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Extraction of cadmium and tolerance of three annual cut flowers on Cd-contaminated soils

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Abstract

To evaluate the production potential and Cd removal by three flower crops, viz.: marigold (*Tagetes erecta*), chrysanthemum (*Chrysanthemum indicum*) and gladiolus (*Gladiolus grandiflorus*), an experiment was conducted on differentially contaminated soils (DTPA-Cd 0.6–68.4 mg kg^{-1}). Biotoxicity of Cd lead to reductions in growth and flower yield of marigold at DTPA-Cd $\geqslant$ 7.9 mg kg^{-1} soil, while the productivity of chrysanthemum and gladiolus was sustained up to 21.2 mg kg^{-1}. DTPA-Cd for 50% yield reduction (C_{50}) was 85, 106 and 215 mg kg^{-1} soil for marigold, chrysanthemum and gladiolus, respectively, that indicates a better Cd-tolerance in gladiolus. The uptake of Cd increased with contents in soils and the maximum accumulation occurred in leaves. Among the economic parts, gladiolus spikes accumulated the highest Cd (7.2) followed by flowers of marigold (6.5) and chrysanthemum (4.0 mg kg^{-1}). But, because of higher biomass, the total Cd removal was the maximum with chrysanthemum (8.3) followed by gladiolus (6.0) and the minimum (2.6 mg m^{-2}) with marigold. Gladiolus with highest tolerance and Cd-content in saleable part holds potential to clean up the moderately contaminated soils.

Keywords: Cadmium toxicity; Phyto-remediation; Marigold; Chrysanthemum; Gladiolus

1. Introduction

Indiscriminate dumping of urban and industrial effluents along with solid wastes often lead to toxic accumulation of heavy metal ions, which not only impair soil productivity but also cause health hazards by entering in to food chain via soil–plant–animal/human route (Bingham et al., 1980; Chaney et al., 1999; Yadav et al., 2002). The problems are being aggravated in developing and underdeveloped countries with fast extending urban agglomeration where raw effluents or effluents with little treatment are disposed on the soils (Minhas and Samra, 2004). The conventional techniques for remediation of the contaminated soils rely heavily on 'dig-and-dump' or encapsulation, none of which addresses the issue of decontamination of soil (Pulford and Watson, 2003). Immobilisation or extraction by physicochemical techniques are typically expensive and often appropriate for small areas where rapid and complete decontamination is required (Martin and Bardos, 1996; Brooks, 1998; Wu et al., 2003; Patel et al., 2005). Phyto-remediation is therefore, being proposed as a cheap, environment friendly and sustainable technology for site restoration (Krammer and Chardonnens, 2001). Plants such as field bindweed (*Convolvulus arvensi*) are available, which could accumulate more than 1500 mg of Cd per kg of dry tissue (Gardea-Torresdey et al., 2004), but have a little economic value. Hyper-accumulator plants like *Thlaspi*, *Brassica* are grown on contaminated sites and harvest is later disposed-off at safer locations (Krishnasamy and Chitdeshwari, 2006). In developing countries, even contaminated sites, which mostly exist to the proximity of urban agglomerations and are the means of livelihood for the poor, cannot be spared

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for such non-economic interventions. Under such situations, a viable and remunerative option could be the cultivation of crops with non-edible part as economic, like tree plantations, cut flowers, aromatic grasses, which will also prevent the entry of pollutants in the food chain. Fast growing trees with short rotation coppice system like Eucalyptus, willow have shown considerable promise in creating green belts around cities (Pulford and Watson, 2003; Minhas and Samra, 2004). However, farmers usually show restraints to spare their lands for these permanent adventures and the information on the other alternatives like cut flowers is meagre. With this in view, three of the cut flowers like gladiolus, marigold, chrysanthemum, were evaluated for their productivity and phyto-remediation capacity on Cd-contaminated soils.

2. Methods

An experiment was conducted to evaluate the production potential and Cd-removal capacity of three annual flower crops, viz. marigold (*Tagetes erecta*), chrysanthemum (*Chrysanthemum indicum*) and gladiolus (*Gladiolus grandiflorus*) at the Research Farm of Central Soil Salinity Research Institute, Karnal, India located at 75°57′E longitude and 29°43′N. The experiment was conducted in a set of 36 lysimters; each $2\ m \times 2\ m \times 2\ m$ in size and provided with drainage outlets at the bottom. Soil filled in these lysimeters was an *Alfisol* as transported from an adjoining field and it was initially stabilised by cultivating rice during monsoon season. The soil was sandy loam (sand 58, silt 29 and clay 13%) in texture with initial pH 7.2, electrical conductivity (EC) 0.23 dS m^{-1}, organic carbon 0.4%, cation exchange capacity (CEC) 9.3 $cmol_c\ kg^{-1}$, exchangeable sodium percentage (ESP) 3.5, Cd 0.6 mg kg^{-1} and calcium carbonate 1.0 g kg $soil^{-1}$. Soils, representative of differential Cd-contaminations, were prepared by the application of Cd at 0, 5, 10, 25, 50 and 100 mg kg^{-1} soil, in the form of cadmium chloride ($CdCl_2$). The salts were thoroughly mixed in surface 0.15 m soil and allowed to equilibrate through two wetting and drying cycles. The resultant diethylene triamine pentaacetic acid (DTPA) extractable-Cd levels were analysed to be 0.6, 4.1, 7.9, 21.2, 32.6 and 68.4 mg kg^{-1} soil, respectively while the total Cd was 1.1, 5.8, 9.7, 23.2, 44.6 and 81.4 mg kg^{-1} soil. Thereafter, a good seedbed was prepared and the corms of gladiolus and seedlings of marigold and chrysanthemum were planted on November 7, 2003. Recommended agronomic practices were followed and the crops were fertilized with 120 kg nitrogen, 35 kg phosphorus and 25 tons of farmyard manure ha^{-1}.

The flowers were plucked at appropriate stages of maturity and monitoring was done for number of flowers/spikes, their diameter, fresh and dry weights. Plant height was also recorded. When bearing of flowers was almost over, the crops of marigold and chrysanthemum were harvested on 18th April; where as gladiolus was harvested on 7th June 2004. The harvested plants were partitioned into flower, leaf and stalks to evaluate the biomass of each component. The corms of gladiolus were also excavated. Sub-samples drawn for each of the parts were washed with distilled water, dried in hot air oven at 70 °C. Dried samples were digested in concentrated HNO_3–$HClO_4$ acids and analyzed for Cd by atomic Absorption Spectrophotometer (AAS). The biomass of gladiolus was apportioned in three components, viz. spike, straw (leaf and stem left after the harvest of spike) and corms. Soil samples (surface 0.15 m) were drawn from each lysimeter at sowing and harvest of crops. These were air dried, mixed, passed through 2 mm sieve and analyzed for DTPA extractable Cd using the method as described by Lindsay and Norvell (1978) and total Cd by following the method of Jackson (1967). The pH (1:2 soil:water), electrical conductivity (1:2 soil: water) and calcium carbonate were estimated by following standard procedures out lined by Jackson (1967). The minimum and maximum temperature during the plant growth averaged 14.7 and 28.8 °C and these ranged between 3.2 and 29.0 and 7.4 and 44.0 °C, respectively. The relative humidity, open pan evaporation rate and sunshine duration recorded were 62.5%; 3.4 mm d^{-1} and 7.5 h, respectively. The crops of marigold and chrysanthemum were irrigated six times while gladiolus received nine irrigations (each 6 cm). In addition, the rainfall received equalled 101 and 232 mm, respectively. The experimental design was randomised block design with four replications. Analysis of variance (ANOVA) was performed using MSTAT statistical programme. Least significant difference, LSD ($p = 0.05$) was used to compare statistically the difference between two treatment means. The cadmium uptake values were derived from Cd content in each part and yield of that component. The data on yield of flowers/spikes and DTPA-Cd were utilized to develop response functions (Maas and Grattan, 1999) of the type; $RY = 1.00/[1 + (Cd/Cd_{50})^P]$. Here RY is the relative yield (%) referenced to yield under non-Cd application conditions, Cd_{50} is the DTPA-Cd at which the yield is reduced by 50% and 'P' defines the decline in RY per unit increase in DTPA-Cd.

3. Results and discussion

3.1. Growth and yield of flowers

The yield of fresh flowers/spikes in case of marigold, chrysanthemum and gladiolus ranged from 819 to 1417, 1597 to 2400 and 648 to 835 g m^{-2} with DTPA-Cd levels varying from 0.6 to 68.4 mg kg^{-1} (Table 1). Fresh flower and dry matter yields of marigold and chrysanthemum and spikes of gladiolus were not affected upto DTPA-Cd of 7.9 mg kg^{-1}. At higher Cd levels, the reduction in yield was the maximum in marigold followed by chrysanthemum and gladiolus. The yield of fresh flowers of marigold declined by 16% at 21.2 mg Cd kg^{-1} while differences were non-significant at this Cd-level in other crops. Reductions in fresh flower yield were 32%, 20% and 18% in marigold,

Table 1
Fresh flower and dry matter yield (g m^{-2}) as affected by DTPA-Cd levels in soils

DTPA-Cd ($mg\ kg^{-1}$ soil)	Flower[a] fresh weight			Dry matter yield											
	MG[b]	CS[c]	GL[d]	Marigold				Chrysanthemum				Gladiolus			
				Flower	Stem	Leaf	Total	Flower	Stem	Leaf	Total	Spike	Straw	Corm	Total
0.6	1417	2400	835	150	239	77	466	351	979	159	1488	193	176	509	878
4.1	1354	2294	820	146	229	80	456	322	978	150	1451	175	171	489	835
7.9	1334	2387	830	147	233	76	457	342	914	158	1414	175	180	481	836
21.2	1192	2316	793	125	213	68	407	328	877	149	1353	175	173	486	834
32.6	966	1918	689	102	173	54	328	284	788	148	1220	171	165	438	774
68.4	819	1597	648	87	164	48	298	256	651	109	1016	136	146	426	708
Mean	1180	1886	769	126	209	67	402	314	865	146	1324	171	169	472	811
LDS ($p = 0.05$)	96	422	95	12	32	14	33	63	199	NS	241	21	20	NS	98

[a] Spikes in case of gladiolus.
[b] MG: marigold.
[c] CS: chrysanthemum.
[d] GL: gladiolus.

chrysanthemum and gladiolus at Cd 32.6 mg kg^{-1}. On an average, dry matter produced per unit area followed the order: chrysanthemum > gladiolus > marigold. The yields of different plant parts showed almost similar trend with enhanced Cd levels as in case of fresh flowers. The decrement in dry matter yield equalled 36%, 32% and 19% in marigold, chrysanthemum and gladiolus, respectively at DTPA-Cd 68.4 mg kg^{-1}. Qadir et al. (2000) and An et al. (2004) have earlier reported similar decline in production of vegetable corps with Cd-toxicity. The functions defining the productivity of economic component i.e. flowers/spikes with DTPA-Cd were represented as follows:

$$\text{Marigold}\quad RY = 1.00/[1 + (Cd/85)^{0.99}]\ R^2 = 0.996$$

$$\text{Chrysanthemum}\quad RY = 1.00/[1 + (Cd/106)^{1.49}]\ R^2 = 0.993$$

$$\text{Gladiolus}\quad RY = 1.00/[1 + (Cd/215)^{0.98}]\ R^2 = 0.996$$

Here, the yields are on relative basis (RY) with a value of one representing maximum yield with non-application of Cd. Gladiolus with Cd_{50} value of 215 mg kg^{-1} was the most tolerant, followed by chrysanthemum (106) and marigold (85 mg kg^{-1}). The rate of decline in yield with Cd was almost equal in gladiolus and marigold (values of P being 0.98 and 0.99). In case of cucumber (*Cucumis sativus*), a crop earlier proposed for phyto-remediation by An et al. (2004), an effective concentration of Cd for 50% reduction in shoot growth was observed to be similar (88 mg kg^{-1} soil). Cadmium tolerant plants such as tomato (*Lycopersicon esculentum*) and cabbage (*Brassica oleracea*) tolerated soil levels of ~170 mg kg^{-1} without exhibiting injury symptoms (Bingham et al., 1975). Synthesis of some metal binding polypeptide was proposed to helpful in better tolerance to Cd (Beltagi et al., 2002).

The crop growth as monitored in terms of plant height also showed marked decline with soil Cd levels (Table 2). Plant height of marigold and gladiolus was reduced to 77% and 91% of the control at Cd 32.6 mg kg^{-1}. Other plant parameters like flower diameters and spike length were also affected but the overall impacts remained non-significant.

3.2. Cadmium concentration and uptake

Heavy metals have different patterns of behaviour and mobility with in the plant system (Page et al., 1981; McBride, 1995). Plants take up heavy metals if present in the growth medium but a major portion of metals like Pb, Cr and Cu are immobilised and held up in the root tissue where as Cd, Ni and Zn are easily translocated to aerial tissues (Pulford and Watson, 2003). The mobility of Cd to

Table 2
Growth and yield attributing parameters as affected by DTPA-Cd levels in soils

DTPA-Cd (mg kg soil)	Marigold			Chrysanthemum			Gladiolus			
	Height (cm)	Flowers (no. m^{-2})	Flower diameter (cm)	Height (cm)	Flowers (no. m^{-2})	Flower diameter (cm)	Height (cm)	Spikes (no. m^{-2})	Spike length (cm)	Floret diameter (cm)
0.6	49.2	201	6.4	135.3	913	6.5	120.1	13	50.1	11.6
4.1	49.2	200	6.0	128.6	816	6.3	118.0	13	51.0	11.5
7.9	44.1	224	5.9	126.0	818	6.2	115.8	13	50.9	11.6
21.2	44.3	189	6.1	118.8	830	6.2	116.0	13	51.8	11.6
32.6	37.8	179	5.9	120.6	725	6.5	108.9	12	43.8	10.8
68.4	36.2	163	5.4	101.7	549	6.3	106.0	12	44.0	10.2
LSD ($p = 0.05$)	5.3	NS	NS	NS	175	NS	5.5	NS	NS	NS

Table 3
Concentration and uptake of Cd in different plant parts as affected by soil Cd

DTPA-Cd (mg kg soil)	Cd concentration (mg kg^{-1})				Uptake of Cd (mg $lysimeter^{-1}$)			
	Flower	Stem	Leaves	Total	Flower	Stem	Leaves	Total
Marigold								
0.6	2.4	1.1	1.8	1.6	1.4	1.0	0.6	3.0
4.1	5.3	2.8	8.3	4.6	3.0	2.6	2.6	8.2
7.9	5.2	3.2	12.3	5.4	3.0	3.0	3.8	9.8
21.2	6.5	4.5	16.2	7.0	3.2	3.9	4.4	11.5
32.6	7.2	6.4	28.8	10.3	3.0	4.4	6.2	13.6
68.4	9.3	7.7	36.8	12.8	3.2	5.0	5.0	13.2
Mean	6.0	4.3	17.4	7.0	2.8	3.4	4.2	10.2
LSD ($p = 0.05$)	1.4	1.1	8.2	1.8	0.4	0.4	1.2	0.6
Chrysanthemum								
0.6	2.3	0.9	0.7	1.2	3.2	3.4	0.4	7.2
4.1	4.2	5.5	6.3	5.3	5.4	21.4	3.8	30.6
7.9	3.9	5.8	10.4	5.9	5.6	21.2	6.6	33.2
21.2	4.1	6.7	19.1	7.4	5.4	23.4	11.4	40.2
32.6	4.1	6.9	32.7	9.4	4.6	21.8	19.4	45.8
68.4	5.6	7.7	39.9	10.6	5.8	20.0	17.4	43.0
Mean	4.0	5.6	19.4	6.6	5.0	18.6	10.6	33.2
LSD ($p = 0.05$)	1.6	1.7	8.5	2.1	1.0	4.8	7.0	8.8
	Spike	Straw	Corm	Total	Spike	Straw	Corm	Total
Gladiolus								
0.6	1.9	0.5	1.6	1.5	1.4	0.4	3.4	5.2
4.1	4.8	4.8	6.1	5.6	3.4	3.2	11.8	18.6
7.9	6.0	5.8	7.3	6.7	4.2	4.2	14.0	22.4
21.2	7.4	7.5	8.3	8.0	5.2	5.2	16.2	26.6
32.6	8.3	8.5	11.0	9.9	5.6	5.6	19.4	30.6
68.4	14.7	9.4	16.3	14.5	8.0	5.6	27.6	41.2
Mean	7.2	6.1	8.4	7.7	5.0	4.2	15.4	24.0
LSD ($p = 0.05$)	3.4	1.7	3.2	3.5	0.6	0.6	2.6	3.8

different plant parts was also evident from the uptake data (Table 3). The accumulations followed the order: leaves > flower > stem in marigold and chrysanthemum. Cd content in flowers, stem and leaves of marigold ranged between 2.4 and 9.3, 1.1 and 7.7 and 1.8 and 36.8 mg kg^{-1}, respectively with mean values of 6.5, 4.3 and 17.4 mg kg^{-1}. Almost similar trend was recorded in chrysanthemum. In a hydroponic experiment, Kirkham (1978) recorded increased but similar Cd contents in leaf and stem of chrysanthemum with Cd $\leqslant$ 1.0 mg l^{-1}. In the present study, contents in stem and leaves increased with Cd levels but the rate was similar up to 7.9 mg Cd kg^{-1}. The average Cd content in different parts of gladiolus followed the trend: corms (8.4) > spikes (7.2) > straw (6.1 mg kg^{-1}). Amongst saleable portions, gladiolus spikes showed the highest accumulations of Cd (7.2 mg kg^{-1}) followed by flowers of

Table 4
Soil Cd concentration (mg kg^{-1}) in surface 0–15 cm layer and per cent of total soil Cd taken by crops

Initial			After harvest						Cd uptake (% of total Cd)		
Applied	Total Cd	DTPA-Cd	Total-Cd			DTPA-Cd					
			MR	CS	GL	MR	CS	GL	MR	CS	GL
0.0	1.1	0.6	0.6	0.7	0.6	0.3	0.4	0.5	0.31	0.74	0.53
5.0	5.8	4.1	3.9	4.1	4.7	1.3	2.5	1.1	0.16	0.60	0.36
10.0	9.7	7.9	6.5	7.2	7.9	4.2	5.3	6.1	0.11	0.39	0.26
25.0	23.2	21.2	18.7	17.3	18.6	11.7	12.2	9.5	0.06	0.20	0.13
50.0	44.6	32.6	35.3	30.2	36.0	19.5	20.1	18.7	0.03	0.12	0.08
100.0	81.4	68.4	69.5	70.7	73.4	48.0	47.9	41.2	0.02	0.06	0.06
Mean	27.6	22.5	22.4	21.7	23.5	14.2	14.7	12.9	0.12	0.35	0.24
LSD ($p = 0.05$)			5.5	8.1	10.9	7.2	5.9	8.5	0.01	0.04	0.02

MG, marigold; CS, chrysanthemum; GL, gladiolus.

marigold (6.2) and chrysanthemum (4.0). Cd concentrations in the harvestable parts were similar in the three crops at low Cd levels but gladiolus accumulated more Cd at higher levels. Higher accumulation of Cd in leaf tissue compared with the seed, fruit and tuber tissue has earlier been reported for several crops (Davis, 1984; Qadir et al., 2000). Others (Jiang et al., 2001; Patel et al., 2005; Wang et al., 2007) reported increased accumulation of Cd in roots, stem and leaves along with decrease in plant biomass of garlic (*Allium sativum*), *Colocassia esculentum* and maize (*Zea mays*) with increase in soil Cd.

The total Cd in harvestable parts of marigold ranged between 3.0 and 13.6 mg $lysimeter^{-1}$ with leaves contributing about 40% and the flowers another 28% (Table 3). The total uptake was higher in chrysanthemum (7.2–45.8) and gladiolus (5.2–41.2 mg $lysimeter^{-1}$). The corms of gladiolus contributed 64% towards total uptake. When calculated on hectare basis, Cd removal would range between 7.6 and 38.2, 17.8 and 114.3 and 12.9 and 103.0 g for marigold, chrysanthemum and gladiolus, respectively. These Cd-removals are comparable with the removal by plants like *Thlaspi* (35 ± 11 g ha^{-1} yr^{-1}) that is being recommended for remediation of Cd-contaminated soils (Greger, 1999). When the Cd uptake was converted to the fraction of total soil Cd removed, the values averaged 0.11%, 0.40% and 0.25% at moderate Cd-contaminations (5–25 mg kg^{-1}), for marigold, chrysanthemum and gladiolus, respectively (Table 4). These values are also similar to the mustard (0.4%), another crop being propagated for phyto-remediation (Wu et al., 2003). The plant species those have both the high biomass production and can tolerate and accumulate high levels of contaminants of interest are rated to be ideal for remediation. Such combinations are rarely possible since the most of the hyper-accumulators are small and slow growing (Pulford and Watson, 2003). Therefore as a trade-off, Keller et al. (2003) recommended that the higher biomass produced by crops could compensate their lower Cd contents when compared with hyper-accumulating but producing lower biomass. Considering the high biomass production by the crops under study and that too the non-hazardous, these can act as viable candidates for phyto-remediation.

It was concluded that toxic accumulations of Cd in plants, though affected the growth and productivity of the three flower crops but their tolerance (Cd_{50} 87–215 mg kg^{-1}) and Cd-removals (7.6–114.3 g ha^{-1}) were comparable with the crops recommended for phyto-remediation. Therefore with the advantage of secondary economically viable use, the flower crops particularly gladiolus have a potential to be raised as an alternate crop for phyto-remediation and other environmental benefits like minimising the risks of Cd entering into the food chain.

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