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POTENTIAL OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) FOR PHYTOREMEDIATION OF NICKLE (Ni) AND LEAD (Pb) CONTAMINATED WATER

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Abstract

Heavy metals are contaminants of much environmental apprehension, as they are hazardous to human being and other biota. Buildup of heavy metals in crop plants is of great concern due to the probability of food contamination through the soil-root interface. For this purpose, a hydroponic study was conducted to evaluate the efficacy of sunflower plant to phytoremediate Pb and Ni contaminated water in the absence and presence of synthetic chelator. Results showed that application of Ni and Pb reduced the dry weights of shoot and root (up to 55.1 and 38.3%; 50.5 and 33.6%), shoot and root length (up to 64.5 and 58.1%; 64.1 and 55.8%), chlorophyll content (up to 63.8 and 54.4%), and photosynthetic activity (up to 66.1 and 62.7%), respectively with EDTA as compared to control. While, maximum concentration of Ni and Pb in shoot and root (up to 18.43 and 20.73 mg kg^{-1}; 12.82 and 18.67 mg kg^{-1}), total accumulation (up to 55.82 and 72.28 mg kg^{-1}), and proline content (up to 128.2 and 98.3%) were recorded in the presence of EDTA respectively as compared to control. Generally, it was observed that concentration and total accumulation of Pb was more than Ni in sunflower plant. The study concludes that the use of synthetic chelator increased the uptake and translocation of heavy metals in plant biomass that could enhance the phytoremediation of Ni and Pb from contaminated water.

Introduction

In the present scenario, heavy metals are an increasing ecological threat which commonly results from human activities. In crop plants, accumulation of heavy metals is of great alarm because of food contamination through the soil root crossing point (Qadir *et al*., 1998; Boominathan *et al*., 2004). Although heavy metals i.e., Cd, Pb and Ni etc., are not necessary for plant growth, however they are passionately taken up and accumulated by plants up to toxic levels (Mussarat & Bhatti, 2005; Yang *et al.*, 2006; Akguc *et al*., 2008). But toxicity of heavy metals and salts stress depending upon their physicochemically prevailing states and metal concentration which cause pollution of soil and groundwater which directly affect the biological diversity in the soil and water environments (Hao *et al*., 2004; Afzal *et al*., 2006; Wahla & Kirkham, 2008) with vanishing of the plant life and resulting irritated risk, caused by metal contaminated soil to the surrounding area and its population.

In public attitude, phytoremediation technology is more favorable due to its potential for cleaning up environment and the overall aesthetic perfection of the contaminated sites (Chen & Cutright. 2002; Fayiaga *et al*., 2004). However, the tribulations of contaminated soils and water can be resolved by the application of phytoremediation. The improvement of contaminated soil and water through this technology, based on the use of plants, has

the objective to extract or stabilize the metals in soil (Silveira *et al*., 2003; Wahla & Kirkham, 2008). Although phytoextraction, the plant accumulation to above ground tissue of pollutants from soil and water, is a simple, inexpensive remediation technology (Baker *et al.,* 1994; Chaney *et al*., 1997).

In big cities of Pakistan, untreated sewage water and the industrial effluents are discharged directly into canals and the polluted water is used for growing crops particularly vegetables and fodder in the hinterland of big cities (Khan *et al*., 2003). City effluents are one of the potential sources of metal pollution which is used for growing crops in the pre-urban areas of Pakistan (Ghafoor *et al.,* 1994; Mussarat *et al*., 2007).

In Pakistan, many different industries have been established in various cities. Tanning is one of the oldest industries in Pakistan and is also one of the potential sources of heavy metals which pollute water and soil environments. Wet process of tanning is the main source of generating waste water. Water consumption per kg of raw hides varies from tannery to tannery which should not go beyond 50 L kg^{-1}. However, the tanneries are generally consuming more water than required. Likewise, sugar and textile industries are also main sources of pollutants to surface water bodies.

It is very perceptive that the majority of the cities and industries are lacking of waste water treatment facilities, in Pakistan. Large quantities of untreated municipal sewage and industrial effluents are entered directly to surface water causing in rigorous pollution mainly due to heavy metals.

Keeping in view the heavy metals exposure directly to the water environments, a study was planned under green house conditions with the objectives to evaluate the efficacy of a synthetic chelator in the uptake and translocation of heavy metals as well as on the total biomass of sunflower plant.

Materials and Methods

A hydroponic study was conducted to evaluate the efficacy of sunflower (*Helianthus annus* L.) for phytoremediation of Ni and Pb contaminated water. Initially sunflower seeds were grown in trays (sand culture) in a green house illuminated with natural light. For irrigation distilled water was used during growth of sunflower seedlings. Finally 10 days old sunflower seedlings of similar size were transplanted in to plastic troughs (containing solution culture) in their individual compartment to initiate the green house experiment. Half strength modified Johnson's nutrient solution (Johnson *et al.,* 1957) was applied to fulfill nutrients demand of the plant. The pH of the solution was checked and maintained in between 6.0 to 6.5 by using 0.1N HCl and 0.1N NaOH. In the hydroponic system, sunflower plants were tested at 0, 15 and 30 mg L^{-1} of Ni and Pb in the absence and presence of EDTA @ 0.265 g L^{-1}. All the plastic troughs were aerated gently with an air compressor for 24 h. The average temperature of green house was approximately 28 ± 1^{o}C in the day time and 20 ± 1^{o}C at night. Moisture and nutrients were provided by watering the plants with a standard hydroponic nutrient solution. This experiment was carried out to determine the role of organic acid (EDTA) on growth and up take of Ni and Pb by sunflower.

Sunflower plants were harvested after 30 days exposure of seedlings to treatments solution. Plants were harvested early in the morning between 8.0 to 9.0 AM. The collected plant samples were placed in plastic bags, labeled carefully and brought to the laboratory. Soon after harvesting, plants were gently rinsed with 1% HCl and then with distilled water, blotted with tissue paper and separated into roots and shoots. Roots and shoots were weighed to record fresh weight. Polythene tools were used in sampling and

storing the collected matrices to avoid the metal contamination. Roots, leaves and stems were then sectioned and stored in foil. The remaining separated plant matter were dried in an oven at 70°C until the plant tissue were completely dry and were used for further analyses by atomic adsorption spectroscopy (AAS).

Chemical analysis of plant samples: Plant samples was sorted i.e. roots, stems and leaves. The 50 g of each fresh sample dried at 70°C in hot air oven for 48 h. dried plant samples of roots and shoots were ground in fine powder in a mechanical grinder (MF 10 IAK, Werke Germany) to pass through 1mm sieve. The ground samples were mixed uniformly. The samples of water and plant-parts were chemically analyzed for detection of heavy metals i.e., Ni and Pb. Accurately 0.5 g of dry powder of each sample was weighed and digested with concentrated HNO_3, H_2SO_4 and H_2O_2 (2: 6: 6) as prescribed by Saison *et al*., (2004). The blanks were run with set, and the samples were analyzed by atomic adsorption spectroscopy (AAS). The concentration of heavy metals such as Ni and Pb were represented in ppm. Mean values of triplicate sub samples of the water and plant samples were considered.

Physiological parameters: Free proline content in leaf was determined according to the method described by Batels *et al*., (1973). One gram leaf sample was homogenized in 3.0% Sulfosalicyclic acid and filtered through Whatman filter paper No. 2. After the addition of acid ninhydrin and glacial acid, mixture was heated at 100°C for 1 h in water bath and reaction stopped by transferring the reacted mixture into ice bath. The mixture was extracted with toluene and absorbance was recorded at 520nm wavelength. Standard solutions of L-proline were run on spectrophotometer (Evolution LC 300, Cambridge, UK) to calibrate the proline assay. Proline concentration was measured from standard curve and expressed in micromole per gram.

For chlorophyll pigments, 0.1g of leaf sample was homogenized with 80% acetone (*v/v*) and filtered. Absorbance of resulting solutions was taken at λ 663nm for total chlorophyll content (Arnon *et al.,* 1949). The photosynthetic activity was determined by calculating photosynthetic efficiency ($14CO_2$ fixation) using 14C technique as described by Moussa (2001), 80% acetone extract of $14CO_2$ was analyzed using liquid scintillation counter.

Statistical procedures were applied to analyze the data (Steel *et al.,* 1997) and means were compared by Duncan's Multiple Range Tests (Duncan, 1955).

Results

A hydroponic study was conducted to assess the phytoremediation of Ni and Pb from hydroponic medium by sunflower plant. Results regarding shoot dry weight are presented in Table 1. Maximum reduction in shoot dry weight was recorded up to 50.5% in response to 30 ppm Ni + EDTA followed by treatment 15 ppm Ni + EDTA as compared to untreated control. While rest of the treatments also showed significant decrease in shoot dry weight because of Ni and Pb uptake that ranged from 16.5 to 28.5% in comparison with control.

Maximum decrease (up to 23.4% less than control) in dry root weight (Table 1) due to application of 30 ppm Ni + EDTA followed by 15 ppm Ni + EDTA that gave 15.3% less dry root weight, while minimum decrease in dry root weight was observed in response to 5 ppm Pb than untreated control. Other remaining treatments also showed significant reduction in dry root weight ranging from 3.8 to 14.7% as compared to control.

Table 1. Effect of Ni and Pb uptake on fresh and dry weights of shoot and root of sunflower plant under hydroponic conditions.

(Mean of three replications)

Treatments	Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)		Root dry weight (g)	
	No EDTA	EDTA	No EDTA	EDTA	No EDTA	EDTA	No EDTA	EDTA
Control	27.45 a	28.67 a	5.01 a	5.21 a	18.82 a	19.48 a	3.10 a	3.28 a
Ni @ 15 mg L^{-1}	19.95 bc	14.51 ef	3.76 b	2.74 ef	14.58 b	9.96 de	2.38 bc	1.73 f
Ni @ 30 mg L^{-1}	16.23 de	11.91 f	3.03 de	2.25 f	11.52 cd	8.71 e	1.94 e	1.54 f
Pb @ 15 mg L^{-1}	20.11 b	18.66 bcd	3.79 b	3.52 bcd	14.70 b	13.64 bc	2.48 b	2.40 bc
Pb @ 30 mg L^{-1}	19.47 bcd	16.51 cde	3.67 bc	3.09 cde	13.43 bc	11.78 cd	2.22 cd	2.06 de
LSD value	3.232		0.5801		1.969		0.201	

Means sharing similar letter (s) do not differ significantly at p=0.05
EDTA was applied @ 0.265 mg L^{-1}

Table 2. Effect of Ni and Pb uptake on shoot and root growth, and leaf area of sunflower plant under hydroponic conditions.

(Mean of three replications)

Treatments	Shoot length (cm)		Root length (cm)		Leaf area (cm^2)	
	No EDTA	EDTA	No EDTA	EDTA	No EDTA	EDTA
Control	54.17 a	56.16 a	38.92 a	40.91 a	94.87 a	97.31 a
Ni @ 15 mg L^{-1}	42.28 c	22.07 f	31.43 c	16.54 fg	76.72 c	54.61 ef
Ni @ 30 mg L^{-1}	37.62 d	19.22 g	27.39 d	13.99 f	68.68 d	49.93 f
Pb @ 15 mg L^{-1}	47.62 b	26.23 e	34.69 b	19.74 e	85.65 b	67.42 d
Pb @ 30 mg L^{-1}	43.88 c	22.69 f	31.96 bc	17.21 ef	77.79 c	57.42 e
LSD value	2.162		2.831		6.482	

Means sharing similar letter (s) do not differ significantly at p=0.05
EDTA was applied @ 0.265 mg L^{-1}

Result showed that maximum reduction in fresh shoot weight (up to 57.8) caused by 30 ppm Ni + EDTA (Table 1). In remaining treatments also observed significant decrease in fresh shoot weight up to 48.6 % in comparison with untreated control. While, minimum decrease in fresh shoot weight was shown up to 28.8% as result of 15 ppm Pb than control.

Results about fresh root weight revealed that maximum decrease (up to 25.7% less than control) in fresh root weight was observed because of Ni and Pb uptake (Table 1). Minimum fresh root weight was recorded in treatment 30 ppm Ni + EDTA followed by 30 ppm Ni as compared control. Rest of treatments also reduced the root dry weight as a result of Ni and Pb uptake that ranged from 6.3 to 17.8 % as compared to control. Minimum reduction in fresh root weight was observed up to 6.3% in 15 ppm Pb in comparison to control.

In case of leaf area, highest decrease in leaf area caused by 48.2% due to application of 30 ppm Ni + EDTA followed by 15 ppm Ni + EDTA as compared with control (Table 2). Rest of the treatments also showed significant decrease in leaf area because of Ni and Pb uptake that ranged from 9.6 to 39.5% while, minimum leaf area was recorded in response to 15 ppm Pb as compared to control.

Results revealed that maximum decrease (up to 68.0%) in shoot length was observed in treatment 30 ppm Ni + EDTA followed by 15 ppm Ni + EDTA as compared to control (Table 2). While other remaining treatments also decreased the shoot length of sunflower plant because of Ni and Pb uptake that ranged from 13.50 to 60.7% as compared to control.

Same trend was observed in root length as well shoot length (Table 2). Highest reduction in root length was recorded in 30 ppm Ni + EDTA that was up to 54.7% while next treatment was 30 ppm Pb + EDTA in comparison to untreated control. Remaining treatments also exhibited significant decrease in root length ranging from 8.9 to 37.4% less than control.

Results about Ni and Pb contents in shoot are presented in Table 3. Maximum uptakes of Ni and Pb in shoot were recorded up to 18.43 and 20.73 mg kg^{-1} in response to 30 ppm Ni + EDTA and 30 ppm Pb + EDTA, respectively. While, remaining treatments also showed uptake of Ni and Pb contents that was up to 15.08 and 18.80 mg kg^{-1} respectively but in case of control uptake of Ni and Pb contents was below detectable limit.

Data regarding Ni and Pb contents in root revealed that maximum contents of Ni and Pb in root were recorded (up to 12.82 and 18.67 mg kg^{-1}) as a results of 30 ppm Ni + EDTA and 30 ppm Pb + EDTA respectively (Table 3). Remaining treatments also exhibited significant uptake of Ni and Pb ranging from 7.0 to 10.54 mg kg^{-1} and 10.64 to 14.33mg kg^{-1} respectively.

In case of Ni and Pb uptake, leaf portion also showed similar results like root and shoot parts of sunflower. Highest uptake of Ni and Pb contents in leaf was observed (up to 24.56 and 32.88 mg kg^{-1}) in 30 ppm Ni + EDTA and 30 ppm Pb + EDTA, respectively (Table 3). Rest of the treatments had also shown significant uptake of Ni and Pb in leaf as compared to control. Maximum accumulation of Ni and Pb in sunflower plant was recorded (up to 55.82 and 72.28 mg kg^{-1}) in response to 30 ppm Ni + EDTA and 30 ppm Pb + EDTA, respectively (Table 3). Moreover, remaining treatments had shown significant accumulation of Ni and Pb that was observed in the range of 33.39 to 41.17 mg kg^{-1} and 49 to 58.78 mg kg^{-1} respectively. It was noted that less accumulation of Ni content compared to Pb in sunflower plant.

Table 3. Concentration and total accumulation of Ni and Pb in sunflower plant under hydroponic conditions.

(Mean of three replications)

Treatments	Shoot (mg kg^{-1})		Root (mg kg^{-1})		Leaf (mg kg^{-1})		Total accumulation (mg kg^{-1})	
	No EDTA	EDTA	No EDTA	EDTA	No EDTA	EDTA	No EDTA	EDTA
Control	0.00 bdl	0.00 bdl	0.00 bdl	0.00 bdl	0.00 bdl	0.00 bdl	0.00 bdl	0.00 bdl
Ni @ 15 mg L^{-1}	9.95 e	15.08 c	7.00 f	10.54 d	16.44 f	22.33 d	33.39 e	47.95 c
Ni @ 30 mg L^{-1}	12.64 d	18.43 b	9.10 e	12.82 c	19.43 e	24.56 c	41.17 d	55.82 b
Pb @ 15 mg L^{-1}	14.65 c	18.80 b	10.64 d	14.33 b	23.71 cd	25.66 bc	49.00 c	58.78 b
Pb @ 30 mg L^{-1}	17.95 b	20.73 a	14.07 bc	18.67 a	26.72 b	32.88 a	58.73 b	72.28 a
LSD value	1.399		1.323		2.051		4.042	

Means sharing similar letter (s) do not differ significantly at p=0.05
EDTA was applied @ 0.265 mg L^{-1}
bdl: below detection limit

Table 4. Effect of Ni and Pb on chlorophyll content, photosynthetic activity and prolein content in sunflower plant under hydroponic conditions.

(Mean of three replications)

Treatments	Total chlorophyll content (mg g^{-1})		Photosynthetic activity (kbq mg^{-1})		Prolein content (µ mol g^{-1})	
	No EDTA	EDTA	No EDTA	EDTA	No EDTA	EDTA
Control	5.32 a	5.47 a	16.50 a	16.72 a	1.19 d	1.21 d
Ni @ 15 mg L^{-1}	3.51 c	2.41 e	14.89 b	5.96 de	1.51 c	2.34 b
Ni @ 30 mg L^{-1}	2.49 de	1.91 f	12.19 c	5.60 e	1.64 c	2.72 a
Pb @ 15 mg L^{-1}	3.77 b	2.68 d	14.34 b	6.94 d	1.48 c	2.25 b
Pb @ 30 mg L^{-1}	3.61 bc	2.41 e	13.00 c	6.15 de	1.57 c	2.36 b
LSD value	0.222		1.065		0.247	

Means sharing similar letter (s) do not differ significantly at p=0.05
EDTA was applied @ 0.265 mg L^{-1}

Results regarding total chlorophyll content are presented in Table 1. Maximum decrease in total chlorophyll content was recorded (up to 63.8%) as a result of 30 ppm Ni + EDTA as compared to control. While rest of the treatments also showed significant decrease in total chlorophyll content because of Ni and Pb uptake that ranged from 29 to 54.4% as compared to control.

There was maximum decrease up to 66.1% less than control in photosynthetic activity (Table 4) due to application of 30 ppm Ni + EDTA followed by 15 ppm Ni + EDTA that gave 63.9% decrease photosynthetic activity, while minimum decrease (up to 9.8%) in photosynthetic activity was observed in case of 15 ppm Ni than control. Maximum production of prolein content was recorded (up to 128.2% more over control) because of 30 ppm Ni + EDTA (Table 4). The remaining treatments also exhibited significant increase in prolein content that was up to 98.3 % as compared to control.

Discussion

Basic need of life for living thing is land and water which are precious natural assets on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum utilization and severely degraded or contaminated because of human activities. In sequence to sustain good quality of soils and waters, and keep them free from pollution, continuous efforts have been made to build up technologies that are easy to use, sustainable and economically reasonable. Physicochemical advances have been extensively used to remediate and recover contaminated soils and waters, especially on small scale. However, these practices are more intricacy for a large scale of remediation due to high costs and side effects. The use of different plant species for cleaning contaminated soils and waters named as phytoremediation has gained increasing awareness since last decade, as an emerging cheaper technology.

For this study, the sunflower plants were selected and grown under hydroponic conditions to evaluate their potential to uptake Ni and Pb from contaminated water. The shoot and root growth of plants were influenced variably in Ni and Pb contaminated treatments. During study, it was also practically observed that Ni is more toxic for sunflower plants than Pb. Our results are line with Meers *et al*., (2005) who noted that *Helianthus annuus* L., and *Zea mays* L., showed more uptake of Pb in comparison to Ni. From our results, it is also concluded that with increasing metal concentrations in water, root and shoot growth of sunflower plants were decreased gradually as compared to respective control. It was also observed that use of EDTA showed more uptakes of Ni and Pb from water than treatments having no EDTA. Root and shoot growth as well as total biomass of plants were severely affected in those treatments containing EDTA because of metal toxicity. However, Chen *et al*., (2002) showed 50 and 60% decrease in shoot and root biomass of maize plants with EDTA versus no EDTA. In another soil study with sunflower, Turgut *et al*., (2005) exhibited 95% of control biomass with 0.1 g kg^{-1} EDTA and 30% of control biomass with 0.3 g kg^{-1} EDTA. In both studies, an increase in EDTA concentration caused a decrease in biomass. However, many researchers also reported that EDTA increased glutathione reductase activity (Kozdroj & van Elsas, 2001). The exhaustion of glutathione and glutathione reductase due to Cd, Pb and Ni can limit uptake of the metals into the roots, thereby reducing toxicity reactions within the plant (Alkorta *et al*., 2004; Freeman *et al*., 2004). It is possible that the addition of EDTA caused the plants to uptake metals at a toxic rate, causing the metals to be compartmentalized in the roots relatively than the shoots of the plant.

Results of chemical analysis showed that uptake of Pb were more than Ni in sunflower plants from contaminated water (Table 3). It is concluded that high concentration of heavy metals in water can negatively affect plant growth, as these metals interfere with metabolic functions in plants, including physiological and biochemical processes, inhibition of photosynthesis and respiration and degeneration of main cell organelles, even leading to death of plants (Schmidt, 2003; Afzal *et al.,* 2006). Soil contamination with heavy metals may also cause changes in the composition of soil microbial community, adversely affecting soil characteristics (Salt *et al.,* 1995; Raskin et al., 1997; Giller *et al.,* 1998; Akguc *et al.,* 2008).

Total chlorophyll content decreased in sunflower plants under metal (Ni and Pb) treatments, which might have been caused by inhibition of chlorophyll biosynthesis and photosynthetic activity (Table 4). Such decreases in the levels of photosynthetic pigments, including chlorophyll content on exposure to heavy metals have been observed in laboratory analysis. Likewise, decreases in total chlorophyll content have also been reported in many plants under heavy metal stress by Cd, Cu, Hg, Pb and Ni (Choudhury & Panda 2004a; Tremper *et al*., 2004). So, decreased content of chlorophyll is a common symptom of heavy metal toxicity. Significant decrease in total chlorophyll with an increase in Ni accumulation reflects the inhibitory effect of this metal on biosynthesis of pigments, which may be a metal specific action (Panda & Choudhury, 2005). Moreover, it can also block the photosynthetic electron transport chain and thus degrade chlorophyll pigment (Quartacci *et al*., 2001; Patsikka *et al*., 2002).

Physiological significance of prolein accumulation is controversial (Table 4). When a plant faces environmental stresses, it accumulates organic solutes like prolein, which at higher concentration act as a solute for intercellar osmotic adjustment (Silveira *et al.,* 2003). However, accumulation of prolein has also been reported as being a sign of stress in plant (Rai *et al.,* 2003). In our study, we found that prolein concentration is significantly higher when metal stress was increased. This may imply that metals increase the severity of salt stress on plant and thus protein concentration (sign of stress) in sunflower leaves also increased. This is also confirmed from the findings that Han & Lee (2005), where high concentration of protein was observed under salt stress in control as compared to inoculated treatments. So that it is clear from our result that photosynthetic activity and biosynthesis of chlorophyll content decreases under stress while increased the prolein in plant.

From our study, it can be concluded that buildup of heavy metals, such as Ni and Pb, in sunflower induces stress and causes chlorophyll loss. Of these metals, Ni appears to be highly toxic and responsible for chlorophyll degradation. Significant chlorophyll loss in sunflower after the accumulation of Ni and Pb must have occurred because of more rapid injurg in the cell membrane. However, much research work is required in this respect such as metal uptake studies at cellular level including efflux and influx of different metal ions by different cell organelles and membranes.

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