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Bioremediation of Heavy Metals from the Land Application of Industrial Sewage Sludge with Minari (*Oenanthe stolonifer* DC.) Plant

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ABSTRACT

Laboratory experiments for the removal efficiency of heavy metals in land application of sludge, the accumulation and translocation of heavy metals in x plants after transplanting, and the responses of Minari growth with different ratio of land application of sludge were conducted to determine the potential ability of bioremediation with Minari plants. The removal rate and translocation of copper, zinc, lead, and cadmium in soil and plants were compared after transplanting the Minari plants to soil treated with different ratio of sludge. The removal efficiency of heavy metals in soil incorporated with sludge was different with application ratio, but increased with growing periods of Minari plants. The removal efficiency of Cu, Zn, Pb, and Cd ranged from 67 to 74%, from 51 to 63%, from 37 to 71%, and from 15 to 25% after 45 days of transplanting, respectively. The amount removed the copper value, 65.9 mg/kg, observed to be highest in soil incorporated 3% sludge after 45 days. The translocation of Cu, Zn, Pb, and Cd from shoots to roots ranged from 18 to 53%, from 17 to 32%, from 14 to 49%, and from 23 to 38% over growing periods, respectively. In plant responses, it appeared to be inhibited the plant growth in the treatment compared with the control at early stage of growth. However, the fresh weights of Minari plant increased from 12.5 to 62.5% in the sludge application after 45 days relative to the control. Therefore the Minari might play a useful role in bioremediation of Cu, Zn, Pb, and Cd in the land application of sludge.

Key words: bioremediation, removal efficiency, translocation, *Oenanthe stolonifer* DC.

INTRODUCTION

Increased environmental concerns over land application of sludge have stimulated interest in the green remediation technique that accumulates the heavy metals in the growing plants. Land application of sludge can have both beneficial and harmful aspects. Its organic matter constitutes approximately 50 % of solids, may improve soil physical and chemical properties(Bengtson and Cornette, 1973; Epstein, 1974). Currently, 25% of municipal sewage sludge produced in the U. S. is recycled through land application, although most is used at low annual application rates for agricultural production. However, sludge often contain undesirable chemicals which may be toxic to plants and/ or eventually toxic to animals and humans that consume edible parts of the plants(Chaney, 1973). Water hyacinth(*Eichhorina crassipes*), a prolific aquatic weed of world wide distribution, has shown some promise in the biological management of aquatic pollutants because of the plant' s ability to absorb and concentrate a variety of metals which include lead, cadmium, mercury, copper, and nickel etc.(Wolverton and McDonald, 1978; Muramoto and Oki, 1983; Lee and Hardy, 1987). Furthermore, it has been shown that this plant may be used as a bioassay to monitor low levels of aquatic cadmium(Rosas et al., 1984) and mercury(Panda et al., 1988). The resistant plants of heavy metals ware commonly known as a Giant-duckweed(*Lemna polyrhiza* L.) (Lenka et al., 1990), Minari (*Oenanthe Stolonifer* DC.) (Na and kwon, 1995), and Azola (Reddy et al., 1988).

Studies on the removal of heavy metals in the soil treated with sludge have been associated with green remediation and we believe that Minari plant has potential to serve in this capacity.

The objectives of our studies were to determine 1) the removal efficiency of heavy metals in land application of sludge with Minari plants, 2) the accumulation and translocation of heavy metals in Minari plants after transplanting, and 3) the responses of Minari growth with different ratio of land application of sludge.

MATERIALS AND METHODS

The soil was obtained from the Sang-Ji University Research Farm in Woun-Ju, Kangwoun Do. Field crops were grown continuously for more than 10 years prior to sampling soil. Soil was collected from plowable layer, 0 to 10cm and transported to the experimental greenhouse where they were air-dried and sieved through 2mm screen to separate out the larger pebbles and rocks. Sludge used was collected from industrial waste water management plant in MounMark, WounJu at August, 1997. Sludge was taken from air-dried storage pile that had been stored for 3months and the bulk sample was crushed and sieved (< 2mm) prior to analysis and used in this experiments. Chemical characteristics of soil are presented in Table 1 and concentrations of heavy metals of a different ratio of sludge incorporated were shown in Table 2. Nodule of Minari was transplanted in the nursery containing a sterilized sand. Plants from established roots were selected for uniformity and transplanted in perforated 250 *ml* styrofoampots containing a different ratio of sludge applications. Appropriate amounts of the sludge were throughly mixed with soil to be equivalent to 1, 3, 5, and 7% application rate. All pots were irrigated 2 cm deep with deionized water as needed until the date for harvesting. Minari was grown in the plant culture room of department under natural conditions, 14 h of day length cycle, 20,000 Lux of light intensity, 25 ± 2 ℃ of culture room temperature. Following a culturing periods of 15, 30, and 45 days, plants and soil were removed from each pot, and measured the fresh weight of plant after throughly rinsed with deionized water at 3 times. The plant and soil in polyethylene bag were stored in cold room at 4 ℃ for the chemical analysis after placed in a dry oven at 75 ℃ for 3 days. Soil pH (1:5, soil : water) was measured by Expandable Ion Analyzer EA940, organic matter with potassium dichromate oxidation procedure of Walkely and Black (Allison, 1965), exchangeable cations by extracting the soil with NH_4OAC (Chapman and Pratt, 1965) and heavy metals in soil and plant with EPA Method 3050 by atomic absorption spectrophotometer. The experimental design was completely randomized design with treatment classes of 4 sludge application rate and 3 plant culturing periods with four replications. The data were subjected to an analysis of least significant difference and Duncan's multiple range tests were calculated to compare the treatments.

Table 1. Chemical properties of soil used.

pH (1 :5)	E.C (ds/m)	O.M (%)	P_2O_5 (mg/kg)	Exchangeable Cations K	Ca	Mg
				-------cmole/kg-----		
6.94	0.0125	0.18	307	0.17	4.8	9.8

Table 2. Heavy metal concentration in soil mixed with a different ratio of sludge before transplanting.

Treatment	Cu	Zn	Pb	Cd
	------------ mg/kg(d.w) ------------			
Soil	13.30	72.50	20.33	1.35
Sludge-1%	39.93	167.75	23.53	1.58
Sludge-3%	88.08	324.00	26.20	3.03
Sludge-5%	103.90	387.50	31.20	3.53
Sludge-7%	138.53	416.75	35.78	4.63

RESULTS AND DISCUSSIONS

Removal efficiency of heavy metals in soils.

Residues of heavy metals in the soil incorporated with different ratio of sludge after transplanting are illustrated in Fig. 1. There was a significant difference in concentrations of heavy metals to soil incorporated with sludge among the treatments ($P \leqq 0.01$) and days after transplanting ($P \leqq 0.01$). The experiments indicated that heavy metals concentrations were increased with different ratio of sludge

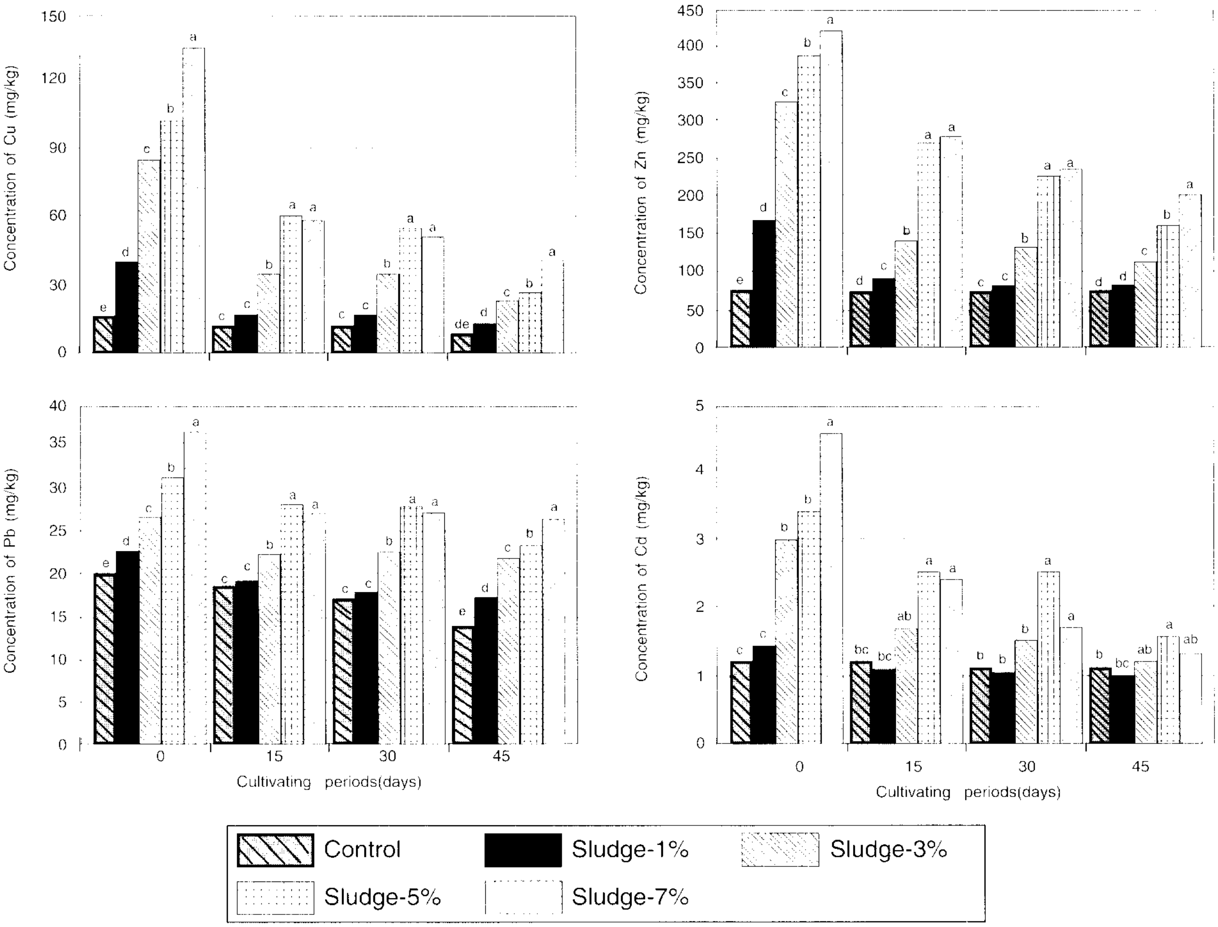

Figure 1. Residues of heavy metals in the soil incorporated with a different ratio of sludge after transplanting, valules with different letters differ signficantly at the 0.05 probability level.

incorporated although no consistently increased among the treatment but the concentrations of Cu, Zn, Pb, and Cd were 138.5, 416.7, 35.8 and 4.6 mg/kg in soil incorporated with 7% sludge, respectively. This trend concur with the results of previous reports although there is a sizable range in heavy metals concentrations reported for different ratios of sludge incorporated (Lee, 1994). In general, the concentrations of heavy metals in the soil incorporated with sludges were greatly decreased with time up to 15 days and slowly from 15 to 30 days after tranplanting (Fig. 1). The Cu and Cd levels might be slightly higher than the regulated heavy metal concentration of soil contamination in the preservative law of soil and environment such as 125, 300, and 4 mg/kg for Cu, Zn, and Cd, respectively (Agriculture ministry, 1997). The amount of Cu, Zn, Pb, and Cd in the soil with 7% sludge application after 15 days were well below the regulated heavy metal concentrations and it was observed to be 58.3, 278.5, 31.8, and 2.4 mg/kg as 58, 33, 11, and 48% for removal efficiency with Minari plants, respectively (Table 3). Generally the removal efficiency was increased with times after transplanting, and shown to be highest as 75, 63, 25, and 71% for Cu, Zn, Pb and Cd in the 3% sludge application after 45 days. The removal efficiency of heavy metals in soil incorporated with sludge was different with application ratio, but increased with growing periods of Minari plants. The removal efficiency of Cu, Zn, Pb and Cd ranged from 67 to 74%, from 51 to 63%,

Table 3. Removal efficiency of heavy metals in soil incoporated a different ratio of Minari plants

Heavy metals	Treatments	After transplanting (days)		
		15	30	45
			%	
Cu	Control	6.9±1.13[a]	10.2±1.82	29.3±0.75
	Sludge 1%	60.3±0.54	64.4±5.68	67.8±4.85
	Sludge 3%	63.5±1.25	63.3±5.34	74.9±5.37
	Sludge 5%	42.9±3.24	47.5±0.64	72.7±2.83
	Sludge 7%	57.9±1.66	63.4±1.38	70.3±1.38
Zn	Control	2.4±0.78	3.7±1.47	16.5±1.08
	Sludge 1%	43.1±3.87	52.5±6.41	54.8±4.17
	Sludge 3%	57.5±3.23	59.3±1.08	63.1±3.17
	Sludge 5%	30.9±0.89	41.4±0.26	57.6±1.04
	Sludge 7%	33.2±3.93	43.3±4.71	51.5±4.96
Pb	Control	7.5±1.14	15.5±1.39	29.0±2.36
	Sludge 1%	19.3±2.85	22.7±2.99	24.7±5.22
	Sludge 3%	12.6±1.78	12.9±1.92	15.9±2.86
	Sludge 5%	3.8±0.52	11.4±5.25	23.6±4.57
	Sludge 7%	11.2±1.27	23.3±1.59	25.5±1.60
Cd	Control	3.0±1.40	11.1±1.70	16.3±1.86
	Sludge 1%	16.3±1.25	29.8±3.96	37.3±1.12
	Sludge 3%	43.6±2.06	49.5±4.25	57.8±3.30
	Sludge 5%	28.9±1.06	30.9±0.36	54.7±5.67
	Sludge 7%	48.4±3.51	52.9±3.48	71.3±4.59

[a] Means are standard deviations of three replications

from 37 to 71%, and from 15 to 25% after 45 days, respectively. The amount removed the copper value, 65.9 mg/kg, observed to be highest in soil incorporated 3% sludge after 45 days.

In view of its possible practical utility, the Minari plants might be put to use for the removal of Cu, Zn, Pb, and Cd from soil incorporated with 3-5% sludge, and therefore can play a useful role in bioremediation of heavy metal pollution.

Accumulation and translocation of heavy metals in plants

Accumulation and translocation of Cu, Zn, Pb, and Cd in the Minari plant after transplanting are illustrated in Table 4 and 5. In view of accumulations of Cu, Zn, Pb, and Cd in plant, they were different with application ratio of sludge, but increased with time after transplanting. The concentrations of Cu, Zn, Pb, and Cd ranged from 17.1 to 27.8mg/kg, from 60.0 to 116.9mg/kg, from 8.0 to 13.3mg/kg, and from 3.0 to 5.7mg/kg in shoots, and from 23.7 to 87.5mg/kg, from 211.8 to 340.3mg/kg, from 13.7 to 49.8mg/kg, and from 3.0 to 5.7mg/kg in roots, respectively. The translocation rate of Cu, Zn, Pb, and Cd from roots to shoots ranged from 18 to 53%, from 17 to 32%, from 14 to 49%, and from 23 to 38% over growing periods.

Copper concentrations in the shoots at 5 and 7% sludge application after 15 and 30 days are observed to be lower than 20% and high in the all pots after 45 days irrespective with incorporated ratio of sludge compared with the untreated pot. Its concentrations in the roots was 87.5mg/kg in the 1% sludge application, 83.0 and 46.0mg/kg in the 7% sludge application after 15, 30, and 45 days, respectively. The translocation rate of copper from the roots to shoots was not significantly different and observed approximately 20% among the application ratios of sludge after 15days, but it is decreased after 30 days with increasing the rate of sludge application.

The zinc concentrations in the shoots are decreased as the rate of sludge applications and date after transplanting are increased. Its concentrations were appeared to be 116.9mg/kg as a most high values in the 1% sludge application

Table 4. Accumlation and translocation of Cu and Zn in the Minari plant after transplanting.

Treatment	Days after transplanting	Cu Tops -mg/kg(d.w)-	Cu Roots -mg/kg(d.w)-	Trans.[a] %	Zn Tops -mg/kg(d.w)-	Zn Roots -mg/kg(d.w)-	Trans. %
Control	15	22.1a	32.3d	40.6	58.1cd	109.0d	34.8
Sludge-1%	15	24.1a	87.5a	22.0	86.9a	339.8a	20.4
Sludge-3%	15	23.7a	84.3a	21.9	74.6b	340.3a	18.0
Sludge-5%	15	17.1b	60.5b	22.0	60.0c	283.7b	17.5
Sludge-7%	15	17.4b	55.8c	23.8	64.0c	212.5c	23.1
Control	30	25.9b	26.0d	49.9	57.4d	115.0c	33.3
Sludge-1%	30	31.9a	51.0c	38.5	77.4a	333.2a	18.8
Sludge-3%	30	25.5b	45.2c	36.1	79.9a	323.5a	19.8
Sludge-5%	30	20.6c	70.0b	27.7	71.3b	281.2b	20.2
Sludge-7%	30	18.6c	83.0a	18.3	66.6c	319.2a	17.3
Control	45	11.8c	22.8d	34.1	30.5e	115.7d	20.9
Sludge-1%	45	27.1a	23.7d	53.4	116.9a	238.2b	32.9
Sludge-3%	45	27.8a	37.7c	42.4	97.3c	211.8c	31.5
Sludge-5%	45	24.3a	42.2b	37.1	104.5b	269.7a	27.9
Sludge-7%	45	18.0b	46.0a	28.1	88.1d	214.0c	29.2

[a] Translocation of heavy metals from the roots to the shoots

a-d Within each cultivating periods, means followed by the same letter are not significantly diffrent at the 0.05 probability level.

Table 5. Accumlation and translocation of Pb and Cd in the Minari plant after transplanting.

Treatment	Days after transplanting	Cu Tops -mg/kg(d.w)-	Cu Roots -mg/kg(d.w)-	Trans.[a] %	Zn Tops -mg/kg(d.w)-	Zn Roots -mg/kg(d.w)-	Trans. %
Control	15	13.0a	17.3d	42.9	2.1a	4.0b	34.4
Sludge-1%	15	8.2c	49.8a	14.1	2.0a	5.7a	26.0
Sludge-3%	15	10.0b	36.8b	21.4	2.1a	5.0ab	29.6
Sludge-5%	15	11.5b	37.7b	23.4	2.1a	4.7b	30.9
Sludge-7%	15	8.0c	28.7c	21.8	1.6b	3.0c	33.3
Control	30	12.4a	28.3c	30.5	2.5a	5.2a	32.5
Sludge-1%	30	9.3bc	33.2b	21.9	2.4a	5.0a	32.4
Sludge-3%	30	10.6b	25.3cd	29.5	1.6b	3.8b	29.6
Sludge-5%	30	11.6ab	33.8b	25.6	1.7b	3.7b	31.5
Sludge-7%	30	12.8a	37.3a	25.6	1.4c	4.7a	23.0
Control	45	9.7b	24.3b	28.5	2.3a	5.0a	31.5
Sludge-1%	45	13.3a	13.7d	49.3	1.6b	3.8b	29.6
Sludge-3%	45	8.7bc	19.2c	31.2	2.0a	3.2b	38.5
Sludge-5%	45	8.1bc	28.8a	22.0	2.0a	3.3b	37.7
Sludge-7%	45	9.2b	30.0a	23.5	1.7b	3.7b	31.5

[a] Translocation of heavy metals from the roots to the shoots

a-d Within each cultivating periods, means followed by the same letter are not significantly diffrent at the 0.05 probability level.

after 45 days compared with the other treatments. This concentrations were higher than 110 mg/kg in the lettuce shoots cultivated from soil incorporated with sludge(Lenka et al., 1990). Its concentrations in the roots after 15 days at 1 and 3% sludge applications were most high as approximately 340mg/kg. However, the tranlocation rate from roots to shoots observed to be low when compared with the control after 45 days(Table 4).

Except for lead concentrations in the shoots at 1% sludge applications after 45 days, the lead and cadmium concentrations were low over the growing periods, compared with the control. Especially, the lead and cadmium concentrations in the 1% of sludge application were 13.3 and 3.8 mg/kg in the shoots after 45 days, and these values were above the concentrations of 10mg/kg for Pb and 3mg/kg for Cd based on the tolerance levels for agronomic crops. Therefore, Minari plant might be described as the tolerance plant for Pb and Cd according to the foliar concentrations of these metals for tolerance limits indicated by Lenka et al(1990).

Responses of Minari plants in soil incorporated with a different sludge ratio.

The fresh weight was consistently high in the 4% of sludge application over the growing periods, but except for this treatment it was low in the other until 30 days of transplanting when compared with the control (Table 6). Overall, the fresh weights of Minari plants were increased from 12.5 to 62.5% after 45 days in a different ratios of sludge applications. The data indicated that they were agreements with decreasing the Cu, Pb, and Cd concentrations in the plants after 30 days with increasing the rate of sludge incorporated.

Table 6. Responses of Minari growth with a different ratio of sludge application to soil.

Treatment	Fresh weight (g/pot)		
	15 DAT	30 DAT	45 DAT[b]
Control	1.2 ± 0.14[a]	3.8 ± 0.16	4.8 ± 0.34
Sludge-1%	0.8 ± 0.17	3.2 ± 0.86	5.4 ± 0.83
Sludge-3%	1.1 ± 0.43	3.4 ± 0.22	5.5 ± 0.54
Sludge-5%	1.7 ± 0.21	4.3 ± 0.62	5.9 ± 0.48
Sludge-7%	2.4 ± 0.69	4.7 ± 0.71	7.8 ± 0.53

[a] Means are standard deviations of four replications

[b] Days after transplanting

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