) pots were set up in a completely randomized manner and each treatment was replicated 4 times. The rate of germination was recorded for one week for both Hysun-33 and FH-533 [10]. The time to 50% seed germination was calculated using the formula of [11] percentage germination [12] germination index [13] vigor index [14]. Three plants from each pot were harvested 20 days after emergence (DAE) and their roots were washed with distilled water. The plants were partitioned into root and shoot, length and fresh weights were determined. Each part was placed in paper bags, labelled and put in preheated oven at 70˚C for 48 h. The dried weight was noted and each sample was grounded to powder form. The 0.5 gsubsamples were digested with 2 mL sulfuric acid and 1 mL hydrogen peroxide [15] at 250˚C on a hot plate for 30 min. The colorless digested samples were filtered and diluted up to 50 mL with deionized distilled water. The resulting filtrate samples were analyzed for Ni, Pb and Cd concentration against standards using Atomic Absorption Spectrophotometer (Aanalyst-330, Perkin Elmer and Germany). The Mn and K contents were analyzed using Flame Photometer. The translocation factor (TF) for Ni, Cd and Pb was calculated for Hysun-33 and FH-533 as metal concentration in shoot by dividing metal concentration in roots [16]. The data was statistically tested and correlated with LSD of 5% using analytical software Statistix(Version 8.1 USA). The correlation was assessed between germination and growth as well as metal concentration and growth attributes.

3. Results

3.1. Germination Indices

Figure 1 shows mean variations for all five germination indices as affected by Ni, Pb and Cd treatments (50, 100, 150 and 200 mM). The final germination (**Figure 1(a)**) was highest for 50 and 100 mM Ni, Pb and Cd treatments (80%) for FH-533. T50 germination (**Figure 1(b)**), total germination count (**Figure 1(c)**), germination index (**Figure 1(d)**) and vigor index (**Figure 1(e)**) were lowest under Cd and Pb. When using T50 germination, 200 mMPb revealed 5% germination of FH-533 and Hysun-33 showed no germination. The T50 germination was lowest 3% under 200 mM Cd for Hysun-33 and 7% for FH-533 respectively (**Figure 1(b)**). The mean vigor index was highest under 50 mM Ni metal for both cultivars while lowest for 200 mM Ni and Cd. All treatments of Pb metal showed lowest mean vigor index as compared to Ni but found better than Cd (**Figure 1(e)**).

3.2. Growth Attributes

The presence of Ni, Pb and Cd in sand culture effects the growth of FH-533 more than Hysun-33, but not statistically significant ($P > 0.05$). The means variation for different treatments of metals on both the cultivars (**Figure 2**) was prominent for growth attributes. Among three different heavy metals, Ni showed positive impact on shoot length (**Figure 2(a)**), root length (**Figure 2(b)**), shoot and root fresh and dry biomass (**Figures 2(c)-(f)**). All the concentration of Ni enhanced the growth attributes of Hysun-33 except 150 mM. The growth of Hysun-33 and FH-533 was unaffected under 50 and 100 mM of Pb and Cd. High doses of 150 and 200 mMPb and Cd negatively affect the growth attributes of both the cultivars (**Figure 2**).

3.3. Translocation Factor of Ni, Pb and Cd and Their Relation with Mn and K

The concentration of Ni and Pb was significant ($P < 0.05$) in whole 20 days old seedlings, shoot and root parts. The concentration of Cd was found non-significant for both two cultivars, Hysun-33 and FH-533. Hysun-33 showed least amount of Ni, Pb and Cd in their plant parts in comparison to FH-533 (**Figure 3**). Increase in the concentration of Ni and Cd with the increasing doses in root parts of Hysun-33 and FH-533. The concentration of Pb was found greater in shoot part of sunflower cultivars.

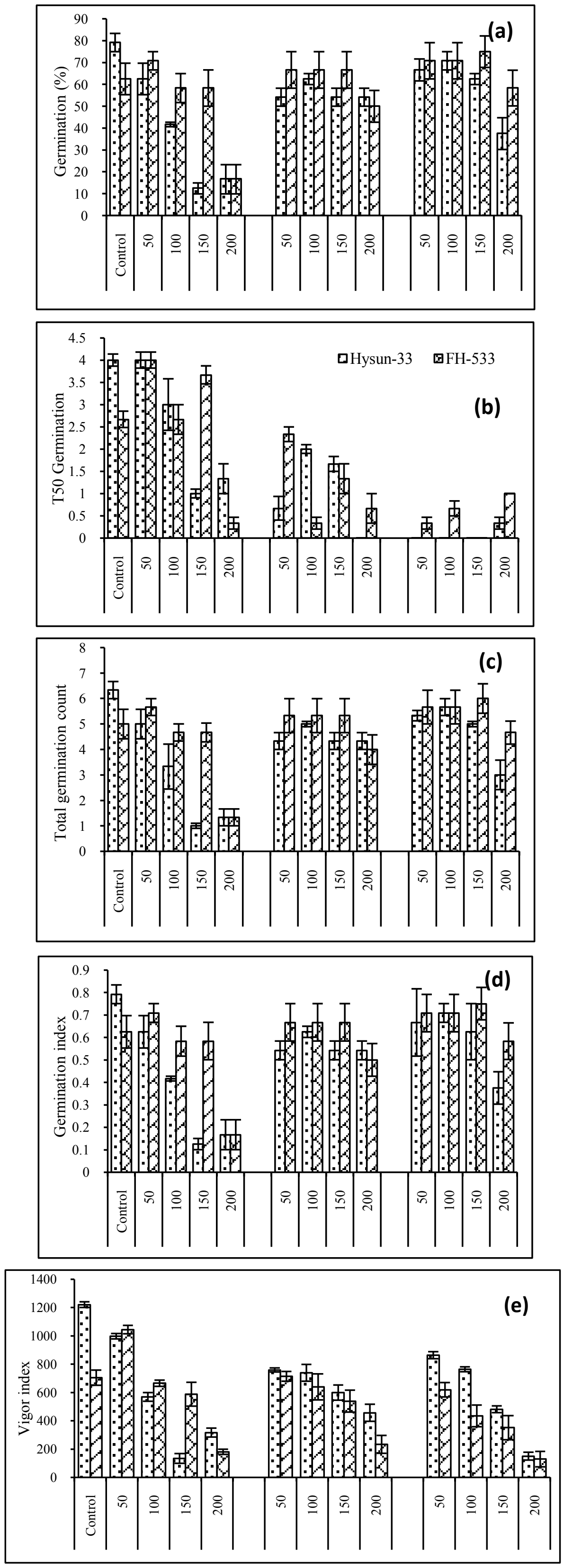
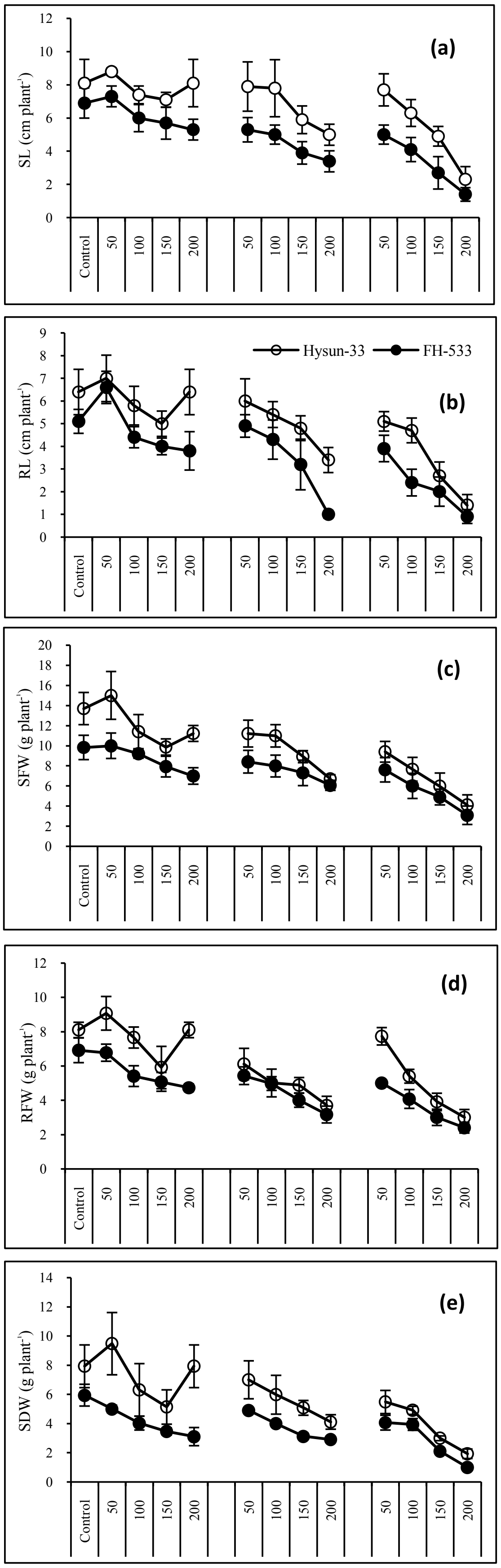


Figure 1. Germination indices as affected by Ni, Pb and Cd (0, 50, 100, 150 200 mM) for Hysun-33 (doted bars) and FH-533 (lined bars). Data presented as Means ± SE.


(a)
SL (cm plant-1)
Control
50
100
150
200
(b)
RL (cm plant-1)
Hysun-33
FH-533
(c)
SFW (g plant-1)
(d)
RFW (g plant-1)
(e)
SDW (g plant-1)

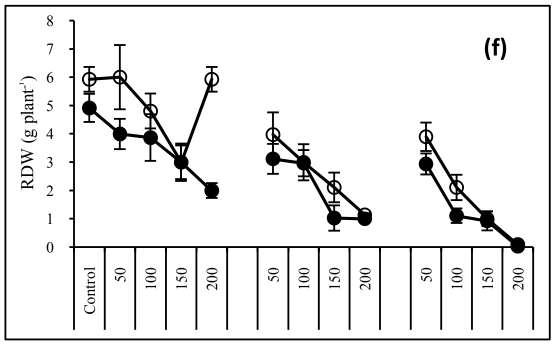


Figure 2. Growth attributes as affected by Ni, Pb and Cd (0, 50, 100, 150 200 mM) for Hysun-33 (white spots) and FH-533 (black spots). SL (shoot length), RL (root length), SFW (shoot fresh weight), SDW (shoot dry weight), RFW (root fresh weight), RDW (root dry weight). Data presented as Means ± SE.

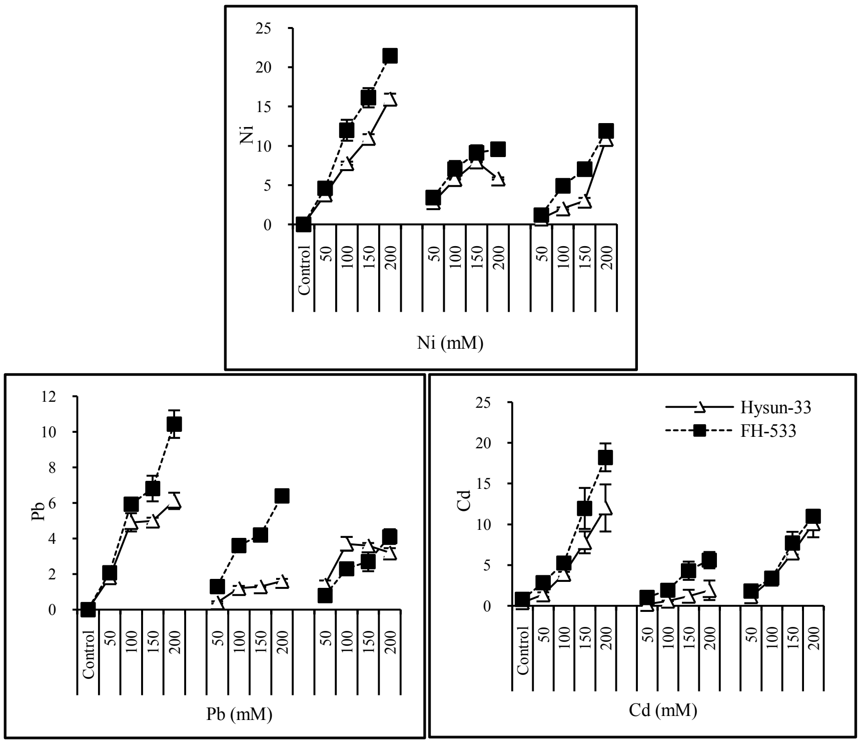


Figure 3. The Ni, Pb and Cd contents (mg·kg^{-1}) in whole plant, shoot and root of Hysun-33 (triangle) and FH-533 (square) under Ni, Pb and Cd (0, 50, 100, 150 200 mM) doses. Data presented as Means ± SE.

The translocation factor (TF) of Ni has expressed a linear sharp decrease for Hysun-33 ($R^2 = 0.8996$) and FH-533 ($R^2 = 0.8325$) with increasing doses of Ni in growth medium (**Figure 4**). Hysun-33 showed a gradual linear decline in Pb with a value of $R^2 = 0.9521$ while translocation of Pb in FH-533 was linear with $R^2 = 1$ with all doses of Pb. The translocation of Cd increase linearly in Hysun-33 ($R^2 = 0.9$) and decrease linearly in FH-533 ($R^2 = 0.86$) with increasing doses of Cd (**Figure 4**).

The Mn contents were statistically significant only for Ni doses for both the sunflower cultivars. The K contents were non-significant under increasing doses of Ni, Pb and Cd for Hysun-33 and FH-533. The means variations in Figure 5 showed more Mn and K contents in Hysun-33 than FH-533. The 150 and 200 mM doses of both Ni and Pb drastically affect the Mn contents of FH-533 The lower doses of Ni (50 and 100 mM) showed more Mn and K contents for both Hysun-33 and FH-533 as compared to higher doses (Figure 5).

3.4. Relationship of Ni, Pb and Cd with Germination, Growth and Nutrients Indices

The correlation analyses were conducted between Ni, Pb, Cd and germination, growth, Mn and K under investigation. The aim was to assess the agreement of each index with each heavy metal.

The Pearson's correlation coefficient (r) values are shown in Table 1.

Ni, Pb and Cd showed negative correlation for Hysun-33 and FH-533 for all the parameters studied. The increase in the doses of Ni, Pb and Cd decrease the germination, growth, Mn and K in both sunflower cultivars. Ni was significantly negative correlation with all germination indices of Hysun-33 while non-significant for shoot, root length and biomass, Mn and K and vice versa for FH-533. Growth attributes were significantly negative correlated with Ni, Pb and Cd with both Hysun-33 and FH-533. Mn and K contents of FH-533 had significant reciprocal relation with Ni, Pb and Cd.

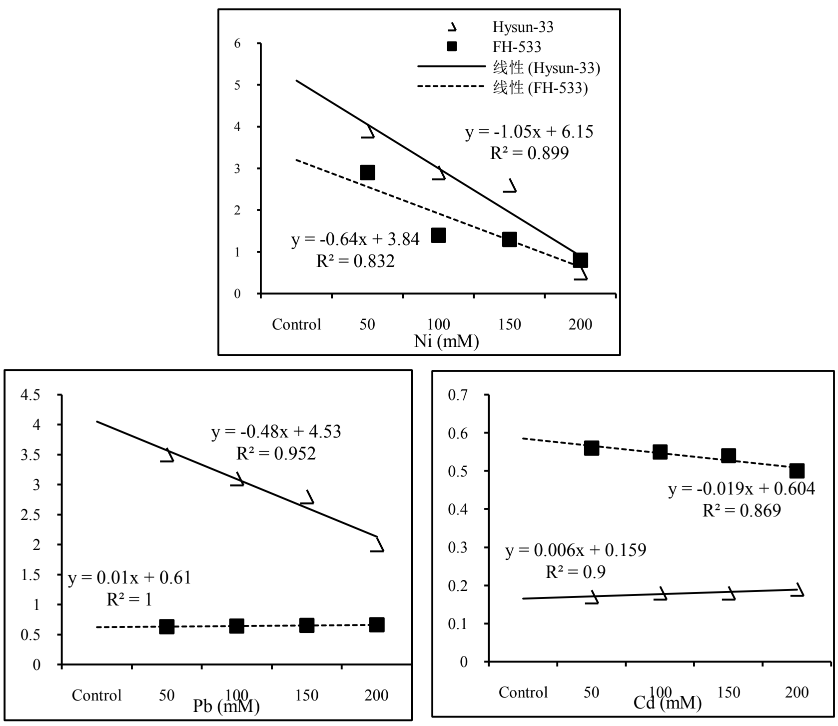


Figure 4. Translocation factor (-) as affected by Ni, Pb and Cd (0, 50, 100, 150 200 mM) for Hysun-33 (triangle) and FH-533 (square). Dotted trend line with regression equation and R-value represents for Hysun-33 and linear black trend line with regression equation and R-value represents FH-533.

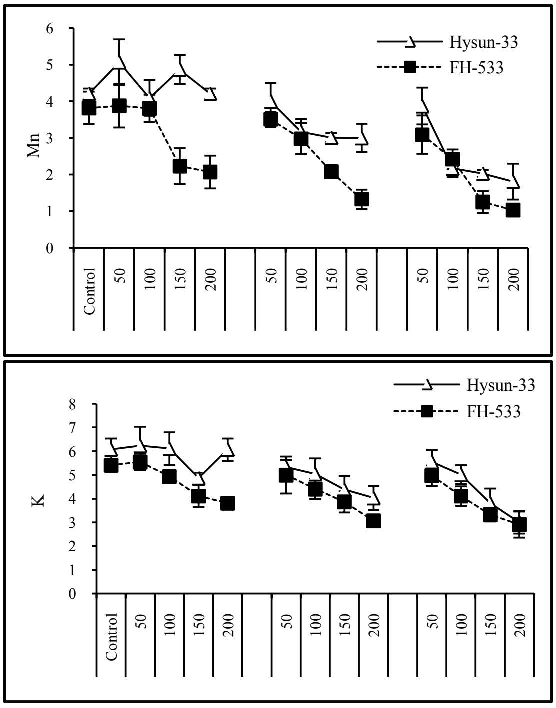


Figure 5. The Mn and K contents (mg·kg^{-1}) in Hysun-33 (triangle) and FH-533 (square) under Ni, Pb and Cd (0, 50, 100, 150 200 mM) doses. Data presented as Means ± SE.

Table 1. Pearson Correlation coefficient (r) between germination, growth and nutrient indices for Hysun-33 and FH-533.

Heavy Metals (mM)	Ni		Pb		Cd	
Parameters	Hysun-33	FH-533	Hysun-33	FH-533	Hysun-33	FH-533
% germination	−0.94*	−0.78ns	−0.70ns	−0.58ns	−0.91*	−0.28ns
T50 germination	−0.89*	−0.56ns	−0.66ns	−0.85ns	−0.49ns	−0.39ns
Total germination count	−0.94*	−0.78ns	−0.70ns	−0.58ns	−0.92*	−0.28ns
Germination index	−0.94*	−0.78ns	−0.70ns	−0.58ns	−0.92*	−0.28ns
Vigorindex	−0.90*	−0.78ns	−0.90*	−0.89*	−0.97**	−0.97**
Shoot length	−0.36ns	−0.93*	−0.77ns	−0.94*	−1.0***	−0.96*
Root length	−0.37ns	−0.76ns	−0.88*	−0.93*	−0.98**	−0.92*
Shoot fresh weight	−0.74ns	−0.94*	−0.89*	−0.97**	−0.90*	−0.94*
Shoot dry weight	−0.37ns	−0.98**	−0.96*	−0.96**	−0.94*	−0.96**
Root fresh weight	−0.38ns	−0.98**	−0.97**	−0.96**	−0.96**	−0.91*
Root dry weight	−0.31ns	−0.96**	−0.97**	−0.90*	−0.92*	−0.85ns
Mn contents	−0.11ns	−0.86ns	−0.98**	−0.96**	−0.86ns	−0.95*
K contents	−0.33ns	−0.94*	−0.94*	−0.99**	−0.99***	−0.96**

4. Discussion

The uptake of Ni at the time of germination affects the vigor of seedling. The lower Ni concentration imposes positive effects on early seedling growth but higher concentrations have been documented to be enormously toxic and impose negative effects on seedling growth [17]. The adverse effects of Ni as well as other metals are predominantly manifested as the reticence of plant growth [18], an index generally used to evaluate the environmental degradation [19]. Higher level of Ni in growth medium more will be the rate of growth inhibition [20]. The root growth is more declined than shoot in excluder plant species which mainly concentrate Ni in their roots [21]. Many reports have also proved toxic effects of higher level of Ni on growth attributes of various plants *i.e.* decrease in radicle and plumule growth and fresh and dry weights [22]. These toxic effects of Ni are the result of its inhibitory effects on enzyme activity involved in breakdown of reserve food material (α- and β-amylase and protease), carbohydrate metabolism, protein synthesis and mobilization of food reserves [23]. In addition, its higher levels have been reported to interfere with essential mineral uptake resulting in altered concentration of essential minerals in germinating seeds and growing seedlings [24].

The magnitude of harmful effects of Pb varies and depends on its concentration and duration of exposure, stage and particular organs of plant. Seedlings sprouting and development is also adversely affected by Pb exposure [25]. It restraints the growth and development of roots and above ground parts even at very low concentration [26]. Root growth is more influenced by Pb toxicity than shoot, which may be interrelated to higher Pb content [27]. Pb toxicity significantly inhibits root elongation in Mesquite (*Prosopis* sp.) [28].

Application of Cd imposed negative effect on all the studied morphological parameters such as shoot, root length, shoot fresh and dry weights and root fresh and dry weights. Although all levels proved toxic for morphological parameters but the highest level of Cd *i.e.* 200 mM affected them more severely. The stunted growth is the most common, but nonspecific indicator of Cd stress [29]. Differences in the extent of phyto-toxicity depend on Cd concentration, duration of exposure and species characteristics. Roots are more affected by Cd stress than shoot because of presence and accumulation of higher Cd contents in roots [30], this can be accredited in part to the reticence of mitosis, damaged Golgi apparatus, reduction in cell-wall components synthesis and altered polysaccharide metabolism [31]. There are also reports about its toxicity *i.e.* alteration in plant metabolism even at very low concentrations [32]. Its presence in growth medium effected the growth of many plant species e.g. chickpea plants [1] and sunflower [33]. Higher Cd concentrations limit the cell growth and at whole plant scale [34]. Similarly fresh weight of mungbean is also affected by higher Cd concentrations [35].

Presence of Ni in growth medium triggered the reduction in nutrients uptake and accumulation. Manganese uptake and translocation was badly affected in pear trees treated with higher levels of Ni [36]. An antagonistic relation between manganese uptake and Ni concentration has also been proved by [37]. The nutrients (K and Mn) uptake and their concentration are significantly reduced by the application of Pb. Pb and K, both ions compete with each other to enter in plant through the common potassium channels. Similarly Pb causes efflux of K ions by effecting the -SH groups of cell membrane proteins and K+ -ATPase [38]. The disturbed nutrients status of plants may be the result of Cd competition with nutrient. Cd travels through the same rout of ZIP and NRAMP members and Ca^{2+} channels which are involved in uptake of various essential nutrients, resulting in nutrients imbalance and retarded growth of plants [39].

The members of ZIP family of proteins (Iron Regulated Transporters/Zinc Regulated Transporters), YSL (Yellow Stripe Like) and NRAMP (Natural Resistance Associated Macrophage Protein) are responsible for Ni transport in different organisms including plants [40]. The Ni chelation with organic acids like citrate and histidine are responsible for tolerance potential of hyperaccumulator plants for this metal. In hyperaccumulator plants, the Ni is accumulated in leaf epidermal cells vacuoles after absorption and transportation through xylem [41]. In addition, in hyperaccumulators the nicotinamaine translocation from leaves to roots facilitates its transport to above ground parts by complex formation [40].

The main pathway for uptake and acuumulation of Pb is through root from soil [38] [42]. The Pb present in soil makes bond with carboxyl groups of uronic avid or make direct interaction with rhizodermis polysaccharides after adsorption onto the roots [43]. It may adopt the passive pathway after entering the roots and track the translocating streams of water. After penetration into root system of plants, it may be concentrated there or may me translocated to the above ground parts of plants. In majority of plant species the Pb absorbed (95% or more) is concentrated in roots, while only a small proportion is transferred to the aerial parts, as reported in *Pisumsativum*and *Viciafaba* [44], *Nicotianatabacum* [45], *Zea mays* [46] and *Allium sativum* [47]. When loaded in the root system, Pb mainly travels through apoplast and trails the water streams unless its access to the endodermis. Many reasons are responsible for limited translocation of Pb into the aerial parts like hindrance due to the presence of negatively charged pectins in cell wall [27], concentration in cell membranes [47] and confiscation on vacuoles of cortical and rhizodermal cells [26]. Pb translocation to the aerial parts requires its movement through xylem [48] and it may be occurs through transpiration pull [49].

Plants respond differently to increased levels of Cd contents in growth medium depending on species uptake and transport potential. Its bioavailability is pH, temperature, redox potential and most importantly concentration dependent. Various phenomena such as carboxylase exudation and rhizosphere acidification are major targets for elevated metals uptake and accumulation [50]. In lettuce roots antagonistic relation between Cd and Zn and their absorption have been reported [51]. The uptake of other nutrients like nitrate, which even have no similar properties with this metal, is significantly affected by Cd. The first target site of Cd is root system [52]. The mechanism responsible for uptake of Cd by roots and its amassing in consumable parts is not fully clear. The difference of electrochemical potential of Cd amongst root apoplast and cytosol wheels the Cd absorption across the root cells plasma membrane. Substantial energy is provided by the hefty membrane potential for Cd uptake to carry on even at little Cd doses Cadmium has the effective efficient potential of penetration through symplastic and apoplastic pathways and hence easily targets the aerial tissues specifically Irrespective of metal mobility its concentration is more dominant in roots than aerial parts of plants. The damaging gradient of Cd is in order: root > leaves > fruits > seeds/grains [52].

5. Conclusion

Our results concluded that Ni in low doses at germination stage of sunflower improved the germination, growth of Hysun-33 while Pb and Cd had vice versa effects.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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