



Journal of Environmental Science and Technology

ISSN 1994-7887

science
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Determination of Heavy Metal Removal Efficiency of *Chrysopogon zizanioides* (Vetiver) using Textile Wastewater Contaminated Soil

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ABSTRACT

A pot culture study was conducted using textile wastewater contaminated soil which was amended with Vermicompost (VC) in various proportions for a period of two months. The plant used for the study was *Chrysopogon zizanioides* (Vetiver) to investigate the accumulation of heavy metals in their roots. Physico-chemical parameters like pH, EC, TKN, P, K, TOC and metals like Pb, Cd and Cu and microbial population of the textile wastewater contaminated soil were analyzed initially (0 day) and finally (60th day). The growth parameters of vetiver like root length, shoot length, fresh weight and dry weight were also recorded initially and finally. Based on the data *C. zizanioides* (Vetiver) tolerated and accumulated the greatest amount of heavy metals. *C. zizanioides* could uptake more lead than the other metals. The effect of vermicompost on the growth of *C. zizanioides* showed that the biomass was increased when the vermicompost concentration was increased. The microbial population like bacteria, actinomycetes and fungi was more in the rhizosphere soil than in non-rhizosphere soil.

Key words: Accumulator, heavy metals, vermicompost, rhizosphere soil, phytoremediation

INTRODUCTION

Tirupur, hosiery capital of India, discharges large quantity of wastewater from dyeing and bleaching units. On the industrial front with over 700 industries the contribution of the industrial discharges in Tirupur is significant. Most of the dyeing and bleaching units located within the city limits discharge their effluents without any treatment either into the Noyyal River or into the agricultural lands in the vicinity of these industries. About 75,000 m³ of effluent is discharged per day (Rajaguru, 1997). In the last decade, metal contamination in urban continental aquatic system has been a growing concern (Sekabira *et al.*, 2010; Mohiuddin *et al.*, 2010). Dyeing industries in Tirupur use numerous synthetic dyes and dye intermediate chemicals such as caustic soda, soda ash, hydrochloric acid, heavy metals, sulphuric acid, peroxides, hypo chlorites etc. Urbanization impacts on metal contamination concern watersheds inhabiting more than 50% of the world population (Meybeck, 2003).

The study of river sediment is a valuable method of studying environmental pollution with heavy metals. Rivers play major roles to the community especially in the fishing industry and source of water supply for people residing within the vicinity of the area. River contamination either directly or indirectly will affect humans as a final consumer. Although some of heavy metals are required as micronutrients, it can be toxic when present higher than the minimum

requirements (Bastami *et al.*, 2011). These chemicals may destroy the soil microflora and fauna in which the existence of man depends. Impacts of anthropogenic activities on metal contamination in a watershed area far from being insignificant (Igbinosa and Okoh, 2009). Attempts made to remediate the contaminated soil were inconclusive.

Heavy metal-tolerant plant species are used for removing heavy metals from the soil and translocating them to the above ground biomass which is subsequently harvested and utilized (Roongtanakiat and Chairaj, 2001). Phytoremediation is an emerging technology, environmental friendly method for large-scale cleanup of contaminated water and soil (Brooks, 1998; Chaudry *et al.*, 1998). It is important to select an appropriate pioneer plant species for successful site reclamation and in phytoremediation efforts to ensure a self-sustainable vegetative cover. Shu *et al.* (2002) showed successful establishment and colonization of vetiver grass as pioneering plant species on Pb/Zn mine soils in China and concluded that this plant should be considered as one of the plants to be used for mine site revegetation. Vetiver (*Chrysopogon zizanioides*) can grow normally in water and is powerful to remove nitrogen and phosphorous from water and, therefore, is a good plant for purifying eutrophic water; (Xia and Shu, 2001) and garbage leachates (Xia *et al.*, 1999). Here the phytoremedial efficiency of vetiver in textile wastewater contaminated soil was evaluated. Since the vetiver grass (*C. zizanioides*) is a versatile plant capable of growing in different conditions, it was attempted for the first time to find out its role in remediation. The objective of the present study was to find out the efficiency of vetiver (*C. zizanioides*) in removal of heavy metals from the textile wastewater contaminated soil and to find an effective method to alleviate heavy metal contamination in the environment.

MATERIALS AND METHODS

Study area: Textile wastewater contaminated soil was collected from Orathupalayam area near Tirupur in Coimbatore District, during 2006-2007. The soil was used for pot culture study with the combination of Vermicompost (VC). The vermicompost was collected from the vermicompost park in the Department of Environmental Sciences, Bharathiar University campus.

Pot culture study: The pot culture studies were carried out in the green house for 60 days. The pots were filled with 5 kg of Orathupalayam field Soil (OS) and Vermicompost (VC) at different ratios. The culms of *C. zizanioides* (vetiver grass) with root (10 cm) and shoot (10 cm) were selected for the pot culture study. Two healthy culms were planted in each pot. Each treatment was made in triplicate (Table 1).

Physico-chemical analysis: The physico-chemical parameters of the soil samples were analysed on initial and final day of the pot culture study. The pH and EC of the soil samples were recorded

Table 1: Pot culture study using textile wastewater contaminated soil (Orathupalayam field soil)

Treatments	Combinations	Ratio
ST1	Control (OS) + <i>C. zizanioides</i>	1:0.0
SR2	OS+VC+ <i>C. zizanioides</i>	1:0.1
ST3	OS+VC+ <i>C. zizanioides</i>	1:0.2
ST4	OS+VC+ <i>C. zizanioides</i>	1:0.3
ST5	OS+VC+ <i>C. zizanioides</i>	1:0.4
ST6	OS+VC+ <i>C. zizanioides</i>	1:0.5

by ELCO L1 163 pH meter and ELCO CM-183 EC meter (Jackson, 1973). Total Kjeldhal Nitrogen was estimated on initial and final day using Kjeldahl Nitrogen Analyser Model:ZDDN-II. Similarly phosphorous and potassium also estimated by Flame photometric method using ELCO CL-22D (Tandon, 1993). Organic Carbon (OC) (Walkley and Black, 1934) was checked on 0th day and 60th day of the study. Heavy metals like Cadmium (Cd), Lead (Pb), Zinc (Zn), Copper (Cu), Iron (Fe) and Manganese (Mn) were analyzed. Heavy metals concentration in the soil was determined initially and finally by an Atomic absorption spectrophotometer (AAS, Perkin Elmer atomic absorption spectrophotometers (model 370)) following digestion with the concentrated nitric acid and 30% H₂O₂ (APHA, 1995).

Characterization of *C. zizanioides*: The growth parameters like Root Length (RL), Shoot Length (SL), Fresh Weight (FW), Dry Weight (DW), total chlorophyll were analyzed on 0th day and on 60th day.

Root length and shoot length: The root length and shoot length of *C. zizanioides* were measured on 0th day and 60th day and mean values were expressed in cm.

Fresh weight and total dry weight: The fresh weight and dry weight of the whole plant was taken on 0th day and 60th day. After the termination of the experiment on 60th day, the plants were kept in a hot air oven at 60°C for 48 h and then weighed. The values were expressed in g culm⁻¹.

Total chlorophyll content: On 0th day and 60th day, the chlorophyll content was analyzed in Hitachi UV-VIS Spectrophotometer at 663 and 645 nm.

Estimation of microbial population in textile wastewater treated soil samples: Pour plate technique Allen (1953) was employed to enumerate the bacteria, actinomycetes and fungal population in the Rhizosphere (R) and Non-Rhizosphere (NR) of *C. zizanioides* grown in textile wastewater contaminated soil.

Data analysis: Statistical analysis of data was conducted using SPSS statistical package programme (Ver.10). Data were subjected to homogeneity of variance. A value of p<0.05 was considered to be statistically significant.

RESULTS

C. zizanioides can tolerate wide range of pH, EC, Total Kjeldhal Nitrogen (TKN), phosphorous, potassium and Total Organic Carbon (TOC).

pH: Initial pH was found to be 8.6 and final was 7.8 in ST1. In all the other treatments also the pH was reduced during the growth of the plant. The minimum reduction was noticed in ST2 and ST3 whereas moderate levels of reduction of pH were observed in ST6. In ST4 and ST5, the pH was reduced from 8.6 to 7.8 in Table 2. Statistical analysis of pH showed that there is reduction in pH and significant at 0.05 level.

Electrical conductivity: Electrical Conductivity (EC) in ST6 was reduced from 1.34 to 0.22 in ST6. The moderate levels of decrease in ST1 to ST5 were recorded except ST4. In ST4, it was 1.31

Table 2: pH, EC of textile wastewater contaminated soil with *C. zizanioides*

Treatments	pH		EC (dS m ⁻¹)	
	Initial	Final	Initial	Final
ST1	8.6±0.2	7.8±0.1	1.45±0.2	0.39±0.1
ST2	8.2±0.1	7.9±0.2	1.36±0.1	0.32±0.2
ST3	8.1±0.1	7.8±0.2	1.35±0.1	0.32±0.1
ST4	8.3±0.2	7.8±0.1	1.31±0.1	0.22±0.1
ST5	8.3±0.1	7.8±0.2	1.35±0.1	0.28±0.1
ST6	8.2±0.2	7.8±0.1	1.34±0.2	0.22±0.1

Values given in the table are Mean±SD

Table 3: N, P, K and OC of textile wastewater contaminated soil treated with *C. zizanioides*

Treatments	N (%)		P (%)		K (%)		OC (g kg ⁻¹)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
ST1	8.76±1	1.26±0.1	4.8±2	1.01±0.2	3.4±1	0.18±0.01	7.6±1	3.1±2
ST2	8.81±2	1.14±0.2	5.6±1	1.02±0.1	3.2±2	0.19±0.02	7.6±1	3.0±2
ST3	8.31±2	0.83±0.1	5.4±1	0.96±0.1	3.1±1	0.18±0.01	8.3±2	2.0±1
ST4	8.54±1	0.68±0.1	5.7±2	1.02±0.2	3.3±2	0.17±0.01	8.2±2	2.4±2
ST5	8.84±1	0.53±0.2	5.8±1	0.84±0.2	3.2±1	0.16±0.02	8.8±1	1.8±1
ST6	8.85±2	0.53±0.2	5.9±2	0.81±0.1	3.4±1	0.2±0.02	9.4±1	1.6±2

Values given in the table are Mean±SD

initially and 0.22 on the final day (Table 2). Statistical analysis for EC of textile wastewater treated soil is significant at 0.05 level.

TKN (Total Kjeldahl Nitrogen): Initial value of Nitrogen was 8.85% in ST6 and it was reduced to 0.53% on the final day. There was not much difference found between ST6 and in ST5 (Table 3). Analysis of variance showed that there is a reduction in the nitrogen on the final day. It is highly significant at 0.05 levels.

Phosphorus and potassium: In the same way phosphorous was also found to be higher in ST6 (5.9%) initially and 0.81 % finally. The percentage of potassium was also drastically reduced from 3.4 to 0.18% in ST1. In ST6, the percentage of potassium was reduced from 3.4 to 0.2% in ST6. The TOC on 60th day was 1.6% in ST6. It was reduced from 8.8 to 1.8% in ST5, whereas in ST3 and ST4 it was from 8.3 to 2% and 8.2 to 2.4%, respectively. Statistical analysis for nitrogen, phosphorus and potassium of textile wastewater treated soil was significant at 0.05 levels (Table 3).

Metals: Metals like Pb, Cd, Cu, Fe, Zn, Mn, were also reduced on 60th day in all the treatments. The metal uptake by the plant was prominent in ST6 (Table 4). The uptake of Fe by the plant was prominent in ST6, followed by, Mn, Zn, Pb, Cu and Cd. The comparison was made in all the treatments and it was observed that Pb has been absorbed by the plant more in ST6 (1.30±0.01 and 0.28±0.02) followed by ST5, ST4, ST2, ST1 and ST3. The Cd uptake was very prominent in ST2. It was reduced from 0.339±0.02 to 0.041±0.01. In ST6, it was 0.221±0.01 initially and reduced to 0.029±0.02 followed by ST5 (from 0.229±0.02 to 0.032±0.02). The absorption of Cu was more in ST6 (0.69±0.02 to 0.01±0.02) followed by ST5. In ST4 and ST3, there was not much variation in the

Table 4: Heavy metals content (ppm/kg⁻¹) in textile wastewater contaminated soil treated with *C.zizanioides*

Treatment	Pb		Cd		Cu		Zn		Fe		Mn	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
ST1	1.57±0.01	0.55±0.02	0.244±0.01	0.044±0.02	0.78±0.02	0.07±0.01	1.10±0.02 ^d	0.63±0.01 ^d	6.66±0.02 ^d	3.01±0.02 ^d	2.64±0.01 ^c	0.44±0.02 ^c
ST2	1.55±0.02	0.53±0.01	0.339±0.02	0.041±0.01	0.76±0.01	0.05±0.02	1.08±0.01 ^c	0.45±0.01 ^c	6.62±0.02 ^c	2.84±0.02 ^c	2.34±0.01 ^c	2.34±0.02 ^c
ST3	1.49±0.01	0.48±0.02	0.233±0.01	0.039±0.02	0.74±0.02	0.04±0.02	1.05±0.02 ^b	0.33±0.01 ^b	6.55±0.02 ^c	2.62±0.01 ^c	2.32±0.02 ^c	0.32±0.01 ^c
ST4	1.42±0.02	0.40±0.02	0.231±0.01	0.035±0.01	0.73±0.02	0.04±0.01	1.04±0.02 ^b	0.32±0.01 ^b	6.49±0.02 ^b	2.35±0.02 ^b	2.29±0.02 ^c	0.25±0.02 ^c
ST5	1.36±0.02	0.32±0.01	0.229±0.02	0.032±0.02	0.69±0.01	0.03±0.02	1.03±0.01 ^a	0.29±0.02 ^a	6.38±0.02 ^b	2.28±0.01 ^b	2.27±0.02 ^b	0.14±0.01 ^b
ST6	1.30±0.01	0.28±0.02	0.221±0.01	0.029±0.02	0.69±0.02	0.01±0.02	1.02±0.02 ^a	0.28±0.01 ^a	6.41±0.02 ^a	2.21±0.02 ^a	2.29±0.02 ^a	0.12±0.02 ^a

Values given in the table are Mean±SD. Values with different letter(s) are significantly different (p<0.05) within the same column

Table 5: Morphological parameters of *C.zizanioides* grown in different treatments (textile wastewater contaminated soil and VC)

Treatments	RL (cm)		SL (cm)		FW (g)		DW (g)		Total chlorophyll (mg g ⁻¹)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
ST1	10±0.4	18.2±0.2	10±0.1	43.0±0.2	6.2±0.1	180.0±0.1	2.1±0.1	58.10±0.1	0.123±0.1	0.197±0.1
ST2	10±0.2	42.0±0.2	10±0.2	51.1±0.1	6.2±0.2	195.2±0.1	2.0±0.2	65.10±0.1	0.216±0.2	0.297±0.1
ST3	10±0.1	48.2±0.1	10±0.4	55.2±0.1	6.2±0.1	210.3±0.1	2.1±0.2	68.66±0.1	0.262±0.1	0.410±0.1
ST4	10±0.2	51.3±0.2	10±0.3	58.1±0.2	6.2±0.1	252.5±0.2	2.3±0.1	82.80±0.1	0.257±0.2	0.624±0.2
ST5	10±0.1	69.4±0.1	10±0.1	59.7±0.1	6.2±0.1	260.0±0.2	2.2±0.2	88.14±0.2	0.272±0.3	0.871±0.2
ST6	10±0.3	75.2±0.1	10±0.4	65.3±0.2	6.2±0.2	273.1±0.1	2.1±0.2	92.58±0.2	0.269±0.1	0.981±0.2

Values given in the table are Mean±SD

absorption of Cu. In ST1 also the absorption of Cu was prominent (0.78±0.02 to 0.07±0.01). The uptake of Zn, Fe and Mn were more in T6 compared to other treatments in Table 4. The metal uptake by the plant was prominent in ST6 and significant at 0.05 level.

Growth parameters: The growth parameters like root length, shoot length, fresh weight, dry weight and total chlorophyll content of *C. zizanioides* were recorded on 0th day and 60th day. There was an increase in all the growth parameters in ST6. There was an increase in root and shoot length until harvest in all the treatments. However, the growth of *C. zizanioides* was prominently greater in ST6 and ST5. The Fresh Weight (FW) of the plant in ST6 (273.1±0.1 g) on the final day was higher than in ST1 (180.0±0.1 g). The dry weight was also increased considerably. It was 58.1±0.1 g in ST1 and 92.58±0.2 in ST6 (Table 5). The growth parameters were analyzed statistically and it was significant at 0.05 level.

Microbial population: The microbial population was enumerated in all the treatments (ST1-ST6). Bacterial, fungal and actinomycetes populations in rhizosphere were found to be more than the non-rhizosphere of all the treatments. It was recorded that bacteria was 121.5×10⁵ cfu g⁻¹, fungi was 16×10⁴ cfu g⁻¹ and actinomycetes was 9×10⁴ cfu g⁻¹ on the 60th day rhizosphere soil in ST6. There was a prominent increase in rhizosphere population than non-rhizosphere in ST5 also. It was 78×10⁵ cfu g⁻¹ initially and 108×10⁵ cfu g⁻¹ finally. Similarly fungal and actinomycetes population were found moderate in ST5 compared to ST6. In all the other treatments a gradual increase of microbial population was noticed (Table 6). The microbial population in all the treatments in the rhizosphere soil was analyzed and it was significant at 0.05 level.

Table 6: Microbial populations in Rhizosphere and Non – Rhizosphere soil taken from different treatments

Treat-ment	Bacteria ($\times 10^5$ cfu g^{-1})				Fungi ($\times 10^4$ cfu g^{-1})				Actinomycetes ($\times 10^4$ cfu g^{-1})			
	R		NR		R		NR		R		NR	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
ST1	15.5	22.5	14	22.0	5	6.0	3	5	3	6	2	2
ST2	22.5	57.5	22	40.0	7	9.0	5	7	3	5	5	5
ST3	40.5	73.0	33	56.5	8.5	10.0	2	4	2	5	5	3
ST4	57.5	91.5	41	61.0	9.5	11.5	2	6	7	4	4	2
ST5	78.0	108.0	53	98.5	10	13.0	3	7	7	4	6	2
ST6	86.0	121.5	67	109.0	13	16.0	6	9	5	9	2	4

R: Rhizosphere and NR: Non-Rhizosphere

DISCUSSION

Most of the districts in Tamil Nadu are experiencing severe environmental problems due to rapid industrialization. This phenomenon is very common where the polluting industries like textile dyeing, leather tanning, paper and processing, sugar manufacturing etc. thrive as clusters. The effluent discharged by these industries leads to serious problems like pollution of surface water sources, ground water and soil and ultimately affects the livelihood of the poor. Some researchers supported that the discharged effluents are known to generate a considerable amount of metal to the environment through various pathways including atmospheric particles (Zhu *et al.*, 2009), urban runoff (Igwe *et al.*, 2008), industrial and wastewater effluents (Nwuche and Ugoji, 2008). In general, amendments such as application of organic manure or inorganic fertilizer are necessary for establishment of plants on contaminated or metal accumulated soil (Yang *et al.*, 2003; Roongtanakiat *et al.*, 2008; Johnson *et al.*, 2009).

In the present study it was observed that physico-chemical condition of the soil samples collected from Tirupur textile water contaminated site revealed a high load of pollution indicators.

The initial pH was slightly alkaline and EC was slightly high than the normal soil by the addition of chemicals and the mixture of dyes used in textile industries. The values of pH and EC decreased in all the treatments from initial to final day of the experiment when treated with *C. zizanioides* and vermicompost. This may be due to the higher organic decomposition rate resulted in CO₂ and acid production which finally lowered the pH of the soil and EC as the mineralization of free ions present in the compost and soil.

The heavy metals can be extracted by phytoextraction. As *C. zizanioides* (vetiver plant) has been found to be highly tolerant to extreme soil condition including heavy metal contamination, the experiment was conducted to check the reduction level of all contaminants of textile wastewater treated soil on the plant's ability to tolerate toxic levels of Manganese (Mn), Zinc (Zn), Copper (Cu) and Lead (Pb) and on the ability to accumulate these heavy metals in roots and shoots. Vetiver grass can tolerate extreme soil conditions including elevated levels of heavy metals (Zheng *et al.*, 1998; Khan, 2001). It can grow in all types of soil regardless of pH or salinity. It tolerates wide range of pH, salinity, sodicity, acidity and heavy metals such as Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb) and Zinc (Zn) (Xia and Shu, 2001).

It was commonly known that vetiver could absorb higher nitrogen, phosphate and potassium. The NPK levels in the textile wastewater contaminated soil when amended with vermicompost were increased than those of control soil. It was found that vetiver could grow well in the treatments of

ST2-ST6, due to the supplementation of vermicompost. According to Karmegam *et al.* (1997), the application of organic manures increased the NPK contents of the soil. Sharma *et al.* (1988) observed that farmyard manure registered higher organic carbon and available phosphorous content in the soil. All the above findings substantiate our present study very well.

The present study demonstrated that vermicompost when added to textile wastewater contaminated soil, greatly enhanced the growth of *C. zizanioides*. The increase of growth parameters like root length, shoot length and chlorophyll content was noticed. Growth stimulation was more pronounced with vermicompost supplement by root initiation, root elongation, root biomass and rooting percentage. Vermicompost loosen the contaminated soil and make the soil texture suitable for the root penetration. The results showed that *C. zizanioides* had better growth performance (RL, SL, FW, DW and total chlorophyll content) in textile wastewater contaminated soil even though the heavy metals like Pb, Cd, Zn and Cu were present. Similar study was carried out by Roongtanakiat and Chairroj (2001) and he observed that the concentration of heavy metals in shoots increased as the amount of the applied heavy metals increased. Increasing the amount of heavy metals applied to soils did not affect the growth and dry weight of the vetiver grass. Yang *et al.* (2003) and Roongtanakiat *et al.* (2007) proved that heavy metals in soil even at the higher-level growth have no negative effect on *C. zizanioides* growth but the roots accumulated higher concentration than shoots. The finding in the present study was supported by the above mentioned statements.

Microbial population and organic humus content were found to be higher in treated soil than the control soil due to the VC treatment. This finding was supported by Kale *et al.* (1991), that the earthworm casts contain maximum microbial population. The activity of microbial population was higher in rhizosphere of vetiver grass than non-rhizosphere. This observation was also noted by Siripin *et al.* (2000). Moreover soil microbes and their activities have the important role in transformation on plant nutrients to available form and also have many metabolisms related to soil fertility improvement. Microorganisms often invade the surface tissue of roots, where they may cause a number plant link for nutrient transport between the plant and the soil, while the roots excrete soluble organic carbon compound, "polysaccharide" for soil microbial metabolism and adaptation. The soil microorganisms associated with vetiver root are nitrogen fixing bacteria, phosphate-solubilizing microbes, mycorrhizal fungi and cellulolytic microorganisms (Siripin *et al.*, 2000). The substances in the exudates of vetiver root served as nutrients and energy sources for the growth of microorganisms in the rhizosphere (Lynch, 1990). Their findings supported the results of the present work. Cultivation of *C.zizanioides* in the contaminated soils is a new and innovative phytoremedial technology and it has potential to meet all the criteria.

CONCLUSION

The textile waste water contaminated land can be pre-treated with vermicompost which makes the soil loose textured for easy root penetration. Since it is rich in soil nutrients and microbes it enhances the growth of *C.zizanioides*. The use of this plant is to improve the soil quality, create an authentic land examination, restoring the ecosystem and to gain the economic benefits from biomass which could be used for making handicraft, biofuel etc. Application of the *C. zizanioides* (vetiver grass) for contaminated soils is a new and innovative phytoremedial technology for the removal of heavy metals and it has the potential to meet all the criteria.

ACKNOWLEDGMENT

The authors are thankful to the Bharathiar University and the Department of Environmental Sciences for providing necessary laboratory facilities. We gratefully acknowledge the CARISM Department, SASTRA University, TamilNadu, for helping us to analyze the samples for heavy metals. We are thankful to Nehru Arts and Science College for moral support and encouragement.

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