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Effect of chelators and mercury on growth and development of *Catharanthus roseus* (L). G Don.

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Mercury posses a major environmental and human health threat because of its constant release through anthropogenic activity. phytoremediation, the use of plants to extract contaminants from soils and groundwater has revealed great potential synthetic chelators that expressed a positive effects in enhancing heavy metal extraction through phytoremediation, but they also revealed a vast number of negative side-effects. The objective of this research was to investigate the use of humic acid and sulfur as an alternative to synthetic chelators. Humic acid and sulfur were applied to a mercury-contaminated soil at various dosages separately, and the uptake of mercury in to *Catharanthus roseus* (nithya kalyani) was determined by ICP-ES. Humic acid and sulfur added at a rate of 2 g/kg and 1.5 g/kg increased the mercury concentration in the shoots about 30.32 mg/kg dw and 10.56 mg/kg dw, respectively amending mercury with humic acid and sulfur can increase the mercury phytoextraction. This may improve phytoextraction as well as reduce environmental pollution in agriculture.

Key words: Phytoextraction, humic acid, Sulphur, contaminated soil

Introduction

Phytoremediation, that use of green plants to decontaminate Hg and other heavy metals in soils, is an emerging technique with advantages of being in situ, cost-effective and environmentally sustainable (Chaney *et al.*, 1997; Cunningham *et al.*, 1997; Salt *et al.*, 1998). The availability of metal in the soil for plant uptake is one important limitation for successful phytoremediation (Blaylock *et al.*, 1997). Mercury (Hg) is a global environmental pollutant that is present in soil, water, air and biota. Hg enters the environment as a result of natural and human activities. Exposures to Hg, e.g. breathing Hg-contaminated air, eating Hg-contaminated food products (especially fish) eating and touching

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Hg contaminated soil may result in devastating neurological damage, kidney damage, and even death (Tchounwou *et al*., 2003).

Specifically, this study was conducted to determine whether amendments of chelates such as humic acid and sulfur can enhance the solubility of Hg and make it more bioavailable for root uptake. An Hg-HA complex are mobile in soils (Wallschlager et al., 1998b) and HA has been demonstrated to enhance both Hg bioavailability in soils and Hg uptake by organisms (Hinton, 2002). Elemental sulphur (S) is used as a fertilizer and has been reported to increase the solubility of cadmium (Cd) in soils and to enhance plant uptake of Cd (Tichy *et al*., 1997, Kayser *et al*., 1999, Cui *et al*., 2004). This study aimed at investigating the effects of chelators addition on Hg availability in the soil and Hg phytoextraction by *C. roseus.*

Materials and methods

Forty days old healthy plantlets were transplanted into the pots (five plants per pot), containing 4 kg of red soil, sand and manure in the ratio of 2:1:1. The soil mixture was air dried and passed through 2 mm sieve. The different levels of $HgCl_2$ (10, 25, 50 and 75 mg/kg soil) was used to screen the sub-lethal concentration of mercury chloride. After screening the sub-lethal concentration forty days old plants were planted into mercury (25 mg) containing soil for 10 days and then chelators (humic acid and sulphur) were added as follows, 0.5, 1, 1.5 and 2 g/kg of HA and 0.5, 1, 1.5 and 2 g/kg of S, respectively. Plants were grown in green house with controlled light and temperature, with 12 h light period at a light intensity of 400 µmol m^{-2} s^{-1} 30/25 $^{\circ}$C day/night temperature, 60-70% humidity. Plants were watered regularly to avoid drought stress. The experiments were repeated three times.

Plant height (cm) of shoot and root, fresh and dry weights (g/plant) were determined at 20 days growth.

The following biochemical estimations were carried out in chelator treated plant samples and the control plants of *Catharanthus roseus* (L.) G. Don. Estimation of chlorophyll pigment, carotenoids, free amino acid and proline were done at 20 days plants and total protein and mercury content was analyzed in 45 days plant dry powder.

Chlorophyll a and b and total chlorophyll were calculated using the following formula (Arnon, 1949). Carotenoid content was estimated and the concentration of free amino acids was determined using L-Glycine as the standard (Troll and Canan, 1953). Proline was measured as described by Bates *et al*. (1973).

Protein content in dry powder was quantitatively estimated according to Bradford (1976). Samples (0.1 g) of dried shoot powder were vortexed with 1

ml borate buffer (pH 8.8), centrifuged and the supernatants were collected in fresh tube. Supernatant was used to estimate total protein.

Hg concentrations were determined on an Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES). Harvested whole plants were washed thoroughly with double distilled water and shoots and roots were separated, blotted and oven dried at 80 °C for 2 d. Hg estimations were done by digesting the plant shoot powder in $HClO_4$:HNO_3 (1:3 v/v) at 100 °C and then diluted them with double distilled water to a known volume then it was subjected to ICP-AES.

The data presented for each treatment was calculated the mean with standard error (X ± S.D.) by standard statistical methods (Mahajan, 1997). The plant survivability, morphological and biochemical characters were analyzed in control, mercury and chelator treated plants at three times.

Results

The effect of increasing rates of Hg (10, 25, 50 and 75 mg/kg) treatments in *Catharanthus roseus* was observed on plant viability. The increase in concentration of heavy metal decreased plant growth and leaded to death. From this, the sub-lethal concentration (25 mg/kg soil) moderately reduced (51%) the plant growth compared with control and other Hg treatments (Table-1).

Shoot and root length of *C. roseus* increased, on humic acid and sulphur treatment in the mercury contaminated soil. Shoot and root length was increased maximum at 2 g and 1.5 g levels of humic acid and sulphur treatments, respectively. Sulphur at higher level (2 g) inhibited the root and shoot growth as can be seen in Table (2). As like the results of shoot and root length, the fresh and dry weight also increased with the increasing level of chelators (Table 3). But of sulphur at 2 g has decreased the fresh and dry weight compared to other sulphur treatments.

Chlorophyll a, b and total chlorophyll were increased with increasing concentration of chelators (humic acid and sulphur). These pigments were significantly increased at 2 g of humic acid and 1.5 g of sulphur treatment. Control plant grown in Hg (25 mg) soil significantly reduced the amino acid content in the leaves of *C. roseus*. All humic acid and sulphur treated plants showed a slight decrease in proline than the positive control. 2 g of humic acid and 1.5 g of sulphur was highly reduced the proline accumulation (Table-5).

In humic acid treated plants, 2 g of humic acid concentration highly increased the accumulation of mercury (30.32 g/kg dw) than the Hg treated and Hg with other humic acid concentration. In sulphur treated plants, 1.5 g of sulphur concentration increased the accumulation of mercury (10.32 g/kg dw) than the Hg treated and Hg with different rates of Sulphur treated plants (Table-6, Figure-1, 2)

Discussion

Increasing concentration of mercury decreased plant growth. Similar result was observed in seed germination and growth of *C. roseus* when plant was exposed to different concentrations of heavy metals like $CdCl_2$ and $PbCl_2$ with a view to observe their bioaccumulation efficiency (Pandey *et al*., 2007). The significant increase in fresh and dry weight was observed with increasing chelators. Application organic chelating agents to heavy metal contaminated soils significantly decreased dry weight of sunflower and maize (Turan and Angin, 2004).

The application of chelators maintained the amino acid content in *C. roseus* better than the Hg treated plants. Amino acid content was increased with the increasing rate of chelators. The amino acid content might be reduced due to the reduction of nitrogen content in plants grown under heavy metal stress. Nitrogen is a precursor for the synthesis of amino acids. Since the nitrogen content of the metal treated plants was reduced, ultimately, amino acids and protein contents of the plants were also reduced as there would be only limited availability of nitrogen for the synthesis of amino acids.

Increasing protein with increased chelators addition was coincide with Costa and Spitz (1997) who also reported a decrease in soluble protein content under high concentration of heavy metals in *Lupinus albus*. The mercury affected plants gradually increase the protein content with the increasing concentration of chelators.

Proline, an amino acid, is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress (Ahmad *et al*. 2006). Proline accumulation in shoots of *Brassica juncea*, *Triticum aestivum* and *Vigna radiata* in response to cadmium toxicity was demonstrated by Dhir *et al*. (2004) but they found that praline accumulation decreased with the exposure to cadmium in hydrophytes (*Ceratophyllum*, *Wolffia*, and *Hydrilla)*.

Forty five (45) days old chelator treated plants were dried and powdered and the mercury content was analyzed. In this study chelators increase the accumulation of mercury in *C. roseus*. Similar work was also carried out in *B. juncea* for induced Hg accumulation using humic acid and sulphur containing ligands (Morena, 2004).

Amending mercury with chelators treatment to the plants enhanced the phytoaccumulation of Hg. When compared to sulphur treatment, humic acid was found to be more effective than the sulphur treatment for phytoextraction of mercury.

Table 1. Percentage of *C. roseus* (L.) G. Don in mercury treatments.

.No	Mercury treatment (mg)	Plant survivability rate (%)
1	Control	100 ±0.00
2	10	91±5.5
3	25	51±3.7
4	50	2±4.1
5	75	0±1.90

Values are averages of three replicates ± SE.

Table 2. Effect of chelators on shoot and root length of *C. roseus* (L.) G. Don in 20 days.

S.No	Chelators treatment (g)	Length of shoot (cm) 20 d	Length of root (cm)20 d
1.	C_1	15±1.29	6±2.23
2.	C_2	33±1.56	12±2.31
3.	HA_1	21±1.23	8±1.20
4.	HA_2	24±1.90	10±2.31
5.	HA_3	26±1.87	12±1.76
6.	HA_4	31±1.12	11±1.89
7.	S_1	20±2.21	7±1.13
8.	S_2	24±2.01	9±1.98
9.	S_3	26±2.12	10±1.85
10.	S_4	21±2.09	8±2.10

Values are averages of three replicates ± SE.,
C_1- Mercury treated, C_2-Mercury untreated, HA_1- Hg + 0.5 g humic acid, HA_1- Hg + 0.5 g humic acid, HA_2- Hg + 1 g humic acid, HA_3- Hg + 1.5 g humic acid, HA_4- Hg + 2 g humic acid, S_1- Hg + 0.5 sulphur, S_1- Hg + 1g sulphur, S_3- Hg + 1.5 g sulphur, HA_4- Hg + 2 g sulphur.

Table 3. Effect of chelators on fresh and dry length of *C. roseus* (L.) G. Don in 20 days.

S.No	Chelators treatment (g)	Fresh weight (g)	Dry weight (g)
1.	C_1	8.2±.98	0.79±0.38
2.	C_2	13.62±1.22	1.82±.0.94
3.	HA_1	9.88±1.28	1.20±0.59
4.	HA_2	10.2±1.54	1.27±0.97
5.	HA_3	11.12±1.73	1.35±1.79
6.	HA_4	12.8±1.61	1.42±1.31
7.	S_1	10.82±1.43	1.13±0.43
8.	S_2	12.20±1.39	1.20±0,97
9.	S_3	11.70±1.69	1.28±1.12
10.	S_4	10.95±1.75	1.23±1.08

Values are averages of three replicates ± SE.
C_1- Mercury treated, C_2-Mercury untreated, HA_1- Hg + 0.5 g humic acid, HA_1- Hg + 0.5 g humic acid, HA_2- Hg + 1 g humic acid, HA_3- Hg + 1.5 g humic acid, HA_4- Hg + 2 g humic acid, S_1- Hg + 0.5 sulphur, S_1- Hg + 1g sulphur, S_3- Hg + 1.5 g sulphur, HA_4- Hg + 2 g sulphur.

Table 4. Influence of chelators on chlorophyll and carotenoid contents of mercury treated *C .roseus* (L.) G. Don in 20 days.

S.No	Chelators treatment (g)	Cholorophyll content (mg/g fw)			Carotenoid Content
		Chl a	Chl b	Total Chlorophyll	(mg/g fw)
1.	C1	3.46±1.71	0.70±0.61	5.20±1.20	1.54±1.37
2.	C_2	3.98±1.86	1.18±0.16	5.90±1.73	1.91±1.22
3.	HA_1	2.98±1.54	0.82±0.87	4.30±1.48	1.34±1.05
4.	HA_2	2.73±1.85	0.76±0.93	4.00±1.56	1.02±1.00
5.	HA_3	2.75±2.01	0.87±0.39	4.14±1.93	1.17±1.02
6.	HA_4	3.08±1.46	2.59±0.79	4.60±1.89	1.45±1.32
7.	S_1	2.86±1.35	0.70±0.47	4.19±2.94	1.13±1.32
8.	S_2	4.20±1.91	0.99±0.98	5.94±2.31	1.28±1.45
9.	S_3	3.70±1.04	0.98±0.59	5.38±2.79	1.37±1.04
10.	S_4	4.15±2.18	0.46±0.12	5.35±2.67	1.20±1.03

Values are averages of three replicates ± SE.

C_1- Mercury treated, C_2-Mercury untreated, HA_1- Hg + 0.5 g humic acid, HA_1- Hg + 0.5 g humic acid, HA_2- Hg + 1 g humic acid, HA_3- Hg + 1.5 g humic acid, HA_4- Hg + 2 g humic acid, S_1- Hg + 0.5 sulphur, S_1- Hg + 1g sulphur, S_3- Hg + 1.5 g sulphur, HA_4- Hg + 2 g sulphur.

Table 5. Influence of chelators on free amino acid, proline and total protein content of mercury treated *C .roseus* (L.) G. Don.

S. No	Chelators treatment (g)	Amino acid (mg/g fw)	Proline (µg/g fw)	Total protein (mg/g dw)
		20 days	20 days	45days
1.	C_1	0.21±0.15	2.1±1.01	0.49±0.25
2.	C_2	0.58±0.42	1.3±1.84	1.11±1.01
3.	HA_1	0.43±1.54	1.9±0.87	0.98±1.01
4.	HA_2	0.49±1.85	1.7±0.93	1.03±1.48
5.	HA_3	0.54±2.01	1.6±0.39	1.05±1.56
6.	HA_4	0.57±1.46	1.4±0.79	1.09±1.93
7.	S_1	0.52±0.76	2±1.61	1.01±1.57
8.	S_2	0.47±0.39	1.8±1.02	1.04±1.18
9.	S_3	0.52±0.86	1.5±1.40	1.09±0.97
10.	S_4	0.48±0.56	1.6±01.03	1.03±0.95

Values are averages of three replicates ± SE.

C_1- Mercury treated, C_2-Mercury untreated, HA_1- Hg + 0.5 g humic acid, HA_1- Hg + 0.5 g humic acid, HA_2- Hg + 1 g humic acid, HA_3- Hg + 1.5 g humic acid, HA_4- Hg + 2 g humic acid, S_1- Hg + 0.5 sulphur, S_1- Hg + 1g sulphur, S_3- Hg + 1.5 g sulphur, HA_4- Hg + 2 g sulphur.

Table 6. Effect of chelators on mercury acccumulation in control and chelators treated *C.roseus* (L.) G. Don in 45 days.

S.No	Chelators treatment (g)	Mercury concentration(mg/kg)
1.	C_1	6.32±1.11
2.	C_2	0.00±3.00
3.	HA_1	9.51±0.81
4.	HA_2	15.70±0.93
5.	HA_3	22.68±1.02
6.	HA_4	30.32±1.41
7.	S_1	6.88±0.79
8.	S_2	7.54±01.07
9.	S_3	10.56±1.18
10.	S_4	9.65±1.12

Values are averages of three replicates ± SE.

C_1- Mercury treated, C_2-Mercury untreated, HA_1- Hg + 0.5 g humic acid, HA_1- Hg + 0.5 g humic acid, HA_2- Hg + 1 g humic acid, HA_3- Hg + 1.5 g humic acid, HA_4- Hg + 2 g humic acid, S_1- Hg + 0.5 sulphur, S_1- Hg + 1g sulphur, S_3- Hg + 1.5 g sulphur, HA_4- Hg + 2 g sulphur.

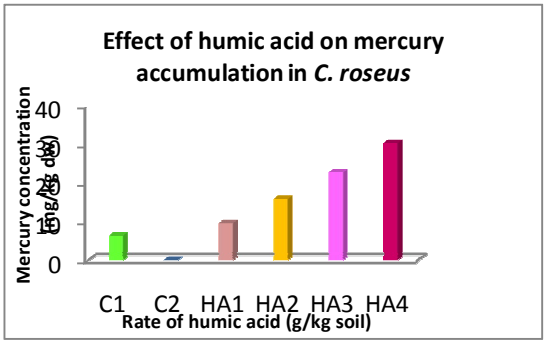

Fig 1. Effect of humic acid on mercury Accumulation in *C. Roseus.*

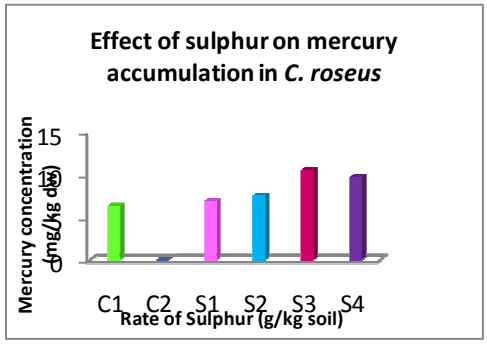

Fig 2. Efect of on mercury accumulation in *C. Roseus.*

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