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EFFECT OF HEAVY METALS (Cd, Cr, Ni and Pb) ENRICHED SEWAGE SLUDGE ON PHYTOEXTRACTION OF HEAVY METALS BY MARIGOLD (*Tagetes* sp.)

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Abstract: A pot experiment was conducted in the net house to study the effect of heavy metals (Cd, Cr, Ni and Pb) enriched sewage sludge on phytoextraction of heavy metals by marigold (*tagetes* sp.). The growth parameters like plant height and dry matter yield of crop was recorded. The dried plant sample was used for analyzing heavy metals (Cd, Cr, Ni, and Pb) following the standard methods. Application of sewage sludge had significant effect on Cd, Cr, Ni and Pb content in marigold crop. The uptake of Cd, Cr, Ni and Pb in marigold also increased with application of sewage sludge. The maximum value recorded in marigold was 0.27, 0.047, 0.49 and 0.116 mg pot⁻¹ for Cd, Cr, Ni and Pb, respectively. Application of sewage sludge in soil provides essential nutrients for plant growth and it reduces environmental and economic considerations that limit disposal in landfills or incineration.

Keywords: Heavy metals, sewage sludge, marigold, phytoextraction and soil contamination.

Introduction: Phytoextraction is the process where plant roots uptake metal contaminants from the soil and translocate them to their above soil tissues as different plant have different abilities to uptake and withstand high levels of pollutants many different plants may be used. This is of particular importance on sites that have been polluted with more than one type of metal contaminant. Once the plants grown and absorbed the metal pollutants they are harvested and disposed of safely. This process is repeated several times to reduce contamination to acceptable levels. Metal compounds that have been successfully phytoextracted include zinc, copper and nickel, but there is promising research being completed on lead and chromium absorbing plants. Some of plant species are used for the phytoextraction purpose e.g. spinach, sunflower and marigold etc.

Marigold (*Tagetes* sp.) is a horticultural plant with a good ability to absorb heavy metals phytoremediation. It also provides economic benefits to the remediators through marketing flowers. Therefore, marigold is considered as a potential economic crop for phytoremediation^[1]. The metal accumulating property of marigold is

appeared to be a good source to be exploited to phytoremediate heavy metal contaminated soil^[2].

Materials and Methods

The pot experiment was conducted to study the effect of heavy metal contaminated sewage sludge on growth, yield and uptake of heavy metals in the marigold and spinach, during last week of November to last week of March 2011, in the Net house of Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, B.H.U. Varanasi U.P. To conduct the pot experiment, the soil sample was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Soil was air dried gently, ground to pass through a 2 mm sieve, homogenized and stored dry. The bulk sample of sewage sludge was collected from Sewage Treatment Plant (STP) at Bhagwanpur Varanasi, dried and analyzed for various chemical properties and heavy metal content. Aqueous solution of four dominant heavy metals presents in sewage sludge i.e. Cd as $3 \text{ CdSO}_4 \cdot 8\text{H}_2\text{O}$ salt, Pb as PbSO_4 salt, Ni as $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ salt and Cr as $\text{K}_2\text{Cr}_2\text{O}_7$ were added to sewage sludge. These

metals were added at the rate of 40 mg kg⁻¹ of Cd, 100 mg kg⁻¹ of Ni, 300 mg kg⁻¹ of Pb and 300 mg kg⁻¹ of Cr in sewage sludge. The sewage sludge irrigated to field capacity and kept in the net house for one month to allow added metals (Cd, Cr, Ni and Pb) to equilibrate before planting. Treatments comprises of Soil (control) T₁, Soil + uncontaminated sewage sludge (10 t ha⁻¹) T₂, Soil + uncontaminated sewage sludge (20 t ha⁻¹) T₃, Soil + uncontaminated sewage sludge (40 t ha⁻¹) T₄, Soil + uncontaminated sewage sludge (80 t ha⁻¹) T₅, Soil + contaminated sewage sludge (10 t ha⁻¹) T₆, Soil + contaminated sewage sludge (20 t ha⁻¹) T₇, Soil + contaminated sewage sludge (40 t ha⁻¹) T₈ and , Soil + contaminated sewage sludge (80 t ha⁻¹) T₉.

Ten kg. of air dried soil was taken into earthen pots and Fifteen days old plant seedling were transplanted and moisture was adjusted 30-40% of WHC. The pots were replicated three times. Amounts of uncontaminated and metal contaminated consummated sewage sludge which were applied to the soil in pots were equivalent to 10, 20, 40 and 80 t ha⁻¹. Required quantities of fertilizers for 10 kg soil was calculated and applied in liquid form using urea, KH₂PO₄ and MOP as source of N, P and K, respectively. The recommended doses for marigold and spinach 50: 45: 45 Kg N: P₂O₅: K₂O ha⁻¹, 80: 60: 45 Kg N: P₂O₅: K₂O ha⁻¹, respectively as full dose (100%). Half dose of N and full dose of P₂O₅ and K₂O was applied as basal dose. Left over dose of nitrogen was added at 30 days after transplanting in each pot. Soil samples were collected from each treatment after harvesting of crop. Three seedling were transplanted on 21st November 2010, one of them maintained and allow to grow to maturity pots were timely irrigated with deionized water at regular intervals to maintain moisture of the soil. Crop was harvested on 20th February 2011. The harvested plant material was put in paper bags, air dried and oven dried at 65± 2 °C to a constant weight. Thereafter dry weight of above ground plant parts was recorded. The plants were harvested at maturity, washed sequentially with 0.2% detergent solution, 0.1 N HCl and finally with double distilled water. The plant material was dried at 60°C for 48 h in a hot air oven.

The content of Cd, Cr, Pb and Ni in the plant sample was digested and determined by using atomic absorption spectrophotometer (UNICAM – 969) as per procedure outlined by Sparks *et al.* (1996). The raw data observed during the whole experiment, was put for

statistical analysis by following the Complete Randomized Design (CRD) to draw the valid differences among the treatments using SPSS software

Results and Discussion

Data on plant height recorded at 30, 60 and 90 DAS of marigold as influenced by sewage sludge levels are presented in Table 1. It is apparent from the data that various nutrient levels had significant influence on plant height at different stages of crop growth of observations. All treatments recorded significantly taller plants at all the stages of observations over control. Further, application of 80 t ha⁻¹ sewage sludge produced tallest plants which were comparable to 20 t ha⁻¹ sewage sludge and 40 t ha⁻¹ sewage sludge these treatments were found significantly superior to rest of the treatments. The increase in plant height due to 80 t ha⁻¹ sewage sludge and 80 t ha⁻¹ metal contaminated sewage sludge was to the tune of 48, 33 per cent at 30 DAS, 36, 28 per cent at 60 DAS and 28, 16 per cent at 90 DAS over control, respectively.

Further scanning of the data presented in Table 1 clearly indicated that there was a significant difference in dry matter production pot⁻¹ due to various sewage sludge levels at 90 DAS. Significantly higher dry matter was accumulated pot⁻¹ in marigold under all sewage sludge levels as compared to control which had the lowest amount of dry matter pot⁻¹. Application of 80 t ha⁻¹ sewage sludge accumulated maximum dry matter pot⁻¹ which was statistically at par with 40 t ha⁻¹ sewage sludge and significantly superior to rest of the other levels under study at 90 DAS. The increase in dry matter pot⁻¹ due to 80 t ha⁻¹ sewage sludge and 40 t ha⁻¹ heavy metal contaminated sewage sludge was to the tune of 33 and 16 per cent at 90 DAS over control, respectively.

The data pertaining to Cd content in marigold (Table 2) showed a significant effect with graded application sewage sludge. Cadmium content in marigold varied from 0.3 to 14.1 mg kg⁻¹. The maximum (14.1 mg kg⁻¹) was recorded in 80 t ha⁻¹ metal contaminated sewage sludge and minimum (0.84 mg kg⁻¹) which was observed in control. Application of heavy metal contaminated sewage sludge increased Cd content in marigold 47 times over control and in treatment 80 t ha⁻¹ sewage sludge sewage sludge application increased Cd content in marigold 35 times over control. Wheat grown in sewage sludge (2–10 kg m⁻²) amended Sandy Loam and Silty Loam soils of USA showed significant

higher concentrations of Zn, Cd and Ni in wheat grains at increasing sewage sludge amendment rates in soil [3]. The data pertaining to Cr content in marigold (Table 2) showed the significant increase with the application of graded levels of sewage sludge. Chromium content in marigold varied from 0.1 to 2.5 mg kg⁻¹. Maximum (2.5 mg kg⁻¹) was recorded in 80 t ha⁻¹ metal contaminated sewage sludge and minimum (0.1 mg kg⁻¹) in control. Treatment 80 t ha⁻¹ sewage sludge and 80 t ha⁻¹ metal contaminated sewage sludge increased Cr content in marigold by 90 and 95 percent over control. The normal range in plants is considered 0.03-14.00 mg kg⁻¹, while the toxic concentrations fall between 5-30 mg kg⁻¹ [4]. This suggests that plant Cr in this study was in the normal range. The data pertaining to Ni content in marigold (Table 2) revealed that there was a significant effect of application of graded dose of sewage sludge. The Ni content in marigold varied from 8.1 to 25.4 mg kg⁻¹, maximum (25.4 mg kg⁻¹) being in 80 t ha⁻¹ metal

contaminated sewage sludge and minimum (8.1 mg kg⁻¹) in control. Treatments 80 t ha⁻¹ sewage sludge and 80 t ha⁻¹ metal contaminated sewage sludge were 3 times higher over control (8.1 mg kg⁻¹). Pb content in marigold (Table 2) varied from 0.1 to 8.5 mg kg⁻¹. The maximum (8.5 mg kg⁻¹) was observed in 80 t ha⁻¹ metal contaminated sewage sludge and minimum (0.1 mg kg⁻¹) in control. Treatments 80 t ha⁻¹ sewage sludge and 80 t ha⁻¹ metal contaminated sewage sludge increased Pb content in marigold by 98 and 99 percent over control. A significant increase in Pb content in marigold was observed with increase in sewage sludge doses. Sewage sludge amendment rates above 4.5 kg m⁻² though increased the yield of rice [5], but caused risk of food chain contamination as concentrations of Ni and Cd in rice grains were found to be above the Indian safe limits (1.5 mg kg⁻¹) of human consumption above 4.5 kg m⁻² sewage sludge and Pb (2.6 mg kg⁻¹) above 6 kg m⁻² sewage sludge amendment.

Table 1 Effect of heavy metal enriched sewage sludge on plant height and dry matter yield of marigold crop.

Treatments	Plant height (cm)			Dry matter yield (g pot ⁻¹) at harvest
	30 DAS	60 DAS	90 DAS	
T ₁ -Control	7.23	12.73	18.73	14.87
T ₂ -10t/ ha Sludge	9.13	15.13	21.13	17.03
T ₃ -20t/ ha Sludge	12.00	18.00	24.00	18.33
T ₄ -40t/ ha Sludge	13.43	19.63	25.63	20.90
T ₅ -80t/ ha Sludge	13.80	19.80	26.00	22.30
T ₆ -10t/ ha Sludge + Heavy metal	9.03	14.70	20.70	16.20
T ₇ -20t/ ha Sludge + Heavy metal	10.87	16.37	22.37	17.73
T ₈ -40t/ ha Sludge + Heavy metal	10.10	15.60	21.60	16.93
T ₉ -80t/ ha Sludge + Heavy metal	9.53	14.90	20.90	16.03
SEM ±	0.54	0.58	0.59	0.79
CD (P=0.01)	2.12	2.27	2.32	3.11

Table.2 Effect of heavy metal enriched sewage sludge on plant heavy metal content and uptake by marigold crop.

Treatments	Heavy metal content (mg kg ⁻¹)				Heavy metal uptake (mg pot ⁻¹)			
	Cadmium	Chromium	Nickel	Lead	Cadmium	Chromium	Nickel	Lead
T ₁ -Control	0.3	0.1	8.1	0.1	0.01	0.001	0.13	0.001
T ₂ -10t/ ha Sludge	6.4	0.6	15.2	0.7	0.11	0.009	0.26	0.011
T ₃ -20t/ ha Sludge	6.9	0.7	16.9	1.8	0.13	0.012	0.31	0.033
T ₄ -40t/ ha Sludge	8.2	0.8	19.1	3.6	0.17	0.017	0.40	0.074
T ₅ -80t/ ha Sludge	10.4	1.0	21.9	5.2	0.25	0.022	0.49	0.116
T ₆ -10t/ ha Sludge+Heavy metal	11.3	1.2	22.7	5.0	0.13	0.010	0.24	0.074
T ₇ -20t/ ha Sludge+Heavy metal	12.1	1.9	23.7	7.0	0.19	0.031	0.30	0.052
T ₈ -40t/ ha Sludge+Heavy metal	13.5	2.3	24.4	7.8	0.22	0.040	0.33	0.065
T ₉ -80t/ ha Sludge+Heavy metal	14.1	2.5	25.4	8.5	0.27	0.047	0.45	0.095
SEM ±	0.64	0.05	0.81	0.84	0.01	0.0013	0.02	0.01
CD (P=0.01)	2.50	0.21	3.17	3.29	0.05	0.001	0.08	0.03

The accumulation of heavy metals by plants depends on various factors such as soil physico-chemical properties, sewage sludge composition sludge application rate, plant species, climatic factors and chemical speciation of metals [6]. The data on Cd ,Cr, Ni and Pb uptake by marigold as influenced by application of sewage sludge have been presented in Table 2. A significant variation in uptake of Cd by marigold by application of sewage sludge was noticed. The Cd uptake by marigold varied from 0.01 to 0.27 mg pot⁻¹. The maximum uptake

(0.27 mg pot⁻¹) was observed in 80 t ha⁻¹ metal contaminated sewage sludge which showed 27 times more over control. The minimum value (0.01 mg pot⁻¹) was recorded in control. Application of 80 t ha⁻¹ sewage sludge alone resulted in 25 times increase over control. Similar results of increase in uptake pattern of Cd were reported [7].

The data further revealed that Cr uptake in marigold increased from 0.001 to 0.047 mg pot⁻¹. The maximum uptake (0.047 mg pot⁻¹) was recorded in 80 t ha⁻¹ metal contaminated sewage

sludge which was about 47 times higher than control. Application of 80 t ha⁻¹ sewage sludge increased the Cr uptake by marigold 22 times higher than control. The Ni uptake in marigold ranged between 0.13 to 0.49 mg pot⁻¹. Maximum uptake (0.49 mg pot⁻¹) was recorded in 80 t ha⁻¹ sewage sludge which was almost 4 times higher than control. Lowest (0.13 mg pot⁻¹) was recorded in control. Application of heavy metal contaminated sewage sludge with 80 t ha⁻¹ dose resulted 3 times increase in uptake of Ni by marigold over control.

The data showed that the uptake of Pb was very less in marigold. The uptake of Pb in marigold was significantly increased with application of graded doses of sewage sludge. The maximum Pb uptake by marigold was 0.116 mg pot⁻¹. The Pb uptake of marigold in treatments where no sludge was applied (T1) were below detectable limits.

Study clearly indicated that application of sewage sludge slightly increased the concentration and uptake of Ni, Cd, Pb and Cr in marigold except for Pb which was not bio-available. It has also been reported that Ni and Cd could be readily taken up and accumulated by plants depending on soil conditions and especially from soils containing elevated levels of these elements. On the other hand Pb tends not to be taken up and poorly accumulated by plants, and plant uptake, if occurs most of the absorbed Pb is located within the root system of the plant.

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