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# DIFFERENTIAL GROWTH AND PHOTOSYNTHETIC RESPONSES AND PATTERN OF METAL ACCUMULATION IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVARS AT ELEVATED LEVELS OF LEAD AND MERCURY

**SEEMA MAHMOOD*, SHABNAM ISHTIAQ, MUHAMMAD IBRAHIM MALIK AND ALI AHMED**

*Institute of Pure and Applied Biology, Botany Division, Bahauddin Zakariya University Multan. Pakistan P.C. 60800*
*Corresponding author's email: drseemapk@gmail.com*

**Abstract**

Influence of lead and mercury levels (100 and 300 mg/Kg soil) on three sunflower cultivars (DK-4040, Hysun-33 and NK-278) was assessed by analyzing germination, various growth attributes (fresh and dry weights of root and shoot and plant height) and photosynthetic traits (leaf area, chlorophyll *a, b* and total) along with carotenoids. Bioaccumulation of metals in plant tissues were assessed by Atomic Absorption Spectroscopy (AAS). Both lead and mercury at a concentration of 100 mg/Kg of soil did not significantly influence the attributes studied. However, the most elevated level of metals (300 mg/Kg of soil) had caused a significant reduction of growth and pigments. Biomass production, leaf area, photosynthetic pigments and carotenoids content appeared to serve as potential indicators for metal tolerance. The results indicated distinct responses of the cultivars as well as differential effects of the tested metals. Among the cultivars, DK-4040 had consistently showed a better threshold for both metals at all levels as it excelled 11 traits for mercury and 8 for lead. Hysun-33 also showed some tolerance while, NK-278 appeared to be a sensitive cultivar for most of the attributes in the presence of lead and mercury. The elevated levels of lead appeared to be more injurious to plants as compared with mercury for Hysun- 33 and NK-278. The greater toxicity of lead can be attributed to translocation of metal from the roots to the aerial tissues. The ability of DK-4040 for sustainable growth, integrity of chloroplast, existence of non enzymatic defense and restricted transfer of metal to above ground tissue seem to provide a compatible strategy for heavy metal tolerance. Based on the bioaccumulation of lead and mercury the cultivars can be placed among metal excluders.

## Introduction

Soil pollution by heavy metals is a global environmental problem as it has affected about 235 million hectares of arable land worldwide (Giordani *et al*., 2005; Bermudez *et al.,* 2012). Agriculture soils of Pakistan are intensively cropped to meet the increasing demand for food production for rapidly growing population. However, low fertility of soils cannot support economic crop production (Naik *et al.,* 2010; Khan *et al*., 2012). Moreover, high cost and scarcity of chemical fertilizers result in the use of land disposal for agricultural purposes. Though, municipal and industrial wastes serve as a major and economic source of nutrients and organic matter for growing several crops by poor farmers of the country but both treated and untreated waste is heavily loaded with toxic metals including mercury, lead, copper, zinc and nickel (Mushtaq & Khan, 2010). In addition, an incredible increase in automobiles in the country resulted in the release of lead through vehicle exhaust pipes thus it substantially pollutes the environment. Moreover, rapid industriialization in the recent years has become a continuous source of lead pollution. Similarly, mercury is also released from metallurgical and agrochemical industries. Therefore, heavy metal pollution is an ever-increasing threat to agriculture and human health because metals once present in the ecosystem remain persistent (Selin, 2009; Khan *et al.,* 2011; Walter *et al*., 2011; Jalaluddin & Hamid, 2012).

Hyper accumulation of lead and mercury in the environment can pose potential threat to aquatic and terrestrial life forms (Jiang *et al.,* 2008; Garcia-Sanchez *et al.,* 2009) particularly their bioaccumulation into the edible parts of crops is a major hazard to human health (Gopal & Rizvi, 2008; Rossato *et al.,* 2012) and can intimidate entire ecosystem as metals enter the food chain (Sahu *et al.,* 2012).

Both lead and mercury are non essential elements but plant species have shown high tendency for their uptake and translocation within their tissues particularly when high concentrations of metals are present in the soil. These metals are toxicant and injurious and have been reported to cause metabolic disorders in plants (Sharma & Dubey, 2005). They may inhibit various physiological and biochemical processes of fundamental significance (Kosobrukhov *et al.,* 2004) thus causing visible toxicity symptoms and an ultimate reduction of vegetative and reproductive growth (Rossato *et al.,* 2012).

During the last few years, more research has been focused on metal toxicity of lead and mercury on plant growth as their concentrations in soil and water have been increased to a drastic level (Kunhikrishnan *et al.,* 2012). Although, some plant species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds (Niu *et al.,* 2007; Hamadouche *et al.,* 2012). Thus, plant species have a dilemma with respect to balancing the uptake and maintaining concentrations of heavy metals in their tissues to cope with heavy metal stress (Sharma & Subhadra, 2010; Badr *et al.,* 2012). Nevertheless, metal tolerant plants are either grouped as metal accumulators or metal excluder based on the bioaccumulation of metals in their roots or aerial tissues, respectively.

Sunflower (*Helianthus annuus* L.) is of the most promising environmental crop that is being used under diverse environmental situations. In the recent years its importance for edible oil is being increasingly recognized in Pakistan. The country has imported 1.9 million tones of edible oil spending US $ 788 million during the year 1999-2000 (Anon., 2001). The import bill of edible oil (Rs. 36.496 millions) is the second largest after petroleum and consumes more than 75% of the total foreign exchange allocated for the import of food and continued up to year 2010.

Keeping in view the above situation, the present study was aimed to reveal lead and mercury tolerance of sunflower cultivars for the production of this oil crop on metal contaminated soils. The performance of the tested cultivars was assessed using various growth and photosynthetic attributes. Bioaccumulation of metal in the below and above ground tissues of the cultivars was also evaluated. Based on the metal tolerant strategy, we also aimed to place the cultivars among metal excluders or accumulators.

## Material and Methods

Seeds of three sunflower (*Helianthus annuus* L.) cultivars i.e., DK-4040, Hysun-33, and NK-278 were obtained from ICI Seed Corporation Pakistan. The experiment was conducted under natural conditions during March-May, 2009. Garden compost (air dried, sieved (2 mm sieve), texture by hydrometer (Clay 8.50%, Silt 4.75% and Sand 86.82%) was used. Lead acetate Pb $(CH_3COO)_2$ and mercuric nitrate Hg $(NO_3)_2$, (Merck, Germany) were used as a source of lead and mercury. Both metal salts were thoroughly mixed with soil in a ratio of 100mg/Kg and 300mg/Kg and seventy two labeled earthen pots (height 35cm and diameter 16 cm) were filled with 3 kg of soil.

The experiment was arranged in Complete Randomized manner. The treatment levels include control, 100mg/Kg and 300mg/Kg of both lead and mercury and there were made four replicates for each cultivar and level. Six seeds of each variety were then sown at equal depth and distance in each pot. When two young leaves emerged, the seeds were considered to be germinated. The growth was continued for four weeks when seedlings became 8-10 cm tall with 6-8 leaves. Seedlings were thinned to maintain three in each pot and watered gently using a spray gun. When plants were 8 weeks old, harvesting was carried out by taking out whole soil from the pot and plants. Excessive soil was removed by gentle shaking and plants were analyzed for various attributes; germination percentage, plant height (cm), leaf area ($cm^2$), fresh and dry weight (g) (root and shoot). The amount of pigments; chlorophyll *a, b* and total along with carotenoids was measured following Arnon (1949). The bioaccumulation of metals in the roots and aerial tissues was determined by Atomic-Absorption Spectroscopy (Varian AAS, 1475).

**Statistical analysis:** The data was subjected to ANOVA (Two-way analysis of variance with replication) using MS Excel 2007, in order to determine significant effects of varying levels of lead and mercury as well as to determine intraspecific variability. Least significant differences between mean values for cultivars and levels were calculated by employing a multiple range test following Snedecor & Cochran (1989).

## Results and Discussion

The present study considered genotypic variations in sunflower cultivars to reveal their differential tolerance to varying levels of lead and mercury. The stress induced by both lead and mercury adversely affected plant growth in all sunflower cultivars.

Table 1 clearly showed differential influence of the 2 metals ($p<0.01$ for both metals) and variable responses of the cultivars ($p<0.05$ for lead and $p<0.01$ for mercury) on seed germination. Results as mean values for seed germination presented in Fig. 1 depicted that germination inhibition was concentration dependent as it was not much influenced by the presence of lower concentration (100mg/Kg of soil) but 300mg/Kg of both metals had shown inhibitory effects. The decline in germination was more profound (48%) for Hysun-33 followed by NK-278 and DK-4040 which showed 43 and 30% reduction, respectively at 300 mg/Kg lead. Decline in seed germination was not much drastic as it varied from 2 to 10% at 100 mg/Kg of mercury. However, DK-4040 and NK-278 had 24% while Hysun-33 showed 20% decline in germination at 300 mg/Kg mercury. The adverse effect of lead and mercury on seed germination coincides with several other studies in which considerable genotypic variability and toxicity of these metals was reported (Farooqi *et al.*, 2009; Kranner & Colville, 2011).

**Table 1. Significance of F- ratios from a Two Way Analysis of Variance (df= 2 for cultivars and levels, residual df=27 and total df= 35) for different attributes of *Helianthus annuus* L. cultivars after eight weeks of growth at various levels of lead and mercury in soil.**

| | Lead | | Mercury | |
|---|---|---|---|---|
| **Attributes** | **Cultivars** | **Levels** | **Cultivars** | **Levels** |
| Germination percentage | * | ** | ** | ** |
| Root fresh weight (g) | *** | N.S | *** | *** |
| Root dry weight (g) | *** | N.S | *** | *** |
| Shoot fresh weight (g) | *** | ** | *** | *** |
| Shoot dry weight (g) | *** | ** | *** | *** |
| Plant height (cm) | N.S | *** | N.S | *** |
| Leaf area ($cm^2$) | ** | * | ** | *** |
| Chlorophyll *a* (mg/g) | * | N.S | N.S | N.S |
| Chlorophyll *b* (mg/g) | * | N.S | N.S | N.S |
| Total chlorophyll (mg/g) | * | N.S | N.S | N.S |
| Total carotenoids (mg/g) | ** | * | ** | * |
| Root metal contents (μg/g) | N.S | ** | N.S | *** |
| Shoot metal contents (μg/g) | N.S | ** | N.S | ** |

N.S = non-significant, *,** & *** significant at 0.05, 0.01 and 0.001% level of probability, respectively.

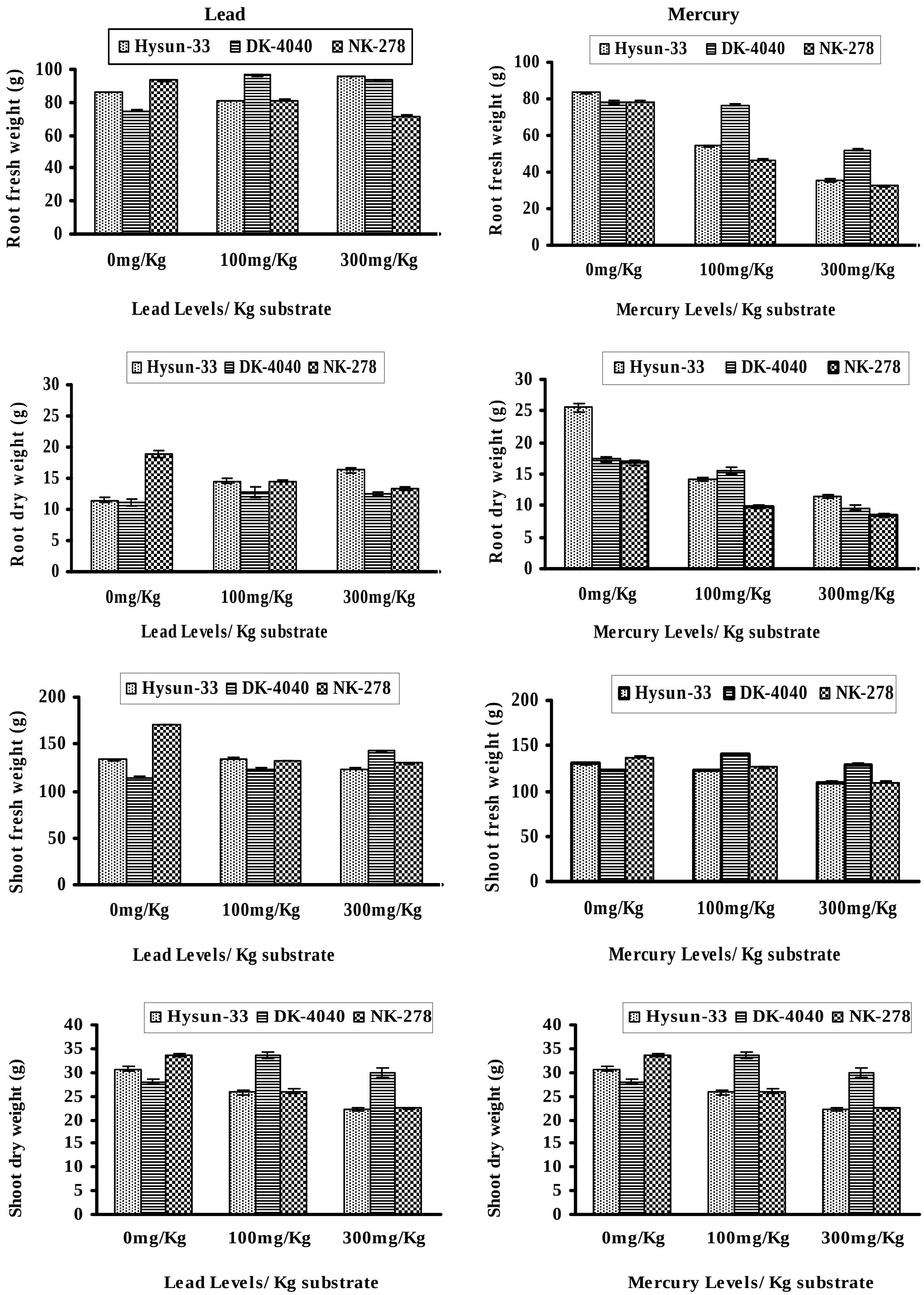


Fig. 1. Mean (± S.E) for various growth attributes in sunflower (*Helianthus annuus* L.) cultivars after eight weeks of growth at various levels of Lead and Mercury in soil.

The adverse effects of lead and mercury on fresh and dry biomass of root and shoot were noticed however, the decline was more evident in cultivar NK-278 as it showed more profound reduction at both levels of metals (Fig. 1). The extent of decline for fresh biomass of root was 15.6 and 26.4% at 100 and 300 mg/kg of lead, respectively. However, the other 2 cultivars did not exhibit any reduction for fresh biomass of root at both levels of lead. The cultivar NK- 278 also showed a pronounced (upto 58.9%) decline in fresh biomass of root in response to 300mg/Kg of mercury. The highest level of mercury also adversely affected fresh biomass of root in Hysun- 33 however, the reduction of this attribute was less drastic (32.4%) in DK-4040. The cultivars exhibited highly significant ($p<0.001$) variability for root fresh and dry biomass for both metals however, mercury levels induced more drastic ($p<0.001$) influence on biomass of root.

With regard to fresh biomass of shoot, the cultivars have highly distinct ($p<0.001$) responses and a significant influence of both lead ($p<0.01$) and mercury ($p<0.001$). The cultivar NK- 278 had the maximum (24.5%) but Hysun- 33 showed the minimum reduction (7.5%) in response to highest level of lead. Biomass of shoot reduced by 5.4-15.5% and 8-20.4% at 100 and 300 mg/kg mercury in Hysun-33 and NK-278, respectively. No reduction of fresh biomass of shoot was observed in DK-4040 over to respective controls at both levels of the tested metals. The tested cultivars exhibited a similar trend for dry weights of the tissues (root and shoot) as shown for fresh biomass.

Zhao & McGrath, (2009) reported that excessive concentrations of metals can cause a considerable decrease in dry weights of plant parts but in certain cases, an apparent increase in dry biomass of plant organs may occur as a result of an increase in the synthesis of cell wall polysaccharides resulting from heavy metal exposure or excessive lignification. In addition, sustainable production of dry biomass without suppression in growth can be ascribed to overall success in total net carbon assimilation as studied by other workers (Roy *et al.,* 2010; Manivasagaperumal *et al.*, 2011).

There was no remarkable contrast among cultivars at both levels of the tested metals for plant height (Table 1) but levels of both metals had a marked ($p<0.001$ for both metals) influence on this attribute. DK-4040 had shown only 3 and 5% reduction in plant height at elevated level of lead and mercury, respectively. Treatment with high concentrations of lead and mercury did not considerably reduce elongation of sunflower plants which suggested that these metals are not associated in causing negative effects on many metabolic processes such as reduced elasticity of cell walls and cell proliferation (Seregin & Kozhevnikova, 2006). Nevertheless, higher sensitivity of growth corresponds with the greater concentration of the metal in plant organs. Thus, the limited translocation of heavy metals to the aboveground parts may be a manifestation of non toxicity. Majid *et al.,* (2012) also demonstrated extremely limited transfer of these metals to aerial parts thus not affecting developing tissues.

All the cultivars had shown marked reduction of the leaf area in response to both lead and mercury. This attribute had drastically been influenced by mercury ($p<0.001$) as compared to lead ($p<0.05$). A differential response of the cultivars also became evident (Table 1) for both metals ($p<0.01$). The reduction in leaf area at the most elevated level of mercury was 46% in Hysun 33 followed by 33% decline in NK- 278 and 31% in DK-4040. By contrast, reduction in leaf area was up to 23% in both Hysun-33 and NK-278 while DK-4040 had the lowest reduction (19%) at 300mg/Kg of lead. Thus, DK-4040 appeared to be less sensitive to both lead and mercury (Fig. 2).

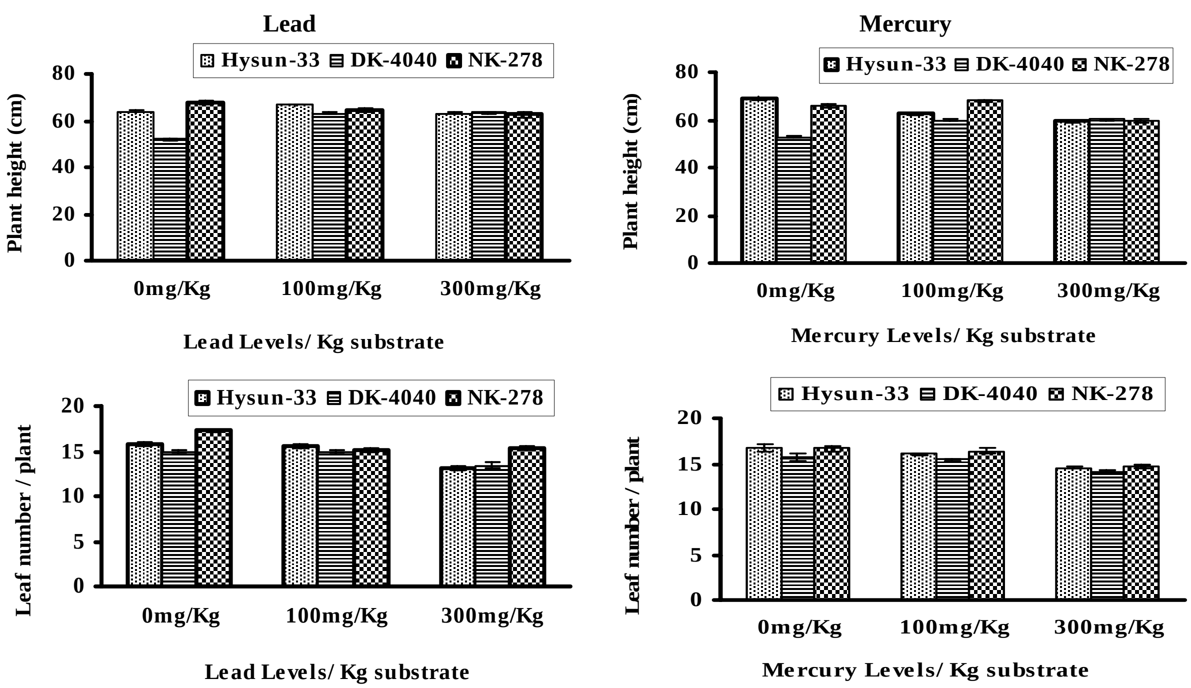


Fig. 2. Mean (± S.E) for various growth attributes in sunflower *(Helianthus annuus* L.*)* cultivars after eight weeks of growth at various levels of Lead and Mercury in soil.

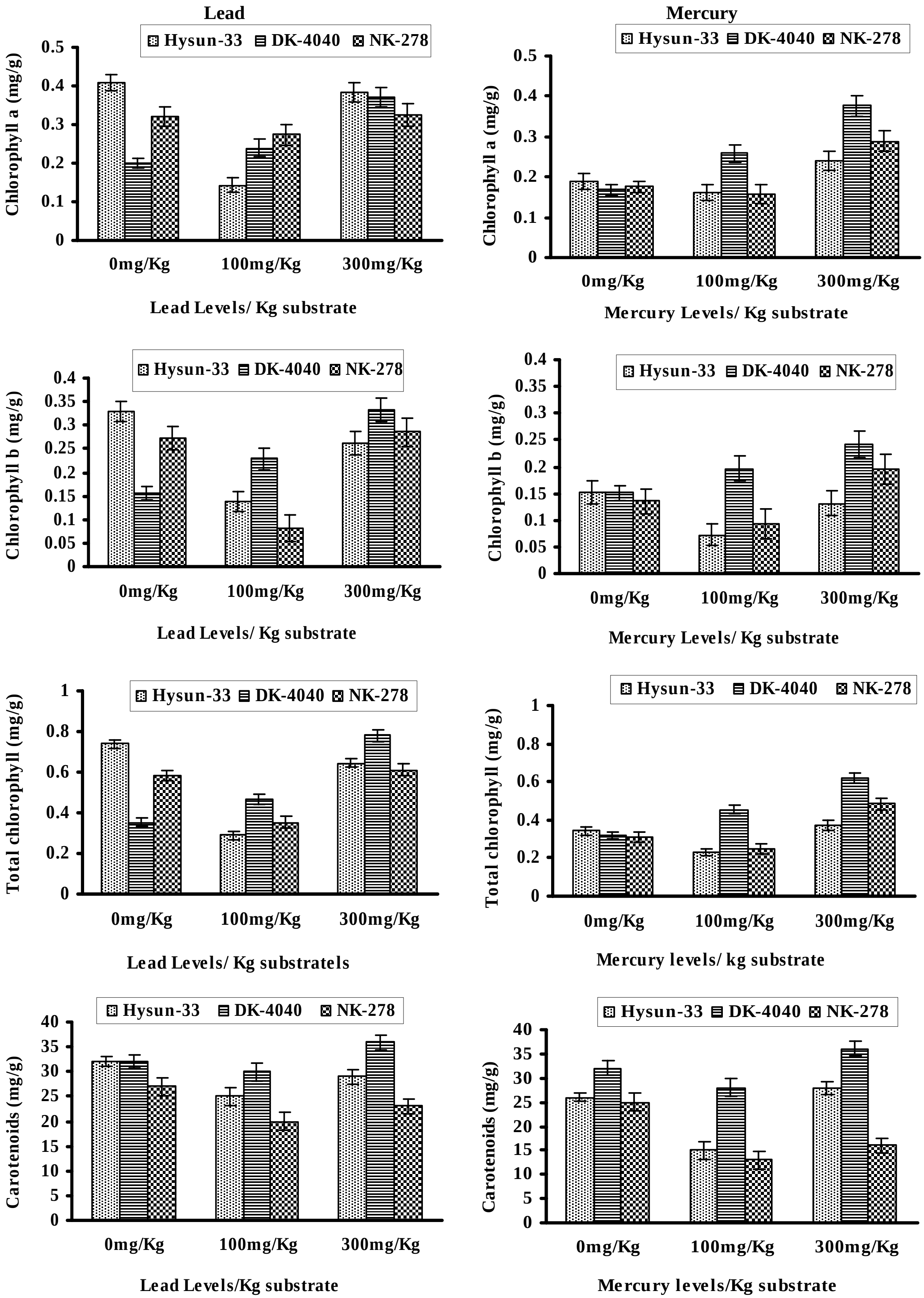


Fig. 3. Mean (± S.E) for pigment content in sunflower (*Helianthus annuus* L.) cultivars after eight weeks of growth at various levels of Lead and Mercury in soil.

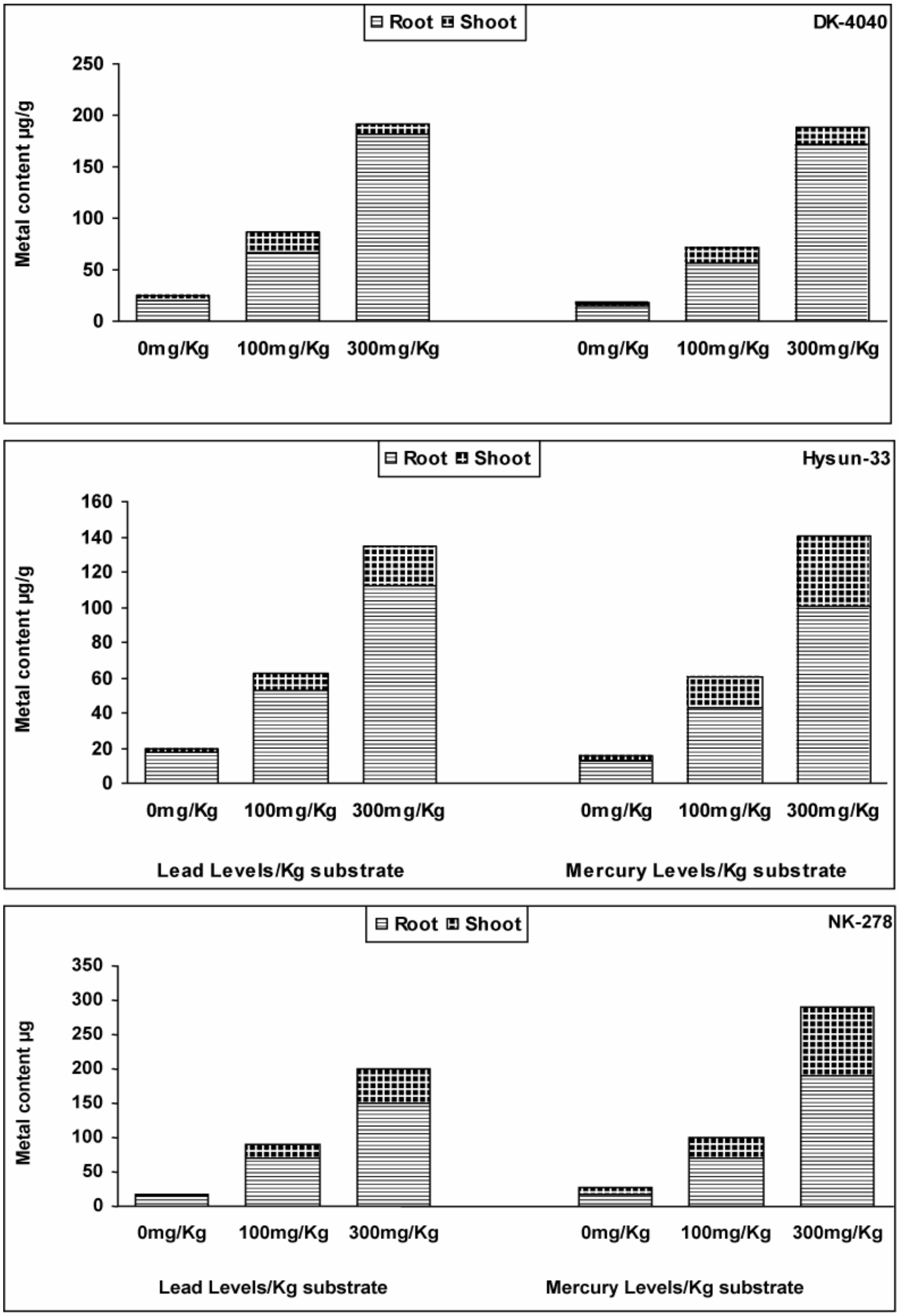


Fig. 4. Metal content in tissues sunflower *(Helianthus annuus* L.*)* cultivars after eight weeks of growth at various levels of Lead and Mercury in soil.

The presence of heavy metals in the growth medium can considerably influence photosynthetic area of the plants that in turn reduces photosynthetic capability and ultimate cause growth reduction (Qurainy, 2009). The sensitivity of the foliage tissues depends on the bioaccumulation of heavy metal ions in the leaves via vascular supply from stem/shoot. The transport is species specific and also depends on the type of metal. Since, mercury has caused more reduction of leaf area because it has been transported more through shoot to the foliage (Fig. 4) rather than its sequestration in the roots. Thus, these findings seem to be supported by workers (Majid *et al.*, 2012; Mellem *et al.*, 2012).

When different levels of lead and mercury were applied and their effect on pigment contents was assessed (Fig. 3) the cultivars did not exhibit any contrast for mercury (Table 1). Similarly, the levels of both tested metals did not vary markedly. Though, it is well established that excessive amounts of toxic heavy metals result in significant decline in photosynthetic pigments of plants that could be attributed to possible degradation of chloroplast or disruption of the thylakoid membrane (Kaur *et al.,* 2012). Reduction may also be caused by the inhibition of chlorophyll biosynthesis in the presence of various metals (Cd, Cr, Pb and Hg) such as activities of important enzymes (ALA-dehydratase and protochlorophyllide reductase) which are associated with chlorophyll biosynthesis are greatly inhibited. In addition, Mg ions present in the tetrapyrole ring of the chlorophyll molecule is substituted by heavy metal ions which affect photosensitization and caused a decrease in the rate of photosynthesis by inhibiting PSII electron transport chain. Moreover, excessive amount of lead and mercury have also been reported to cause irreversible degradation of pigment molecules (Madhu *et al.,* 2008). The results of the study did not exhibited any significant decline in pigment content (chlorophyll a, b, and total ) which can be attributed to the reason that chloroplasts are very robust structures and can not be easily degraded. Some workers (Kosobrukhov *et al.,* 2004; Kaur *et al.,* 2012) also reported that degradation of chloroplast occurs if metals translocate rapidly to photosythetic tissues thus both lead and mercury appeared to be less injurious to photosynthetic pigments. Lower pigment content at 100 mg of both lead and mercury /Kg of soil can be ascribed to the initial sensitivity of the pigments to heavy metals but later on number of chloroplast seems to be maintained by division, thus, no decline in pigment content even at 300 mg/Kg was observed.

It is well known that heavy metals cause an oxidative stress but at the same time, several anti-oxidant enzymes and non enzymatic compliment scavenge oxidative stress (Rossato *et al*., 2012). Though, for this study, we have observed no significant decline in pigment content which might be due to oxidative defense. Although, a non enzymatic defensive component that is carotenoids (Fig. 3) was considered. A significant ($p<0.01$) increase in carotenoid contents in cultivars at the elevated level of both lead and mercury might be an indicative of non enzymatic defense (Mishra *et al.,* 2006) which might provide a greater threshold for both these metals.

The results of the study indicated that the accumulation of lead and mercury was mainly in the roots (Fig. 4) and cultivars had no variable pattern of accumulation of both metals in above and below ground tissues (Table 1). The metal content in the tissue were proportionate to the metal levels in the growth medium as reported by many other workers (Jadia *et al.,* 2008; Badr *et al.,* 2012). A slow translocation was also accomplished at the elevated levels of both lead and mercury. The translocation of heavy metal ions to the aerial tissues occurs because at with plant development, root endoderm may become weak barrier. For this reason, metals easily penetrate xylem and then the above-ground parts of plants (Majid *et al.,* 2012; Mellem *et al.,* 2012). Consequently, plants accumulate higher levels metal in the roots with slow translocation to the shoots. Similar pattern of metal accumulation has been reported for cadmium (Ahmed *et al.,* 2010; Anwer *et al.,* 2012) and Nickel has also been found to accumulate rapidly and preferentially in the roots with little being translocated to the other parts of the plant (Ishtiaq & Mahmood, 2011; Ozyigit, 2012).

## Conclusions

Based on the results of the study, using various biometric, biochemical and metal uptake, it became evident that sunflower cultivars have a threshold for both lead and mercury. However, the 3 cultivars exhibited differential tolerance for the 2 metals as well as differential influence of the metal levels on various attributes. Sunflower cultivar DK-4040 had consistently performed better under both levels of the 2 metals as exhibited greater mean values for most of the attributes studied. Hysun-33 also showed some tolerance to elevated level of lead as compared to mercury while, NK-278 appeared to be sensitive for most of the attributes to both levels of lead and mercury.

The bioaccumulation of both metals in plant tissues of the 3 cultivars was consistent as the cultivars accumulated more metal in their roots than in the shoots. However, at elevated levels of both metals, root threshold for metal content seems to alter thus allowed some of the metal ions to translocate to the aerial tissues. The ability of DK-4040 for sustainable growth, integrity of chloroplast, existence of a non enzymatic defense and restricted transfer of metal to above ground tissue seem to provide a compatible strategy for heavy metal tolerance. Based on the bioaccumulation of lead and mercury in the roots, it would be safe to conclude that these sunflower cultivars can be placed among metal excluders.

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