

Research article

RESPONSE OF SUNFLOWERS (*HELIANTHUS ANNUUS L.*) CULTIVARS TO DIFFERENT LEVELS OF EXOGENOUSLY APPLIED CADMIUM AND ELUCIDATION OF POSSIBLE TOLERANCE MECHANISM

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The effects of different concentrations of Cadmium on the growth of sunflower (*Helianthus annuus L.*) and its accumulation in roots, stem and leaves were investigated using Atomic absorption spectrophotometer. The concentrations of Cadmium chloride used were 0, 6, 9 and 12 mg/kg soil. Seedlings of three sunflower cultivars viz., HI-SUN33, HI-SUN38 and S278 exposed to 9 PPM Cd exhibited substantial growth reduction and all of them died 10 days after treatment application. Growth of roots and shoots was inhibited at concentrations of 9 mg/kg soil and 12 mg/kg. Cd during the entire experiment (20 days). Cadmium accumulation in roots, stems and leaves increased significantly ($p < 0.05$) with increasing Cd concentrations. Cadmium was concentrated mainly in the roots, and variable amounts of Cd were also transported to stem and leaves. Among the three cultivars, HI-SUN38 produced more roots and higher biomass than HI-SUN33 and had a greater ability to accumulate Cd when compared with S278.

Key words: Cadmium, sunflower, *Helianthus annuus*, Atomic absorption, Growth of roots and shoots

Introduction

Cadmium (Cd) a trace heavy metal is considered a hazardous pollutant due to its high toxicity and solubility in water (Lockwood, 1976). At low concentration it has stimulatory effect on root growth of *Allium sativum* (Liu et al., 2003, 2004), but at higher concentration it is toxic and directly or indirectly inhibits physiological processes such as respiration, photosynthesis, cell division, plant-water relationships, metabolism and mineral nutrition, resulting in poor growth and low biomass (Barcelo & Poschenrieder, 1990; Sanita & Gabrielli, 1999; Liu et al., 2003/2004). Cadmium can be easily taken up by plants, transferred through food chains to impart adverse effects on human health (Nordberg, 2003; Wagner, 1994). Emphasis has become more prevalent towards the problems of Cd pollution with the development of

modern industry and agriculture. Anthropogenic activities, such as mining, industry, agriculture and waste disposal has increased since the beginning of the 20th century (Alloway, 1995). Most conventional remediation approaches do not provide an acceptable solution to treating contamination caused by metal pollution. Alternatively, phytoremediation provides an attractive strategy, being low cost and environmentally sustainable (McGrath et al., 2002; Salt et al., 1998). The idea of using rare plants which hyperaccumulate metals to selectively remove and recycle excessive soil metals was introduced by Chancy (1983). Salt et al. (1996) also considered phytoremediation as an emerging technology using selected and engineered metal-accumulating plants for environmental clean-up. Metal-accumulating plants can accumulate unusually high concentrations of heavy metals in both roots and shoots from polluted soil and waters (Kumar et al., 1995; Dushenkov et al., 1995). A few terrestrial plant species e.g. *Thlaspi* sp., (Lombi et al., 2000) and *Arabidopsis halleri* (Bert et al., 2002) were reported to accumulate high concentrations of Cd,

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higher than the hyperaccumulation criterion, 0.01% of shoot dry matter (Baker *et al.*, 2000).

Sunflower (*Helianthus annuus*), a fast-growing crop, has a reasonable tolerance to heavy metals. It has been used for rhizofiltration because it has a high root uptake of metals but shows low efficiency in their translocation from root to shoot (Saxena *et al.*, 1999; Kamnev & Vander Lelie, 2000; Letal, 2003). Few reports on cadmium accumulation by *H. annuus* are available (Madejon *et al.*, 2003; Pena *et al.*, 2006). The aim of this investigation was to study the effects of different Cd concentrations on growth of sunflower. In addition, the accumulation of Cd by sunflower roots, stem and leaves was measured using Atomic absorption spectrophotometer.

Materials and Methods

Plant cultivation and treatment application: Seeds of three sunflower (*Helianthus annuus* L.) cultivars HI-SUN33, HI-SUN38 and S278 used in the present investigation were obtained from Nuclear Institute of Agriculture and Biology (NIAB) Faisalabad. Healthy and equal-sized seeds were chosen from each cultivar, soaked in tap water for 12 h and germinated in earthen pots in the dark at a constant temperature of 25°C for 48 h. Seeds were allowed to grow in vermiculite in a climate chamber with a day/night period 14/10 h, a day/night temperature and humidity regime of 20°C/25°C and 55/75% relative humidity (RH), respectively. After 7 days 20 sunflower seedlings were selected, fixed on a polystyrol-plate in a pot containing 2L of 1/2 modified Hoagland nutrient solution (Stephan & Prochazka, 1989) for 10 days, respectively. The Hoagland's solution consisted of 5 mM Ca(NO₃)₂, 5 mM KNO₃, 1 mM KH₂PO₄, 50 µM Mg₃BO₃, 1 mM MgSO₄, 4.5 µM MnCl₂, 3.8 µM ZnSO₄, 0.3 µM CuSO₄, 0.1 µM (NH₄)₆Mo₇O₂₄ and 10 Cd ranging from 3 PPM to 9 PPM for 20 days. Cadmium was provided as Cadmium chloride. The Cd solutions were prepared in deionized water and were added to the full strength Hoagland nutrition solution. The full strength Hoagland solution without Cd was used for the control plants. The nutrient solutions were continuously aerated and changed regularly every 5 days until the seedlings were harvested.

Macroscopic observations: The lengths of roots and stems were measured after 10, 20, 30 and 40 d, respectively. Ten seedlings from each treatment were harvested based on uniformity in size and color after 20 days of incubation. The seedlings were removed from

solution and washed thoroughly with running tap water for 30 min. and then with deionized water to remove traces of nutrients and Cd ions from root surfaces. The samples were dried for 3 days at 45°C, for 1 day at 80°C, and for 12 h at 105°C.

Estimation of total Cd: All dried plant samples were prepared using a wet-digestion method (Piper, 1942). Tissue Cd concentrations were analyzed using Atomic absorption spectrophotometer.

Statistical analysis: Data were expressed as mean±standard error (SE) and were analyzed through analysis of variance (ANOVA) using Sigma statistical software (Jandel Scientific Corporation, USA). Test of equality of averages using a Mest was applied equally. The statistical significance was set at p<0.05.

Results

Macroscopic symptoms

The effects of Cd on root growth of cultivars varied with Cd concentration. Cadmium inhibited root growth during the entire experiment, when compared with control seedlings. Root growth in sunflower cultivar S278 was inhibited significantly, and root length decreased with increasing Cd concentration and duration of treatment. Seedlings exposed to 9 PPM Cd solution exhibited substantial growth reduction, and root growth even stopped; they appeared thinner and were sparsely branched. The roots became yellow and were slightly decomposed after 2 days of treatment. After 5 days, the roots appeared yellow brown and some were broken. Root growth in the other two cultivars was approximately the same as that of HI-SUN33. Biomass of roots was in the order: S278>HI-SUN38>HI-SUN33. The root system of S278 appeared qualitatively strongest.

The effects of Cd on shoot growth varied with different Cd concentrations. Shoot length in the three cultivars progressively decreased with increasing Cd concentration and duration of treatment. The least shoot growth was in seedlings treated with 9 PPM Cd. Biomass of shoots was in the order: S278>HI-SUN38>HI-SUN33.

Control seedlings had 5 pairs of leaves when they were harvested. The plants of S278 treated with Cd did not grow as large, robust or healthy as the control during the experiment. With increasing Cd concentration, this phenomenon appeared progressively obvious. After 10 days the young laminae exposed to 3 PPM and 6 PPM Cd solutions appeared yellow. After 15 days chlorosis was evident in all young laminae. Toxic symptoms

Table: Cadmium Concentration (mg/kg DW) and changes in roots, stems and leaves of *Helianthus annuus* L.

Cultivars	Treatment mg/kg soil	Total amount (%)	Root (%)	Stem (%)	Leaf (%)
HI-SUN33	Control	17.9	60.78	4.9±0.63	4.6±0.76
	6	786.3	632.2±5.9	72.9±2.49	67.2±2.75
	9	2852.9	1896.6±176.36	607.2±4.49	263.3±21.19
	12	149.7	129.6	113.8	111.3
HI-SUN38	Control	3.6	2.6±0.17	0.6±0.009	1.3±0.13
	6	695.4	552.6±11.12	86.4±1.47	66.4±1.56
	9	2285.6	1319.6±11.38	619.4±2.59	349.4±5.03
	12	107.6	69.11	103.14	89.7
S-278	Control	0.7	0.4±0.07	0.3±0.10	0.1±0.15
	6	468.8	319.3±1.10	73.2±0.63	86.4±2.17
	9	2032.0	1150.1±27.74	514.9±9.63	377.7±2.86
	12	123.7	102.9	99.11	107.8

Values followed by same letters are not significantly different at (p>0.05). Means ± SE.

began in 9 PPM Cd 2 days after treatment and after 5 days chlorosis and necrosis appeared. The shoots wilted and leaf margins and leaf apices also wilted. When compared to HI-SUN33, the toxic effects of Cd on laminae of the other two cultivars were less severe. At 9 PPM Cd, the root and shoot lengths significantly decreased and the seedlings died after 10 days. These phenomena indicated sensitivity of all root and shoot growth to higher concentrations of Cd (9 PPM).

Accumulation of Cd:

The accumulation of Cd in the roots, stem and leaves of the three cultivars increased significantly (p<0.05) with increasing Cd concentration. Cd ions accumulated primarily in roots (Table 1). The Cd concentration in roots of HI-SUN33 was higher than that in HI-SUN38 and S278 at 6 PPM Cd. Cadmium was also transported to stem and leaves (Table 1). The Cd shoot/root ratios increased with increasing Cd concentration. Levels of Cd in the three cultivars treated with 3 PPM and 6 PPM Cd were in the order: roots > stem > leaves, with the exception of roots > leaves > stem at 3 PPM Cd in HI-SUN38. In contrast, HI-SUN33 showed the best capacity to absorb and take up cadmium ions from solution, while the shoot/root ratios of S278 were higher than the other two cultivars (S278 > HI-SUN38 > HI-SUN33).

Discussion

Cadmium pollution has become a major environmental problem with the development of modern industry and agriculture. Salt et al., (1995, 1996) proposed the use of

metal-accumulating plants (e.g., *B. brassica juncea*, *Thlaspi caerulescens*, *Zea mays* and *Helianthus annuus*) to remove toxic metals. According to the accepted shoot concentration defining hyperaccumulation being 0.01% (w/w) of Cd (Baker et al., 2000), the three cultivars of *H. annuus* can be considered metal hyperaccumulators (Table 1). Among three cultivars, Cd concentrations in the stem and leaves of HI-SUN38 were 149.2 µg/g DW (at 3 PPM Cd) and 963.1 µg/g DW (at 6 PPM Cd) respectively, suggesting a greater ability to accumulate Cd when compared with S278. This cultivar, produced more roots and a higher biomass than HI-SUN33, makes it a better choice for remediation of Cd contaminated soils.

The results in the present investigation provide basic information necessary for further development of phytoremediation methods and confirm previous studies (Lin et al., 2003; Madejon et al., 2003; Pena et al., 2006 and Soudek et al., 2006). Localization of Cd in plants depends on the total concentration of contaminant in soil or water. The results from this investigation showed that the Cd content in roots increased with increasing solution concentrations of Cd. Cadmium primarily accumulated in roots, and small amounts were transferred to shoots in the seedlings treated with 3 PPM Cd. The reported differences in root uptake and shoot accumulation might be explained by the fact that one of the normal functions of roots is to selectively remove ions from the soil solution (Salt et al., 1997). Cadmium-tolerant plants must be able to prevent the absorption of excess Cd or detoxify the Cd after it has been absorbed.

A few unfavorable changes in the appearance of the Cd-treated seedlings of *H. annuus* in the present investigation were observed, for example browning roots, the decreased number of roots, and growth inhibition of roots and shoots. Chlorotic phenomena in seedlings treated with Cd in this investigation were also found, which agrees with the findings of Das et al., (1997).

It is likely that Cd tolerance mechanisms may differ depending on the species. More work still needs to be done to determine the mechanism of high uptake and accumulation of Cd by *H. annuus*. In our investigation, sunflower accumulated substantial amounts of Cd at 6 PPM Cd, but seedling growth was severely decreased. Therefore, it is important to select suitable species with potential for high uptake and accumulation of Cd without the plant undergoing severe damage.

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