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Phytoremediation of Heavy Metal Ions and Contaminants from Municipal Waste Dump Soil using Tagetus Erecta

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Abstract: ***Phytoremediation refers to a diverse collection of plant based technologies that use either naturally occurring or genetically engineered, plants to clean contaminated environments. Topsoil samples (20-50cm) were taken at various locations of landfill depot, the metals analyzed were Cr, Cu, Zi, Ni, Mn, Fe and Co. The results showed that, the percentage conc. of metals were Cu (72-73 %), Zn (87-88 %), Cr (89-90 %) and Ni (87 %) showed higher in waste soil (WS) as compared to control soil (CS). The T. erecta grown in this soil during the 2 months period these plants have ability to accumulate more amounts of metals from waste dump soil. Data showed that this plant have a ability to absorb, stabilize, volatilize and extract Cu (90-95 %), Zn (70-72 %), Cr (74-75 %) and Ni (43-44 %) form WS. This data showed that the potential of plant for remediation it hyperaccumulate Cu > Cr > Zn from waste soil. Vigorous growth of Tagetus erecta has been observed in waste soil than control with respect to biomass production, chlorophyll contents and anatomy of plant.***

Keywords: Phytoremediation, heavy metal, soil contamination, compost and landfill Depot.

1. Introduction

In Indian cities, solid waste generation rate is continuously goes on increasing day by day in most of states, metropolian and large cities. The annual waste generation has observed to increase in proportion to the rise in population and urbanization and issues related to disposal have become challenging, as more land has needed for the ultimate disposal of these solid wastes [11]. In 1995, EPRIT [7], Hyderabad showed that 23 big Indian cities generated 11 million tons (million tons) of solid waste every year. The solid waste management (SWM) in state of Maharashtra, particularly in its major cities, is of serious concern. Per capita MSW generation in various towns of the state ranges between 100 and 600 gm/day. In total, over 16000 tons per day (TPD) of MSW has generated, of which around 50 per cent in three cities, namely Mumbai, Thane and Pune only (as in 2001-02). Mining, manufacturing and the use of synthetic products (eg. pesticides, paints, batteries, industrial waste and land application of industrial or domestic sludge) can result in heavy metals contamination of urban and agricultural soils. Heavy metals such as Copper (Cu), Chromium (Cr), Iron (Fe), Manganese (Mn), Nickel (Ni), Mercury (Hg), Cadmium (Cd), Lead (Pb) etc., accumulation in soil is toxic to human and other animals. Exposure to heavy metals is normally chronic (exposure over a longer period), due to food chain transfer.

The Sukali compost and landfill depot selected for study, which is situated outskirt of the Amravati city (**Fig. 1)** near village Sukali. The samples collected from different points in their respective sampling area. Amravati city has a population of 6, 78,192 (2008) including 1, 28,682 rural and 5, 59,510 urban persons. The Sukali landfill site is a highly disturbed area, because of the constant turning of soil due to solid waste dumping. About 79 % of land in Sukali compost depot are totally filled up only remain 21 % of land for further loading of waste material, it also filled up in upcoming year. Hence, it is necessary study critically present situation of solid waste of Sukali compost and landfill depot, Amravati and to suggest possible solutions for its safe management for disposal.

Research on phytoremediation has demonstrated that plants are effective in cleaning up contaminated soil [20]. Phytoremediation is a general term for using plants to remove, degrade soil pollutants such as heavy metals, pesticides, solvents, crude oil, polyaromatic hydrocarbons and landfill leacheates viz., prairie grasses can stimulate breakdown of petroleum products, wildflowers had recently used to degrade hydrocarbons from an oil spill [4]. A plant has used to stabilize or remove metals from soil and water. Depending on the contaminants, the site conditions, the level of clean up required and the types of plants.

2. Material and Methods

1. Experimental plant description:
Tagetes erecta plant belongs to the family Compositae. The plants were immensely popular annual flower crop widely grown throughout the world. The plant was studied based on well distinguishing features with the help of local flora **(Fig. 2)**.

2. Site of selection and sample collection
Soil sample were collected, in 2009-2011 from the Sukali compost depot which is situated outskirt of the Amravati city near village Sukali. The samples collected from different points in their respective sampling area. An aerial photo and a zoomed sampling area are shown in figure (Fig.1). Soil samples were collected from the site in summer season; three pits of 20-50 cm depth were dug for soil sampling at selected points.

Figure 1: Compost and landfill depot

Figure 2: *Tagetus erecta* plant grow in control soil and waste soil

3. Collection and germination of Seed: - *Tagetus erecta* seeds were collect from different sites in Campus Government Vidarbha Institute of Science and Humanities, Amravati in Year 2009. Seed germinated in control soil collect from garden and waste soil from Sukali compost and landfill depot. Each bag contains 5 kg of soil sample. Seeds will be sown in the pots. Total 25 seeds sown in each bag and count the % of germination.

4. Physicochemical Analysis:-Moisture Content result of soil analysis was to be calculated on the basis of oven dried sample weight [6]. Soil texture was indicated by the proportion of different sized particles which make it [2]. pH of the soil was measured potentiometrically in a 1:2 or 1:5 soil- water suspension or in saturates soil paste. Use pH meter Systronics µ pH System 361. Electrical Conductivity measured in 1:2 or 1:5 soil-water suspensions with the help of conductivity meter Model Systronics Conductivity Meter 306 µc. Measured the temperature using mercury thermometer. Colour notations indicated by using Munsell`s soil colour chart.

5. Mineral and Salt Analysis:-Na, K and Ca ions were determined by flame photometer [10]. Organic Carbon The organic carbon in the sample was oxidized with potassium dichromate and sulphuric acid [19]. Calcium carbonate by titrimetric method [17]. The chloride content of the soil was directly measured by titrimetric method [18].

6. Metal Analysis:- Detection and Analysis of metal ions such as Cu, Zn, Cr, Ni, Fe, Mn and Co, for the release of minerals elements from soil and sediments, wet oxidation of sample was carried out. Wet oxidation employs oxidizing acids like HNO_3 –$HCLO_4$ di-acid mixture [12]. Detection of metals from Plant Material by dry ashying, this process was simple, non-hazardous and less expensive [5]. Filtrate was used for estimation of metals was carried out using software and protocol of atomic absorption spectrophotometer (AAS 303). Readings were recorded in triplicates for each sample and mean value of readings was calculated in ppm then it was converted in to mg/g.

7. To study effect of pollutants on Anatomy:-Sections were stained with saffranin and light green. These sections were examined under the inverted microscope (Axiovert 40c) for detail study of cell structure. Staining sections were photographed with camera.

8. To study effect of pollutants on Chlorophyll Contents of Plant: - Chlorophyll was extracted in 80% acetone and absorbance at 663 nm and 645 nm were read in a UV-Visible spectrophotometer (Elico SL 164). Leaves samples with control and treatments were estimated following the procedure of Whatley and Arnon [21].

9. Statistical analysis

The extent of variation can be study from the statistical analysis. Standard deviation gives the deviation of actual value from the average value. Along with standard deviation, percent coefficient variance and standard error were using to measure the control tendency of variations of the particular value [22].

3. Result and Discussion

1. Germination of seed

The results of percentage of seed germination are tabulated in **Table 1**. Percent germination of seeds in the CS (68 %) and WS (84 %), significant increase in the seed germination in WS was notice as compared to CS.

2. Biomass and growth performance

After completion of 30 - 60 days, (**Fig.2),** the plants of each pots were harvested to study growth parameters and biomass. The length, breadth, number of leaves per plant, number of branches and plant height was studied **(Table 1)**. All the growth parameters were significantly greater in waste soil in relation to plant height (38.96 cm), number of branches per plant (5), number of leaflet (16), number of pinna (780), length of leaflet (15.44 cm) and breadth of leaves (5.9 cm) after 60 days plant growth as compared to control. The number of branches in waste soil was equal to control soil after 30 days but there was in number of branches (5) increase after 60 days. Fresh weight of plant grown in waste soil (37.25 gm) after 30 days and after 60 days it again increase up to (83.05 gm) i.e. 50-60 % increase than control.

3. Chlorophyll contents

The *Tagetes erecta* plants grown in waste soil showed significantly higher chlorophyll content (Chl. a, Chl. b and total Chl.) than control **(Table 1)**. However, relation between chl. a and chl. b content after 60 days in waste soil showed higher quantity of chl. a (1.03 mg chl./gm) than chl. b (0.29 mg chl./gm) over the control. The total chlorophyll contents continue to increase in waste soil growing plant as compared to control. Relation to total chlorophyll amounts after 30-60 days, the maximum amount of chl. content in waste soil (0.91 mg chl./gm) was recorded after 30 days and (1.24 mg chl./gm) after 60 days these values are (75-90 %) higher over control **(Table 1)**. Analysis of variance showed that the effect of WS treatment on total chl. production of plants was statistically significant in $p < 0.2$ and $p < 0.05$ percent level after 30 days and $p < 0.05$ and $p < 0.01$ percent level after 60 days **(Table 1)**. The results of present study showed that the highest amount of Mn stored in *T. erecta* plants, this plants contain higher amount of chlorophyll. Manganese is also essential for the biosynthesis of chlorophyll (through the activation of specific enzymes), aromatic amino acids (tyrosine), secondary products, like lignin and flavonoids [13].

Table 1: Growth Performance of *Tagetus erecta* in field trial

Parameters	**After 30 Days**		**After 60 Days**	
	CS	**WS**	**CS**	**WS**
Seed germination (%) (After 7 days)	68	84****		
Plant height (cm)	23.7±0.59	33.62±1.95*	27.16±0.85	38.96±1.38*
Number of branches	3±0.28	3±0.20	3±0.34	5±0.40**
Number of leaflet	10±0.49	13±0.62***	12±0.49	16±0.63
Number of pinna	107±24.48	215±2.26**	154±.8.66	780±15.34****
Length of leaflet in (cm)	3.9±0.33	11.64±1.29**	6.34±0.26	15.44±0.65**
Breadth of leaflet in (cm)	2.32±0.12	5.94±0.37*****	2.76±0.17	5.9±0.23****
**Biomass (gram)	10.46	37.25***	14.74	83.05**
*Chl. a (mg chl./gm tissue)	0.41	0.51	1.00	1.03**
*Chl. b (mg chl./gm tissue)	0.08	0.10*****	0.26	0.29**
*Total chl. (mg chl./gm tissue)	0.50	0.67*****	1.26	1.33**

CS - Control Soil, WS - Waste Soil
NS - Non significant, $^{* ****}p < 0.2$, $^{****}p < 0.1$, $^{***}p < 0.02$, $^{**}p < 0.05$, $^{*}P < 0.01$
***Leaves, ** plant sample**

4. Anatomy: Ultra structural effects of waste soil on *T. erecta*

Observations on the anatomical traits of root and stem of WS and CS growing plants showed that, due to pollutants compressed the vascular tissues and breakage occurs at pith portion **(Fig. 3)**. Transverse section of stem consists of single layered epidermis ruptured in some portions in waste soil growing plant. Cortex 6-8 layered, made up of paranchymatous cells, xylem vessels were very well developed in waste soil growing plant as compared to control. In WS, conc. of heavy metals 40-88 % higher over CS **(Table 3)**. High heavy metal concentrations in the soil exert negative effects on the plants [8], [15]. Increase in vascular tissues especially xylem may be due to higher conc. of nutrients and metals present in WS, which are beneficial for plant growth. Anatomy of root showed, epidermis single layered, just below the epidermis 4-10 layered cortical tissues. Secondary tissues were greatly extended in waste soil growing plant, epidermal portion ruptured and pith tissues reduced as compared to control.

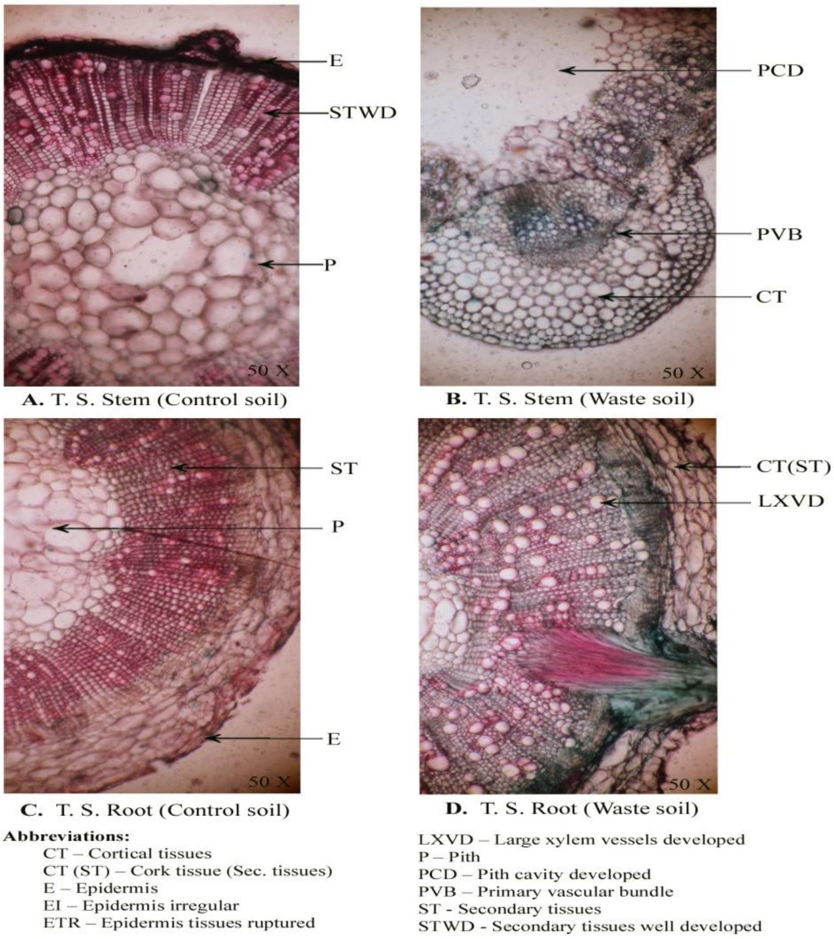

Figure 3: Microscopic structure of *Tagetus erecta*

5. Physicochemical analysis of soil (rainy season)

The results of physicochemical analysis were tabulated in **Table 2**. The initial temperature of CS and WS soil was found to be same (25.5ºC). The pH of WS (8.34) it was higher as compared to CS (7.25). The pH was mostly towards the alkaline side, no significant change before and after the experiment. The colour of the soil is a useful indicator, it determine quality of soil, the colour of waste soil grayish brown, this colour indicate, waste soil had very poor drainage or suffer from waterlogged. Moisture content in waste soil was found to be (4.63 %) this value 82-83 % higher as compared to control (47.306 %). The organic carbon content in initial WS (27.76 %) it was 10-15 % lower as compared to CS (34.72 %). The electrical conductivity of initial WS ($1.472X10^6$) i.e. 80-90 % higher than CS ($0.192X10^6$). In initially collected WS the amount of Na (14 mg/kg), K (410 mg/kg) and Cl (113.6 mg/kg). The percent conc. of Na (80-81 %), K (91-92 %) and Cl (62-63 %) are higher in WS as compared to CS **(Table 2)**. During the experiment period, *T. erecta* plants absorbed Na (18-19 %), K (31-32 %) and Cl (46-47 %) from the WS. Concentration of Ca was found to be very low in WS (60 mg/kg) as compared to CS (820 mg/kg). The levels of $CaCO_3$ was (117.6 %) in initially collected WS it was 53-54 % higher over the control (55.2 %) this value also again reduce 8-9 % due to *T. erecta* grown in WS. About the different minerals and nutrients i.e. Na, K, $CaCO_3$ and Cl contents was much higher in WS, from these ions some acts as chelating agents of different metals for metal transporter inside and outside of the cell. There all minerals and nutrients are responsible for better growth of *T. erecta* in waste soil.

Growth of leaves depends upon different kind's growth factors of plants i.e. growth hormones, nutrients and minerals, it affect on photosynthetic activities of these plants. In WS various nutrients were found in greater quantity, it showed stimulating effect on the growth and biomass production. Anikwe and Nwobodo (2001) [1] showed municipal wastes of urban agriculture in Abakaliki region, increase the nitrogen, pH, cation exchange capacity (K, Na, Cl etc.), percentage of base saturation and organic matter. Highest amount of K recorded in initially collected WS it was 13-15 times higher than the CS, during the experimental period 50-60 % K^+ absorbed by *T. erecta* plant from WS. Belorkar *et al.* (1992) [3] reported that the beneficial effect of K^+ in promoting the growth of marigold plants, this may be due to the fact that potassium involved in synthesis of peptide bond, proteins, carbohydrate metabolism, participates in rapid cell division and differentiation. The presence of nutrients like, nitrogen, potassium, phosphorous, calcium, sulphate and magnesium in the diluted effluents, which are at optimum level, may promote the germination through enhanced cell division, expansion and differentiation [14].

Table 2: Physicochemical analysis of soil (*T. erecta* grown in experimental field trial)

Parameters	Initial Analysis		After two month soil analysis	
	CS	WS	CS	WS
Temperature (^{O}C)	25.5	25.5	25	25.5
pH	7.25	8.34	8.47	8.37
Colour	Dark reddish brown	Grayish brown	Dark reddish brown	Grayish brown
Moisture content (%)	47.306	4.63	10.13	3.24
Moisture correction factor	1.473	0.11	1.10	1.03
Soil texture	Sandy loam	Sandy	Sandy loam	Sandy
Organic Carbon (%)	34.72	27.76	37.67	18.02
Chlorides (mg/Kg)	42.6	113.6	28.4	213.0
Conductivity µmho/m	$0.192 X10^6$	$1.472X10^6$	$0.128 X10^6$	$0.448 X10^6$
Na (mg/Kg)	6	14	7	5.2
K (mg/Kg)	34	410	10	280
Ca (mg/Kg)	820	60	700	40
$CaCO_3$ (%)	55.2	117.66	22	107.12

6. Comparative study of metal phytoremediation through *T. erecta*

The results of metal analysis Cu, Zn, Cr, Ni, Fe and Mn in the waste soil are given in **Table 3.** The results showed that, highest concentration of heavy metals was recorded in initial waste soil Cu (3.650 mg/g), Zn (14.324 mg/g), Cr (2.090 mg/g) and Ni (0.970 mg/g) than the control. These all percentage conc. of metals were Cu (72-73 %), Zn (87-88 %), Cr (89-90 %) and Ni (87 %) showed higher in WS as compared to CS. However, concentration of Fe (20.72 mg/g) i.e. 9 % and Mn (3.604 mg/g) i.e. 25-26 % lower in WS compared to CS. The *T. erecta* grown in this soil during the 2 months period these plants have ability to accumulate more amounts of metals from waste dump soil.

Absorptive uptake of metals from soil through *T. erecta*

T. erecta was a chosen as a more effective plant regarding as a phytoremediation or heavy metal absorption **(Table 3)**. Data showed that this plant have a ability to absorb, stabilize, volatilize and extract Cu (90-95 %), Zn (70-72 %), Cr (74-75 %) and Ni (43-44 %) form WS. This data showed that the potential of plant for remediation it hyperaccumulate Cu > Cr > Zn from waste soil. With increasing pollutants load in dump soil, where *T. erecta* plant can grow, there is a greater tendency for their bio- accumulation. *T. erecta* has a capacity to hyperaccumulate Cu ion form WS. Due to higher amount of Cu in waste soil it significantly involve in growth of plant, due to this, number of branches, leaflet, length and breadth of leaves increases many times than control soil growing plants. Peralta *et al*. (2001) [16] reported that Cu is an important constituent of several proteins and enzymes involved in photosynthesis and respiration. Hall and Williams (2003) [9] reported that Cu is an integral component of certain electron transfer proteins in photosynthesis (e.g. plastocyanin) and respiration (e.g. Cytochrome C oxidase) it significant role in growth.

Table 3: Comparative study of metal phytoremediation through *T. erecta*

Sr. No.	Type of Soil	Metals Ions mg/ g					
		Cu^{2+}	Zn^{2+}	Cr^{4+}	Ni^{2+}	Fe^{2+}	Mn^{4+}
1.	Initial control soil (I-CS)	1.003	1.804	0.228	0.125	22.59	4.517
2.	Plant absorbs metals in control soil (60 days) (PAM-CS)	0.360	1.601	0.095	0.097	8.482	0.375
3.	Total metals remains in control soil (TMR-CS)	0.690	4.140	0.479	0.007	23.25	4.814
4.	Initial waste soil (I-WS)	3.650	14.324	2.090	0.970	20.72	3.604
5.	Plant absorbs metals in waste soil (60 days) (PAM-WS)	0.346	1.550	0.079	0.233	5.707	0.382
6.	Total metals remains in waste soil (TMR-WS)	0.290	4.075	0.523	0.547	22.56	2.92

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