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Utilization of Vetiver Grass (*Vetiveria zizanioides*) for Removal of Heavy Metals from Industrial Wastewaters

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ABSTRACT: The experiment was conducted to investigate the ability of vetiver grass (*Vetiveria zizanioides*) to uptake heavy metals from industrial wastewater. Three vetiver ecotypes, Kamphaeng Phet-2, Sri Lanka and Surat Thani, were hydroponically cultured in four samples of industrial wastewater taken from a milk factory (W1), a battery manufacturing plant (W2), an electric lamp plant (W3) and an ink manufacturing facility (W4). The results indicated that the vetiver could grow well in these wastewater sources; however, the concentration of heavy metals in wastewater played an important role in vetiver growth. The vetiver grown in W1 had the best growth, while the vetiver grown in W4, highly contaminated with Mn, Fe and Cu, had the worst growth. The three vetiver ecotypes absorbed Fe>Mn>Zn>Cu>Pb, and they concentrated these metals more in roots than in shoots. The total uptake of Fe and Zn were highest in vetiver grown in W1, while the highest total uptake of Mn and Cu occurred in vetiver grown in W4. Also the vetiver grown in W2, with the highest concentration of Pb, had the highest total Pb uptake. The vetiver grown in W1 had the highest Mn, Fe, Zn and Pb removal efficiencies of 33.72, 27.63, 52.73 and 8.94 %, respectively, whereas, the vetiver grown in W4, had an efficiency of up to 87.5% Cu removal from the wastewater, although it showed a symptom of Cu toxicity on root growth. Among the three vetiver ecotypes, the Sri Lanka had the best growth and the highest heavy metal removal efficiencies.

KEYWORDS: Vetiver, Wastewater, Heavy metal, Phytoremediation.

INTRODUCTION

Thailand has been recognised as an agricultural country with rice, agricultural products and processed agricultural products being the major exports. However, the country has seen rapid industrialization since the late 1980s and this has been a key factor for the economic and social progress that have eventuated. Of late, industry has become Thailand's main source of GDP[1].

Water is a very important commodity for industry and it is difficult to imagine any type of industry in which water is not used. Water is required as a direct raw material and as an ingredient of the product itself. In other cases, water is used as an indirect raw material, in washing, heating, cooling or as a part of the manufacturing process.

The World Indicators Report (WIR) indicated that water for industrial use represents approximately 22 % of global fresh water use[1]. In 1995, the water volume used by industry was 752 litres/year and was estimated to be 1170 litres/year in 2025[2]. Despite industries requiring good quality water for manufacture, the wastewater produced from industry is usually contaminated with nutrients and heavy metals such as Pb, Fe, Mn, Cu and Zn. As these pollutants are toxic to humans and have impact on the environment, treatment before discharging to a natural or storage water body is essential. A study in 1989 showed that 18 major rivers in Thailand were contaminated with heavy metals and a subsequent study in 1994 found that 25 of the total 43 rivers in Thailand were contaminated[3]. According to preliminary estimates, water pollution is estimated to cost between 0.6 and 1% of GDP, annually[4].

Efficient wastewater treatment can be achieved in several ways, physical, chemical and biological. Phytoremediation is one of the biological wastewater treatment methods which is simple, environmentally friendly and consumes less energy. Suitable plant species used for phytoremediation should have high uptake of both organic and inorganic pollutants, grow well in polluted water and be easily controlled in quantitatively propagated dispersion. Vetiver grass (*Vetiveria zizanioides* recently reclassified as *Chrysopogon zizanioides*) belongs to the gramineae family and was first used for soil and water conservation purposes. Due to its unique morphological and physiological characteristics, and tolerance to high levels of heavy metal and adverse conditions, vetiver has also been successfully used in the field of environmental protection[5]. It is excellent for the removal of heavy metals from contaminated soil[6,7] and rehabilitating landfills[8]. Even though it is not an aquatic plant, vetiver can be established and survive under hydroponic conditions[9]. It can purify eutrophic water[10], garbage leachates[9] and wastewater from pig farms[11]. Therefore, vetiver has high potential to be used for industrial wastewater treatment. This experiment was conducted to compare the growth of three vetiver ecotypes grown in different sources of industrial wastewater. The amount of major plant nutrients and heavy metals absorbed by vetiver grass are also investigated.

Materials and Methods

The experiment was conducted in the greenhouse at the Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University. Three vetiver ecotypes, Kamphaeng Phet-2, Sri Lanka and Surat Thani, were hydroponically cultured in four sources of industrial wastewater. The water was collected from a milk factory (W1), a battery manufacturing plant (W2), an electric lamp plant (W3) and an ink manufacturing facility (W4). The 3 x 4 factorial experiment in Completely Randomized Design with 3 replications was performed. The pH values of the four wastewater sources were adjusted to 7 before using them for vertiver cultivation. Twenty-centimeter tall vetiver seedlings from tissue culture propagation were used. One seedling was planted in a plastic cup filled with sand. The bottom of each cup was streaky cut across to allow wastewater to soak the sand. The five cups were fitted on foam sheet floating in each bucket. The plants were pruned to 15 cm high before cultivation which lasted for 120 days. During cultivation, weeds were thoroughly removed and the solution volume in the buckets was maintained at 20 liters by weekly the addition of distilled water.

Plant height was measured at 30, 60, 90 and 120 days. After harvesting, shoot and root were separated and thoroughly rinsed for dry matter measurement and analysis of major plant nutrients and heavy metals. Total nitrogen and phosphorus were analyzed using Kjeldahl and colorimetric methods, respectively, while atomic absorption method[12] was used to analyze potassium, manganese, iron, zinc, copper and lead.

Before the experiment, the industrial wastewater was analyzed for biological oxygen demand (BOD) by dilution method and chemical oxygen demand (COD) by the closed reflux method. Water hardness was determined by titration method with ethylenediamine tetraacetic acid (EDTA). The wastewater samples were evaporated at 103-105°C for one hour to measure the total dissolved solids (TDS). Suspended solids (SS) were measured by filtration through glass fibre paper[13]. For qualitative and quantitative analysis of heavy metals in industrial wastewater, a non-destructive and multi-element analysis technique was performed by energy dispersive X-ray fluorescence spectroscopy. In addition, the atomic absorption spectroscopy was used to analyze low concentration of heavy metals which were beyond the detectable limits of X-ray florescence spectroscopy.

Results and Discussion

Industrial Wastewater Quality before Vetiver Culture

The pH values of the wastewaters used in this experiment were within the limits of industrial effluent standards, except W2, which had a pH of 1.35 (Table 1). The contents of BOD, COD and SS in the four samples of wastewater were very much higher than the industrial effluent standards, especially W4 which had the highest content of BOD, COD and SS probably due to the sediment from printing ink production facility. The hardness of the W4 sample could not be determined by titration method with EDTA because of its dark blue color, even though it had been diluted 200 times. Of major plant nutrient elements (nitrogen, phosphorous and potassium), the W4 sample had nitrogen content

Table 1. Pre-experimentation properties and major plant nutrient contents of the four sources of industrial wastewater, in comparison to the industrial effluent standards[14].

Wastewater source[1]	Properties of industrial wastewater						Nutrient content		
	pH	BOD	COD	Hardiness	TDS	SS	N	P	K
		(mg L^{-1})							
W1	6.58	960	3050	168	118	1018	68.0	9.3	37.3
W2	1.35	710	2448	116	473	636	30.7	5.3	25.0
W3	5.97	650	2897	92	258	612	39.8	96.4	20.3
W4	8.13	1170	29154	*	343	2045	453.0	4.0	25.6
Industrial effluent std.	5.5–9.0	≤ 20	≤ 120	-	≤ 3000	≤ 50	≤ 100	-	-

[1] W1 = Milk factory
W2 = Battery manufacturing plant
W3 = Electric lamp plant
W4 = Ink manufacturing facility

* = Not available
- = Not indicated

above the industrial effluent standard. The W3 sample, however, had the highest phosphorous content among the four studied sources while the four sources had potassium content in the range of 20.3-37.3 mg L^{-1}.

The wastewater from W1 had all the investigated heavy metal concentrations within the limit of the industrial effluent standards (Table 2). Both Zn and Pb are usually used in electronics production. It could be seen that the highest concentration of Pb (11.11 mg L^{-1}) was in the wastewater from W2. Besides Pb pollutant, the W3 wastewater had a very high Zn concentration of 43.69 mg L^{-1}. The W4 sample contained the highest Cu concentration due to the presence of Cu in the ink pigment. It also had the highest Mn and Fe concentrations of 8.34 and 126.39 mg L^{-1}, respectively.

It is obvious that all the industrial wastewater samples used in this study were contaminated with both organic and inorganic pollutants, especially heavy metals. These wastewaters definitely require treatment before discharging them into the waterways.

Growth of Vetiver Cultivated in Industrial Wastewater

The average heights of the three vetiver ecotypes at 30, 60, 90 and 120 days after growing them in the four sources of industrial wastewater are shown in Fig. 1. At 30 days after planting, the height of Kamphaeng Phet-2 ecotype was significantly higher than those of the Sri Lanka and the Surat Thani ecotypes. In the middle stage (60, 90 days) of the growth period, the Sri Lanka ecotype grew better and gave the highest plant height. At harvest (120 days), the Sri Lanka and Kamphaeng Phet-2 ecotypes were significantly taller than the Surat Thani. As expected, vetiver grown in W1 gave the highest plant height and were significantly higher than those grown in other tested wastewater sources (Table 3).

All three vetiver ecotypes had higher shoot dry weight compared to the root dry weight (Table 4). The shoot, root and total dry weights of the Sri Lanka ecotype were highest overall. The vetiver grown in the four tested wastewater, W1, W2, W3 and W4, had the total dry weights of 45.19, 12.73, 19.29 and 11.73 g, respectively. This could indicate that heavy metal played an important role in attenuating vetiver growth. The vetiver grown in W1 had the best growth (Figure 2) due to less content of heavy metals, while the worst growth was found in W4 which was not only badly contaminated with Mn, Fe and Cu but also had a very high COD.

Heavy Metal Concentration in Shoots and Roots of Vetiver

The concentrations of heavy metals in shoots and roots of vetiver are shown in Figure 2. Among all studied wastewater sources, the vetiver grown in W1 had the lowest heavy metal concentrations in shoots and roots.

Table 2. Heavy metal concentration in industrial wastewater from the milk factory (W1), battery manufacturing plant (W2), electric lamp plant (W3) and ink manufacturing facility (W4), in comparison to the industrial effluent standards (Pollution Control Department, 2003).

Wastewater source	Heavy metal concentration ± SE (mg L^{-1})				
	Mn	Fe	Cu	Zn	Pb
W1	0.49 ± 0.02*	16.15 ± 2.64	0.06 ± 0.01*	4.09 ± 0.14	0.05 ± 0.01*
W2	0.16 ± 0.01*	13.81 ± 1.66	1.24 ± 0.11	10.41 ± 0.56	11.11 ± 0.32
W3	3.54 ± 2.31	18.84 ± 4.00	0.05 ± 0.01*	43.69 ± 1.83	6.83 ± 1.40
W4	8.34 ± 3.68	126.39 ± 5.35	118.92 ± 4.03	0.23 ± 0.01*	0.70 ± 0.53
Industrial effluent std.	≤ 5.0	-	≤ 2.0	≤ 5.0	≤ 0.2

* By atomic absorption analysis   - = Not indicated

Table 3. Height of three vetiver ecotypes at 120 days after planting in the wastewater sources from milk factory (W1), battery manufacturing plant (W2), electric lamp plant (W3) and ink manufacturing facility (W4).

Wastewater source	Height (cm) of vetiver ecotype[1]			Mean
	Kamphaeng Phet-2	Sri Lanka	Surat Thani	
W 1	109.1 a	102.1 a	87.7 a	99.6 a
W 2	61.4 c	60.8 c	56.5 b	59.6 c
W 3	73.0 b	78.4 b	51.1 b	67.5 b
W 4	64.0 c	64.0 c	38.1 c	54.4 c
Mean[2]	76.3 a	76.3 a	58.4 b	70.3

[1]Figures in the same column with a common letter are not significantly different at 0.05 probability by DMRT.
[2]Figures in the same row with a common letter are not significantly different at 0.05 probability by DMRT.

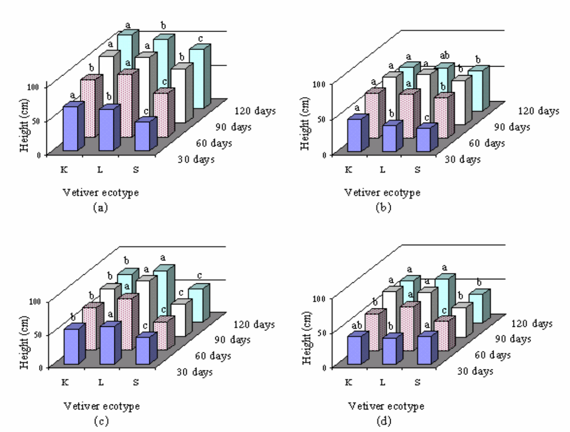

Fig 1. Average heights (cm) of Kamphaeng Phet-2 (K), Sri Lanka (L) and Surat Thani (S) vetiver ecotypes at 30, 60, 90 and 120 days after planting in industrial wastewater from milk factory (a), battery manufacturing plant (b), electric lamp plant (c) and ink manufacturing facility (d) [Histograms in the same row in each graph associated with a common letter are not significantly different at the 0.05 probability by DMRT.]

The vetivers absorbed more heavy metals from the polluted wastewater (W2-W4). The highest Pb concentration in shoots and roots was found in vetiver grown in W2, while the shoots and roots of vetiver grown in W4 had the highest Mn, Fe and Cu concentrations. For Zn, its concentration was highest in vetiver shoots grown in W4 and in the vetiver roots grown in W2. In general, the three vetiver ecotypes absorbed Fe>Mn>Zn>Cu>Pb. The Sri Lanka ecotype could absorb all heavy metals at significantly higher concentrations in both shoots and roots than those of other ecotypes.

All five heavy metals distributed more in vetiver roots than in shoots, especially Fe and Pb as seen from

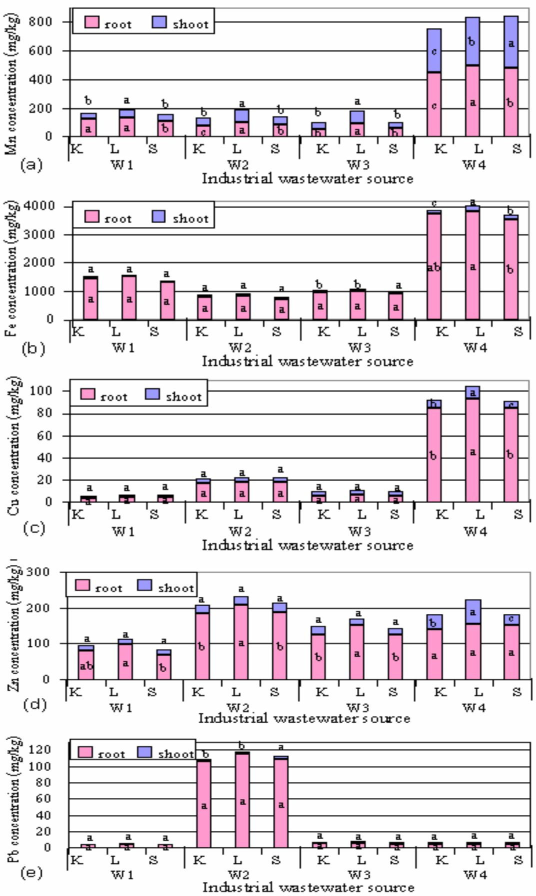

Fig 2. Concentrations of Mn (a), Fe (b), Cu (c), Zn (d) and Pb (e) in shoot and root of Kamphaeng Phet-2 (K), Sri Lanka (L) and Surat Thani (S) vetiver ecotypes grown in industrial wastewater from milk factory (W1), battery manufacturing plant (W2), electric lamp plant (W3) and ink manufacturing facility (W4). [Histograms of the same plant parts and same sources of wastewater with a common letter are not significantly different at the 0.05 probability by DMRT.]

Table 4. Dry weight of Kamphaeng Phet-2 (K) Sri Lanka (L) and Surat Thani (S) vetiver ecotypes grown in industrial wastewater from milk factory (W1), battery manufacturing plant (W2), electric lamp plant (W3) and ink manufacturing facility (W4).

Plant part	Vetiver ecotype	Vetiver dry weight (g) grown in[1]				Mean
		W1	W2	W3	W4	
Shoot	K	30.12 a	7.28 a	12.39 b	7.29 ab	14.27 a
	L	30.21 a	7.29 a	13.09 a	7.51 a	14.53 a
	S	29.17 b	6.95 b	12.00 c	7.13 b	13.81 a
Mean[2]		29.83 a	7.17 c	12.49 b	7.31 c	14.20
Root	K	15.40 ab	5.72 a	6.69 b	4.47 ab	8.07 ab
	L	15.66 a	5.82 a	7.14 a	4.73 a	8.34 a
	S	15.00 b	5.14 b	6.54 c	4.07 b	7.69 b
Mean[2]		15.35 a	5.56 c	6.79 b	4.42 d	8.03
Total	K	45.51 a	12.99 a	19.08 b	11.76 ab	22.34 ab
	L	45.88 a	13.10 a	20.24 a	12.24 a	22.86 a
	S	44.17 b	12.09 a	18.54 b	11.20 b	21.50 b
Mean[2]		45.19 a	12.73 c	19.29 b	11.73 c	22.23

[1]Figures in the same column of each category with a common letter are not significantly different at 0.05 probability by DMRT.
[2]Figures in the same row of each category with a common letter are not significantly different at 0.05 probability by DMRT.

Table 5. Distribution of heavy metals in shoots and roots of vetiver grown in industrial wastewater from milk factory (W1), battery manufacturing plant (W2), electric lamp plant (W3) and ink manufacturing facility (W4).

Heavy metal	Wastewater source	Concentration of heavy metal (mg kg^{-1}) in		
		Shoot	Root	Shoot/Root (%)
Mn	W1	48.12	121.55	39.59
	W2	64.76	88.65	73.05
	W3	58.24	68.73	84.74
	W4	30.26	473.21	69.66
	Average	**125.35**	**188.04**	**66.66**
Fe	W1	62.31	1430.07	4.36
	W2	83.13	791.18	10.51
	W3	64.02	977.36	6.55
	W4	165.75	3688.30	4.49
	Average	**93.80**	**1721.73**	**5.45**
Cu	W1	2.45	4.30	56.98
	W2	4.07	17.95	22.67
	W3	4.23	5.88	71.94
	W4	8.46	87.54	9.66
	Average	**4.80**	**28.92**	**16.61**
Zn	W1	14.27	82.31	17.34
	W2	25.28	192.76	13.11
	W3	18.97	134.76	14.08
	W4	46.58	148.90	31.28
	Average	**26.28**	**139.68**	**18.81**
Pb	W1	0.69	4.50	15.33
	W2	3.76	109.57	3.43
	W3	2.02	5.51	36.66
	W4	2.25	4.88	46.11
	Average	**2.18**	**31.12**	**7.01**

the average concentration ratio of 5.45% and 7.01%, respectively, while Cu and Zn were at moderate levels of 16.61% and 18.81%, respectively (Table 5). For Mn, it was translocated at relatively high level to shoot (66.66%) as an essential nutrient element that is mobile in plant.

Heavy Metal Uptake and Removal Efficiency of Vetiver

The concentration of heavy metal in plant, and total dry weight, are the two factors involving the amount of heavy metal uptake. Since plant growth and heavy metal absorption of the three vetiver ecotypes grown in four industrial wastewater sources were different, the total uptake of studied heavy metals were distinctly different (Figure 3). The best plant growth was found in vetiver plant grown in the W1; whereby, it showed the highest uptake of Fe and Zn. For W2, with the highest concentration of Pb, the grown vetiver plant had the highest Pb uptake. The same was true for the vetiver grown in the W4 with the highest concentrations of Mn and Cu. Its Mn and Cu uptakes were higher than those grown in the other wastewater sources.

The removal efficiency (%) is defined as the ratio of heavy metal uptake to the amount of original heavy metal in wastewater. The vetiver grown in W1 with the highest plant growth, had the highest Mn, Fe, Zn and Pb removal efficiencies of 33.72, 27.63, 52.73 and 8.94 %, respectively (Figure 4), whereas the vetiver grown in W4 had efficiency up to 87.5% Cu removal from the wastewater. However, the vetiver grown in W4 appeared unhealthy with stunted plant, few tillers and whitish-yellow old leaves. Roots were stunted, cracked and brown. This was probably caused by Cu toxicity as its principal effect is on root growth[15,16]. Among the three vetiver ecotypes, the Sri Lanka had the highest removal efficiencies for all studied heavy metals (Figure 5).

Conclusions

Phytoremediation for industrial waste water polluted with heavy metals could be carried out using vetiver technology. The quality of wastewater, and especially the concentration of heavy metals, had a significant effect on vetiver growth and the uptake of heavy metals. The Sri Lanka ecotype performed best in heavy metal uptake among the three studied vetiver ecotypes. In practice, vetiver could be grown in a hydroponic system using a floating platform, such as a bamboo or PVC pipe raft, to achieve heavy metal uptake to the shoot and root. This system enables the shoot and root to be easily harvested. The vetiver shoot may be used for Bio-fuels or handicraft products, while the root part could be a source of essential oils. High

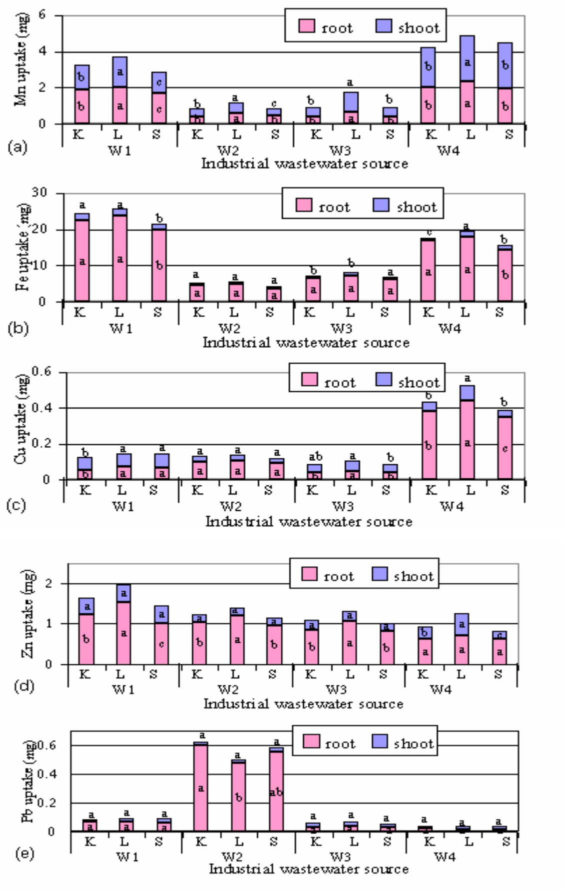

Fig 3. Uptake of Mn (a), Fe (b), Cu (c), Zn (d) and Pb (e) in shoot and root of Kamphaeng Phet-2 (K), Sri Lanka (L) and Surat Thani (S) vetiver ecotypes grown in industrial wastewater from milk factory (W1), battery manufacturing plant (W2), electric lamp plant (W3) and ink manufacturing facility (W4). [Histograms of the same plant parts and same sources of wastewater with a common letter are not significantly different at the 0.05 probability by DMRT.]

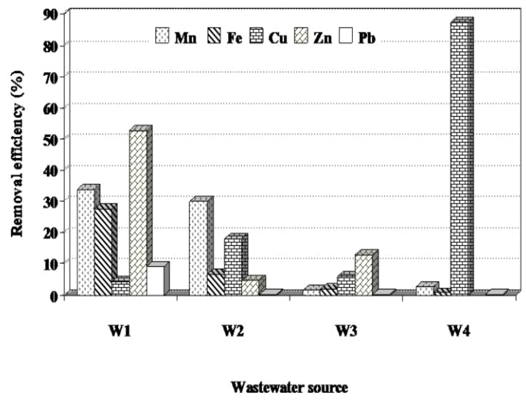

Fig 4. Average Mn, Fe, Cu, Zn and Pb removal efficiencies of vetiver grown in industrial wastewater taken from milk factory (W1), battery manufacturing plant (W2), electric lamp plant (W3) and ink manufacturing facility (W4).

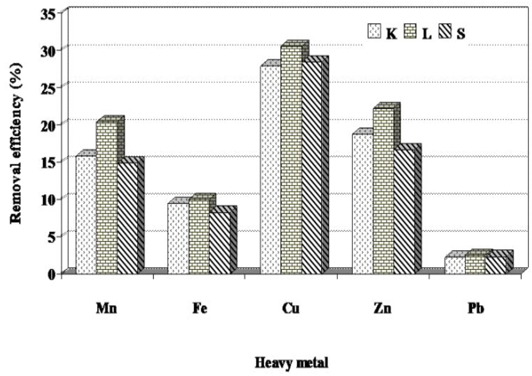

Fig 5. Average Mn, Fe, Cu, Zn and Pb removal efficiencies of three vetiver ecotypes, Kamphaeng Phet-2 (K), Sri Lanka (L) and Surat Thani (S) vetiver ecotypes grown in industrial wastewater.

concentrations of heavy metals could also affect plant growth and cause low removal efficiency, therefore, this method would be suitable after pre-treatment or in low-medium heavy metal polluted wastewaters. The number of vetiver plants used for the clean-up of wastewater should be of a sufficient quantity to reduce the concentrations of heavy metals to the industrial effluent standards, otherwise, re-cultivation may be necessary to achieve the good results.

Acknowledgments

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